Dietary interventions to reduce Advanced Glycation Endproducts (AGEs)

Nicole J Kellow
Bachelor of Science (1996)
Master of Nutrition and Dietetics (1998)
Graduate Certificate in Diabetes Education & Health Care (2009)

A thesis submitted for the degree of Doctor of Philosophy at
Monash University in 2016

Department of Public Health & Preventive Medicine
Monash University

&

Glycation, Nutrition & Metabolism Laboratory
Baker IDI Heart & Diabetes Institute

Copyright notice

Abstract:

Dietary interventions to reduce Advanced Glycation End-products (AGEs)

Advanced glycation endproducts (AGEs) are formed inside the body when reducing sugars attach to proteins. AGE formation and/or accumulation is increased in people with elevated blood glucose levels, excessive inflammation and oxidative stress or impaired kidney function. Elevated AGE levels in the bloodstream and in body tissues may contribute to the development of obesity, type 2 diabetes and cardiovascular disease. Many highly heated processed foods contain large concentrations of AGEs, which are partially absorbed by the gastrointestinal tract, contributing to the body's total AGE content.

A proportion of diet-derived AGEs reach the colon and could be fermented by gut bacteria. Therapeutic manipulation of the gut microbiota composition and restoration of beneficial microbial species could potentially reduce circulating AGE levels and improve the metabolic health of individuals at risk for the development of type 2 diabetes. Regular dietary consumption of prebiotics (non-digestible plant-derived fibres) promotes the growth of beneficial gut microbiota and may represent a treatment strategy for individuals with excessive AGE accumulation who are at risk for chronic disease.

This PhD project aimed to investigate the effect of diet on advanced glycation in humans, and determine whether consumption of a prebiotic dietary supplement can reduce circulating AGE levels, improve glucose control and biochemical markers of inflammation and oxidative stress in adults with pre-diabetes. First, a review of the current evidence base was performed to explore the relationship between dietary AGE

intake and chronic low-grade inflammation, and avenues for future nutrition research were suggested. Second, a systematic review of RCTs investigating the effect of low-AGE diets on circulating AGE levels and markers of metabolic health was performed. This review concluded that there is insufficient evidence at present to recommend dietary AGE restriction to any patient group. This was due to the poor quality of most existing trials, their short duration, participant heterogeneity and use of non-validated methods to measure serum and food-derived AGEs.

Third, a cross-sectional study was undertaken to ascertain whether habitual dietary and lifestyle factors influence long-term tissue AGE accumulation in healthy adults. The study found positive associations between tissue AGE levels and chronological age, cigarette smoking, waist circumference and dietary intake of meat and meat products. Further research is required to determine whether frequent consumption of foods containing large quantities of dietary AGEs contribute to tissue AGE deposition in healthy individuals.

Fourth, a systematic review of high quality trials exploring the cardiometabolic benefits of dietary prebiotic supplementation was conducted. The review found that dietary prebiotic consumption in healthy adults was associated with subjective improvements in satiety and significant reductions in postprandial glucose and insulin levels. These findings lead to the hypothesis that prebiotic-induced changes in the human gut microbiota may improve metabolic health which could subsequently reduce endogenous AGE production and exogenous AGE absorption.

Finally, a randomised, placebo-controlled crossover trial was undertaken to determine the effect of twelve-week consumption of a prebiotic dietary supplement on circulating and urinary AGE levels, insulin resistance and inflammatory biomarkers in 27 adults with pre-diabetes. Prebiotic consumption significantly reduced waist circumference, urine albumin excretion and increased HDL-cholesterol levels. There were no significant changes in serum or urinary AGE levels, insulin resistance or markers of inflammation. Longer term intervention studies are required to determine whether this is sufficient to prevent or slow the development type 2 diabetes.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature:

Print Name: Nicole Kellow

Date: 20/10/16

Thesis including published works General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 4 original papers published in peer reviewed journals and 2 original submitted publications. The core theme of the thesis is reduction of Advanced Glycation Endproducts (AGEs) by dietary factors. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Public Health & Preventive Medicine (Monash University) and the Glycation, Nutrition & Metabolism Laboratory (Baker IDI) under the supervision of Professor Christopher Reid and Associate Professor Melinda Coughlan. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 2, 3, 4, 5, 6 and 7 my contribution to the work involved the following:

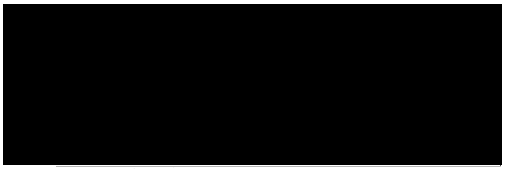
Thesis chapter	Publication title	Publication status	Nature and extent (%) of students contribution
2	Effect of diet derived advanced glycation endproducts on inflammation.	Published	Conducted literature search and drafted 70% of manuscript (75%)
3	Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review.	Published	Conducted literature search, quality assessment of trials, statistical analysis and drafted manuscript (99%)
4	Association between habitual dietary and lifestyle behaviours and skin autofluorescence (SAF), a marker of tissue accumulation of advanced glycation endproducts (AGEs), in healthy adults.	Under Review	Study design, ethics application, participant recruitment, data collection, statistical analysis and drafted manuscript (95%)
5	Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials.	Published	Conducted literature search, quality assessment of trials, statistical analysis and drafted manuscript (99%)
6	Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial	Published	Study design, conducted literature search and drafted manuscript (95%)
7	Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes. A randomised placebo-controlled crossover trial.	Submitted	Study design, ethics application, participant recruitment, data collection, laboratory analyses, statistical analysis and drafted manuscript (85%)

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 20/10/16

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student and co-authors' contributions to this work.

Main Supervisor signature: **Date:** 07/11/16



Acknowledgements

To my PhD supervisors Professor Chris Reid and Associate Professor Melinda Coughlan: thank you for your dedication to student teaching, it was indeed a privilege to work with you both. I have been very fortunate to have such excellent research role models, and learn from the best! I am grateful you were always calm when I needed reassurance, and always provided options whenever I had problems.

Thank you to Dr Karly Sourris and the rest of the team at the Glycation, Nutrition & Metabolism Laboratory at Baker IDI. You all welcomed me into the lab and were always happy to answer my millions of questions and laugh at my jokes.

To my husband Brendan Kellow: thank you for your love, humour, grace, understanding, patience and encouragement. You are my moral compass, my practical advice giver and awesome pancake cooker. I am so grateful to have had you by my side to ride the highs and lows of this PhD with me, while remaining unfailingly supportive and inspiring.

To Barbara McRae (1952-2016): a very dear friend and mentor who shared her passion for diabetes care, believed in me and encouraged me to undertake further study and commence diabetes-related research. Thank you for your enthusiasm, time and wisdom. Although you were unable to see me complete my PhD, you never doubted my ability to get there in the end.

Thank you to the National Health and Medical Research Council (NHMRC) for supporting me with a Postgraduate Public Health Scholarship APP1039709 (2012-2015).

This PhD thesis is dedicated to my parents, Kaye & Barrie Hahne,
for giving me opportunities they could only dream of

Table of Contents:

Figures & Tables:

List of Abbreviations:

AGE: Advanced Glycation Endproduct

BMI: Body mass index

CML: Carboxymethyllysine

CRP: C-reactive protein

ELISA: Enzyme-linked immunosorbent assay

FOS: Fructo-oligosaccharide

GLP: Glucagon-like protein

GPR: G-protein coupled receptor

GI: Glycemic index

HDL: High density lipoprotein

HOMA-IR: Homeostasis model assessment – insulin resistance

LC-MS/MS: Tandem mass spectrometry – high performance liquid chromatography

LDL: Low density lipoprotein

LPS: Lipopolysaccharide

MG: Methylglyoxal

RAGE: Receptor for Advanced Glycation Endproducts

ROS: Reactive oxygen species

SAF: Skin autofluorescence

SCFA: Short chain fatty acid

T2DM: Type 2 diabetes mellitus

TG: Triglyceride

Chapter 1:

Introduction

Advanced glycation endproducts (AGEs) are formed by the Maillard Reaction endogenously and in heated foods when aldehyde groups of reducing sugars non-enzymatically react with amino moieties on proteins, phospholipids and nucleic acids [(1)]. The initial glycation products formed during these reactions (known as Schiff bases and Amadori products) are potentially reversible, and include well-known intermediates such as HbA1c and fructosamine. Over time however, a series of further oxidation, degradation, condensation and reduction reactions occur to form late-stage, irreversible AGEs [(2)]. Apart from the Maillard Reaction, glucose auto-oxidation and lipid peroxidation pathways generate highly reactive dicarbonyl compounds (such as glyoxal, methylglyoxal and 3-deoxyglucosone) [(3)], which then react with proteins to form AGEs.

The AGE family are a group of heterogeneous, largely uncharacterised compounds which include carboxymethyllysine, CML (a pre-melanoidin which is not coloured, not fluorescent and does not cross-link with other proteins), carboxyethyllysine, CEL (not fluorescent, not cross-linked), vesperlysine (fluorescent, cross-linked), pyrraline (not fluorescent, not cross-linked), pentosidine (a melanoidin which is yellow-brown in colour, fluorescent and cross-linked), crossline (fluorescent, cross-linked) and methylglyoxal-hydroimidazalone, MG-H1 (not fluorescent, cross-linked) [(4)].

The concentration of AGEs in the human body increases with aging, and AGE modification of proteins is considered a normal consequence of cellular aging [(5)]. It is thought that AGE modification of proteins represents a mechanism of identification of senescent proteins which require degradation [(6)]. However, elevated AGE levels are known for their role in the development of diabetes-related complications. Brownlee

(2001) hypothesised that hyperglycemia-induced mitochondrial superoxide overproduction increased the accumulation of glycolytic intermediates, which feed into the AGE pathway (Figure 1) [7]. Excessive glucose can also be converted to fructose (a potent reducing sugar) via the polyol pathway, promoting further AGE formation. Indeed, high blood glucose levels associated with poor glycemic control in individuals with diabetes provides additional reducing sugars to encourage protein glycation [8]. More rapid pathways to AGE formation also exist which do not require increased glucose concentrations. Oxidation of glucose and fatty acids to form reactive carbonyl compounds (AGE precursors) occur in situations of increased inflammatory, oxidative and carbonyl stress (Figure 2) [9]. Moreover, hyperlipidemia increases the fatty acid substrate available for AGE generation.

Figure 1: The unifying theory of diabetes complications (Brownlee, *Nature* 2001)[7]

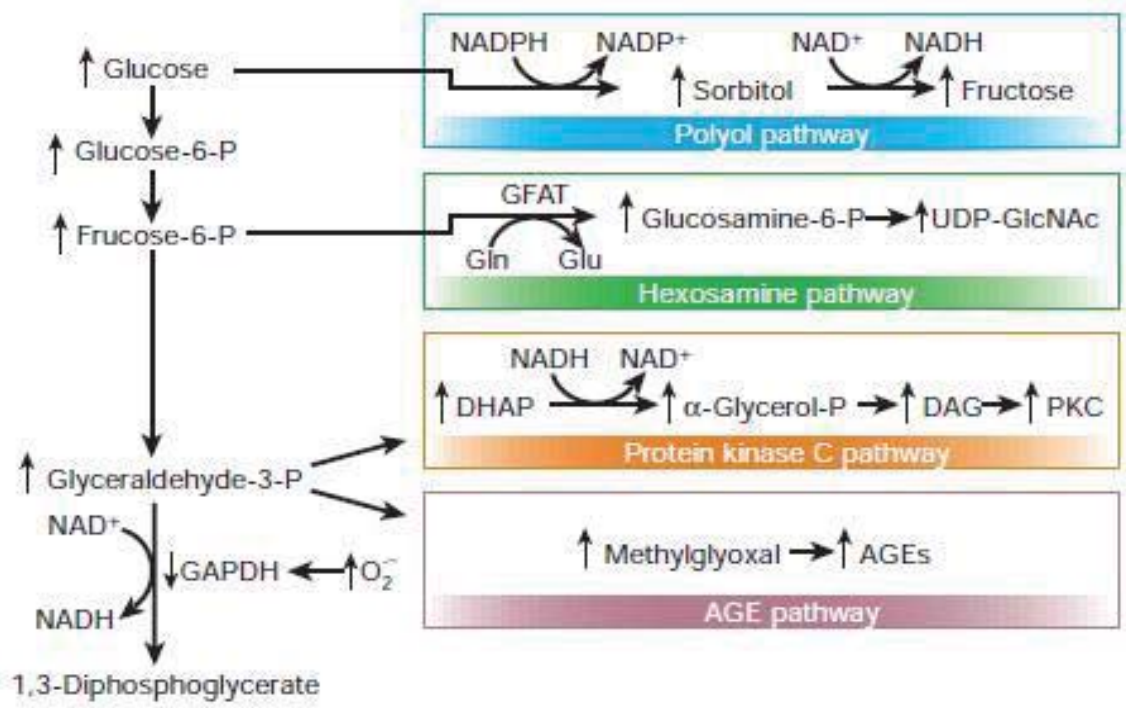
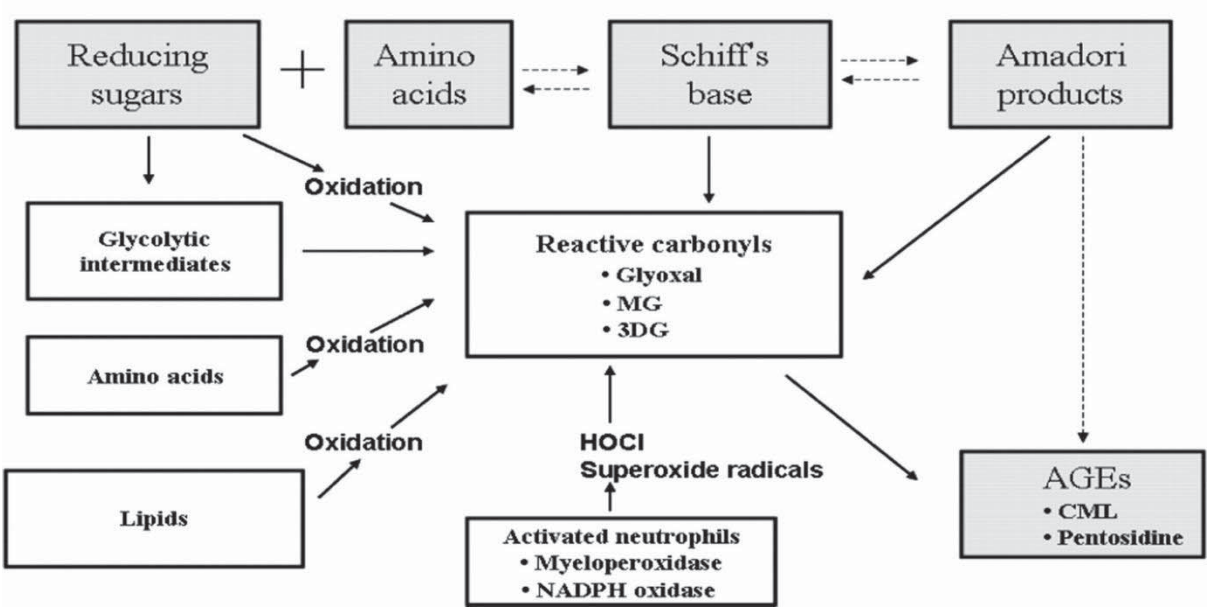


Figure 2: Endogenous AGE generation (Uribarri *et al*, *Clin J Am Soc Nephrol* 2006)[9]. Dotted lines depict the classical Maillard reaction pathway.



Excessive AGEs contribute to the development of vascular complications in people with diabetes through a variety of mechanisms. Firstly, by reacting with intra- and extracellular proteins, AGEs alter protein structure and function. AGE modification of proteins can impair wound healing, reduce tissue elasticity, alter cellular movement and adhesion properties and disrupt communication between cells [10, 11]. AGE-induced crosslinking of extracellular matrix proteins increases the stiffness and rigidity of vessels contributing to sclerosis of renal glomeruli, thickening of the capillary basement membrane and the development of atherosclerosis [12]. AGEs on vascular matrix proteins mediate defects in the vasodilatory response by inactivating nitric oxide [13]. The amino groups of DNA bases adenine and guanine are also susceptible to glycation. AGE formation on DNA can induce depurination, single strand breaks and an increased frequency of mutations [14].

The interaction of AGEs with the cell surface receptor for AGEs (RAGE) has been extensively studied. RAGE is expressed in a range of cell types, including endothelial cells, neurons, smooth muscle cells and macrophages. Ligation of high molecular weight AGEs to RAGE generates intracellular reactive oxygen species, which trigger the sustained activation of nuclear factor-kappa B (NF-κB) [15]. NF-κB translocates from the cytoplasm to the nucleus, stimulating an intracellular cascade of reactions which upregulate the expression of pro-inflammatory cytokines (interleukin-1, interleukin-6, tumour necrosis factor-alpha), chemokines (monocyte chemoattractant protein-1), pro-coagulants (thrombomodulin), growth factors (transforming growth factor-β1, connective tissue growth factor, VEGF) and adhesion molecules (E-selectin, vascular cell adhesion molecule-1, intracellular adhesion molecule-1) [16]. The ligation of AGEs to RAGE also results in NADPH oxidase and mitochondrial ROS generation. Increased ROS production and inflammation as a consequence of AGE-RAGE interaction in turn stimulates further AGE production, creating a vicious cycle of cellular dysfunction. Finally, AGEs themselves are able to act as stable active sites for catalysing the formation of free radicals [17], contributing to tissue damage and acceleration of the aging process.

Exogenous AGEs

Outside of the body, AGEs formed during the curing of tobacco enter the body during cigarette smoking, which is thought to contribute to the increased risk of atherosclerosis observed in smokers [18]. AGEs generated by the Maillard reaction during the heat treatment of food products containing sugars and/or lipids and proteins enter the body when these foods are consumed. Browning of food during cooking is used to enhance quality, flavour, colour and aroma [19]. Factors which enhance AGE

formation in foods include high lipid and protein content, low water content during cooking, elevated pH and the application of high temperatures over a short time period [20]. More AGEs are generated in foods exposed to dry heat (grilling, frying, roasting, baking and barbecuing) than foods cooked at lower temperatures for longer time periods in the presence of higher water content (boiling, steaming, poaching, stewing or slow cooking) [21].

Heat processing is often used by the food industry during food production in order to improve food safety and prolong shelf-life by destroying harmful bacteria [22]. Foods thought to contribute large quantities of AGEs and carbonyl compounds (AGE precursors) to the diet include powdered milk and cheese, meats, fish and chicken cooked by dry heat [23], heat processed or alkaline-treated cereal-based products (bread, biscuits, bakery products, extruded breakfast cereals) [3], sweet sauces [24], honey [25] and carbonated soft drinks containing high fructose corn syrup [26].

The AGE content of a range of commonly consumed foods has been estimated using immunoassay (ELISA based on an anti-CML antibody) [21] and tandem mass spectrometry-high performance liquid chromatography (LC-MS/MS) [23, 27] techniques. These values have been used to generate databases which enable the estimation of dietary AGE intake (Table 1). The specificity and reliability of various AGE measurement techniques is currently unknown, and different methods of dietary AGE quantification have produced conflicting results [28]. Databases also report AGE values in different units, making them difficult to compare.

Table 1: Comparison of high vs low AGE diets (created using database in Hull, *Food Chemistry* 2012)[27]

	High-AGE Meal Plan	CML Content (mg)	Low-AGE Meal Plan	CML Content (mg)
Breakfast	Instant coffee with sugar Cornflakes cereal (30g) Whole milk (1 cup)	0.15 1.01 0.14	Roasted coffee with sugar Natural Swiss muesli (30g) Skim milk (1 cup)	0.02 0.19 0.05
M/Tea	Full fat fruit yoghurt (200g)	2.77	Low fat fruit yoghurt (200g)	1.08
Lunch	Toasted wholemeal bread (80g) Cheddar cheese (50g) Tinned salmon in brine (90g) Salad (1/2 cup)	1.93 0.61 5.92 0.01	Soft brown bread roll (80g) Scrambled egg (50g) Tinned tuna in brine (90g) Salad (1/2 cup)	0.29 0.33 0.45 0.01
A/Tea	Roasted peanuts (50g) Orange (60g)	1.25 0.02	Raw almonds (50g) Apple (60g)	0.09 0.00
Dinner	Pork sausages, grilled (180g) Potato chips, microwave (80g) Mixed vegetables (100g) Chocolate cake (100g)	4.45 0.11 0.02 1.85	Lamb chops, grilled (180g) Mashed potato (80g) Mixed vegetables (100g) Cheesecake (100g)	0.53 0.03 0.02 0.59
Supper	Shortbread biscuit (40g)	2.18	Popcorn (1 cup)	0.22
	Total CML (mg): 22.42		**Total CML (mg): 3.90**	

Food AGE values can only be considered estimates as different foods vary in their amino acid composition, resulting in the formation of many different types of AGEs. Most research groups have traditionally measured CML as a surrogate marker for total AGE concentration, which is likely to underestimate the AGE content of foods containing low levels of lysine, such as wheat-based breads and cereals. Moreover, CML is derived from both glycation and lipid oxidation reactions, which overestimates the extent of glycation in foods with a high fat content [4].

Digestion, absorption and excretion of dietary AGEs

In order to be digested and absorbed, dietary peptides must be broken down by gastrointestinal and cell-surface peptidases into their constituent amino acids,

dipeptides and tripeptides before being absorbed across intestinal cell membranes by simple diffusion or via specific transporters [29]. Low molecular weight (<1000 Da) dietary AGEs and carbonyls are able to be gastrointestinally absorbed, whereas high molecular weight AGEs require further proteolytic degradation before becoming available for absorption. Highly crosslinked dietary AGEs demonstrate reduced protein digestibility as their structure is resistant to the action of intestinal enzymes [30].

It has been estimated that approximately 10-30% of dietary AGEs consumed are intestinally absorbed [31], with variable quantities of ingested AGEs subsequently excreted in urine and faeces [32]. In AGE balance studies, some dietary AGEs are almost fully recovered in urine and faeces [33], whereas others show minimal elimination and accumulate in tissues such as the kidney [34]. Plasma AGE concentration appears to be directly influenced in the short-term (1-4 hours after ingestion) by dietary AGE intake [35], but whether AGE ingestion is reflected in the circulation over the longer term continues to be a subject of debate [36, 37]. Urinary excretion of AGEs may therefore be a better indicator of dietary AGE absorption than plasma levels. Individuals with renal insufficiency demonstrate decreased urinary excretion of dietary AGEs, and plasma AGE levels inversely correlate with renal function [38].

Undigested AGEs pass through the gastrointestinal tract to the colon, where they are fermented by the resident microbiota [39]. Preliminary research suggests that AGE-modified proteins in the colon promote the growth of detrimental bacteria [40], diminish the growth of beneficial micro-organisms [41], and reduce microbial species diversity in

the large intestine [42]. Further research is required to further elucidate the effect of poorly digested AGEs on the composition of the gut microbiota.

AGEs in the pathogenesis of diabetes

While the association between elevated AGE formation and diabetes complications is well established, there is emerging evidence supporting the role of AGEs and their precursors in the pathogenesis of diabetes itself. Hyperglycemia is not the only driver of protein glycation, and significant AGE production can be induced by excessive oxidative stress, inflammation, hypoxia and dyslipidemia in the absence of increased blood glucose levels. Indeed, approximately twenty percent of individuals already have early stage retinopathy at the time of type 2 diabetes diagnosis [43], suggesting that pathological pathways involved in vascular complications have already developed prior to the onset of frank hyperglycemia. In type 1 diabetes, HbA_{1c} and diabetes duration explained only 11% of the variation in complications [44]. Moreover, individuals with prediabetes have a similar cardiovascular risk profile to those with established type 2 diabetes [45], implying that production of AGEs and other toxic mediators is not solely dependent on the current level of glycemia. The addition of methylglyoxal to the drinking water of euglycemic mice was sufficient to induce endothelial inflammation and atherogenesis to a similar extent as that observed in mice with diabetes and hyperglycemia [46].

Cross sectional studies show correlations between circulating AGE concentrations and insulin resistance [47, 48], impaired insulin secretion [49], inflammation [50], cardiovascular disease [51] and even incidence of type 2 diabetes [52]. However, other research groups have found no such associations [53]. Intervention studies in humans

and animal models demonstrate that reduction of AGE production (by either AGE inhibitors or an AGE-restricted diet) has ameliorated beta-cell destruction [54], insulin resistance [55] and inflammation [56] and delayed the onset of the metabolic syndrome [57].

Gut microbiota in health and disease

Over the last ten years, there has been renewed interest in the role of the gut microbiota in the development of chronic disease, with the finding that the composition of intestinal bacteria differs between lean and obese individuals [58], and between people with and without diabetes [59]. Alterations in the bacterial composition of the gut microbiota and reduced species diversity is now considered one of the contributors to the development of metabolic disturbances such as obesity, insulin resistance and type 2 diabetes.

Prebiotics are non-digestible plant-derived soluble fibres which confer health benefits to the host by acting as a fermentation substrate for bacteria in the colon, stimulating the preferential growth and activity of a limited number of beneficial microbial species [60]. Recent dietary prebiotic intervention studies have demonstrated positive effects on metabolic health by reducing low-grade inflammation, post-prandial blood glucose and insulin concentrations, insulin resistance, oxidative stress and dyslipidemia [61]. However, it is not known whether prebiotic-induced reductions in AGE concentrations provided some of these benefits.

While the interactions between advanced glycation, diet, intestinal microbiota and host metabolism are still being elucidated, no studies have investigated the effect of diet-

induced changes in gut bacteria on plasma, urinary and faecal AGE concentrations. This PhD project was designed to examine the influence of diet on advanced glycation in humans, and to investigate the effect of a prebiotic dietary supplement on AGE accumulation in adults diagnosed with prediabetes.

Research questions:

What is the effect of diet on advanced glycation in humans?

Can dietary consumption of a prebiotic supplement improve biomarkers of metabolic health and reduce circulating AGE levels in adults with pre-diabetes?

Subsidiary questions:

- What is the effect of dietary AGEs on chronic low grade inflammation?

- Do habitual dietary and lifestyle factors influence long-term tissue AGE accumulation in a large sample of healthy adults?

- Is there currently sufficient evidence to recommend therapeutic AGE-restricted diets in healthy or overweight individuals, people with diabetes or those with renal impairment for the improvement of glucose control, endothelial function and the reduction of biomarkers of inflammation and oxidative stress?

- What is currently known about the effects of dietary prebiotic supplementation on human cardiometabolic health?

- What is the effect of 12-week consumption of a prebiotic dietary supplement on circulating AGE levels, glucose control and inflammatory biomarkers in adults diagnosed with pre-diabetes?

Chapter 2:

Are dietary AGEs harmful to human health?

There is convincing evidence to support a pathological role for dietary AGEs and their dicarbonyl precursors in both the development and progression of age-related chronic diseases. Rodents receiving high-AGE diets for as little as two weeks have demonstrated significant endocrine and metabolic defects when compared to low-AGE diet controls. In animal models, short-term high-AGE and/or high-methylglyoxal diets upregulate inflammatory pathways associated with the progression of non-alcoholic fatty liver disease (NAFLD) [62], polycystic ovarian syndrome (PCOS) [63], atherosclerosis [64], insulin resistance [65], dysregulation of appetite [66], impaired adipose tissue signalling [67], cognitive deficits [57] and colonic inflammation [68]. In contrast, other murine studies have found either no pathogenic outcomes [69] or beneficial effects [70] associated with AGE consumption, indicating that longer-term dietary interventions using defined synthetic Maillard reaction products is required in order to fully investigate their metabolic fate and function.

Human dietary AGE trials have produced contradictory results. Short-term AGE restricted diets have improved insulin sensitivity in healthy subjects [71], overweight individuals [55, 72] and people with type 2 diabetes [73]. Low-AGE dietary interventions have reduced circulating markers of inflammation in healthy people [73, 74] and those with type 2 diabetes [73, 75], while others found no such improvement [76, 77]. Serum markers of endothelial dysfunction and oxidative stress either decreased [73, 77] or showed no change [56, 76, 78, 79] after an AGE-restricted diet. The discrepancies in data are likely explained by the different AGE quantification and outcome measurement techniques utilised, the heterogeneity of patient populations studied, diversity of the test diets administered and variations in study quality between trials [80]. Additionally, dietary AGEs may have minimal effects on healthy individuals with the capacity to

detoxify or excrete them, and may only exert pathological effects in people with pre-existing metabolic dysfunction or renal impairment.

Reduction of AGE accumulation by pharmacological agents and nutritional compounds

AGE production can be inhibited by a number of endogenous mechanisms and exogenous agents, which are capable of preventing or delaying the glycation process at different stages of AGE formation and function (Tables 2 and 3). These mechanisms include:

1. Improved glycemic control

A reduction in circulating blood glucose attenuates AGE formation by lowering the concentration of reducing sugars available to react with amino groups. Hyperglycemia also generates reactive oxygen species (ROS) and inhibits the action of endogenous antioxidative mechanisms. Therefore, many glucose-lowering pharmacological agents currently used in the management of diabetes (Metformin, thiazolidinediones, insulin) are considered effective inhibitors of AGE accumulation [81-83]. Metformin also appears to play a role in improving the composition of the gut microbiota in people with type 2 diabetes [84], which may indirectly reduce AGE formation by lowering oxidative stress and inflammation.

2. Free radical scavenging

The generation of reactive carbonyl or dicarbonyl compounds is related to oxidative metabolism. A number of pharmacological agents and food-derived antioxidants scavenge hydroxyl and superoxide radicals to attenuate oxidative stress and reduce

the generation of reactive carbonyls, subsequently inhibiting glycation. Medications which demonstrate free radical scavenging capabilities include the antidiabetic agent metformin, anti-hypertensive agent classes angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), aspirin, aminoguanidine, pyridoxamine (a Vitamin B6 derivative) and benfotiamine (a pro-drug of thiamine monophosphate) [85]. Studies involving the administration of the antioxidant drugs Stobadine, Trolox and butylated hydroxytoluene (BHT) to animals with diabetes prevented or delayed the development of glycation-related complications such as cataracts, skin collagen crosslinking and lipid abnormalities [86-88]. Soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) may also exert antioxidative effects [89].

Trials of naturally occurring free radical scavengers including vitamins (Vitamin C, Vitamin E, thiamine), antioxidants (alpha-lipoic acid, inositol) and flavonoids (epicatechin, genistein) have produced contradictory results [24, 90, 91] and further research, particularly in humans is required.

Lactic acid bacteria in the gut, which includes genera such as *Lactobacillus* and *Lactococcus* are capable of reducing inflammation by detoxifying peroxide radicals, and may have the capacity to eliminate specific dietary AGEs prior to intestinal absorption. *In vitro* studies have revealed that fructoselysine, the primary AGE in pasteurised milk, is degraded when incubated with the intestinal microbiota [92].

Table 2: Pharmacological agents with AGE-lowering properties

Agent
Aspirin
Antihypertensive agents (ACE inhibitors, ARBs)
Glucose-lowering agents (Metformin, Thiazolidinediones, Insulin)
Vitamin derivatives (Pyridoxamine, Benfotiamine)
Antioxidants (Stobadine, BHT, Trolox)
Aminoguanidine
AGE breakers (Alagebrium)
Metal ion chelators (TETA, Penicillamine)
Soluble RAGE
Lipid-lowering agents (Statins)

3. Carbonyl group trapping

During the glycation process, interaction with or blocking of the carbonyl groups on reducing sugars, Schiff bases or Amadori products can inhibit glycation. Aminoguanidine (Pimagedine) is a hydrazine-like molecule which was the first AGE-inhibitor studied [93]. Aminoguanidine is a potent carbonyl trap, however clinical trials in humans were discontinued due to safety concerns. Trial subjects experienced side effects including flu-like symptoms, liver function test abnormalities, gastrointestinal disturbances and anaemia [94].

The biguanide Metformin is able to trap reactive carbonyls due to its guanidine functional group, and pyridoxamine quenches 3-deoxyglucosone reactive carbonyl intermediates, protecting lysine residues from glycation.

High doses of thiamine and benfotiamine moderate vascular complications of diabetes in animal models [95] and reduce AGEs and markers of oxidative stress in people with diabetes consuming a high-AGE diet [96]. These compounds are able to scavenge both

Amadori products and reactive dicarbonyls. They have also been found to decrease the formation of reducing sugars and intermediates of the polyol pathway [97]. Thiamine and benfotiamine maintain their function in heated foods which makes them attractive for use by the food industry [98], but long-term studies are required to confirm their therapeutic value.

Inhibitors of the renin angiotensin system (RAS), angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) reduce carbonyl compounds, inhibit oxidative stress and chelate metal ions [99].

Carnosine (a dipeptide of the amino acids beta-alanine and histidine), effectively sequesters reactive aldehydes and subsequently protects protein amino groups from glycation [100]. Carnosine supplementation of up to two grams per day improved insulin sensitivity and appeared to be well tolerated in a pilot human trial [101].

4. Metal ion chelation

AGEs can be produced in the presence of transition metal ions, which catalyse oxidation reactions. Indeed, metal ion chelators successfully inhibit AGE formation *in vitro* [102]. Aspirin inhibits the formation of pentosidine by chelation of copper and other transition metals [103], but side effects limit its clinical use at high doses. Aspirin also acetylates the amino groups on proteins, competing with reducing sugars such as glucose and galactose.

Table 3: Nutritional compounds with AGE-lowering properties

Ingredient	Food sources	Mechanism of action
Vitamins: Thiamine (Vitamin B1)	Liver, yeast extract, wholegrain breads & cereals	Carbonyl trap
Vitamin C	Fruit, vegetables, juices	Free radical scavenger
Vitamin E	Avocado, nuts & seeds	Free radical scavenger
Phytochemicals: Chlorogenic acid	Coffee beans, potato peel	Free radical scavenger, metal ion chelator
Rutin	Citrus fruits, green tea, buckwheat	Free radical scavenger, metal ion chelator
Epicatechin	Cocoa, tea, grapes	Reduces lipid peroxidation
Ferulic acid	Wheat & barley bran, rye, flaxseed, corn	Free radical scavenger
Soy isoflavones (genisten, genistein)	Soy beans, lupin	Free radical scavenger
Antioxidants: Alpha-lipoic acid	Kidney, heart, liver, spinach, broccoli, yeast extract, dietary supplements	Free radical scavenger
Taurine	Seafood, fish, poultry	Free radical scavenger, glucose lowering
Mangiferin	Mango, dietary supplements	Free radical scavenger, metal ion chelator, increases Glyoxalase-1 activity
Fish oil	Oily fish, dietary supplements	Anti-inflammatory, free radical scavenger
Inositol	Oranges, dietary supplements	Free radical scavenger
Carnosine	Meat, fish, dietary supplements	Free radical scavenger, metal ion chelator, carbonyl trap
Gallic acid	Rhubarb, berries, tea, pomegranate	Free radical scavenger, reduces lipid peroxidation
Citric acid	Citrus fruits and juices	Metal ion chelation
Herbs & Spices: Cinnamon (bark) *Cinamomum cassia*	Cinnamon	Free radical scavenger, carbonyl trap
Curcumin root (turmeric) *Curcuma longa*	Curcumin	Anti-inflammatory, glucose-lowering
Aged Garlic extract *Allium sativum*	Garlic	Inhibits auto-oxidation, metal ion chelator
Rosemary (leaves) *Origanum officinalis*	Rosemary	Free radical scavenger
Other: Tea (leaves) *Camellia sinensis*	Green tea, black tea	Reduces lipid peroxidation, metal ion chelator, glucose-lowering and insulin sensitising properties
Onion (skin) *Allium cepa*	Onion	Free radical scavenger
Tomato (paste)	Tomatoes	Reduces LDL oxidation
Lactic acid bacteria	Fermented foods, dietary probiotic supplements	Free radical scavenger, anti-inflammatory, deamination of fructoselysine in milk

Penicillamine is a copper chelator used in the treatment of rheumatoid arthritis and has been shown to inhibit AGE formation *in vitro* [104]. Common side effects of penicillamine include pruritis, mouth ulcers and taste changes, which render it unsuitable for widespread use. Additionally, Vitamin E was found to be more effective than penicillamine at protecting cells from AGE-induced toxicity [105].

Triethylenetetramine (TETA) is a highly selective chelator of copper ions, capable of reducing the accumulation of AGEs and their precursors by suppressing copper-mediated oxidative stress and upregulating copper-catalysed antioxidant defence mechanisms [106]. TETA has been used in proof-of-principle human studies without resulting in major side effects or copper deficiency [107], and clinical trials are now anticipated.

Dietary supplementation of citric acid inhibited ketosis and prevented the accumulation of AGEs in the lens of diabetic rats, delaying the development of cataracts [108].

5. Potentiators of endogenous defence and detoxification systems

Chronic renal hypoxia is associated with increased oxidative stress and subsequent AGE production. Cobalt mitigates oxidative stress and advanced glycation in the diabetic rat kidney *in vivo* by inhibiting the degradation of hypoxia inducible factor (HIF) [109], a molecule which activates a variety of genes involved in the defence against and adaptation to hypoxia.

The glyoxalase system is an endogenous complex which catalyses the detoxification of a variety of dicarbonyls, including methylglyoxal, by conversion to D-lactate. The

glyoxalase system consists of the enzymes glyoxalase-1 and glyoxalase-2, and glutathione, a cofactor required for glyoxalase 1 activity [110]. Glycation of ROS scavenging enzymes by AGEs is thought to deplete intracellular levels of glutathione. In addition NF-κB, activated by AGE-RAGE ligation, binds to glyoxalase-1, suppressing its capacity to detoxify dicarbonyls [111]. Mangiferin is a plant polyphenol derived from the mango tree (*Mangifera indica*), which was found to upregulate glyoxalase-1 activity, attenuate oxidative stress and increase glutathione levels *in vitro* and *in vivo* in diabetic rats [112].

There is evidence to support the function of polyphenols, flavonoids, carotenoids and omega-3 fatty acids as natural activators of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which increases the production of specific antioxidant enzymes involved in the cellular protection against glycation [113, 114].

6. AGE cross-link breakers

AGEs form non-reversible covalent cross-links within and between extracellular matrix proteins, causing vascular and myocardial stiffness. These cross-links are highly resistant to proteolysis, resulting in collagen accumulation and fibrosis. Alagebrium chloride (ALT-711) is able to cleave the crosslinking structures, enabling the clearance of glycated proteins by scavenger proteins or renal excretion [99]. Alagebrium shows promise as a pharmacological agent for use in hypertension and heart failure, and human clinical trials are required [2].

7. RAGE antagonism

Inhibition of the AGE-RAGE axis can repress the occurrence of oxidative stress, inflammation and further AGE generation, which inhibits downstream glycation events and protects against long-term vascular complications. Low molecular weight heparin is a RAGE agonist, and dose-dependently prevents nephropathy in diabetic mice [115].

Thiazolidinediones (TZDs) are insulin sensitizing agents and ligands of the peroxisome proliferator-activated receptor-gamma (PPAR-γ), used in the management of type 2 diabetes. The TZD rosiglitazone has been identified as a RAGE antagonist and also downregulates RAGE expression [116], however concerns about its safety and side effects have placed a number of restrictions on its use and it is now rarely prescribed in Australia [117].

Anti-RAGE antibodies have been produced which have prevented some of the deleterious outcomes of RAGE activation *in vitro* and in animal models [118]. Incretin therapies (glucagon-like peptide-1 agonists and dipeptidyl peptidase-4 inhibitors) which increase circulating glucagon-like peptide-1 (GLP-1) concentrations may reduce the expression of RAGE [119]. Soluble RAGE (sRAGE), endogenous secretory RAGE (esRAGE) and miniRAGE are truncated forms of RAGE which circulate in plasma and body fluids, competing with full-length RAGE for ligands. As they lack a transmembrane domain, AGE ligation to these RAGE isoforms fails to trigger signal transduction. Administration of sRAGE to diabetic mice has reduced the development of nephropathy and improved renal function [120]. Rosiglitazone and ACE inhibitors also increase the production of sRAGE [121].

9. Lipid lowering

Statins reduce the lipid reactants involved in AGE formation and inhibit lipid peroxidation through their antioxidant properties [122]. Human clinical trials investigating the efficacy of statins for AGE reduction have produced contradictory results, and further studies are needed to determine the utility of statins for AGE inhibition.

The effects of dietary AGEs and their precursors on human health will depend on the degree of digestion, absorption and elimination of these compounds, as well as the efficiency of endogenous defence and detoxification mechanisms. Genetics and the presence of chronic illness and disease influence the capacity of the body to process and excrete dietary AGEs. External factors such as diet quality, nutritional antioxidant intake, pharmaceutical agents, physical activity levels and exposure to environmental toxins are also likely to affect protection against glycation. Finally, the composition of the gut microbiota may play a role in the degradation of exogenous AGEs, their degree of absorption and the ability of the immune system to attenuate AGE-related pathology.

Although numerous *in vitro* and experimental studies (in rodents) have demonstrated the AGE-inhibiting and effects of various pharmacological and nutritional interventions, the potential clinical value of many of these agents is yet to be established. The cost of anti-hypertensive, glucose-lowering and lipid-lowering agents is not covered by the Australian Pharmaceutical Benefits Scheme (PBS) when prescribed to individuals without existing hypertension, diabetes or hypercholesterolemia, limiting their use as preventative agents in people with early metabolic perturbations. Side effects and

safety concerns associated with vitamin derivatives, aminoguanidine, AGE breakers and metal ion chelators restrict their use in humans.

Nutritional compounds with anti-glycation effects *in vitro* have great potential for use by individuals in supplement form to reduce endogenous AGE formation, and by the food industry to limit the AGE content of processed foods. However, human trials have not been conducted for the majority of reported nutritional anti-glycation agents, so the effective dose, interactions with other nutrients and potential side effects of these compounds are still to be determined. Administration of high doses of a single compound is unlikely to be a successful strategy for AGE reduction, and ideal treatments will combine multiple agents which target different stages of AGE formation and pathology. Moreover, compounds capable of reducing AGE formation at physiological temperatures (37°C) may not be suitable for use in heated foods. While polyphenols such as epicatechin and rutin are effective free radical scavengers and reduce glycation *in vitro*, they are degraded at high temperatures and lose their anti-glycation capacity when added to foods during cooking [(123)]. Further research is required on the thermal stability of many antioxidants before they can be used by the food industry to inhibit glycation reactions.

The following article summarises human and animal studies investigating the effects of dietary AGEs on inflammatory markers, and offers some suggestions for future research into AGE inhibition.

Article: Kellow NJ, Coughlan MT. Effect of diet derived advanced glycation endproducts on inflammation.

Nutr Rev 2015; 73: 737-759.

Lead Article

Effect of diet-derived advanced glycation end products on inflammation

Nicole J. Kellow and Melinda T. Coughlan

Advanced glycation end products (AGEs) formed via the Maillard reaction during the thermal processing of food contributes to the flavor, color, and aroma of food. A proportion of food-derived AGEs and their precursors is intestinally absorbed and accumulates within cells and tissues. AGEs have been implicated in the pathogenesis of diabetes-related complications and several chronic diseases via interaction with the receptor for AGEs, which promotes the transcription of genes that control inflammation. The dicarbonyls, highly reactive intermediates of AGE formation, are also generated during food processing and may incite inflammatory responses through 1) the suppression of protective pathways, 2) the incretin axis, 3) the modulation of immune-mediated signaling, and 4) changes in gut microbiota profile and metabolite sensors. In animal models, restriction of dietary AGEs attenuates chronic low-grade inflammation, but current evidence from human studies is less clear. Here, the emerging relationship between excess dietary AGE consumption and inflammation is explored, the utility of dietary AGE restriction as a therapeutic strategy for the attenuation of chronic diseases is discussed, and possible avenues for future investigation are suggested.

INTRODUCTION

Advanced glycation end products (AGEs) are a heterogeneous class of compounds reported to play a pathogenic role in the development and progression of aging-related chronic diseases. AGEs and their precursors are slowly produced throughout the body during the normal process of aging but are also widely distributed in heat-treated food and in cigarette smoke, providing an additional avenue for AGE accumulation. While excessive endogenous AGE production within the body has been associated with proinflammatory processes underlying conditions such as insulin resistance,[1,2] atherosclerosis,[3,4] and the vascular complications of diabetes,[5,6] less is known about the activity and metabolic fate of diet-derived AGEs. Here, the current state of knowledge about the formation of AGEs and their precursors within food; the digestion, absorption, and excretion of dietary AGEs; and the potential role of dietary AGEs in the etiology of chronic inflammation is presented. In addition, dietary intervention studies involving restriction of AGEs in animals and humans, along with possible strategies for reducing the formation of AGEs in the food supply, are outlined. An enhanced knowledge of how diet-derived AGEs negatively regulate cell metabolism is critical for understanding the links between excess consumption of highly processed diets and development of chronic disease.

Affiliation: *N.J. Kellow* and *M.T. Coughlan* are with the Glycation, Nutrition and Metabolism Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia, and the Department of Epidemiology & Preventive Medicine, School of Public Health and Preventive Medicine, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria, Australia. *M.T. Coughlan* is with the Department of Medicine, Central Clinical School, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria, Australia.

Correspondence: *M.T. Coughlan,* Glycation, Nutrition & Metabolism Laboratory, Baker IDI Heart & Diabetes Institute, PO Box 6492, St Kilda Rd Central, Melbourne, 8008, Australia. E-mail: melinda.coughlan@bakeridi.edu.au. Phone: +61-3-8532-1278.

Key words: advanced glycation end products, diet, dicarbonyls, gut, inflammation, Maillard reaction products.

doi: 10.1093/nutrit/nuv030

THE MAILLARD REACTION

AGEs are formed via the nonenzymatic reaction of amino acids with sugars, termed the Maillard reaction, which consists of a complex network of degradative reactions.[7] In general, within the early stage of the Maillard reaction, the first stable products to be formed are the Amadori products, generated as a result of condensation reactions and subsequent chemical rearrangement of carbonyl groups of reducing sugars (such as, but not limited to, glucose, fructose, maltose, and lactose) and amino groups of amino acids, peptides, or proteins (see Henle[7] for a more comprehensive review). During the intermediate stages of the Maillard reaction, Amadori products undergo degradation and are subsequently converted to α-dicarbonyl compounds such as 1-deoxyglucosone, 3-deoxyglucosone, glyoxal, and methylglyoxal. Several chemical reactions can produce these highly reactive intermediates, including Schiff base oxidation, degradation of triosephosphates (during glycolysis), and lipid peroxidation (recently reviewed by Rabbani and Thornalley[8]). Subsequently, in the advanced stages of the Maillard reaction, dicarbonyls, which are potent glycating agents, react with lysine or arginine side chains of proteins to form stable peptide-bound amino acid derivatives, or AGEs. Well-known AGEs include carboxymethyllysine (CML), carboxyethyllysine, pyrraline, pentosidine, crossline, vesperlysine, glucosepane, and hydroimidazolone adducts.[9]

FACTORS AFFECTING FORMATION OF ADVANCED GLYCATION END PRODUCTS IN FOOD

Food-derived AGEs and their carbonyl precursors are present within highly heated foods and beverages.[7,10–14] The yellow-brown pigment produced by the Maillard reaction contributes to the organoleptic properties of food, such as appearance, flavor, aroma, and texture. In fact, the Maillard reaction is utilized by the food industry to obtain favorable sensory attributes of foods, thereby increasing palatability. Improvements in aroma, taste, and color are the key characteristics provided by AGEs. The majority of the effects of the Maillard reaction within foods are considered desirable to the food industry, such as the golden brown colors or the caramel aromas that develop upon heating.[15] Studies have shown that the Maillard reaction can also give rise to compounds that may exert an antioxidative activity, such as protecting against lipid peroxidation,[16–18] thus potentially promoting stability and longer shelf life.

Since the Maillard reaction represents many thousands of individual reactions, each is influenced by different factors that include temperature, time, water activity, pH, and composition of the system.[15] Factors that enhance food-derived AGE formation include low water content during cooking, increased pH, and the application of high temperatures over a short time period. An increase in temperature increases the rate of Maillard browning. More AGEs are generated in foods exposed to dry heat (grilling, frying, roasting, baking, and barbecuing) than foods cooked at lower temperatures for longer time periods in the presence of higher water content (boiling, steaming, poaching, stewing, or slow cooking).[11] Microwaving does not generate the AGE levels observed during conventional cooking methods because it cooks foodstuffs over a relatively short time period and does not achieve a sufficiently dry environment to result in excessive browning.[19] The addition of sodium bicarbonate (baking soda) during the production of bakery foodstuffs increases the pH of the mixture, enhancing AGE formation and associated browning, whereas the addition of vinegar or lemon juice reduces the pH (increases the acidity) of the cooking environment, lowering the potential for AGE generation.[20]

The chemical structure of individual sugars present within food also determines the level of reactivity to the Maillard reaction, with pentose sugars, such as ribose, reacting more readily than hexoses, such as glucose. In contrast, disaccharides such as lactose are less reactive.[15] Amino acids or peptides with free amino groups must also be present within the food to enable the Maillard reaction to take place. As a result of structural and processing factors, foods thought to contribute large quantities of AGEs and AGE-precursors to the Western diet include powdered milk and cheese; meats, fish, and chicken cooked by dry heat[11,12]; heat-processed or alkaline-treated cereal-based products (bread, biscuits, bakery products, extruded breakfast cereals)[7]; sweet sauces[21]; and carbonated soft drinks containing high-fructose corn syrup.[22] As microorganisms readily produce and release AGEs and their carbonyl precursors, alcoholic drinks and fermented foods also contain significant levels of AGEs.[9]

MEASUREMENT OF ADVANCED GLYCATION END PRODUCTS

A variety of different analytical techniques have been used to measure the AGE content of foods. Most studies have estimated the CML concentration in foods using methods based on an enzyme-linked immunosorbent assay (ELISA). Although ELISA is rapid and inexpensive, most of the available CML ELISAs previously utilized have not been fully validated, and therefore the accuracy and reliability of reported AGE levels based on this technique have been questioned.[23] Incomplete characterization of antibody epitope recognition is the main problem in utilizing immunological techniques to quantify AGE content.[24] The most extensive food AGE

database currently available lists the CML content of over 500 foods[11,12] and was developed using an ELISA technique based on an anti-CML antibody. An additional disadvantage of this ELISA method is that it only allows the AGE concentration in foods to be expressed in arbitrary units (kilounits of AGE), making comparisons with other analytical techniques difficult. Importantly, the ELISA-derived database contains food AGE information originating from only one laboratory and will therefore require confirmation by other research groups in the future.

More-specific methods of dietary AGE estimation, such as ultra-performance liquid chromatography–tandem mass spectrometry, are now widely recommended.[25] One challenge associated with chromatographic techniques is the requirement for an acid hydrolysis step, which destroys a number of acid-sensitive AGEs such as pyrraline and hydroimidazolones.[23] This issue has been addressed by the introduction of enzymatic hydrolysis, which employs the use of proteases, but this method is currently less efficient than acid-based techniques. An open-access food AGE database, based on validated analytical methods[10,26,27] is available through the Dresden University of Technology[28] and is likely to be of benefit to researchers in this field. The use of standardized databases for the quantification of any dietary component is often problematic, and the AGE content of foods will vary according to season, location, preparation technique, degree of heating, and preservation method. However, an AGE database can be a useful tool for the general classification of food and beverages into high-, medium-, or low-AGE categories.

No validated questionnaires or standardized surveys currently exist to enable the estimation of dietary AGE intake among large groups of individuals. The development of an AGE Food Frequency Questionnaire has recently been reported,[29] but this tool is probably more useful in research settings than in clinical practice because it is based on the ELISA-derived food AGE database.

DIGESTION, ABSORPTION, AND EXCRETION OF DIETARY ADVANCED GLYCATION END PRODUCTS

There is a paucity of knowledge regarding the metabolic fate of diet-derived AGEs. It has been estimated that humans consume up to 1200 mg of Amadori products and up to 75 mg of AGEs in food and fluids daily.[30] In food, glycated amino acids are bound in protein and cannot be absorbed intestinally until the proteins are digested by gastric and intestinal peptidases into peptides and free amino acids. AGE-modified peptides are able to penetrate the gastrointestinal mucin layer, where they undergo further proteolytic cleavage into di- and tripeptides at the intestinal brush-border in order to facilitate their absorption.[31] Low-molecular-weight AGEs (AGEs on free amino acids and those bound to di- and tripeptides) are likely to be well absorbed by either simple diffusion or by peptide transporter proteins such as peptide transporter 1.[32] However, crosslinking low-molecular-weight AGEs are less available for absorption because of their resistance to digestive enzymes. Moreover, most high-molecular-weight AGEs also escape digestion in the upper gastrointestinal tract, primarily as a result of crosslinking and protein aggregation, and pass through to the large intestine, eventually being excreted in feces and/or acting as a fermentation substrate for colonic microorganisms.[33] Following bacterial fermentation, amino acids may become available as substrates for the formation of further toxic metabolites.[33]

Kinetic studies have estimated that 10%–30% of diet-derived AGEs consumed are absorbed intestinally and enter the circulation.[34,35] Studies involving the administration of radioactively labeled AGEs indicate that, in addition to a generalized whole-body distribution, absorbed AGEs accumulate preferentially in renal and hepatic tissue.[36,37] Whether different postabsorptive AGEs and carbonyl compounds demonstrate unique binding affinities for distinct body proteins is currently unknown.

Currently, limited data are available concerning bioavailability and intestinal absorption of dicarbonyl compounds. Daily dietary intake of 3-deoxyglucosone and methylglyoxal has been estimated to range between 20 and 160 mg and between 5 and 20 mg, respectively.[10] Recent in vitro studies suggest that dicarbonyls can react with digestive enzymes, leading to a reduction in bioavailability.[38] However, this reduction in dicarbonyl concentration may indicate degradation during the process of de novo AGE formation, after which absorption could occur.

A number of human studies have investigated the relationship between dietary AGE intake and circulating AGE levels, with conflicting results. Some studies have found moderate to strong correlations between dietary intake and circulating AGE levels,[39–41] whereas others have found no association.[42–44] The conflicting results of these studies are likely due to differences in methods of measuring dietary intake and serum or plasma AGEs, inconsistencies in study duration, variation in the types and preparation of foods administered, and the physiological diversity of research subjects involved. In particular, it is well known that changes in renal function lead to enhanced urinary excretion of AGEs.[45] Urinary excretion of AGEs may also be a more informative marker of whole-body AGE accumulation,[45] although it is difficult

to differentiate the AGEs generated endogenously from comorbid conditions from those consumed via the diet.

Determining the relative contributions of diet-derived and endogenously produced AGEs to the total AGE content in body fluids and tissues is of particular interest. Urinary CML measurement is often used as a marker of food AGE absorption because it responds rapidly to short-term changes in dietary AGE intake in individuals with efficient renal function.[46,47] In serum, the concentration of free CML has been suggested to be a better correlate of dietary AGE intake than protein-bound CML.[48] The background AGE content of the diet also appears to influence the efficiency of AGE absorption from food. Urinary CML output appears to correlate with AGE intake but reaches a saturation point at high levels of dietary AGE consumption,[49] and fecal CML excretion also increases in proportion to dietary CML intake.[47,50] One obvious problem associated with the measurement of CML in body fluids is that it does not provide any indication of the metabolic fate of the many other dietary AGEs entering the body. Future studies investigating the intestinal transit and absorption of dietary AGEs and their dicarbonyl precursors need to be carried out using synthetic AGEs of known molecular weights in order to exclude the confounding effects of food processing and interactions with other dietary components.

The fate of the remaining 70%–90% of dietary AGEs that escape digestion and absorption in the human small intestine warrants further investigation. Since amino acids molecularly modified by heat are more likely to escape digestion in the upper gut,[51] a significant proportion of dietary Maillard reaction products (MRPs) reach the colon, where they may modulate gut microbial growth.[35,52]

GUT HOMEOSTASIS

In vitro studies have shown that glycated proteins encouraged the preferential growth of greater numbers of detrimental colonic bacteria (clostridia, bacteroides, and sulfate-reducing bacteria) when exposed to colonic microbiota derived from patients with ulcerative colitis.[53] Recent studies indicate that consumption of a high-AGE diet for 2 weeks is sufficient to alter the colonic bacteria profile in humans. A 2-week randomized crossover trial in which healthy male adolescents were fed a diet high in MRPs resulted in a decrease in lactobacilli, enterobacteria, and copy numbers of the *Escherichia/Shigella* group, while the intake of Amadori products (AGE precursors) was negatively correlated with bifidobacterial growth.[54] Parallel studies in male rats fed a diet high in MRPs for 3 months revealed inhibition of the growth of cecal lactobacilli.[54]

In contrast, Anton et al.[50] found that a highly heat-treated diet improved inflammatory bowel disease in the dextran-sulfate-sodium–induced colitis mouse model. The authors speculated that these MRPs might have encouraged the growth of microorganisms beneficial to the host. Indeed, Borrelli and Fogliano[55] found that MRPs derived from bread crust promoted the growth of species of beneficial bacteria in in vitro studies designed to mimic hindgut conditions. Limitations associated with this area of research include the use of in vitro models unable to replicate the intestinal environment, the large number of different MRPs and their intermediates that can act as potential substrates for bacterial fermentation, and, in human in vivo studies, the use of stool samples, which can indicate colonic bacterial growth but may not adequately reflect microbial activity in the proximal large intestine.[56]

Low-molecular-weight AGEs and their reactive dicarbonyl precursors are most likely absorbed from the diet. However, it is conceivable that, in circumstances of increased intestinal epithelial cell permeability, greater quantities of dietary AGEs may be able to gain entry into the systemic circulation. Indeed, elevated levels of circulating AGEs are frequently observed in individuals with diabetes.[57] Moreover, diabetes is associated with an increased prevalence of upper and lower gastrointestinal symptoms[58,59] and an increase in intestinal permeability,[60–62] lending weight to the concept that dietary MRPs may gain access to the circulation through disrupted gastrointestinal physiology. Interestingly, inflamed gut biopsy tissue collected from patients with inflammatory bowel disease demonstrated upregulation of the receptor for advanced glycation end products (RAGE) in tandem with activation of nuclear factor–κB (NF-κB), a major driver of inflammation,[63] suggesting that AGE accumulation within the gut can lead to inflammation. A recent study found that the reactive dicarbonyl methylglyoxal mediated glycation of tight junction proteins, which impaired protein function, albeit within the blood–brain endothelial barrier.[64] These studies indicate that MRPs/AGEs are likely to have disparate effects within the gastrointestinal tract, which may affect the gut barrier homeostasis.

A recent study found that small intestine segments from streptozotocin-induced diabetic rats had greater AGE protein content, measured by immunohistochemistry, compared with nondiabetic controls, whereas RAGE was increased in the small intestine and the ganglia of the colon.[65] Intestinal AGE–RAGE interactions are likely to stimulate localized production of proinflammatory cytokines and reactive oxygen species (ROS), both of which have been shown to compromise tight junctions between epithelial cells, thereby disrupting the integrity of the intestinal barrier. Increased

gastrointestinal permeability may enable not only AGEs but also other toxic compounds to translocate from the gut into the circulation, activating host immune responses and amplifying inflammatory signals throughout the body. Some studies have demonstrated that a high-AGE diet results in increased intestinal inflammation and reduced antioxidant activity,[66] while others have shown a significant anti-inflammatory effect of AGEs.[18] Exploration of the effect of dietary AGEs on colonic inflammatory processes is also complicated by the production and secretion of AGEs by intestinal bacteria, which are thought to be capable of inducing an inflammatory response in vitro.[67]

One focus of current research is the potential of diet-derived AGE moieties to induce proinflammatory gastrointestinal changes early in the pathogenesis of a number of chronic diseases.[68] Glycation of epithelial intestinal cell tight junction proteins such as zonulin and occludin, as well as excess generation of ROS, may contribute to increased gastrointestinal permeability and inflammation. Additionally, by acting as a substrate for microbial growth in the colon, dietary AGEs may negatively influence the intestinal balance of gut microbiota, resulting in reduced bacterial production of anti-inflammatory short-chain fatty acids and increased movement of luminal endotoxins into the host circulation.

ADVANCED GLYCATION END PRODUCTS IN THE PATHOGENESIS OF INFLAMMATION AND CHRONIC DISEASE

Inflammation is an immune response to the presence of harmful stimuli, such as pathogens, toxins, or damaged cells. Acute inflammation is distinguished by redness, swelling, heat, pain, and loss of function and is mediated by a tightly regulated system of complex cell types, with the ultimate aim of promoting healing and recovery. In contrast, chronic inflammation is a maladaptive response to cellular insult that occurs when inflammatory signals and processes are allowed to remain unregulated within the body, giving rise to further damage, stress, and disease. Chronic low-grade inflammation is characterized by elevated circulating levels of inflammatory markers and activated immune cells and is frequently observed in individuals with conditions such as obesity, the metabolic syndrome, diabetes, and cardiovascular disease.[69]

AGEs and their reactive dicarbonyl precursors are generated endogenously as a normal consequence of metabolism and aging, but their formation is accelerated upon increased availability of substrates for AGE generation, such as in the context of hyperglycemia and dyslipidemia. AGE production is also intensified in the presence of increased oxidative stress, which is frequently observed in obese individuals or those with the metabolic syndrome, diabetes, or cardiovascular disease.[70] Excess ROS generate dicarbonyl compounds by interrupting cellular glycolysis, resulting in the accumulation of glycolytic intermediates that feed into the AGE pathway, increasing the production of methylglyoxal.[71] ROS also promote the oxidation of glucose (autoxidation) and polyunsaturated lipids (peroxidation), generating AGE precursors that include glyoxal and methylglyoxal.[72] The products of oxidation of unsaturated fatty acids and glycation of the amino group of phospholipids are called advanced lipoxidation end products, many of which appear to exert pathological effects in vivo similar to those of AGEs.[73] Moreover, in individuals with impaired renal function, urinary AGE excretion may be diminished, resulting in a greater accumulation of AGEs in the body,[74] although this does not appear to be the case for all AGEs.[45,75] Persistent exposure to elevated levels of endogenous and exogenous AGEs or advanced lipoxidation end products are thought to contribute to the pathogenesis and progression of a variety of chronic conditions associated with immune cell activation and low-grade inflammation,[76] including type 1 diabetes,[77] type 2 diabetes,[78] neurodegenerative conditions,[79] allergy,[80] nonalcoholic steatohepatitis,[81] asthma,[82] inflammatory bowel disease,[83] renal disease,[42] and certain cancers[84] (Figure 1).

The pathophysiological consequences of excessive production and accumulation of AGEs may be broadly categorized into 3 major areas. First, AGE modification of extra- and intracellular proteins results in structural and/or functional changes in these proteins. Glycation of long-lived extracellular matrix proteins results in impaired wound healing, changes in both cellular movement and adhesion properties, and dysregulation of intercellular communication.[85] For example, extracellular collagen modified by AGEs has reduced elasticity and solubility, which results in increased vascular stiffness, disturbed cellular attachment, and reduced turnover, all of which contribute to basement membrane thickening. Common extracellular targets of AGE-induced crosslinking include collagen, elastin, tubulin, myelin, and lens crystallins, all of which contribute to the vascular dysfunction associated with aging and diabetes.[86] Inside the cell, AGE modification of mitochondrial proteins results in aberrant electrolyte transport, increased production of ROS, and mitochondrial dysfunction.[87] Dicarbonyl compounds also glycate guanine bases within DNA, contributing to a reduction in DNA replication and an increased frequency of mutation.[88]

Secondly, AGEs themselves are able to catalyze the formation of ROS and incite oxidative stress and inflammation at sites of AGE accumulation.[89] Protein glycation generates stable active centers for catalyzing

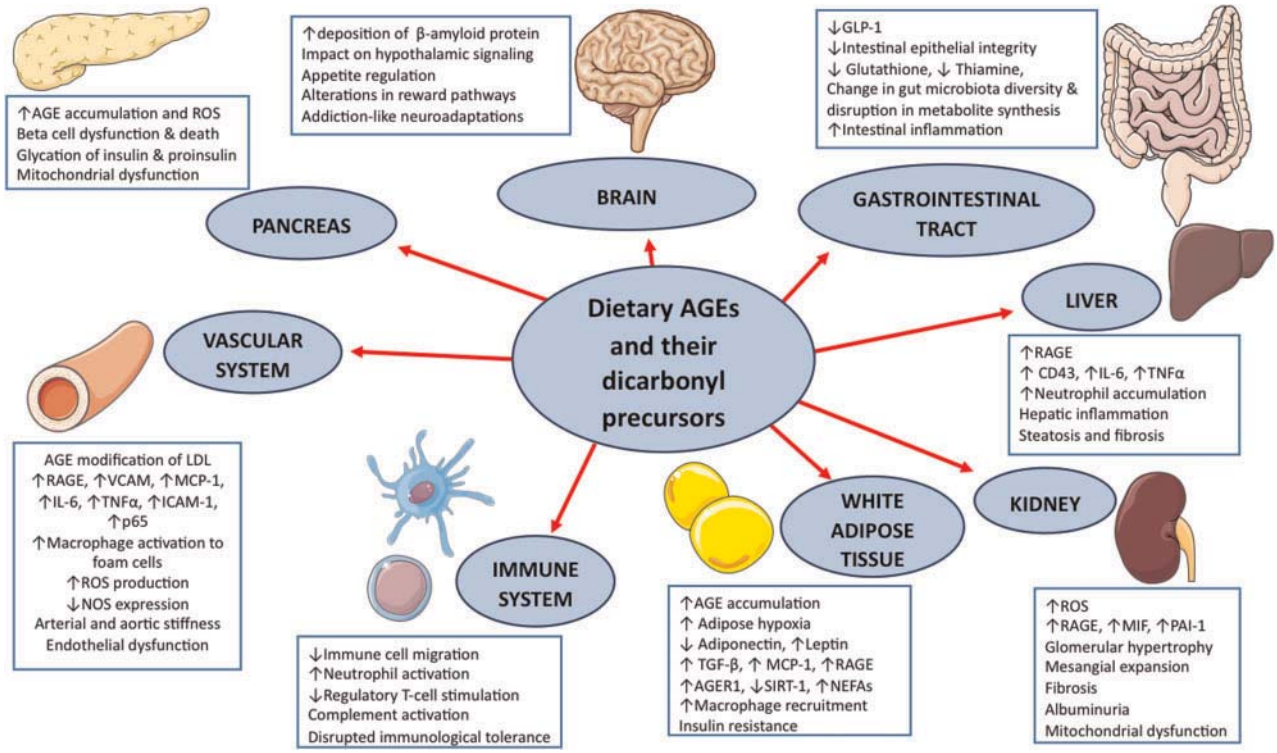


Figure 1 **Putative target sites for dietary AGEs.** Examples of putative target sites in which diet-derived AGEs and their precursors may mediate pathological processes. Illustrations adapted from Servier Medical Art by Servier, used under Creative Commons Attribution 3.0 Unported License. *Abbreviations*: AGER-1, advanced glycation end product 1; GLP-1, glucagon-like peptide 1; ICAM-1, intracellular adhesion molecule 1; IL-6, interleukin 6; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein 1; MIF, macrophage migration inhibitory factor; NEFAs, nonesterified fatty acids; NOS, nitric oxide synthase; PAI-1, plasminogen activator inhibitor 1; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; SIRT-1, sirtuin 1; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor α; VCAM, vascular cell adhesion molecule.

redox reactions and the subsequent formation of free radicals. The generation of a Schiff base by the cross-linking of dicarbonyls to amino groups of proteins yields ROS as a result of the donation of electrons from the Schiff base to the dicarbonyl compound.[90] Excessive ROS are particularly pathogenic to cells that express low levels of detoxification enzymes or limited antioxidant capacity. Pancreatic β cells are highly susceptible to ROS, with transient elevations in oxidative stress capable of stimulating sustained β-cell dysfunction and death.[91] Indeed, excess consumption of dietary AGEs in rodent models promotes ROS production, leading to defects in insulin secretion as well as β-cell death.[77]

Thirdly, AGEs are able to bind and activate a range of receptors that subsequently trigger a downstream cascade of pathogenic mediators. For example, activation of RAGE promotes sustained activation of NF-κB, with subsequent formation of ROS and upregulation of proinflammatory cytokines such as tumor necrosis factor α (TNF-α), chemokines such as monocyte chemoattractant protein 1 (MCP-1), and profibrogenic mediators such as transforming growth factor-β.[92] Cellular dysfunction and inflammation induced by the AGE-RAGE axis has been implicated in the pathogenesis of multiple chronic conditions, including cardiovascular disease, neurodegenerative disorders, stroke, arthritis, cancer, and complications of diabetes.[93] AGE-RAGE signaling participates in the progression of diabetic nephropathy by enhancing renal mitochondrial production of ROS[87] and glomerular injury, upregulating the expression of transforming growth factor β and other profibrotic factors. It has been argued that metabolic memory, the observation that the reduced risk of diabetes-related complications associated with a period of good glycemic control can extend for many years after glucose control deteriorates, may be partly mediated by the AGE-RAGE pathway.[94] Indeed, individuals who are relatively free of serious vascular complications after having lived with diabetes for at least 50 years demonstrate lower concentrations of AGE accumulation in tissue.[95]

THE AGE–RAGE AXIS

Ligation of AGEs with the cell-surface receptor for AGEs (RAGE, *AGER*) is currently considered the

primary mechanism underlying AGE-induced inflammatory processes. RAGE is a multiligand cell-surface pattern recognition receptor. Its ligands include high-mobility group box 1, S-100/calgranulins, amyloid-β-protein, Mac-1, and phosphatidylserine.[96] RAGE is expressed on a range of cell types, including endothelial cells, neurons, smooth muscle cells, lymphocytes, dendritic cells, and macrophages, and RAGE expression is thought to be upregulated in cells and tissue affected by chronic disease.[97] Binding of high-molecular-weight AGEs to RAGE is associated with long-term cellular damage. Engagement of AGEs with RAGE activates NADPH oxidase to increase intracellular ROS production[87] and triggers the sustained activation of NF-κB.[92] Once activated, NF-κB translocates from the cytoplasm to the nucleus, stimulating gene transcription of proinflammatory cytokines (interleukin 6 [IL-6], TNF-α), C-reactive protein, chemokines (MCP-1), procoagulants (thrombin), growth factors (vascular endothelial growth factor), and adhesion molecules (E-selectin, vascular cell adhesion molecule 1 [VCAM-1], and intercellular adhesion molecule 1 [ICAM-1]).[98] NF-κB maintains its own activation by stimulating production of the NF-κB p65 subunit; it also upregulates RAGE expression at the cell surface, thereby amplifying the inflammatory state.

In addition to the NF-κB–dependent pathways, RAGE activates the transcription factors STAT3, AP-1, and forkhead box O1 as well as phosphoinositide 3-kinase/Akt signaling, Jun N-terminal kinase, p38 kinase, and extracellular signal-regulated kinases.[99] The key molecular signature induced by RAGE signaling is inflammation accompanied by cellular migration.[96] Indeed, RAGE plays a role in the innate immune system because it can recognize and interact with microbial products (i.e., pathogen-associated molecular patterns) as well as with endogenous molecules released in the context of tissue injury and inflammation (i.e., damage-associated molecular pattern molecules). Recent studies indicate that RAGE is involved in crosstalk with Toll-like receptors to coordinate and regulate immune and inflammatory responses.[100]

DEFENSE MECHANISMS AGAINST ADVANCED GLYCATION END PRODUCTS

The glyoxalase system

The human body has evolved a number of detoxification systems to reduce the burden associated with AGEs and their dicarbonyl precursors. The glyoxalase system catalyzes the detoxification of a number of dicarbonyls, including methylglyoxal, by conversion to D-lactate. The glyoxalase system consists of the enzymes glyoxalase 1 and glyoxalase 2, and glutathione, a cofactor required for glyoxalase 1 activity.[101] Under normal circumstances, more than 99% of intracellular methylglyoxal is detoxified by the glyoxalase system,[8] but in circumstances of increased oxidative stress, reactive dicarbonyl compounds accumulate. NF-κB, activated by AGE-RAGE ligation, binds to glyoxalase 1, suppressing its capacity to detoxify dicarbonyls.[102] Additionally, an increase in AGE-stimulated production of ROS is believed to deplete cellular levels of glutathione,[103] and excessive methylglyoxal intake diminishes circulating glutathione concentrations,[104] creating a feed-forward loop of dicarbonyl stress.

It is increasingly appreciated that glucose metabolites can induce inflammation in a variety of settings. In vitro studies have shown that exposure of human vascular endothelial cells to methylglyoxal activates Jun N-terminal kinase and p38 mitogen-activated protein kinase, while glyoxal stimulates cyclooxygenase 2, inflammatory mediators involved in the early pathogenesis of atherosclerosis.[105] Administration of methylglyoxal to the microvasculature of healthy mice leads to an increase in leukocyte recruitment, activation of the NF-κB pathway, and upregulation of endothelial cell adhesion molecules.[106] Oral consumption of methylglyoxal induced hypertension[107] and precipitated inflammatory changes in adipose tissue[108] in rats, and acute treatment with methylglyoxal inhibited the contractility of isolated blood vessels.[109] Additionally, infusion of methylglyoxal resulted in impaired glucose-stimulated insulin secretion from isolated rat pancreatic islets.[78] Chronic dietary intake of methylglyoxal impaired the serine/threonine protein kinase (Akt) pathway in rats, a signaling cascade necessary for myocardial recovery following cardiac ischemia.[110]

A recent study showed that administration of dietary methylglyoxal increased vascular adhesion and augmented atherogenesis in normoglycemic *apoE* knockout mice.[111] The effects of methylglyoxal were mediated only partly by RAGE, since deletion of RAGE was able to reduce, but not completely prevent, inflammation and atherogenesis associated with methylglyoxal exposure. Overexpression of glyoxalase 1 in diabetic rats reduced intracellular glycation, inflammation, and endothelial dysfunction and attenuated both the loss of podocytes in the glomerulus and the renal excretion of early markers of diabetic nephropathy.[112] Recently, it was shown that genetic deletion of glyoxalase 1 in mice recapitulated several features of diabetic kidney disease,[113] suggesting that methylglyoxal accumulation is a pivotal mediator of renal disease pathogenesis. Whether diet-derived methylglyoxal can induce end-organ injury is yet to be determined. However, early studies in mice suggest that oral methylglyoxal administration induced

amyloid-β and AGE deposits in the brain by suppressing neural sirtuin 1 (SIRT-1) activity, contributing to cognitive and motor deficits.[114]

Nrf2 pathway

Another endogenous defense system against inflammatory, oxidative, and carbonyl stress involves the nuclear factor erythroid 2–related factor 2 (Nrf2), a transcription factor that increases the production of specific antioxidant enzymes involved in the cellular protection against glycation. Nrf2 activates genes containing an antioxidant response element (ARE) in their promoter and is expressed throughout the body, with particularly high levels found in the intestine, liver, and kidney.[115] Under basal conditions, Nrf2 is complexed with the adaptor molecule Kelch-like erythroid cell-derived protein with Cap "N" Collar (CNC) homology-associated protein 1 (Keap1) within the cytosol, resulting in the degradation of Nrf2 by the proteasome.[116] Upon stimulation with oxidants or electrophiles, Nrf2 is released from Keap1 and is translocated to the nucleus, where it forms a heterodimer with Maf and binds to the ARE in the upstream promoter region of many cytoprotective genes, initiating their transcription.[117] Enzymes involved in the metabolism of reactive dicarbonyls that are activated by Nrf2 include glutathione reductase, glutathione *S*-transferase, glutathione peroxidases, aldehyde reductases, and aldehyde dehydrogenases.

Recently, Xue et al.[118] demonstrated that dicarbonyl stress is countered by upregulation of glyoxalase 1 via the Nrf2 pathway, providing protein and DNA protection and preserving cell function. Highlighting the importance of adequate dietary fruit and vegetable intake, natural small-molecule activators of Nrf2 include isothiocyanates and indoles (sulforaphane and indole-3-carbinol, present in cruciferous vegetables), polyphenols and flavonoids (resveratrol in grape juice), carotenoids (lycopene in tomatoes), triterpenoids (found in apples and grapes), and oxidized n-3 fatty acids.[119–121] Monascin and ankaflavin, compounds derived from beni-koji (red yeast rice, a Chinese fermented food), are able to abrogate methylglyoxal-induced toxicity through the activation of Nrf2.[122–124] Dietary polyphenols have also been reported to reduce the expression of the Nrf2-negative regulator, Keap1.[125] The Nrf2/keap1/ARE system also increases the expression of proteins involved in the autophagic degradation of long-lived proteins such as p62/sequestome-1,[120] suggesting that it may be involved in the turnover of AGEs. While AGEs have been shown to induce the upregulation of Nrf2-dependent antioxidant genes in endothelial cell culture,[126] the effects of dietary AGEs on the Nrf2/keap1/ARE system have not been described.

AGE-binding proteins

There are other RAGE isoforms that bind AGEs and appear to dampen inflammation. Soluble RAGE (sRAGE) is a soluble isoform of RAGE generated by proteolytic cleavage by matrix metalloproteinases.[127] As it lacks a membrane-spanning region and a cytosolic intracellular domain, sRAGE is incapable of catalytic activity and may function in the circulation as a decoy AGE receptor, reducing the pool of serum AGEs and other RAGE agonists available to engage with full-length RAGE. Moreover, one of the mechanisms whereby angiotensin-converting enzyme inhibitors are thought to achieve AGE reduction and renoprotection is through increased sRAGE production.[128]

Because sRAGE sequesters RAGE ligands and acts as a cytoprotective agent in vitro, multiple studies have explored the relationship between sRAGE and inflammation. Subjects with types 1 and 2 diabetes, particularly those with coronary artery disease or renal dysfunction, display elevated sRAGE levels in comparison with nondiabetic controls.[129,130] Some studies have found sRAGE concentrations to positively correlate with circulating inflammatory markers such as MCP-1 and TNF-α in people with diabetes.[131] In contrast, reduced serum levels of sRAGE have been found in individuals with inflammatory conditions such as obesity, atherosclerosis, rheumatoid arthritis, and chronic obstructive pulmonary disease.[132–135] The contradictory findings in serum sRAGE levels between people with diabetes and those with other inflammatory conditions might be explained by increased levels of matrix metalloproteinases. Elevated circulating AGEs (frequently seen in people with diabetes) are associated with enhanced expression and production of matrix metalloproteinases, thereby increasing the proteolytic cleavage of sRAGE from the cell surface.[136] Subjects with diabetes may also be affected by varying degrees of renal impairment, which is known to positively correlate with sRAGE concentrations.[137] Individual genetic polymorphisms in *RAGE* or glyoxalase genes may also result in altered sRAGE levels.[138]

Administration of recombinant sRAGE to rodents with experimentally induced inflammatory and autoimmune conditions has successfully suppressed the development of micro- and macrovascular complications of diabetes, reduced the expression of proinflammatory cytokines, and attenuated vascular dysfunction.[139] Therapeutic administration of sRAGE also reduced inflammation and disease progression in murine models of inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis.[140] However, sRAGE levels are

undetectable in wild-type mice and rats, so it is not yet known whether results in rodent systems have any application in human disease.

Endogenous secretory RAGE is a truncated splice variant of the *RAGE* gene that is secreted from cells and appears to confer protection against low-grade inflammation, with cross-sectional studies finding inverse correlations between endogenous secretory RAGE concentrations and ROS, the metabolic syndrome, atherosclerosis, and microvascular complications of diabetes.[141–143] However, because it is present in very low levels in serum, it is questionable whether this soluble form of RAGE is able to effectively reduce ligand concentration. It may instead simply have a use as biomarker of risk.[144]

The advanced glycation end product receptor 1 (AGER1, oligosaccharyltransferase subunit 48 [OST48], or dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit [DDOST]) is a cell-surface AGE receptor involved in binding AGE ligands and facilitating their degradation.[74] AGER1 may reduce AGE-induced intracellular ROS generation and downregulate RAGE-mediated inflammatory signaling.[145] In healthy individuals, elevated levels of AGEs and oxidative stress in the circulation enhance AGER1 expression, which in turn reduces the expression of both RAGE and the oxidative stress-dependent epidermal growth factor receptor.[146] Moreover, AGER1 transgenic mice were protected from inflammation and oxidative stress after a high-fat diet and femoral artery injury.[147] AGER1 is downregulated in states of chronic oxidative stress, such as diabetes, cardiovascular disease, and renal disease,[148] and by prolonged consumption of a high-AGE diet.[149] In contrast, mice receiving an AGE-restricted diet maintained normal levels of AGER1 and did not experience increased oxidative stress with aging.[150] Similarly, suppressed AGER1 expression in peripheral mononuclear cells at baseline was partially restored by a low-AGE dietary intervention in individuals with type 2 diabetes.[151] While inverse correlations exist between the AGER1:RAGE ratio and the levels of oxidative stress in both mice and humans,[152] a well-defined role for AGER1 as a clearance receptor involved in attenuating the detrimental effects of AGEs is not yet known.

EMERGING ADVANCED GLYCATION END PRODUCT-INDUCED INFLAMMATORY PATHWAYS

Immune-mediated signaling

AGEs and their precursors are likely to modulate both innate and adaptive immune responses. AGE modification induces conformational shape changes in proteins; these modified proteins may be subsequently detected by the body as foreign structures, stimulating an autoimmune response.[153,154] Dendritic cells play an important role in the initiation and regulation of inflammatory immune responses by sampling antigen and directing appropriate T-cell proliferation. Dendritic cells exposed to methylglyoxal showed a dose-dependent loss in their capacity to stimulate primary proliferation of allogeneic T cells,[155] suggesting a pathogenic role for dicarbonyls in the immunological changes associated with diabetes. Methylglyoxal also altered the expression of interferon γ, TNF-α, and interleukin 10 in both myeloid and T cells.[156] The reduced T-cell stimulation and impaired cytokine responses potentiated by methylglyoxal exposure may explain, in part, why people with diabetes exhibit lower resistance to infection.

Complement is a key component of the innate immune system. Under normal conditions, complement is tightly regulated by a number of fluid-phase and cell-surface proteins.[157] When complement is hyperactivated, as occurs in autoimmune diseases or in individuals with dysfunctional regulatory proteins, it drives a severe inflammatory response.[158,159] There is some evidence that the glycation pathway can modulate complement. AGE-modified low-density lipoproteins are able to bind to immunoglobulin G antibodies to form stable immune complexes capable of stimulating the complement system.[160] CD59, a regulatory protein that limits complement activation and the formation of the membrane attack complex, is inactivated by glycation.[161] Glycated CD59 was found in the urine of patients with diabetes[161] and also in the kidneys, nerves, and vasculature of patients with diabetes.[162] Preliminary laboratory data indicates that dietary MRPs can activate the complement pathway, since excess MRP consumption in rodents for 6 months led to an increase in complement C3 in the circulation and to urinary excretion of C5a, a key mediator of inflammation, in the context of renal dysfunction, inflammation and oxidative stress (authors' unpublished observations). These data provide a direct link between dietary AGEs and immune-mediated inflammation. Interestingly, the complement component C3a is a high-affinity RAGE ligand, providing an additional mechanism for the promotion of RAGE-mediated inflammatory signaling.[163]

Metabolite-sensing G-protein–coupled receptors

An emerging area of nutrition research is the role of diet and bacterial metabolites in regulating gut homeostasis and inflammation (recently reviewed[164]). Diet-related metabolites engage metabolite-sensing G-protein–coupled receptors, including GPR41 and GPR43, which are expressed on a variety of cell types,

including gastrointestinal, adipose, and immune cells. These metabolites also include short-chain fatty acids produced by the microbial fermentation of dietary fibers in the colon.[165] Functions of GPR41 and GPR43 include the regulation of energy intake and expenditure, modulation of glucose metabolism, and the resolution of inflammatory responses via, for example, activation of the NLRP3 inflammasome.[166,167] Short-chain fatty acids can also exert anti-inflammatory effects by inhibiting histone deacetylases, thereby regulating gene transcription and the post-translational modification of proteins such as NF-κB.[168]

It is plausible that dietary AGEs, by inhibiting the growth of short-chain fatty acid–producing bacterial species in the colon, may contribute to heightened inflammatory signals in the gastrointestinal tract and a variety of other body tissues. AGEs have also been found to selectively increase the in vitro expression of histone deacetylases known to be upregulated in the pathogenesis of diabetes complications.[169] It is widely appreciated that this area of research is still in its infancy, and further studies must explore whether dietary AGEs have the capacity to negatively regulate metabolite sensors in the gut.

Incretin axis

The interaction between the incretin and glycation pathways is less studied. Incretins are gut-derived glucoregulatory hormones secreted postprandially from intestinal cells. Glucagon-like peptide 1 signals the glucose-dependent secretion of insulin from pancreatic β cells and thus acts to lower blood glucose. Glucagon-like peptide 1 also slows gastric emptying and enhances satiety signals in the brain.[170] Another incretin hormone secreted in response to food intake, glucose-dependent insulinotropic polypeptide, regulates energy storage through direct actions on adipose tissue and stimulates β-cell proliferation and insulin secretion.[171] Both glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide have been reported to demonstrate anti-inflammatory properties through their effects on the advanced glycation pathway. In addition, they have both been found to downregulate AGE-induced ROS generation and subsequent RAGE expression in vitro by enhancing the generation of cyclic AMP.[172,173] A 24-week high-AGE dietary intervention in rats reduced plasma levels of glucagon-like peptide 1 and impaired insulin secretion in comparison with a low-AGE dietary intervention in rats,[174] suggesting that dietary AGEs play an important role in the early endocrine dysfunction that precipitates the development of diabetes. In an in vitro study, AGEs upregulated endothelial cell production of DPP-4[175] (dipeptidyl peptidase-4, an enzyme that degrades glucagon-like peptide 1 soon after its secretion from intestinal cells), but the precise mechanism by which dietary AGEs modulate incretin signaling is still unknown.

DIETARY ADVANCED GLYCATION END PRODUCTS AND INFLAMMATION: ANIMAL INTERVENTION STUDIES

Dietary AGEs have been shown to potentiate inflammation in vitro. Food-derived AGEs (prior to ingestion) increased proinflammatory cytokine production and depleted antioxidant levels in human endothelial cells in culture.[176] Whether the toxic effects of dietary AGEs outside of the body are replicated in vivo is still being determined. Experimental AGE-restricted diets have prevented or arrested inflammatory processes in animal models and have reduced markers of inflammation in human trials.[177] However, further studies are required to confirm the long-term benefits of dietary AGE restriction in humans.[178]

Animal models represent a unique opportunity for the investigation of the health effects of dietary AGE consumption. Selected studies involving dietary AGEs and their inflammatory effects in animals are shown in Table 1.[42,50,66,81,108,111,114,149,150,179–190] Animal models are less expensive than human trials, and large numbers of animals can be followed for extended time periods. Moreover, dietary intake can be strictly controlled, and MRPs can be provided in food at concentrations that are several orders of magnitude greater than those provided to controls. Other advantages of the study of dietary AGEs in animal models include the ease of profiling proinflammatory genes in target organs and cells and the use of genetically modified mouse models with genetic deletion or overexpression to better elucidate the effects of dietary AGEs on inflammatory pathways. Animal studies have successfully demonstrated clear relationships between dietary AGE consumption and many of the low-grade inflammatory processes associated with both the pathogenesis and the long-term complications of noncommunicable chronic diseases.

Despite the advantages of animal models, it is uncertain whether the investigation of excessive AGE consumption in animals is applicable to human health. High-AGE diets consumed by experimental animals have generally contained 3 to 10 times the AGE content of standard or control diets. In comparison, low-AGE diets in human studies contain only 40% to 50% fewer AGEs than the standard or high-AGE diet in order to maintain palatability and dietary compliance over relatively short time periods (2–16 wk) (Table 1). Accordingly, a major caveat when examining AGE-overfeeding studies is the administration of supraphysiological doses of AGEs or dicarbonyls, which

Table 1 **Summary of animal intervention studies examining the relationship between dietary AGE intake and inflammatory markers**

Reference	Animal model	Dietary intervention (length of intervention)	Dietary AGE ratio (high-AGE to low-AGE)	Effect of dietary intervention on inflammatory markers
Anton et al. (2012)[50]	Wild-type mice With DSS-induced colitis	High-MRP diet vs standard diet (3 wk)	5:1 CML	High-MRP diet resulted in: ↓ Colonic lesions ↓ MPO activity in colonic tissue
Cai et al. (2007)[150]	Male wild-type mice	Regular (high-AGE) diet vs low-AGE diet (96 wk)	2:1 CML	Low-AGE diet resulted in: ↑ GSH/GSSG ratio, ↓plasma 8-isoprostanes, ↑ AGER1, ↓ RAGE and ↓ p66shc expression in spleen, ↑ lifespan
Cai et al. (2012)[149]	Wild-type mice	Standard (high-AGE) diet vs low-AGE diet supplemented with synthetic methylglyoxal vs low-AGE diet (4 generations of mice)	2:1.5:1 CML 2:2.5:1 MG (standard-AGE to MG-supplemented, low-AGE to low-AGE)	Low-AGE, MG-supplemented diet resulted in: ↓ AGER1, ↓ SIRT-1, ↑ RAGE expression in white adipose tissue
Cai et al. (2014)[114]	Wild-type mice	Standard (high-AGE) diet vs low-AGE diet supplemented with methylglyoxal vs low-AGE diet (72 wk)	2:1.5:1 CML 2:2.5:1 MG (standard-AGE to MG-supplemented, low-AGE to low-AGE)	Low-AGE, MG-supplemented diet resulted in: ↓ SIRT-1 and ↓ PPARγ expression in brain, ↑ amyloid-β and AGE deposits in brain, ↑ cognitive and motor deficits, ↑ metabolic syndrome
Cassese et al. (2008)[179]	Female wild-type mice	High-AGE diet vs low-AGE diet (20 wk)	3:1 CML	High-AGE diet resulted in: ↑ Formation of RAGE-PKCα-Src in skeletal muscle, ↑ insulin resistance in skeletal muscle
Chatzigeorgiou et al. (2013)[180] Kandaraki et al. (2012)[181]	Female Wistar rats	High-AGE diet vs low-AGE diet (12 wk)	58:1 CML 2:1 Fructoselysine 2:1 Furosine	High-AGE diet resulted in: ↑ Glucose, ↑ insulin, ↓ testosterone, ↓ estradiol, and ↓ progesterone in serum ↓ RAGE expression in PBMCs ↓ GLO-1 activity in ovarian tissue
Feng et al. (2007)[182]	Rats (5/6 nephrectomy, sham-operated controls)	High-AGE diet vs low-AGE diet vs standard rodent diet (5–13 wk)	7:1:2 CML (high-AGE to low AGE to standard AGE)	Low-AGE diet resulted in: ↓ Macrophage infiltration of remnant kidney, ↓ MCP-1, ↑ glutathione peroxidise activity
Guo et al. (2012)[183]	CD-1 mice	High-AGE diet vs low-AGE diet (4 wk)	5:1 AGE	High-AGE diet resulted in: ↑ MPO activity in lung, ↑ soluble TNF receptor II in plasma following gastric aspiration.
Harcourt et al. (2011)[42]	Wild-type *RAGE* knockout mice	High-AGE, high-fat Western diet vs standard diet (16 wk)	5:1 CML	High-AGE diet resulted in: ↑ RAGE and ↑ MIF expression in kidney, ↓ plasma MIF
Leung et al. (2014)[184]	Male Sprague-Dawley rats	High-AGE methionine- and choline-deficient diet vs standard methionine- and choline-deficient diet (6 wk)	4:1 CML	High-AGE diet resulted in: ↑ CD43, ↑ IL-6, and ↑ TNF-α expression in liver of rats with hepatic steatosis
Lin et al. (2002)[185]	*apoE*-deficient mice with femoral artery injury	High-AGE diet vs low-AGE diet (1 wk prior to artery injury and 4-wk post injury)	10:1 CML	Low-AGE diet resulted in: ↓ Neointimal formation, ↓ macrophage infiltration of neointimal lesions, ↔ plasma cholesterol

(continued)

Table 1 **Continued**

Reference	Animal model	Dietary intervention (length of intervention)	Dietary AGE ratio (high-AGE to low-AGE)	Effect of dietary intervention on inflammatory markers
Lin et al. (2003)[186]	*apoE*-deficient mice with STZ-induced diabetes	High-AGE diet vs low-AGE diet (8 wk)	5:1 CML	Low-AGE diet resulted in: ↓ RAGE expression, ↓ VCAM, ↓ MCP-1, ↓ macrophage infiltration of atherosclerotic lesions, ↔ Cholesterol, ↔ TG, ↔ HDL, ↔ glucose
Matafome et al. (2012)[108]	Wistar-wild type rats with GK-type 2 diabetes)	Drinking water supplemented with MG vs regular drinking water (14 wk)	MG-supplemented water contained MG at 50–75 mg/kg/d	MG supplementation resulted in: ↑ Plasma NEFAs, ↓ serum adiponectin, ↑ urinary 8-isoprostanes, ↑ adipose hypoxia, ↑ macrophage infiltration of adipose tissue, ↑ TGF-β and ↑ MCP-1 expression in adipose tissue
Patel et al. (2012)[81]	Mice	High-AGE diet vs regular AGE diet (39 wk)	3:1 AGE Low-fat diet	High-AGE diet resulted in: Liver neutrophil infiltration present at week 26, in the absence of steatosis. Neutrophil infiltration had resolved at week 39, and steatosis was present
Peppa et al. (2003)[187]	Mice with diabetes (1-cm full-thickness skin wound)	High-AGE diet vs low-AGE diet (12 wk)	5:1 CML	Low-AGE diet resulted in: ↓ Time to full wound closure, ↓ inflammatory cell infiltration of wound at day 21
Sena et al. (2012)[188]	Wistar wild-type rats with GK-type 2 diabetes	Drinking water supplemented with MG vs standard drinking water (12 wk)	MG-supplemented water provided MG at 50–75 mg/kg/d	MG supplementation resulted in: ↑ RAGE expression in vascular wall and ↑ MCP-1 expression in aorta in both wild type and diabetic rats
Shangari et al. (2007)[66]	Rats	High-AGE diet vs regular-AGE diet (10 wk)	9:1 AGE	High-AGE diet resulted in: ↓ Glutathione, ↓ thiamin, ↑ macrophage infiltration of the colon, ↑ nitrotyrosine present in colon, plasma, and liver
Tikellis et al. (2008)[189]	Wild-type *RAGE* knockout mice	High-AGE, high-fat Western diet vs standard diet (16 wk)	3:1 AGE 3:1 fat	High-AGE diet resulted in: ↑ IL-6, ↑ TNF-α, ↑ ICAM-1, ↑ MCP-1, ↑ p65 expression in cardiac tissue. Expression was reduced in RAGE knockout mice on high-AGE diet
Tikellis et al. (2014)[111]	*apoE* knockout mice (*RAGE*/*apoE* double knockout)	Drinking water supplemented with MG vs standard drinking water (6 wk)	MG-supplemented water provided MG at 10 mg/kg/d	MG supplementation resulted in: ↑ MCP-1, ↑ ICAM-1 in circulation, ↑ ICAM-1, ↑ tetherin expression in aortic endothelial cells, ↑ macrophage activation in aorta
Zhu et al. (2011)[190]	Wild-type mice (5-mm burn wound)	Diets supplemented with high-AGE food vs diets not supplemented with high-AGE foods (8 d post burn)	High-AGE diet contained 205 more AU of AGEs per day than low-AGE diet	High-AGE diet resulted in: ↓ Wound healing, ↑ HMGB1 expression in WBC, ↑ plasma HMGB1

Abbreviations: AGE, advanced glycation end product; AGER1, advanced glycation end product receptor 1; apoE, apolipoprotein E; AU, arbitrary units; CML, carboxymethyllysine; DSS, dextran sulfate sodium; GK, Goto-Kakizaki (nonobese model of type 2 diabetes); GLO-1, glyoxalase 1; GSH, reduced glutathione; GSSG, oxidized glutathione; HDL, high-density lipoprotein; HMGB1, high-mobility group box 1; ICAM-1, intracellular adhesion molecule 1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; MG, methylglyoxal; MIF, macrophage migration inhibitory factor; MPO, myeloperoxidase; MRP, Maillard reaction product; NEFAs, nonesterified fatty acids; PBMCs, peripheral blood mononuclear cells; PKCα, protein kinase Cα; RAGE, receptor for advanced glycation end products; SIRT-1, sirtuin 1; STZ, streptozotocin; TG, triglyceride; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; WBC, white blood cells; ↓, significantly lower than comparison diet group post intervention; ↑, significantly higher than comparison diet group post intervention; ↔, no significant difference between low-AGE and high-AGE diet groups post intervention.

highlights the need for cautious interpretation of data from animal studies. Moreover, when assessing the numerous cross-sectional studies, further caution is required not to attribute data to the primary cause of pathology. The initiation of pathology would need to be investigated by studies specifically profiling disease progression. There are also major limitations associated with providing heat-treated food to represent a high-AGE diet during feeding studies, because heating may destroy both antioxidants and heat-labile vitamins, and generate other toxic components within food. In order to demonstrate a causal relationship between dietary AGEs and disease, future research should involve the dietary administration of individual synthetically generated AGEs of known molecular weights.[191]

Dietary AGE restriction in mice prone to atherosclerosis demonstrated significant reductions in the inflammation of vascular lesions and reduced endothelial migration of monocytes due to the downregulation of VCAM-1 and MCP-1 expression, likely related to the reduced expression of RAGE.[185,186] These changes occurred in the absence of any differences in serum cholesterol, triglycerides, high-density lipoprotein, or glucose between low-AGE and high-AGE diet groups. This suggests a role for excessive dietary AGEs very early in the vascular inflammatory process, whereby atherosclerosis is enhanced and vascular repair is inhibited well before changes in circulating lipids or glucose occur. Dietary methylglyoxal was found to be equally detrimental to the vasculature in a rat model of type 2 diabetes.[188] AGEs are well known to mediate damage in the diabetic kidney,[192] with high-AGE diets in rats[182] and mice[42] increasing renal inflammation, macrophage recruitment, and RAGE expression. A low-AGE diet increased antioxidant activity to provide a protective function.

Wounded mice consuming a low-AGE diet exhibited faster healing times than those receiving a high-AGE diet,[187,190] and wounds showed reduced macrophage infiltration. Expression of high-mobility group box-1 was increased in the animals consuming the high-AGE diet, implying that AGEs may participate in the perpetuation and amplification of inappropriate inflammatory signals. The effects of dietary AGEs on intestinal inflammation are contradictory. One study in rats found a high AGE intake to be associated with increased colonic oxidative stress and inflammation, along with reduced antioxidant capacity.[66] In contrast, a high-AGE diet in mice with colitis appeared to be protective of the colon and was associated with reduced intestinal inflammation.[50]

Tikellis et al.[189] compared the effect of a 16-week high-AGE Western diet on cardiac dysfunction in wild-type mice with the effect of standard chow in controls. Mice receiving the Western diet showed significant upregulation in the myocardial expression of inflammatory cytokines IL-6 and TNF-α, chemokines ICAM-1 and MCP-1, and the NF-κB subunit p65. Expression of inflammatory proteins was significantly reduced in RAGE knockout mice on the Western diet compared with that in the wild-type mice, implicating the AGE-RAGE axis as the primary mediator of cardiac inflammation in response to a high-AGE, high-fat diet. In many of the animal studies performed, high-AGE diets contained large quantities of fat, suggesting that the pathogenic endpoints observed might also relate to the fat content rather than to the AGE content alone. Indeed, high-fat feeding in mice has been shown to upregulate proinflammatory cytokine and Toll-like receptor production in white adipose tissue,[193] and the saturated fatty acid palmitate is known to activate NF-κB.[194,195]

Due to their preferential accumulation in the liver and their contribution to RAGE activation, AGEs are thought to induce hepatic injury and contribute to the progression from steatosis to nonalcoholic fatty liver disease.[196] A high-AGE dietary intervention in mice was associated with increased hepatic neutrophil accumulation in the absence of steatosis,[81] implicating food-derived AGEs in the early pathogenesis of liver inflammation. Leung et al.[184] reported that a high-AGE diet increased hepatic AGE accumulation in rats, stimulated NADPH-dependent oxidative stress, and induced liver inflammation and fibrosis. These effects were mediated by RAGE and ROS, as the blockade of NADPH oxidase and RAGE prevented hepatic cellular injury.

Mice consuming a high-AGE diet exhibited impaired insulin action in skeletal muscle prior to any increase in fasting glucose,[179] which was potentiated by an AGE-induced upregulation of protein kinase Cα activity. High-AGE diets in androgenized female rats were associated with hormonal dysregulation[181] and reduced ovarian glyoxalase 1 activity, lending support to the hypothesis that excess dietary toxins might contribute to polycystic ovarian syndrome and its sequelae.

A low-AGE diet supplemented with methylglyoxal induced adipose tissue expansion and insulin resistance and reduced AGER1 expression over 4 generations of mice,[149] suggesting a role not only for AGEs but also for dicarbonyls in the perpetuation of inflammatory stress.

DIETARY ADVANCED GLYCATION END PRODUCTS AND INFLAMMATION: HUMAN INTERVENTION STUDIES

Population studies examining the relationship between dietary AGE intake and chronic inflammatory diseases are limited, primarily because there are currently no

validated survey tools available to assess long-term dietary AGE consumption. A cross-sectional study in humans with type 2 diabetes found positive correlations between habitual high dietary AGE consumption and biomarkers of inflammation such as interleukin 1α (IL-1α), TNF-α, and MCP-1.[197] Another study of healthy adults found associations between elevated dietary AGE consumption and increased levels of high-sensitivity C-reactive protein and VCAM-1.[198]

Dietary intervention trials exploring the effects of dietary AGE restriction on inflammatory markers in humans are summarized in Table 2.[42,151,198–212] These studies have been performed in healthy overweight individuals, women with polycystic ovarian syndrome, people with types 1 and 2 diabetes, and patients with chronic kidney disease or end-stage renal failure. Dietary interventions involving a single high-AGE meal or beverage showed no change in NF-κB DNA-binding activity in peripheral blood mononuclear cells from healthy adults,[206] increased circulating plasminogen activator inhibitor 1 in healthy adults and people with diabetes,[211] and increased serum VCAM-1 and ICAM-1 in people with type 2 diabetes.[202–204,213] These results suggest that a single high-AGE meal could potentially activate certain inflammatory processes. A 2-week low-AGE dietary intervention improved inflammatory markers in overweight males[42] and in overweight people with type 1 or 2 diabetes.[212] A high-AGE diet was capable of influencing the oxidative status of young healthy volunteers within only 4 weeks.[199] Excessive AGE consumption increased oxidative stress and reduced the levels of vitamins and fatty acids known for their anti-inflammatory effects. However, apart from differences in AGE intake, there were also considerable differences in macro- and micronutrient intakes between the study groups.

The inability to avoid significant differences in fat and carbohydrate consumption between low-AGE and high-AGE groups may have also been a potential confounder in a 4-week study involving overweight women, in whom dietary AGE restriction improved insulin sensitivity.[201] A 6-week intervention study that provided isocaloric, nutrient-equivalent diets to healthy individuals on a high-AGE or low-AGE diet found no differences between groups in circulating markers of endothelial function or inflammation.[207] An 8-week high-AGE diet increased insulin resistance, testosterone levels, and oxidative stress in women with polycystic ovarian syndrome.[208] These abnormal parameters subsequently improved after the women were changed to an 8-week low-AGE diet, indicating the potential contribution of dietary AGEs to the hormonal and metabolic pathology associated with polycystic ovarian syndrome. Longer-term studies of 16 weeks' duration in people with diabetes[151] and in healthy individuals[198] favored a low-AGE diet for reducing the production of proinflammatory cytokine TNF-α, reducing the expression of RAGE, and upregulating the anti-inflammatory proteins AGER1 and SIRT-1 in peripheral mononuclear cells. It is thought that low-molecular-weight AGEs do not interact with RAGE,[57,214] so it is uncertain how consumption of a high-AGE meal (during which only low-molecular-weight AGEs are absorbed) can result in a subsequent increase in RAGE activation. It is possible that dicarbonyls could form high-molecular-weight complexes in the circulation immediately following a high-AGE meal, but this requires confirmation by future research.

It has recently been hypothesized that dietary AGEs might increase appetite and energy intake due to enhanced sensory-stimulating properties of MRPs in food.[215] Sebekova et al.,[216] in a recent study in rats, showed that consumption of a diet high in MRPs (in the form of bread crust) for 3 weeks led to increases in food intake and circulating leptin and adiponectin, along with increased expression of hypothalamic and olfactory bulb leptin receptor in the context of an increase in neuronal activity in brain areas involved in the central regulation of food intake and energy homeostasis.[216] Other research, however, fails to support this theory, with a single high-AGE meal having no effect on hunger, appetite hormone responses, or subsequent food intake in overweight individuals.[205] More research is required to determine whether dietary AGEs modulate the processes driving food consumption, with the effects of food-derived AGEs on central appetite regulation and energy homeostasis at the level of the hypothalamus being an exciting new area of investigation. It is possible that the addictive qualities of highly processed foods are mediated by MRPs through effects on the reward centers of the brain.

LIMITATIONS OF DIETARY ADVANCED GLYCATION END PRODUCT STUDIES IN HUMANS

The results of human dietary AGE intervention studies must be interpreted with caution. Humans consume foods rather than individual nutrients, and the proinflammatory effects attributed to AGEs in food may be the result of other detrimental food components generated during the cooking process. A variety of food components commonly found in the Western diet have been shown to correlate with biomarkers of inflammation and endothelial dysfunction,[69,217] and AGEs are likely to be only one of many dietary factors capable of adversely effecting human health. The simple act of eating has been shown to activate NF-κB, regardless of the AGE content of the food consumed.[206] Additionally,

Table 2 **Summary of human intervention studies examining the relationship between dietary AGE intake and inflammatory markers**

Reference	Study location and characteristics of participants	Study design/blinding	Dietary intervention	Method of dietary AGE measurement	Effect of dietary intervention on circulating inflammatory markers
Birlouez-Aragon et al. (2010)[199]	France, n=64 healthy volunteers, 32 M and 32 F (mean age, 19 y; mean BMI, 21.8 kg/m^2)	Crossover Food provided Not blinded	Random assignment to 4-wk low-MRP diet (2.2±0.9 mg CML/d) or 4-wk standard MRP diet (5.4±2.3 mg CML/d) before crossover	GC–MS/MS to CML	High-AGE diet resulted in: ↓ vitamin C, ↓ vitamin E, ↓ total n-3 fatty acids, ↑ ubiquinol
Harcourt et al. (2011)[42]	Australia, n=11 healthy overweight or obese males (mean age, 30 y; mean BMI, 31.8 kg/m^2)	Crossover Food provided Outcome assessors blinded	Random assignment to 2-wk low-AGE diet (3302 kU CML/d) or 2-wk high-AGE diet (14 090 kU CML/d) before crossover	AGE database based on ELISA to CML	Low-AGE diet resulted in: ↓ cystatin C, ↓ MCP-1, ↑ MIF
Luevano-Contreras et al. (2013)[200]	Mexico, n=26 nonsmoking patients with type 2 DM, 3 M and 23 F (mean age, 47.3 y; mean BMI, 29.3 kg/m^2; mean HbA1c, 8.6%; mean DM duration, 5.2 y)	Parallel Participants instructed on food choices and preparation methods Outcome assessors blinded	Random assignment to 6-wk low-AGE diet (n=13; 3996 kU CML/d) or 6-wk high-AGE diet (n=13; 12 214 kU CML/d)	AGE database based on ELISA to CML	Low-AGE diet resulted in: ↓ TNF-α, ↔ CRP
Mark et al. (2014)[201]	Denmark, n=73 women aged 20–50 y with BMI 25–40 kg/m^2	Parallel Participants instructed on food choices and preparation methods. Some food provided Outcome assessors blinded	Random assignment to 4-wk low-AGE diet (n=36) or 4-wk high-AGE diet (n=37). AGE content of diets not reported	LC–MS/MS to CML	High-AGE diet resulted in: ↑ Fasting insulin, ↑ HOMA-IR, ↔ fasting glucose, ↔ postprandial glucose, ↔ GLP-1
Negrean et al. (2007)[202] Stirban et al. (2007)[203]; (2008)[204]; (2008)[213]	Germany, n=20 patients with type 2 DM, 14 M and 6 F (mean age, 55.9 y; mean BMI, 29.5 kg/m^2; mean HbA1c, 8.9%; mean DM duration, 8.7±1.7 y)	Crossover Food provided Outcome assessors blinded	Random assignment to single low-AGE meal (2750 kU CML) or single high-AGE meal (15100 kU CML) before crossover. Measurements at 2, 4, and 6 h postprandial	ELISA to CML	Low-AGE meal resulted in: ↑ Adiponectin, ↑ leptin, ↓ VCAM-1, ↔ fibrinogen, ↔ CRP, ↔ TNF-α, ↔ IL-6
Poulsen et al. (2014)[205]	Denmark, n=19 overweight subjects, 3 M and 16 F (mean age, 34.8 y; mean BMI, 31.3 kg/m^2)	Crossover Food provided Outcome assessors blinded	Assignment to either a single low-AGE meal (2.8 mg CML) or a single high-AGE meal (5.0 mg CML) before crossover to the alternative meal 2 wk later. Measurements at 45, 90, 180, and 300 min postprandial	LC–MS/MS to CML	High-AGE meal resulted in: ↑ Urinary isoprostanes, ↑ VCAM-1, ↔ CRP, ↔ TNF-α, ↔ IL-6, ↔ ICAM-1
Schiekofer et al. (2006)[206]	Germany, n=9 healthy nonsmoking male volunteers (mean age, 32 y; mean BMI, 24 kg/m^2)	Crossover Food provided Not blinded	Assignment to single low-AGE meal (250 g minimally AGE-modified casein, 104.2±23.3 ng CML/mg casein) before crossover to single high-AGE meal (250 g highly AGE-modified casein, 301.7±49.2 ng CML/mg casein). Measurements at 2 h postprandial	ELISA to CML	High-AGE meal resulted in: ↔ NF-κB activation
Semba et al. (2014)[207]	USA, n=24 healthy volunteers (mean age, 59 y; mean BMI, 26 kg/m^2)	Parallel Food provided Outcome assessors blinded	Random assignment to 6-wk low-AGE diet (n=12) or 6-wk high-AGE diet (n=12). AGE content of diets not reported	Database based on ELISA to CML	High-AGE diet resulted in:↔ VCAM-1, ↔ hsCRP, ↔ TNF-α receptors, ↔ IL-6

(continued)

Table 2 **Continued**

Reference	Study location and characteristics of participants	Study design/blinding	Dietary intervention	Method of dietary AGE measurement	Effect of dietary intervention on circulating inflammatory markers
Tantalaki et al. (2014)[208]	Greece, n=23 women with polycystic ovarian syndrome (mean age, 24 y; mean BMI, 26 kg/m^2)	Crossover Participants instructed on food choices and preparation methods Not blinded	Assignment to an 8-wk high-AGE diet (16 000 kU CML/d) followed by an 8-wk low-AGE diet (5700 kU CML/d). No washout	Database based on ELISA to CML	Low-AGE diet resulted in: ↓ Oxidative stress (lipid peroxide concentration)
Uribarri et al. (2003)[209] Peppa et al. (2004)[210]	USA, n=18 patients with nondiabetic end-stage renal failure on peritoneal dialysis (mean age and BMI not reported)	Parallel Participants instructed on food choices and preparation methods Not blinded	Random assignment to either 4-wk low-AGE diet (5500 kU CML/d) or 4-wk high-AGE diet (17 000 kU CML/d)	Database based on ELISA to CML	Low-AGE diet resulted in: ↓ CRP, ↓ PAI-1, ↓ TNF-α, ↓ VCAM-1
Uribarri et al. (2007)[211]	USA, n=44 subjects with DM, 36 M and 8 F (mean age, 51 y; mean HbA1c, 8.6%), and n=10 healthy subjects, 5 M and 5 F (mean age, 43 y)	Pre and post test Beverage provided	Single oral challenge of a high-AGE beverage containing 1800 kU CML. Measurements taken before and 90 and 150 min after beverage consumption	ELISA to CML	High-AGE oral challenge resulted in: DM and healthy subjects: ↑ PAI-1, ↔ VCAM-1
Uribarri et al. (2011)[151]	USA, n=36 subjects, 18 with type 2 DM and 18 without DM (mean age, 64 y; mean BMI, 29.8 kg/m^2; HbA1c in patients with DM not reported)	Parallel Participants instructed on food choices and preparation methods Not blinded	Random assignment to 16-wk standard diet (>20 000 kU CML/d) or 16-wk AGE-restricted diet (<10 000 kU CML/d)	Database based on ELISA to CML	Low-AGE diet resulted in: Type 2 DM subjects: ↑ adiponectin, ↓ leptin, ↓ TNF-α in circulation, ↑ AGER1 mRNA, ↑ SIRT-1 mRNA, and ↓ RAGE mRNA in MNCs Healthy subjects: ↓ TNF-α in circulation
Vlassara et al. (2002)[212]	USA, n=11 subjects, 2 with type 1 DM and 8 with type 2 DM (mean age, 52 y; mean HbA1c, 7.8%; mean BMI, 28 kg/m^2)	Crossover Food provided Not blinded	Random assignment to 2-wk low-AGE diet (3670 kU CML/d) or 2-wk high-AGE diet (16 300 kU CML/d) before crossover	Database based on ELISA to CML	High-AGE diet resulted in: ↑ TNF-α, ↑ VCAM-1, ↔ CRP
Vlassara et al. (2002)[212]	USA, n=13 subjects, 4 with type 1 DM and 9 with type 2 DM (mean age, 62 y; mean HbA1c, 7.2%; mean BMI, 30 kg/m^2)	Parallel Food provided Not blinded	Random assignment to either 6-wk low-AGE diet (3670±1200 kU CML/d) or 6-wk high-AGE diet (16 300±3700 kU CML/d)	Database based on ELISA to CML	High-AGE diet resulted in ↑ CRP, ↑ TNF-α Low-AGE diet resulted in ↓ CRP, ↓ VCAM-1
Vlassara et al. (2009)[198]	USA, n=30 healthy subjects and 9 patients with CKD	Parallel Healthy participants instructed on food choices and preparation methods Food provided to participants with CKD Not blinded	Healthy subjects: random assignment to 16-wk regular diet (>13 000 kU CML/d) or 16-wk low-AGE diet (<5500 kU CML/d). CKD patients: random assignment to these same groups, but duration of intervention was 4 wk	Database based on ELISA to CML	Low-AGE diet resulted in: Healthy subjects: ↓ AGER1 mRNA, ↓ RAGE mRNA in MNCs, ↓ TNF-α, ↓ VCAM-1, ↔ CRP in circulation CKD subjects: ↓ TNF-α, ↔ VCAM-1 in circulation, ↑ AGER1 mRNA, ↔ RAGE mRNA in MNCs

Abbreviations and symbols: AGE, advanced glycation end product; AGER-1, advanced glycation end product receptor 1; BMI, body mass index; CKD, chronic kidney disease; CML, carboxymethyllysine; CRP, C-reactive protein; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; GC–MS/MS, gas chromatography coupled to tandem mass spectrometry; GLP-1, glucagon-like peptide 1; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment – insulin resistance; hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; kU, kilounits; LC–MS/MS, liquid chromatography coupled to tandem mass spectrometry; MCP-1, monocyte chemotactic protein 1; MIF, macrophage migration inhibitory factor; MNCs, mononuclear cells; mRNA, messenger ribose nucleic acid; MRP, Maillard reaction product; NF-κB, nuclear factor–kappa B; PAI-1, plasminogen activator inhibitor 1; RAGE, receptor for advanced glycation end products; SIRT-1, sirtuin 1; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; ↓, significantly lower than comparison diet group postintervention; ↑, significantly higher than comparison diet group postintervention; ↔, no significant difference between low-AGE and high-AGE diet groups postintervention.

the benefits associated with a low-AGE diet may be more closely related to a higher antioxidant concentration in the food (which would have otherwise been destroyed during the cooking process) rather than a reduced AGE content.

Dietary AGEs are thought to exert their negative effects over many years, thereby limiting the conclusions that can be drawn from short-term human intervention trials that range in length from a single meal to 16 weeks. Longer-term studies might be problematic because many individuals will find it difficult to adhere to a low-AGE diet, which may be less palatable than the high-AGE Western diet to which they are accustomed. Many of the trials contained very low numbers of participants, and most were likely to be underpowered. A number of the trials did not provide participants with food throughout the study, but instead provided instructions to each subject about appropriate food choices and cooking methods. Reduced dietary compliance may have been an issue during these studies, particularly during the later stages of the trials. Some trials administered dietary AGEs at concentrations that far exceeded normal physiological intake, so the results should be interpreted with caution.

Twelve of the 15 dietary AGE intervention studies listed in Table 2 either utilized an ELISA technique to measure the CML content of food or referred to an AGE database that was developed using this technique.[12] While this database was very useful when information about the potential toxicity of food-derived AGEs was still in its infancy, more accurate and validated methods of AGE measurement are now available and should be utilized where possible. To date, most studies have focused on the assessment of CML in food, body fluids, and tissue. CML is only one of many AGEs, most of which have not yet been fully characterized. More information about dietary AGEs other than CML is required, as other AGE moieties are likely to have a variety of different effects on human health. Some AGEs in the body may merely be markers of inflammation rather than causal agents, and others might be capable of exerting beneficial antioxidant activity.

REDUCING ADVANCED GLYCATION END PRODUCT INTAKE

There is currently insufficient evidence to recommend reducing dietary AGE intake as a therapeutic strategy for the prevention and/or management of inflammatory conditions in humans. However, a variety of agents that inhibit the formation or action of AGEs have been identified. Exposure to AGEs could be minimized by substances that prevent the generation of AGEs (by reducing glucose and/or ROS, acting as dicarbonyl traps, or chelating metal ions), interfere with the action of AGEs (by putative crosslink breakers or RAGE antagonists), or prevent exposure to AGEs (by AGE detoxification or reduced environmental contact with AGEs), as suggested in a recent review.[218] A mitochondria-targeted reagent, MitoG, that assesses the levels of mitochondrial dicarbonyls has recently been developed.[219] Since this compound sequesters methylglyoxal, it may hold promise for targeting intracellular AGE formation.

Strategies to limit the intake of diet-derived AGEs have been previously discussed.[12,220] The phosphate binder sevelamer carbonate is frequently used in patients with chronic kidney disease and, in addition to phosphate, is known to bind to a number of compounds in the gut, such as bile acids, bacterial endotoxins, and AGEs, thus preventing their absorption.[221] Independent of its phosphate-lowering ability, sevelamer is thought to attenuate coronary artery calcification, atherosclerosis, and endothelial damage in chronic kidney disease patients by reducing serum glucose, cholesterol, ROS, and inflammatory markers.[222] Exploratory studies indicate that sevelamer's capacity to sequester intestinal AGEs is sufficient to reduce circulating AGEs[223,224] and may prove to be a useful tool in reducing the absorption of dietary AGEs.

Although the absolute content of individual AGE moieties in food is the subject of controversy, there is a general consensus that bakery products, fried meats, and other highly heated foods contain a high proportion of AGEs. Limiting the dietary intake of foods that have undergone browning by the application of intense heat, particularly food and beverages containing high levels of protein or carbohydrate derived from simple sugars, would significantly reduce AGE consumption (see Figure 2). Modification of cooking methods, such as the replacement of frying and baking (where food is exposed to intense, dry heat) with steaming, boiling, and stewing (where food has a higher water content and is cooked at lower temperatures), reduces the formation of AGEs, though achieving maximum palatability and desired texture may be problematic. Alternatively, the addition of natural products (such as green tea extract, garlic, or vitamin C) known to inhibit AGE formation during the cooking process may offer a solution.[225,226] Previous studies have suggested that acidic ingredients such as lemon juice or vinegar reduce AGE formation by lowering the pH of the food.[20] Thiamin (vitamin B_1) and its derivatives are able to scavenge dicarbonyl compounds, thereby inhibiting AGE formation.[227,228] Rutin (a flavonoid) and α-tocopherol (vitamin E) are powerful antioxidants capable of neutralizing ROS and reducing AGE production associated with oxidative stress.[229,230] The Mediterranean diet has been suggested as a potential dietary strategy in the prevention of AGE toxicity because it

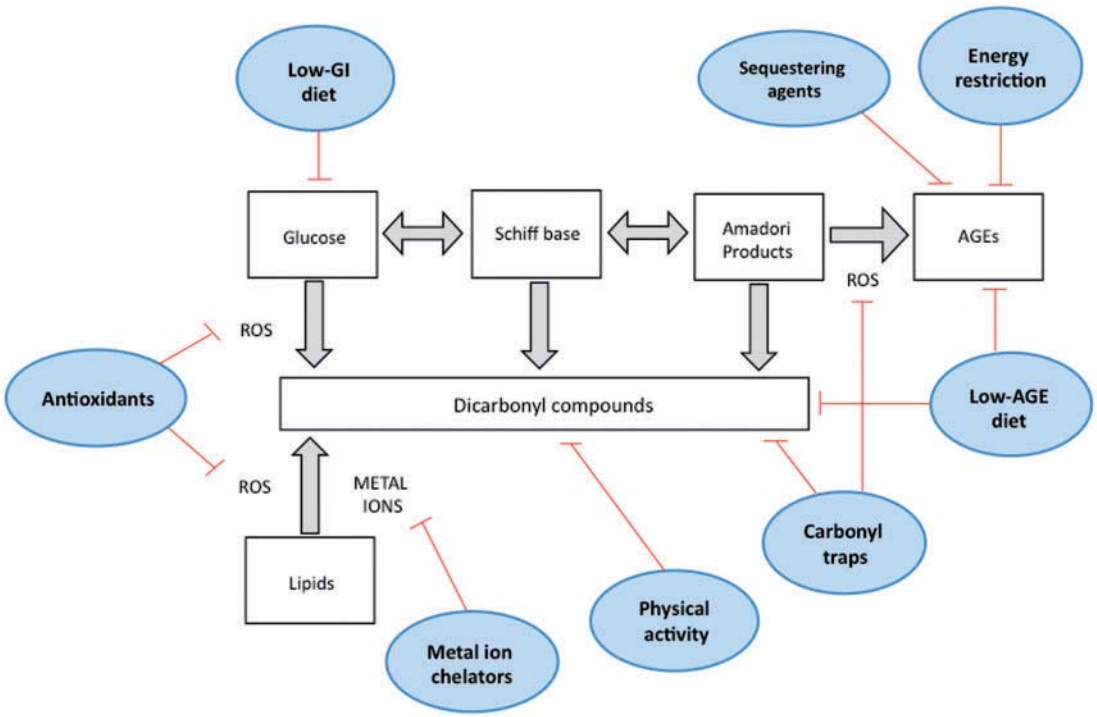


Figure 2 **Potential dietary and lifestyle strategies for the reduction of AGE-induced toxicity.** Potential dietary and lifestyle strategies hypothesized to attenuate AGE-induced pathology. Antioxidants (α-tocopheral, ascorbic acid, flavonoids, lipoic acid), metal ion chelators (carnosine, citric acid), carbonyl traps (vitamin B_1, vitamin B_6), sequestering agents (phosphate binders), low-AGE diet (dietary AGE restriction, Mediterranean diet). *Abbreviations*: GI, gastrointestinal; ROS, reactive oxygen species.

is lower in AGEs and higher in antioxidants than the typical Western diet.[79,231]

Interestingly, Nagai et al.[232] demonstrated that citrate (a derivative of citric acid) also inhibited the formation of CML and *N*ε-carboxyethyllysine in the lens of diabetic rats. Citric acid is a dietary chelator found in citrus fruits and drinks, and the authors suggested that it may inhibit AGE formation, possibly by limiting the uptake or promoting the excretion of metal ions through chelating activity. Diabetic rats fed n-3 polyunsaturated fatty acids while receiving a high-fat thermolyzed diet demonstrated increased antioxidant superoxide dismutase activity and reduced lipoperoxidation and AGE levels in the liver compared with controls.[233] However, the high fat content of the diets may have contributed to the liver toxicity observed. Physical activity has also been hypothesized to reduce AGE formation by increasing the body's capacity to detoxify methylglyoxal. Exercise increases activation of the Nrf2/ARE pathway and stimulates glutathione synthesis, which may attenuate the accumulation of dicarbonyls.[234] However, much of the research involving AGE-inhibiting compounds is limited to in vitro or animal studies, and therefore it is not yet known whether these interventions will have clinical applications.

Additionally, in the small number of human trials specifically designed to determine the effectiveness of AGE-reducing agents, serum AGE levels have been measured as the primary endpoint. This is problematic because it has not yet been fully established whether a reduction in circulating AGEs translates into improved health outcomes. At this time, it appears that adherence to a diet based on current dietary guidelines and maintenance of a healthy body weight, avoidance of cigarette smoking, and engaging in regular physical activity offers the best strategy to minimize the effects of AGEs.

Current evidence indicates that, in order to limit dietary intake of dicarbonyl compounds such as 3-deoxyglucosone and methylglyoxal, consumption of a diet based on fresh fruits, vegetables, and milk products would be advantageous.[10] Increased consumption of sugar-rich products such as sugar beet syrup and high fructose corn syrup, together with the consumption of fruit juices and beer, may increase dicarbonyl intake. However, as discussed earlier, the paucity of knowledge regarding bioavailability and intestinal absorption of dicarbonyl compounds prevents conclusions about the impact of excess dicarbonyl consumption in humans.

FUTURE DIRECTIONS

Long-term dietary studies are required to more accurately determine the effect of food-derived AGEs on chronic

low-grade inflammatory conditions. As adherence to a low-AGE diet might be challenging for many individuals, the use of agents that sequester dietary AGEs in the intestine in order to reduce their absorption from food may have future therapeutic potential for the inhibition of inflammatory pathways.[222] The effect of dietary AGEs on the composition and function of the gut microbiota and its metabolites requires intensive research. Diets aimed at favorably altering the bacterial composition within the colon could potentially reduce intestinal precursors to MRPs, decrease MRP absorption, or minimize pathogenic microorganisms in the gut able to generate and secrete AGEs.

The transient postprandial hyperglycemia induced by food intake (independent of its AGE content) has been shown to induce microinflammatory processes within the body.[235] The use of short-term intermittent fasting requires further exploration, as it may be more beneficial than dietary AGE restriction for the attenuation of inflammation. An additional advantage of intermittent fasting is the increased likelihood of weight reduction, which has been shown to reduce circulating AGE levels.[236] In a society where many individuals continuously "graze" rather than maintain specific mealtimes, there may be benefits in simply limiting the number of eating occasions on any given day. Consumption of a low-glycemic-index diet is associated with reductions in postmeal blood glucose and insulin excursions as well as lower levels of circulating inflammatory markers and advanced glycation end products.[237] High-glycemic-index diets in mice are associated with a 3-fold increase in pathological AGE accumulation in tissue,[238] supporting further exploration of low-glycemic-index diets for attenuation of AGEs in humans.

CONCLUSION

It is clear that the role of AGEs in the pathogenesis of chronic inflammatory conditions requires more research. AGEs and their precursors are ubiquitous in the highly processed foods characteristic of typical Western diets, and it is plausible that AGEs may contribute significantly to the inflammatory milieu associated with many chronic noncommunicable diseases, though the potential of diet-derived AGEs to facilitate inflammation is still unresolved. AGE accumulation can be manipulated via a number of nutritional components to reduce protein glycation, alter dietary metabolites, or enhance dicarbonyl detoxification systems. However, additional targeted research is required to determine the long-term consequences of chronic AGE overconsumption and the optimal strategies for minimizing AGE-related pathology.

Acknowledgments

Funding. This work was completed with support from the National Health and Medical Research Council of Australia (NHMRC) (MC grant no. 1043753) and the Victorian Government's Operational Infrastructure Support Program. N.K. holds an NHMRC postgraduate scholarship grant, no. 1039709. M.T.C. holds an Australian and New Zealand Society of Nephrology Career Development Award. Neither the NHMRC nor the Victorian government had a role in the design, analysis, or writing of this article.

Declaration of interest. The authors have no relevant interests to declare.

Author contributions. M.T.C. conceived the article topic, and M.T.C. and N.J.K. researched and wrote the article.

REFERENCES

1. Tahara N, Yamagishi S, Matsui T, et al. Serum levels of advanced glycation end products (AGEs) are independent correlates of insulin resistance in nondiabetic subjects. Cardiovasc Ther. 2012;30:42–48.
2. Tan KC, Shiu SW, Wong Y, et al. Serum advanced glycation end products (AGEs) are associated with insulin resistance. Diabetes Metab Res Rev. 2011;27:488–492.
3. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res. 2004;63:582–592.
4. Nin JW, Jorsal A, Ferreira I, et al. Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. Diabetes Care. 2011;34:442–447.
5. Coughlan MT, Forbes JM. Temporal increases in urinary carboxymethyllysine correlate with albuminuria development in diabetes. Am J Nephrol. 2011;34:9–17.
6. Milne R, Brownstein S. Advanced glycation end products and diabetic retinopathy. Amino Acids. 2013;44:1397–1407.
7. Henle T. Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. Amino Acids. 2005;29:313–322.
8. Rabbani N, Thornalley PJ. Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino Acids. 2012;42:1133–1142.
9. Vistoli G, De Maddis D, Cipak A, et al. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic Res. 2013;47(suppl 1):3–27.
10. Degen J, Hellwig M, Henle T. 1,2-dicarbonyl compounds in commonly consumed foods. J Agric Food Chem. 2012;60:7071–7079.
11. Goldberg T, Cai W, Peppa M, et al. Advanced glycoxidation end products in commonly consumed foods. J Am Diet Assoc. 2004;104:1287–1291.
12. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc. 2010;110:911.e12–916.e12.
13. Ahmed N, Mirshekar-Syahkal B, Kennish L, et al. Assay of advanced glycation endproducts in selected beverages and food by liquid chromatography with tandem mass spectrometric detection. Mol Nutr Food Res. 2005;49:691–699.
14. Gensberger S, Glomb MA, Pischetsrieder M. Analysis of sugar degradation products with alpha-dicarbonyl structure in carbonated soft drinks by UHPLC-DAD-MS/MS. J Agric Food Chem. 2013;61:10238–10245.
15. Ames JM. Control of the Maillard reaction in food systems. Trends Food Sci Technol. 1990;1:150–154.
16. Chuyen NV. Maillard reaction and food processing. Application aspects. Adv Exp Med Biol. 1998;434:213–235.
17. Chuyen NV, Ijichi K, Umetsu H, et al. Antioxidative properties of products from amino acids or peptides in the reaction with glucose. Adv Exp Med Biol. 1998;434:201–212.
18. Dittrich R, Dragonas C, Kannenkeril D, et al. A diet rich in Maillard reaction products protects LDL against copper induced oxidation ex vivo, a human intervention trial. Food Res Int. 2009;42:1315–1322.
19. Reineccius GA, Whorton C. Flavor Problems Associated with the Microwave Cooking of Food Products. Vol. 1. Basel: Birkhauser Verlag; 1990.

20. O'Brien J, Morrissey PA. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci Nutr. 1989;28:211–248.
21. Bengmark S. Advanced glycation and lipoxidation end products – amplifiers of inflammation: the role of food. JPEN J Parenter Enteral Nutr. 2007;31:430–440.
22. Tan D, Wang Y, Lo CY, et al. Methylglyoxal: its presence in beverages and potential scavengers. Ann N Y Acad Sci. 2008;1126:72–75.
23. Ames JM. Determination of *N* epsilon-(carboxymethyl)lysine in foods and related systems. Ann N Y Acad Sci. 2008;1126:20–24.
24. Rabbani N, Thornalley PJ. Dicarbonyls linked to damage in the powerhouse: glycation of mitochondrial proteins and oxidative stress. Biochem Soc Trans. 2008;36(pt 5):1045–1050.
25. Assar SH, Moloney C, Lima M, et al. Determination of *N* epsilon-(carboxymethyl)lysine in food systems by ultra performance liquid chromatography-mass spectrometry. Amino Acids. 2009;36:317–326.
26. Foerster A, Henle T. Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline. Biochem Soc Trans. 2003;31(pt 6):1383–1385.
27. Hull GLJ, Woodside JV, Ames JM, et al. *N* epsilon-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet. Food Chem. 2012;131:170–174.
28. Dresden University of Technology. AGE Database, 2012. Dresden, Germany. http://tu-dresden.de/die_tu_dresden/fakultaeten/fakultaet_mathematik_und_naturwissenschaften/fachrichtung_chemie/lc/forschung/age_intro?set_language=en. Updated March 23, 2015. Accessed March 22, 2014.
29. Luevano-Contreras C, Durkin T, Pauls M, et al. Development, relative validity, and reliability of a food frequency questionnaire for a case-control study on dietary advanced glycation end products and diabetes complications. Int J Food Sci Nutr. 2013;64:1030–1035.
30. Henle T. AGEs in foods: do they play a role in uremia? Kidney Int Suppl. 2003;63:S145–S147.
31. Hellwig M, Matthes R, Peto A, et al. N-epsilon-fructosyllysine and N-epsilon-carboxymethyllysine, but not lysinoalanine, are available for absorption after simulated gastrointestinal digestion. Amino Acids. 2014;46:289–299.
32. Hellwig M, Geissler S, Matthes R, et al. Transport of free and peptide-bound glycated amino acids: synthesis, transepithelial flux at Caco-2 cell monolayers, and interaction with apical membrane transport proteins. Chembiochem. 2011;12:1270–1279.
33. Tuohy KM, Hinton DJ, Davies SJ, et al. Metabolism of Maillard reaction products by the human gut microbiota – implications for health. Mol Nutr Food Res. 2006;50:847–857.
34. Koschinsky T, He CJ, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci U S A. 1997;94:6474–6479.
35. Faist V, Erbersdobler HF. Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. Ann Nutr Metab. 2001;45:1–12.
36. He C, Sabol J, Mitsuhashi T, et al. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. Diabetes. 1999;48:1308–1315.
37. Bergmann R, Helling R, Heichert C, et al. Radio fluorination and positron emission tomography (PET) as a new approach to study the in vivo distribution and elimination of the advanced glycation endproducts N epsilon-carboxymethyllysine (CML) and N epsilon-carboxyethyllysine (CEL). Nahrung. 2001;45:182–188.
38. Papetti A, Mascherpa D, Marrubini G, et al. Effect of in vitro digestion on free alpha-dicarbonyl compounds in balsamic vinegars. J Food Sci. 2013;78:C514–C519.
39. Uribarri J, Peppa M, Cai W, et al. Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. Am J Kidney Dis. 2003;42:532–538.
40. Cai W, He JC, Zhu L, et al. High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. Circulation. 2004;110:285–291.
41. Uribarri J, Cai W, Peppa M, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci. 2007;62:427–433.
42. Harcourt BE, Sourris KC, Coughlan MT, et al. Targeted reduction of advanced glycation improves renal function in obesity. Kidney Int. 2011;80:190–198.
43. Piroddi M, Palazzetti I, Quintaliani G, et al. Circulating levels and dietary intake of the advanced glycation end-product marker carboxymethyl lysine in chronic kidney disease patients on conservative predialysis therapy: a pilot study. J Ren Nutr. 2011;21:329–339.
44. Semba RD, Ang A, Talegawkar S, et al. Dietary intake associated with serum versus urinary carboxymethyl-lysine, a major advanced glycation end product, in adults: the Energetics Study. Eur J Clin Nutr. 2012;66:3–9.
45. Coughlan MT, Patel SK, Jerums G, et al. Advanced glycation urinary protein-bound biomarkers and severity of diabetic nephropathy in man. Am J Nephrol. 2011;34:347–355.
46. Tessier FJ, Birlouez-Aragon I. Health effects of dietary Maillard reaction products: the results of ICARE and other studies. Amino Acids. 2012;42:1119–1131.
47. Delgado-Andrade C, Tessier FJ, Niquet-Leridon C, et al. Study of the urinary and faecal excretion of N (epsilon)-carboxymethyllysine in young human volunteers. Amino Acids. 2012;43:595–602.
48. Roncero-Ramos I, Delgado-Andrade C, Tessier FJ, et al. Metabolic transit of N(epsilon)-carboxymethyl-lysine after consumption of AGEs from bread crust. Food Funct. 2013;4:1032–1039.
49. Alamir I, Niquet-Leridon C, Jacolot P, et al. Digestibility of extruded proteins and metabolic transit of N epsilon -carboxymethyllysine in rats. Amino Acids. 2013;44:1441–1449.
50. Anton PM, Craus A, Niquet-Leridon C, et al. Highly heated food rich in Maillard reaction products limit an experimental colitis in mice. Food Funct. 2012;3:941–949.
51. Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and implications for the pathophysiology of heart failure. Curr Heart Fail Rep. 2012;9:107–116.
52. Rai V, Toure F, Chitayat S, et al. Lysophosphatidic acid targets vascular and oncogenic pathways via RAGE signaling. J Exp Med. 2012;209:2339–2350.
53. Mills DJ, Tuohy KM, Booth J, et al. Dietary glycated protein modulates the colonic microbiota towards a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis subjects. J Appl Microbiol. 2008;105:706–714.
54. Seiquer I, Rubio LA, Peinado MJ, et al. Maillard reaction products modulate gut microbiota composition in adolescents. Mol Nutr Food Res. 2014;58:1552–1560.
55. Borrelli RC, Fogliano V. Bread crust melanoidins as potential prebiotic ingredients. Mol Nutr Food Res. 2005;49:673–678.
56. Helou C, Marier D, Jacolot P, et al. Microorganisms and Maillard reaction products: a review of the literature and recent findings. Amino Acids. 2014;46:267–277.
57. Penfold SA, Coughlan MT, Patel SK, et al. Circulating high-molecular-weight RAGE ligands activate pathways implicated in the development of diabetic nephropathy. Kidney Int. 2010;78:287–295.
58. Bytzer P, Talley NJ, Leemon M, et al. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. Arch Intern Med. 2001;161:1989–1996.
59. Stevens JE, Jones KL, Rayner CK, et al. Pathophysiology and pharmacotherapy of gastroparesis: current and future perspectives. Expert Opin Pharmacother. 2013;14:1171–1186.
60. Visser J, Rozing J, Sapone A, et al. Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. Ann N Y Acad Sci. 2009;1165:195–205.
61. Vaarala O. Leaking gut in type 1 diabetes. Curr Opinion Gastroenterol. 2008;24:701–706.
62. Carratu R, Secondulfo M, de Magistris L, et al. Altered intestinal permeability to mannitol in diabetes mellitus type I. J Pediatr Gastroenterol Nutr. 1999;28:264–269.
63. Andrassy M, Igwe J, Autschbach F, et al. Posttranslationally modified proteins as mediators of sustained intestinal inflammation. Am J Pathol. 2006;169:1223–1237.
64. Li W, Maloney RE, Circu ML, et al. Acute carbonyl stress induces occludin glycation and brain microvascular endothelial barrier dysfunction: role for glutathione-dependent metabolism of methylglyoxal. Free Radic Biol Med. 2013;54:51–61.
65. Chen P, Zhao J, Gregersen H. Up-regulated expression of advanced glycation end-products and their receptor in the small intestine and colon of diabetic rats. Dig Dis Sci. 2012;57:48–57.
66. Shangari N, Depeint F, Furrer R, et al. A thermolyzed diet increases oxidative stress, plasma α-aldehydes and colonic inflammation in the rat. Chem Biol Interact. 2007;169:100–109.
67. Cohen-Or I, Katz C, Ron EZ. AGEs secreted by bacteria are involved in the inflammatory response. PLoS One. 2011;6:e17974. doi:10.1371/journal.pone.0017974.
68. Kellow NJ, Coughlan MT, Savige GS, et al. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial. BMC Endocr Disord. 2014;14:55. doi:10.1186/1472-6823-14-55.
69. Calder PC, Ahluwalia N, Brouns F, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106(suppl 3):S5–S78.
70. Caballero AE. Metabolic and vascular abnormalities in subjects at risk for type 2 diabetes: the early start of a dangerous situation. Arch Med Res. 2005;36:241–249.
71. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615–1625.
72. Thornalley PJ. Dicarbonyl intermediates in the Maillard reaction. Ann N Y Acad Sci. 2005;1043:111–117.
73. Han L, Li L, Li B, et al. Review of the characteristics of food-derived and endogenous Nε-carboxymethyllysine. J Food Prot. 2013;76:912–918.
74. Vlassara H, Uribarri J, Cai W, et al. Advanced glycation end product homeostasis: exogenous oxidants and innate defenses. Ann N Y Acad Sci. 2008;1126:46–52.

75. Schwenger V, Morath C, Schonfelder K, et al. An oral load of the early glycation compound lactuloselysine fails to accumulate in the serum of uraemic patients. Nephrol Dial Transplant. 2006;21:383–388.
76. Sebekova K, Boor P, Valachovicova M, et al. Association of metabolic syndrome risk factors with selected markers of oxidative status and microinflammation in healthy omnivores and vegetarians. Mol Nutr Food Res. 2006;50: 858–868.
77. Coughlan MT, Yap FY, Tong DC, et al. Advanced glycation end products are direct modulators of β-cell function. Diabetes. 2011;60:2523–2532.
78. Dhar A, Dhar I, Jiang B, et al. Chronic methylglyoxal infusion by minipump causes pancreatic β-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. Diabetes. 2011;60:899–908.
79. Steele M, Stuchbury G, Munch G. The molecular basis of the prevention of Alzheimer's disease through healthy nutrition. Exp Gerontol. 2007;42:28–36.
80. Ilchmann A, Burgdorf S, Scheurer S, et al. Glycation of a food allergen by the Maillard reaction enhances its T-cell immunogenicity: role of macrophage scavenger receptor class A type I and II. J Allergy Clin Immunol. 2010;125: 175–183.e171–111.
81. Patel R, Baker SS, Liu W, et al. Effect of dietary advanced glycation end products on mouse liver. PLoS One. 2012;7:e35143. doi:10.1371/journal.pone.0035143.
82. Watanabe T, Asai K, Fujimoto H, et al. Increased levels of HMGB-1 and endogenous secretory RAGE in induced sputum from asthmatic patients. Resp Med. 2011;105:519–525.
83. Kato S, Itoh K, Ochiai M, et al. Increased pentosidine, an advanced glycation endproduct, in urine and tissue reflects disease activity in inflammatory bowel diseases. J Gastroenterol Hepatol. 2008;23(suppl 2):S140–S145.
84. Rojas A, Figueroa H, Morales E. Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis. Carcinogenesis. 2010;31:334–341.
85. Smit AJ, Lutgers HL. The clinical relevance of advanced glycation endproducts (AGE) and recent developments in pharmaceutics to reduce AGE accumulation. Curr Med Chem. 2004;11:2767–2784.
86. Schalkwijk CG, Miyata T. Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. Amino Acids. 2012;42:1193–1204.
87. Coughlan MT, Thorburn DR, Penfold SA, et al. RAGE-induced cytosolic ROS promote mitochondrial superoxide generation in diabetes. J Am Soc Nephrol. 2009;20:742–752.
88. Murata-Kamiya N, Kamiya H, Kaji H, et al. Mutations induced by glyoxal and methylglyoxal in mammalian cells. Nucleic Acids Symp Ser. 2000;(44):3–4.
89. Yim MB, Yim HS, Lee C, et al. Protein glycation: creation of catalytic sites for free radical generation. Ann N Y Acad Sci. 2001;928:48–53.
90. Kalapos MP. The tandem of free radicals and methylglyoxal. Chem Biol Interact. 2008;171:251–271.
91. Jaganjac M, Tirosh O, Cohen G, et al. Reactive aldehydes – second messengers of free radicals in diabetes mellitus. Free Radic Res. 2013;47(suppl 1):39–48.
92. Bierhaus A, Schiekofer S, Schwaninger M, et al. Diabetes-associated sustained activation of the transcription factor nuclear factor–κB. Diabetes. 2001;50:2792–2808.
93. Nedic O, Rattan SI, Grune T, et al. Molecular effects of advanced glycation end products on cell signalling pathways, ageing and pathophysiology. Free Radic Res. 2013;47(suppl 1):28–38.
94. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a "glycoxidation-centric" point of view. Nutr Metab Cardiovasc Dis. 2013;23:913–919.
95. Sun JK, Keenan HA, Cavallerano JD, et al. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: the Joslin 50-Year Medalist Study. Diabetes Care. 2011;34:968–974.
96. Ramasamy R, Yan SF, Schmidt AM. The diverse ligand repertoire of the receptor for advanced glycation endproducts and pathways to the complications of diabetes. Vascul Pharmacol. 2012;57:160–167.
97. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE, the receptor for advanced glycation end products. J Mol Med (Berl). 2005;83:876–886.
98. Ramasamy R, Yan SF, Schmidt AM. Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications. Ann N Y Acad Sci. 2011;1243:88–102.
99. Barlovic DP, Thomas MC, Jandeleit-Dahm K. Cardiovascular disease: what's all the AGE/RAGE about? Cardiovasc Hematol Disord Drug Targets. 2010;10:7–15.
100. Ibrahim ZA, Armour CL, Phipps S, et al. RAGE and TLRs: relatives, friends or neighbours? Mol Immunol. 2013;56:739–744.
101. Thornalley PJ. Glyoxalase I – structure, function and a critical role in the enzymatic defence against glycation. Biochem Soc Trans. 2003;31(pt 6):1343–1348.
102. Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. Diabetes. 2010;59:249–255.
103. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107:1058–1070.
104. Ankrah NA, Appiah-Opong R. Toxicity of low levels of methylglyoxal: depletion of blood glutathione and adverse effect on glucose tolerance in mice. Toxicol Lett. 1999;109:61–67.
105. Yamawaki H, Saito K, Okada M, Hara Y. Methylglyoxal mediates vascular inflammation via JNK and p38 in human endothelial cells. Am J Physiol Cell Physiol. 2008;295:C1510–C1517.
106. Su Y, Lei X, Wu L, et al. The role of endothelial cell adhesion molecules P-selectin, E-selectin and intercellular adhesion molecule-1 in leucocyte recruitment induced by exogenous methylglyoxal. Immunology. 2012;137:65–79.
107. Vasdev S, Ford CA, Longerich L, et al. Role of aldehydes in fructose induced hypertension. Mol Cell Biochem. 1998;181:1–9.
108. Matafome P, Santos-Silva D, Crisóstomo J, et al. Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity. Arch Physiol Biochem. 2012;118:58–68.
109. Mukohda M, Yamawaki H, Nomura H, et al. Methylglyoxal inhibits smooth muscle contraction in isolated blood vessels. J Pharmacol Sci. 2009;109:305–310.
110. Crisóstomo J, Matafome P, Santos-Silva D, et al. Methylglyoxal chronic administration promotes diabetes-like cardiac ischaemia disease in Wistar normal rats. Nutr Metab Cardiovasc Dis. 2013;23:1223–1230.
111. Tikellis C, Pickering RJ, Tsorotes D, et al. Dicarbonyl stress in the absence of hyperglycemia increases endothelial inflammation and atherogenesis similar to that observed in diabetes. Diabetes. 2014;63:3915–3925.
112. Brouwers O, Niessen PM, Miyata T, et al. Glyoxalase-1 overexpression reduces endothelial dysfunction and attenuates early renal impairment in a rat model of diabetes. Diabetologia. 2014;57:224–235.
113. Giacco F, Du X, D'Agati VD, et al. Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. Diabetes. 2014;63:291–299.
114. Cai W, Uribarri J, Zhu L, et al. Oral glycotoxins are a modifiable cause of dementia and the metabolic syndrome in mice and humans. Proc Natl Acad Sci U S A. 2014;111:4940–4945.
115. Sauer T, Raithel M, Kressel J, et al. Activation of the transcription factor Nrf2 in macrophages, Caco-2 cells and intact human gut tissue by Maillard reaction products and coffee. Amino Acids. 2013;44:1427–1439.
116. Itoh K, Wakabayashi N, Katoh Y, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev. 1999;13:76–86.
117. Itoh K, Chiba T, Takahashi S, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–322.
118. Xue M, Rabbani N, Momiji H, et al. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. Biochem J. 2012;443:213–222.
119. Ma Q, He X. Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. Pharmacol Rev. 2012;64:1055–1081.
120. Thornalley PJ, Rabbani N. Dietary and synthetic activators of the antistress gene response in treatment of renal disease. J Ren Nutr. 2012;22:195–202.
121. Palsamy P, Subramanian S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. Biochim Biophys Acta. 2011;1812:719–731.
122. Lee BH, Hsu WH, Chang YY, et al. Ankaflavin: a natural novel PPARγ agonist upregulates Nrf2 to attenuate methylglyoxal-induced diabetes in vivo. Free Radic Biol Med. 2012;53:2008–2016.
123. Lee BH, Hsu WH, Huang T, et al. Effects of monascin on anti-inflammation mediated by Nrf2 activation in advanced glycation end product-treated THP-1 monocytes and methylglyoxal-treated Wistar rats. J Agric Food Chem. 2013;61:1288–1298.
124. Hsu WH, Lee BH, Chang YY, et al. A novel natural Nrf2 activator with PPARγ-agonist (monascin) attenuates the toxicity of methylglyoxal and hyperglycemia. Toxicol Appl Pharmacol. 2013;272:842–851.
125. Tanigawa S, Fujii M, Hou DX. Action of Nrf2 and Keap1 in ARE-mediated *NQO1* expression by quercetin. Free Radic Biol Med. 2007;42:1690–1703.
126. He M, Siow RC, Sugden D, et al. Induction of HO-1 and redox signaling in endothelial cells by advanced glycation end products: a role for Nrf2 in vascular protection in diabetes. Nutr Metab Cardiovasc Dis. 2011;21:277–285.
127. Zhang L, Bukulin M, Kojro E, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. J Biol Chem. 2008;283:35507–35516.
128. Forbes JM, Thorpe SR, Thallas-Bonke V, et al. Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. J Am Soc Nephrol. 2005;16:2363–2372.
129. Nakamura K, Yamagishi S, Adachi H, et al. Elevation of soluble form of receptor for advanced glycation end products (sRAGE) in diabetic subjects with coronary artery disease. Diabetes Metab Res Rev. 2007;23:368–371.
130. Nin JW, Ferreira I, Schalkwijk CG, et al. Levels of soluble receptor for AGE are cross-sectionally associated with cardiovascular disease in type 1 diabetes, and this association is partially mediated by endothelial and renal dysfunction and by low-grade inflammation: the EURODIAB Prospective Complications Study. Diabetologia. 2009;52:705–714.
131. Nakamura K, Yamagishi S, Adachi H, et al. Serum levels of sRAGE, the soluble form of receptor for advanced glycation end products, are associated with inflammatory markers in patients with type 2 diabetes. Mol Med. 2007;13: 185–189.

132. Norata GD, Garlaschelli K, Grigore L, et al. Circulating soluble receptor for advanced glycation end products is inversely associated with body mass index and waist/hip ratio in the general population. Nutr Metab Cardiovasc Dis. 2009;19:129–134.
133. Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscl Thromb Vasc Biol. 2005;25:1032–1037.
134. Prasad K, Dhar I. Oxidative stress as a mechanism of added sugar-induced cardiovascular disease. Int J Angiol. 2014;23:217–226.
135. Pullerits R, Bokarewa M, Dahlberg L, et al. Decreased levels of soluble receptor for advanced glycation end products in patients with rheumatoid arthritis indicating deficient inflammatory control. Arthritis Res Ther. 2005;7:R817–R824.
136. Prasad K. Low levels of serum soluble receptors for advanced glycation end products, biomarkers for disease state: myth or reality. Int J Angiol. 2014;23:11–16.
137. Kalousova M, Hodkova M, Kazderova M, et al. Soluble receptor for advanced glycation end products in patients with decreased renal function. Am J Kidney Dis. 2006;47:406–411.
138. Yan SF, Ramasamy R, Schmidt AM. Soluble RAGE: therapy and biomarker in unraveling the RAGE axis in chronic disease and aging. Biochem Pharmacol. 2010;79:1379–1386.
139. Ramasamy R, Yan SF, Schmidt AM. RAGE: therapeutic target and biomarker of the inflammatory response – the evidence mounts. J Leukoc Biol. 2009;86:505–512.
140. Maillard-Lefebvre H, Boulanger E, Daroux M, et al. Soluble receptor for advanced glycation end products: a new biomarker in diagnosis and prognosis of chronic inflammatory diseases. Rheumatology. 2009;48:1190–1196.
141. Katakami N, Matsuhisa M, Kaneto H, et al. Serum endogenous secretory RAGE level is an independent risk factor for the progression of carotid atherosclerosis in type 1 diabetes. Atherosclerosis. 2009;204:288–292.
142. Piarulli F, Lapolla A, Ragazzi E, et al. Role of endogenous secretory RAGE (esRAGE) in defending against plaque formation induced by oxidative stress in type 2 diabetic patients. Atherosclerosis. 2013;226:252–257.
143. Yang L, Wu Q, Li Y, et al. Association of the receptor for advanced glycation end products gene polymorphisms and circulating RAGE levels with diabetic retinopathy in the Chinese population. J Diabetes Res. 2013;2013:264579. doi:10.1155/2013/264579.
144. Kislinger T, Fu C, Huber B, et al. N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. J Biol Chem. 1999;274:31740–31749.
145. Lu C, He JC, Cai W, et al. Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. Proc Natl Acad Sci U S A. 2004;101:11767–11772.
146. Cai W, He JC, Zhu L, et al. Advanced glycation end product (AGE) receptor 1 suppresses cell oxidant stress and activation signaling via EGF receptor. Proc Natl Acad Sci U S A. 12 2006;103:13801–13806.
147. Torreggiani M, Liu H, Wu J, et al. Advanced glycation end product receptor-1 transgenic mice are resistant to inflammation, oxidative stress, and post-injury intimal hyperplasia. Am J Pathol. 2009;175:1722–1732.
148. He CJ, Koschinsky T, Buenting C, et al. Presence of diabetic complications in type 1 diabetic patients correlates with low expression of mononuclear cell AGE-receptor-1 and elevated serum AGE. Mol Med. 2001;7:159–168.
149. Cai W, Ramdas M, Zhu L, et al. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. Proc Natl Acad Sci U S A. 2012;109:15888–15893.
150. Cai W, He JC, Zhu L, et al. Reduced oxidant stress and extended lifespan in mice exposed to a low glycotoxin diet: association with increased AGER1 expression. Am J Pathol. 2007;170:1893–1902.
151. Uribarri J, Cai W, Ramdas M, et al. Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. Diabetes Care. 2011;34:1610–1616.
152. Uribarri J, Cai W, Pyzik R, et al. Suppression of native defense mechanisms, SIRT1 and PPARγ, by dietary glycoxidants precedes disease in adult humans; relevance to lifestyle-engendered chronic diseases. Amino Acids. 2014;46:301–309.
153. Virella G, Thorpe SR, Alderson NL, et al. Autoimmune response to advanced glycosylation end-products of human LDL. J Lipid Res. 2003;44:487–493.
154. Vytasek R, Sedova L, Vilim V. Increased concentration of two different advanced glycation end-products detected by enzyme immunoassays with new monoclonal antibodies in sera of patients with rheumatoid arthritis. BMC Musculoskelet Disord. 2010;11:83. doi:10.1186/1471-2474-11-83.
155. Price CL, Sharp PS, North ME, et al. Advanced glycation end products modulate the maturation and function of peripheral blood dendritic cells. Diabetes. 2004;53:1452–1458.
156. Price CL, Hassi HO, English NR, et al. Methylglyoxal modulates immune responses: relevance to diabetes. J Cell Mol Med. 2010;14:1806–1815.
157. Noris M, Remuzzi G. Overview of complement activation and regulation. Semin Nephrol. 2013;33:479–492.
158. Walport MJ. Complement. Second of two parts. N Engl J Med. 2001;344:1140–1144.
159. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344:1058–1066.
160. Saad AF, Virella G, Chassereau C, et al. OxLDL immune complexes activate complement and induce cytokine production by MonoMac 6 cells and human macrophages. J Lipid Res. 2006;47:1975–1983.
161. Acosta J, Hettinga J, Fluckiger R, et al. Molecular basis for a link between complement and the vascular complications of diabetes. Proc Natl Acad Sci U S A. 2000;97:5450–5455.
162. Qin X, Goldfine A, Krumrei N, et al. Glycation inactivation of the complement regulatory protein CD59: a possible role in the pathogenesis of the vascular complications of human diabetes. Diabetes. 2004;53:2653–2661.
163. Ruan BH, Li X, Winkler AR, et al. Complement C3a, CpG oligos, and DNA/C3a complex stimulate IFN-α production in a receptor for advanced glycation end product-dependent manner. J Immunol. 2010;185:4213–4222.
164. Thorburn AN, Macia L, Mackay CR. Diet, metabolites, and "Western-lifestyle" inflammatory diseases. Immunity. 2014;40:833–842.
165. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461:1282–1286.
166. Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. Nat Immunol. 2011;12:5–9.
167. Macia L, Thorburn AN, Binge LC, et al. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immunol Rev. 2012;245:164–176.
168. Schilderink R, Verseijden C, de Jonge WJ. Dietary inhibitors of histone deacetylases in intestinal immunity and homeostasis. Front Immunol. 2013;4:226. doi:10.3389/fimmu.2013.00226.
169. Wang X, Liu J, Zhen J, et al. Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy. Kidney Int. 2014;86:712–725.
170. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007;132:2131–2157.
171. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153–165.
172. Ojima A, Matsui T, Maeda S, et al. Glucose-dependent insulinotropic polypeptide (GIP) inhibits signaling pathways of advanced glycation end products (AGEs) in endothelial cells via its antioxidative properties. Horm Metab Res. 2012;44:501–505.
173. Ishibashi Y, Nishino Y, Matsui T, et al. Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. Metabolism. 2011;60:1271–1277.
174. Forbes JM, Cowan SP, Andrikopoulos S, et al. Glucose homeostasis can be differentially modulated by varying individual components of a Western diet. J Nutr Biochem. 2013;24:1251–1257.
175. Ishibashi Y, Matsui T, Maeda S, et al. Advanced glycation end products evoke endothelial cell damage by stimulating soluble dipeptidyl peptidase-4 production and its interaction with mannose 6-phosphate/insulin-like growth factor II receptor. Cardiovasc Diabetol. 2013;12:125. doi:10.1186/1475-2840-12-125.
176. Cai W, Gao QD, Zhu L, et al. Oxidative stress-inducing carbonyl compounds from common foods: novel mediators of cellular dysfunction. Mol Med. 2002;8:337–346.
177. Vlassara H, Striker GE. AGE restriction in diabetes mellitus: a paradigm shift. Nat Rev Endocrinol. 2011;7:526–539.
178. Kellow NJ, Savige GS. Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review. Eur J Clin Nutr. 2013;67:239–248.
179. Cassese A, Esposito I, Fiory F, et al. In skeletal muscle advanced glycation end products (AGEs) inhibit insulin action and induce the formation of multimolecular complexes including the receptor for AGEs. J Biol Chem. 2008;283:36088–36099.
180. Chatzigeorgiou A, Kandaraki E, Piperi C, et al. Dietary glycotoxins affect scavenger receptor expression and the hormonal profile of female rats. J Endocrinol. 2013;218:331–337.
181. Kandaraki E, Chatzigeorgiou A, Piperi C, et al. Reduced ovarian glyoxalase-I activity by dietary glycotoxins and androgen excess: a causative link to polycystic ovarian syndrome. Mol Med. 2012;18:1183–1189.
182. Feng JX, Hou FF, Liang M, et al. Restricted intake of dietary advanced glycation end products retards renal progression in the remnant kidney model. Kidney Int. 2007;71:901–911.
183. Guo WA, Davidson BA, Ottosen J, et al. Effect of high advanced glycation end-product diet on pulmonary inflammatory response and pulmonary function following gastric aspiration. Shock. 2012;38:677–684.
184. Leung C, Herath CB, Jia Z, et al. Dietary glycotoxins exacerbate progression of experimental fatty liver disease. J Hepatol. 2014;16:832–838.
185. Lin RY, Reis ED, Dore AT, et al. Lowering of dietary advanced glycation endproducts (AGE) reduces neointimal formation after arterial injury in genetically hypercholesterolemic mice. Atherosclerosis. 2002;163:303–311.
186. Lin RY, Choudhury RP, Cai W, et al. Dietary glycotoxins promote diabetic atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis. 2003;168:213–220.
187. Peppa M, Brem H, Ehrlich P, et al. Adverse effects of dietary glycotoxins on wound healing in genetically diabetic mice. Diabetes. 2003;52:2805–2813.

188. Sena CM, Matafome P, Crisóstomo J, et al. Methylglyoxal promotes oxidative stress and endothelial dysfunction. Pharmacol Res. 2012;65:497–506.
189. Tikellis C, Thomas MC, Harcourt BE, et al. Cardiac inflammation associated with a Western diet is mediated via activation of RAGE by AGEs. Am J Physiol Endocrinol Metab. 2008;295:E323–E330.
190. Zhu Y, Lan F, Wei J, et al. Influence of dietary advanced glycation end products on wound healing in nondiabetic mice. J Food Sci. 2011;76:T5–T10.
191. Poulsen MW, Hedegaard RV, Andersen JM, et al. Advanced glycation endproducts in food and their effects on health. Food Chem Toxicol. 2013;60:10–37.
192. Goh SY, Cooper ME. Clinical review: the role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab. 2008;93:1143–1152.
193. Kwon EY, Shin SK, Cho YY, et al. Time-course microarrays reveal early activation of the immune transcriptome and adipokine dysregulation leads to fibrosis in visceral adipose depots during diet-induced obesity. BMC Genomics. 2012;13:450. doi:10.1186/1471-2164-13-450.
194. Weigert C, Brodbeck K, Staiger H, et al. Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor–κB. J Biol Chem. 2004;279:23942–23952.
195. Ajuwon KM, Spurlock ME. Palmitate activates the NF-κB transcription factor and induces IL-6 and TNFα expression in 3T3-L1 adipocytes. J Nutr. 2005;135:1841–1846.
196. Zeng S, Feirt N, Goldstein M, et al. Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia and reperfusion injury to the liver in mice. Hepatology. 2004;39:422–432.
197. Chao PC, Huang CN, Hsu CC, et al. Association of dietary AGEs with circulating AGEs, glycated LDL, IL-1α and MCP-1 levels in type 2 diabetic patients. Eur J Nutr. 2010;49:429–434.
198. Vlassara H, Cai W, Goodman S, et al. Protection against loss of innate defenses in adulthood by low advanced glycation end products (AGE) intake: role of the anti-inflammatory AGE receptor-1. J Clin Endocrinol Metab. 2009;94:4483–4491.
199. Birlouez-Aragon I, Saavedra G, Tessier FJ, et al. A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. Am J Clin Nutr. 2010;91:1220–1226.
200. Luevano-Contreras C, Garay-Sevilla ME, Wrobel K, et al. Dietary advanced glycation end products restriction diminishes inflammation markers and oxidative stress in patients with type 2 diabetes mellitus. J Clin Biochem Nutr. 2013;52:22–26.
201. Mark AB, Poulsen MW, Andersen S, et al. Consumption of a diet low in advanced glycation end products for 4 weeks improves insulin sensitivity in overweight women. Diabetes Care. 2014;37:88–95.
202. Negrean M, Stirban A, Stratmann B, et al. Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Clin Nutr. 2007;85:1236–1243.
203. Stirban A, Negrean M, Stratmann B, et al. Adiponectin decreases postprandially following a heat-processed meal in individuals with type 2 diabetes: an effect prevented by benfotiamine and cooking method. Diabetes Care. 2007;30:2514–2516.
204. Stirban A, Negrean M, Gotting C, et al. Dietary advanced glycation endproducts and oxidative stress: in vivo effects on endothelial function and adipokines. Ann N Y Acad Sci. 2008;1126:276–279.
205. Poulsen MW, Bak MJ, Andersen JM, et al. Effect of dietary advanced glycation end products on postprandial appetite, inflammation, and endothelial activation in healthy overweight individuals. Eur J Nutr. 2014;53:661–672.
206. Schiekofer S, Franke S, Andrassy M, et al. Postprandial mononuclear NF-κB activation is independent of the AGE-content of a single meal. Exp Clin Endocrinol Diabetes. 2006;114:160–167.
207. Semba RD, Gebauer SK, Baer DJ, et al. Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. J Nutr. 2014;144:1037–1042.
208. Tantalaki E, Piperi C, Livadas S, et al. Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS). Hormones. 2014;13:65–73.
209. Uribarri J, Peppa M, Cai W, et al. Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. J Am Soc Nephrol. 2003;14:728–731.
210. Peppa M, Uribarri J, Cai W, et al. Glycoxidation and inflammation in renal failure patients. Am J Kidney Dis. 2004;43:690–695.
211. Uribarri J, Stirban A, Sander D, et al. Single oral challenge by advanced glycation end products acutely impairs endothelial function in diabetic and nondiabetic subjects. Diabetes Care. 2007;30:2579–2582.
212. Vlassara H, Cai W, Crandall J, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci U S A. 2002;99:15596–15601.
213. Stirban A, Negrean M, Gotting C, et al. Leptin decreases postprandially in people with type 2 diabetes, an effect reduced by the cooking method. Hormone Metab Res. 2008;40:896–900.
214. Xie J, Reverdatto S, Frolov A, et al. Structural basis for pattern recognition by the receptor for advanced glycation end products (RAGE). J Biol Chem. 2008;283:27255–27269.
215. Vlassara H, Uribarri J. Advanced glycation end products (AGE) and diabetes: cause, effect, or both? Curr Diab Rep. 2014;14:453. doi:10.1007/s11892-013-0453-1.
216. Sebekova K, Klenovics KS, Boor P, et al. Behaviour and hormonal status in healthy rats on a diet rich in Maillard reaction products with or without solvent extractable aroma compounds. Physiol Behav. 2012;105:693–701.
217. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr. 2004;80:1029–1035.
218. Engelen L, Stehouwer CD, Schalkwijk CG. Current therapeutic interventions in the glycation pathway: evidence from clinical studies. Diabetes Obes Metab. 2013;15:677–689.
219. Pun PB, Logan A, Darley-Usmar V, et al. A mitochondria-targeted mass spectrometry probe to detect glyoxals: implications for diabetes. Free Radic Biol Med. 2014;67:437–450.
220. Wu CH, Huang SM, Lin JA, et al. Inhibition of advanced glycation endproduct formation by foodstuffs. Food Funct. 2011;2:224–234.
221. Rastogi A. Sevelamer revisited: pleiotropic effects on endothelial and cardiovascular risk factors in chronic kidney disease and end-stage renal disease. Ther Adv Cardiovasc Dis. 2013;7:322–342.
222. Vlassara H, Cai W, Chen X, et al. Managing chronic inflammation in the aging diabetic patient with CKD by diet or sevelamer carbonate: a modern paradigm shift. J Gerontol A Biol Sci Med Sci. 2012;67:1410–1416.
223. Kakuta T, Tanaka R, Hyodo T, et al. Effect of sevelamer and calcium-based phosphate binders on coronary artery calcification and accumulation of circulating advanced glycation end products in hemodialysis patients. Am J Kidney Dis. 2011;57:422–431.
224. Vlassara H, Uribarri J, Cai W, et al. Effects of sevelamer on HbA1c, inflammation, and advanced glycation end products in diabetic kidney disease. Clin J Am Soc Nephrol. 2012;7:934–942.
225. Elosta A, Ghous T, Ahmed N. Natural products as anti-glycation agents: possible therapeutic potential for diabetic complications. Curr Diabetes Rev. 2012;8:92–108.
226. Franke SI, Muller LL, Santos MC, et al. Vitamin C intake reduces the cytotoxicity associated with hyperglycemia in prediabetes and type 2 diabetes. Biomed Res Int. 2013;2013:896536. doi:10.1155/2013/896536.
227. Karachalias N, Babaei-Jadidi R, Kupich C, et al. High-dose thiamine therapy counters dyslipidemia and advanced glycation of plasma protein in streptozotocin-induced diabetic rats. Ann N Y Acad Sci. 2005;1043:777–783.
228. Kihm LP, Muller-Krebs S, Klein J, et al. Benfotiamine protects against peritoneal and kidney damage in peritoneal dialysis. J Am Soc Nephrol. 2011;22:914–926.
229. Srey C, Hull GL, Connolly L, et al. Effect of inhibitor compounds on Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL) formation in model foods. J Agri Food Chem. 2010;58:12036–12041.
230. Pashikanti S, de Alba DR, Boissonneault GA, et al. Rutin metabolites: novel inhibitors of nonoxidative advanced glycation end products. Free Radic Biol Med. 2010;48:656–663.
231. Dai J, Jones DP, Goldberg J, et al. Association between adherence to the Mediterranean diet and oxidative stress. Am J Clin Nutr. 2008;88:1364–1370.
232. Nagai R, Nagai M, Shimasaki S, et al. Citric acid inhibits development of cataracts, proteinuria and ketosis in streptozotocin (type 1) diabetic rats. Biochem Biophys Res Commun. 2010;393:118–122.
233. de Assis AM, Rech A, Longoni A, et al. Ω3-Polyunsaturated fatty acids prevent lipoperoxidation, modulate antioxidant enzymes, and reduce lipid content but do not alter glycogen metabolism in the livers of diabetic rats fed on a high fat thermolyzed diet. Mol Cell Biochem. 2012;361:151–160.
234. Elokda AS, Nielsen DH. Effects of exercise training on the glutathione antioxidant system. Eur J Cardiovasc Prev Rehabil. 2007;14:630–637.
235. El-Osta A, Brasacchio D, Yao D, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med. 2008;205:2409–2417.
236. Gugliucci A, Kotani K, Taing J, et al. Short-term low calorie diet intervention reduces serum advanced glycation end products in healthy overweight or obese adults. Ann Nutr Metab. 2009;54:197–201.
237. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA. 2002;287:2414–2423.
238. Uchiki T, Weikel KA, Jiao W, et al. Glycation-altered proteolysis as a pathobiologic mechanism that links dietary glycemic index, aging, and age-related disease (in nondiabetics). Aging Cell. 2012;11:1–13.

Chapter 3:

Is dietary AGE restriction beneficial to human health?

The relationship between dietary AGE intake and markers of metabolic dysfunction have been explored in a number of cross-sectional studies. Dietary AGE intake was found to be independently associated with abdominal obesity and hypertriglyceridemia in a large sample (n= 5848) of Iranian adults [124]. However, the authors used a food frequency questionnaire which did not obtain any information from participants about the cooking methods they utilise, potentially neglecting a significant source of dietary AGEs. A study in adults with type 2 diabetes demonstrated a positive correlation between intake of dietary AGEs and oxidative and inflammatory parameters [125]. These associations were absent in healthy controls despite high dietary AGE intakes, suggesting that normal AGE excretion and detoxification mechanisms may be impaired in diabetes. Although dietary AGE intake was assessed using 7-day food records which included information about cooking techniques in this instance, dietary intake over seven days may not adequately reflect habitual food consumption over many years. Dietary AGE consumption in individuals with renal failure has been cross-sectionally related to increased plasma AGE concentrations and inflammatory markers in some studies [38, 74], while others have found no such association [126].

As these investigations were all cross-sectional in nature, it is impossible to implicate dietary AGEs as a causative factor in these metabolic abnormalities. Indeed, dietary AGEs may simply be a marker of other negative dietary components frequently found in highly processed Western diets. High AGE foods tend to be high in fat, low in dietary fibre and lower in micronutrients due to their exposure to heating, which introduces dietary confounders into the equation. All of the aforementioned studies based their estimates of dietary AGE intake on an American AGE food database [21], developed using immunological methods of AGE measurement which are now considered to lack

specificity. Immunological techniques such as ELISA (enzyme-linked immunosorbent assay) have a high risk of matrix interference because the antigen–antibody interaction is dependent on the chemical environment [23].

Methods for dietary AGE reduction

It was first hypothesised in 1997 that dietary AGEs may be at least partially absorbed and induce toxic effects in the body [127]. Since this time, a variety of methods have been proposed to reduce the AGE content of the diet. AGE formation during cooking can be decreased by heating food at lower temperatures in the presence of liquid (stewing, poaching, casseroling, steaming, boiling, slow cooking). The addition of acidic ingredients such as lemon juice and vinegar have been shown to prevent excessive AGE formation in meat [21]. Further dietary AGE intake can be prevented by removing crusts from bread, eating fresh bread instead of toasted bread, and consuming minimally processed breakfast cereals (eg. oats) rather than heat-processed cereals. Consumption of powdered, condensed and evaporated milk consumption should be restricted, with fresh milk used instead. Bakery products (cakes, biscuits, crackers, pastries) and deep-fried take-away foods should be limited due to their high AGE content. Miscellaneous foods containing high levels of AGEs include fried black pudding, peanut sauces, roasted nuts and peanut butter [23, 27].

A diet based on fresh fruit and vegetables, boiled rice and pasta, fresh milk, yoghurt and cheese (all types), minimally processed meats, chicken and fish (not fried), raw nuts, legumes, fresh bread (not toasted, crust removed) and minimally processed breakfast cereals (rolled oats) appears to contain the lowest AGE concentration. This type of diet is consistent with current healthy eating guidelines. Butter, olive oil, jam,

tomato sauce, mayonnaise, wine and coffee contain negligible or very low concentrations of AGEs [23]. Although significant amounts of AGEs can be consumed in breads and cereals due to the large contribution that these products make to most Western diets [19], it would appear to be unwise to avoid breads and cereal products completely. Individuals with ketosis (induced by utilising fat as a primary fuel source while following a very low carbohydrate diet, commonly referred to as “The Atkins” diet) increased methylglyoxal production by more than two-fold from baseline within two weeks of commencing the diet [128]. Serum methylglyoxal levels remained at this concentration (which was significantly greater than that seen in individuals with poorly controlled diabetes) for the duration of the diet [128]. The ketone acetoacetate breaks down into acetone, which then undergoes oxidation to acetol, a methylglyoxal precursor. Rather than exclude breads and cereals from the diet, it may be beneficial to consume low glycemic index (GI) varieties of these products. Low GI carbohydrate-containing foods reduce post-prandial glycemic excursions, reducing the concentration of glucose substrate available for AGE formation. Mice fed a low GI diet had significantly reduced tissue AGE accumulation compared to age-matched controls fed a high GI diet [129].

Consumption of the Mediterranean diet is associated with numerous health benefits [130]. This eating pattern places a primary focus on plant foods, with vegetables, fruit, legumes, nuts and wholegrain breads and cereals considered the foundation of the diet. Olive oil is the principle source of fat in the Mediterranean diet, and red wine is consumed daily with the main meal. The main meal is frequently casseroled or slow-cooked. Dairy products and small quantities of fish and chicken are eaten daily, but red meat is consumed only occasionally. Food is minimally processed, high in dietary

fibre, antioxidants and monounsaturated fat, but low in saturated fat. The diet is also lower in AGEs than Western diets, and has been shown to reduce circulating carboxymethyl-lysine and methylglyoxal levels in elderly individuals [131]. Moreover, consumers of the Mediterranean diet showed reduced RAGE expression and upregulation of AGE defence mechanisms such as Glyoxylase-1 in peripheral mononuclear cells [131].

A reduction in total kilojoule intake resulting in weight loss has successfully lowered serum AGEs [132, 133] or urinary pentosidine levels [134] in non-controlled trials involving non-diabetic subjects. This was likely due to improvements in circulating lipids and decreased inflammation. Randomised controlled trials of longer duration are required to confirm these findings. A diet low in total fat content may be sufficient to attenuate the effects of a high AGE diet in healthy individuals, without the requirement for kilojoule restriction [135].

The phosphate binder Sevelamer carbonate is frequently used in patients with chronic kidney disease and is known to bind to a number of compounds in the gut in addition to phosphate, such as bile acids, bacterial endotoxins and AGEs, thus preventing their absorption [136]. A proof of concept study indicated that the capacity of sevelamer to sequester intestinal AGEs was sufficient to reduce circulating carboxymethyl-lysine, methylglyoxal and markers of oxidative stress in patients with diabetic kidney disease [137]. Sevelamer may be a useful tool for the reduction of intestinal AGE absorption in individuals with chronic kidney disease, who may find in difficult to adhere to an AGE-restricted diet in addition to multiple other dietary restrictions.

The following systematic review aimed to determine whether there was sufficient evidence from human randomised controlled trials to recommend dietary AGE restriction as a dietary intervention for the reduction of circulating AGEs, insulin resistance, and markers of oxidative stress and endothelial dysfunction. At the time this review was performed, more than half of the trials were of low methodological quality, short duration, involved small sample sizes, and the heterogeneity of trial participants and outcome measures made it difficult to pool studies of similar design. In addition, most of the trials measured AGEs in food and body fluids using semi-quantitative immunological techniques, providing limited evidence to support low-AGE diets as therapeutic interventions until further research using LC-MS/MS AGE measurement techniques was undertaken.

Since the publication of this review in early 2013, the evidence supporting dietary AGE restriction to reduce the risk of type 2 diabetes has strengthened, with two high quality RCTs published in overweight individuals [55, 72] demonstrating improved insulin sensitivity after 2-4 weeks consumption of a low-AGE diet. Both trials measured AGEs using LC-MS/MS techniques, and provided isocaloric diets differing only in AGE content to intervention and control groups. In contrast, a 6-week low-AGE diet in people with type 2 diabetes reduced markers of oxidative stress and inflammation, but failed to affect insulin sensitivity [75]. However, this study measured serum and food AGEs based on immunological methods, and provided participants with instructions for preparing low AGE meals rather than supplying food items, which may raise questions about participant dietary compliance.

Longer-term dietary AGE restriction trials (3-4 months) in overweight men [138] and healthy individuals over 60 years of age [139] reduced circulating AGE levels, but used ELISA to measure AGE levels and relied on study participants to follow low-AGE dietary instructions at home. Healthy, middle-aged adults consuming an isocaloric diet differing only in AGE content for 6 weeks showed no difference in markers of endothelial dysfunction and inflammation when compared to controls [76]. On the basis of these recent findings, there is still insufficient evidence to suggest that healthy people who follow an AGE-restricted diet will gain any health benefits. However, overweight individuals with type 2 diabetes risk factors who do not wish to undertake a kilojoule-restricted weight reduction diet may achieve metabolic improvements (particularly increased insulin sensitivity) by reducing the AGE content of their diet. Whether this translates into a reduced risk of developing type 2 diabetes is unknown. Further research is required to determine whether a low-AGE diet needs to be maintained indefinitely, or whether short-term dietary AGE restrictions are sufficient to induce health benefits which persist after the diet is discontinued.

Article: Kellow NJ, Savige GS. Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review.

Eur J Clin Nutr 2013; 67: 239-248.

European Journal of Clinical Nutrition (2013) **67**, 239–248

www.nature.com/ejcn

REVIEW

Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review

NJ Kellow and GS Savige

The benefits of advanced glycation end-product (AGE)-restricted diets in humans are unclear. This review aimed to determine the effect of dietary AGE restriction on the inflammatory profiles of healthy adults and adults with diabetes or renal failure. Eight computer databases were searched for controlled feeding trials published in English between January 1997 and December 2012. Human trials were included if at least one group received an AGE-restricted dietary intervention. A total of 12 trials reporting on 289 participants were included in the review. Five trials (42%) were of high methodological quality. Meta-analysis of two long-term (16 week) trials provided evidence favoring an AGE-restricted diet for the reduction of 8-isoprostanes (standardized mean difference 0.9; 95% confidence interval (CI): 0.3–1.5) and tumor necrosis factor-α (1.3; 95% CI: 0.6–1.9) in healthy adults. Intermediate-term dietary AGE restriction in adults with chronic renal failure reduced serum VCAM-1 (0.9; 95% CI: 0.1–1.7). Individual trials provided some evidence that long-term dietary AGE restriction reduces HOMA-IR (1.4; 95% CI: 0.3–2.6) and AGE-modified low-density lipoprotein (2.7; 95% CI: 1.6–3.9) in adults with type 2 diabetes. Generalisability is limited, as 75% of studies were of less than 6 weeks duration and more than half were of low methodological quality. Evidence quality ranged from low to very low, limiting the conclusions that can be drawn from this review. There is currently insufficient evidence to recommend dietary AGE restriction for the alleviation of the proinflammatory milieu in healthy individuals and patients with diabetes or renal failure. Additional long-term high-quality RCTs with larger sample sizes measuring patient-important outcomes are required to strengthen the evidence supporting the effects of AGE-restricted diets.

European Journal of Clinical Nutrition (2013) **67,** 239–248; doi:10.1038/ejcn.2012.220; published online 30 January 2013

Keywords: systematic review; advanced glycation end product; dietary AGE restriction; diabetes; inflammation; humans

INTRODUCTION

Advanced glycation end products (AGEs) are formed endogenously when the carbonyl groups of reducing sugars non-enzymatically react with the free amino groups on proteins. AGEs are generated *in vivo* as a normal consequence of metabolism, but their formation is accelerated under conditions of hyperglycemia, hyperlipidemia and increased oxidative stress.

Although glucose is relatively slow in reacting with proteins, highly reactive dicarbonyl compounds (generated as a result of glucose auto-oxidation, lipid peroxidation and the interruption of glycolysis by reactive oxygen species) are capable of rapid AGE formation. Dicarbonyls such as glyoxal, methylglyoxal and 3-deoxyglucosone interact with intracellular proteins to form AGEs, and can also diffuse out of the cell and react with extracellular proteins.

Excessive AGE accumulation results in significant cellular dysfunction by inhibiting communication between cells, altering protein structure and interfering with lipid accumulation within the arterial wall.[1] Interaction of AGEs with the receptor for AGEs (RAGE) activates nuclear factor κB, triggering oxidative stress, thrombogenesis, vascular inflammation and pathological angiogenesis,[2] thereby contributing to many of the long-term complications of diabetes. More recently, AGEs have been implicated in the pathogenesis of type 2 diabetes by contributing to the development of insulin resistance and low-grade inflammation known to precede the condition.[3,4]

Apart from endogenous AGE formation, AGEs and their precursors are also absorbed by the body from exogenous sources such as cigarette smoke and through consumption of highly heated processed foods. Browning of food during cooking is used to enhance the quality, flavour, color and aroma of the diet. This process (known as the Maillard reaction) generates large quantities of AGEs.[5] Factors that enhance AGE formation in foods include high lipid and protein content, low water content during cooking, elevated pH and the application of high temperature over a short time period. More AGEs are generated in foods exposed to dry heat (grilling, frying, roasting, baking and barbecuing) than foods cooked at lower temperatures for longer time periods in the presence of higher water content (boiling, steaming, poaching, stewing or slow cooking).[6]

Kinetic studies have demonstrated that approximately 10–30% of dietary AGEs consumed are intestinally absorbed,[7] with only one-third of ingested AGEs excreted in urine and feces. Plasma AGE concentration appears to be directly influenced by dietary AGE intake and the body's capacity for AGE elimination.[8] Individuals with renal insufficiency demonstrate reduced urinary excretion of dietary AGEs, and plasma AGE levels inversely correlate with renal function.[9]

Department of Rural & Indigenous Health, Monash University, Moe, Victoria, Australia. Correspondence: NJ Kellow, Department of Rural & Indigenous Health, Monash University, PO Box 975, Moe, Victoria 3833, Australia.
E-mail: nicolekellow@live.com.au
Received 12 November 2012; revised 19 December 2012; accepted 21 December 2012; published online 30 January 2013

Low-AGE diets in animal studies have been shown to reverse insulin resistance and chronic inflammation, inhibit the progression of atherosclerosis and prevent experimental diabetic nephropathy and neuropathy,[10] but whether these results can be translated to humans is uncertain. Cross-sectional and case–control studies involving humans with impaired renal function or diabetes have demonstrated associations between elevated AGE intakes and serum biomarkers of oxidative stress, endothelial dysfunction, inflammation, hyperlipidemia and hyperglycemia.[11,12] AGEs have also recently been implicated in the dysfunction and death of pancreatic beta cells,[13] leading to the hypothesis that excessive AGE formation and oxidative stress possibly have a role in the development of type 1 and type 2 diabetes.[14,15] Low-AGE diets have been suggested as a possible future therapeutic option for healthy individuals at risk for the development of type 1 or type 2 diabetes.[16]

Through reduced consumption of highly processed heat-treated foods, dietary AGE restriction may represent a relatively simple, noninvasive therapy for the effective treatment of many of the metabolic disturbances attributed to excessive AGE levels. This systematic review sought to determine whether there is sufficient evidence to recommend therapeutic AGE-restricted diets in healthy or overweight individuals, people with diabetes or those with renal impairment for the prevention or attenuation of insulin resistance, the improvement of endothelial function and the reduction of biomarkers of inflammation and oxidative stress.

MATERIALS AND METHODS

Search strategy

A computer database search was undertaken for the time period between 1 January 1 1997 and 1 December 2012, using Medline, CINAHL, EMBASE, Current Contents, PubMed, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and AMED. Databases were not searched before 1997, because the potentially deleterious effects of dietary AGE consumption was first postulated in 1997.[17] Citation tracking was performed using the ISI Web of Science for all trials identified, and the reference lists of all identified trials were hand-searched for relevant studies. The following search terms were used: (1) (diet$ OR food) and (advanced glyc$ OR glycation OR Maillard OR thermal), (2) limit 1 to year = '1997–2012', (3) limit 2 to humans.

Criteria for selecting trials in this review

All full reports of controlled feeding trials were eligible for inclusion if they were published in English between 1 January 1997 and 1 December 2012. Trials were included if they involved human participants aged $\geqslant$18 years and at least one group of participants received an AGE-restricted dietary intervention. For the purposes of this review, we defined a low-AGE dietary intervention as one that contained 30–50% of the measured AGEs or Maillard reaction products (MRPs) present in the standard or high-AGE comparison diet. Trials involving dietary restriction of AGE precursors only (such as Amadori products) were not included.

The outcomes of interest in this review were serum markers of the following: (1) insulin resistance (HOMA-IR), (2) inflammation (tumor necrosis factor-α (TNF-α)), (3) oxidative stress (8-isoprostane), (4) endothelial dysfunction (VCAM-1) and (5) increased cardiovascular disease risk (AGE-modified low-density lipoprotein (LDL)). On the basis of the duration of low-AGE dietary interventions used in the included trials, the length of follow-up of outcomes was categorized as short term (one meal to 6 days after randomization), intermediate term (1–4 weeks after randomization) or long term (more than four weeks after randomization).

Assessment of methodological quality

The two reviewers independently assessed the methodological quality of included trials using the Heyland Methodological Quality Score[18] (Supplementary Table S1). This checklist rates primary research based on the use of allocation concealment during randomization, intention-to-treat analysis, double-blinding, patient selection with minimal risk of bias, comparability of intervention and control groups at baseline, 100% participant follow-up, clearly described treatment protocol and well-defined outcome measurements. Trials scoring $\geqslant$8 out of a possible 14 points are considered to be of high methodological quality. Disagreements between reviewers in assigning methodological quality scores were resolved by discussion until consensus was achieved.

Data extraction and analysis

Trial information regarding the type and number of participants, interventions used and significant findings was extracted from each study by the first author and entered into a standardized computer spreadsheet. As all extracted data were continuous, treatment effects and 95% confidence intervals (CIs) were calculated using the Hedges (adjusted-g) standardized mean difference (SMD).[19] The 'adjusted' statistic was used because it includes an adjustment for bias from small sample sizes. The SMD enables comparison of effect sizes between trials that use different outcome measures.[20] SMDs were calculated from group mean results and s.d's) collected at the time of follow-up. When mean values were not available, trial authors were contacted to provide the appropriate data. When standard errors were reported, these were converted to s.d's as per Cochrane Collaboration Guidelines.[21] SMDs were standardized so that positive values indicated effects favoring the AGE-restricted dietary intervention, and negative values were used to indicate effects favoring the standard diet. SMD values of 0.2, 0.5 and 0.8 were considered to represent small, moderate and large effect sizes, respectively.[22]

Data synthesis

Meta-analysis of pooled data was implemented in cases in which at least two trials contained similar participants (health status), intervention (low-AGE diet), comparison intervention (standard-AGE diet), outcome measures and length of follow-up. Trials with similar characteristics were assessed for statistical heterogeneity, which was indicated by a $P<0.1$ on the χ^2 test and an I^2 statistic greater than 20%.[23] Clinically and statistically homogeneous trials ($I^2<20\%$) underwent a fixed-effects model meta-analysis using RevMan 5.1.[24]

Where meta-analysis was considered not possible because of clinical or statistical heterogeneity, effect sizes and 95% CIs were reported for outcomes within individual trials, and a narrative analysis was performed using the GRADE (Grades of Recommendation, Assessment, Development & Evaluation) approach for collating evidence in systematic reviews[25] (Supplementary Table S2). The GRADE criteria consider randomized controlled trials as high-quality evidence, which can be downgraded to moderate-, low- or very low-quality evidence in the event of limitations to methodological quality (defined in this review as a Heyland Methodological Quality Score <8), inconsistency of results between trials, imprecision of results due to small sample sizes and/or wide CIs, indirectness of results due to the measurement of secondary end points or a high probability of reporting bias.

RESULTS

Description of selected trials

A total of 3855 citations were originally identified at the time of the initial database search, and progressed through each stage of the selection process according to the predefined inclusion criteria (Figure 1). Sixteen articles reporting on 12 controlled trials including 289 participants were ultimately included in the review.[17,26–40] The characteristics of the included trials are outlined in Table 1.

Of the 12 trials included in this review, 4 trials included only healthy participants,[26,28,29,35] 1 trial included only overweight or obese participants,[30] 3 trials included only participants with type 1 or type 2 diabetes[27,31,39] and 1 trial included only participants with nondiabetic renal failure receiving peritoneal dialysis.[37] The remaining three trials included combinations of healthy participants, patients with diabetes and/or patients with chronic renal disease.[17,38,40]

Four trials reported data on short-term low-AGE dietary interventions,[17,29,31,35] five trials presented intermediate-term follow-up data[26,28,30,37,39] and three trials contained long-term follow-up data.[27,38,40]

A variety of post-intervention outcome measures were reported by the trials in this review, including differences in serum AGE

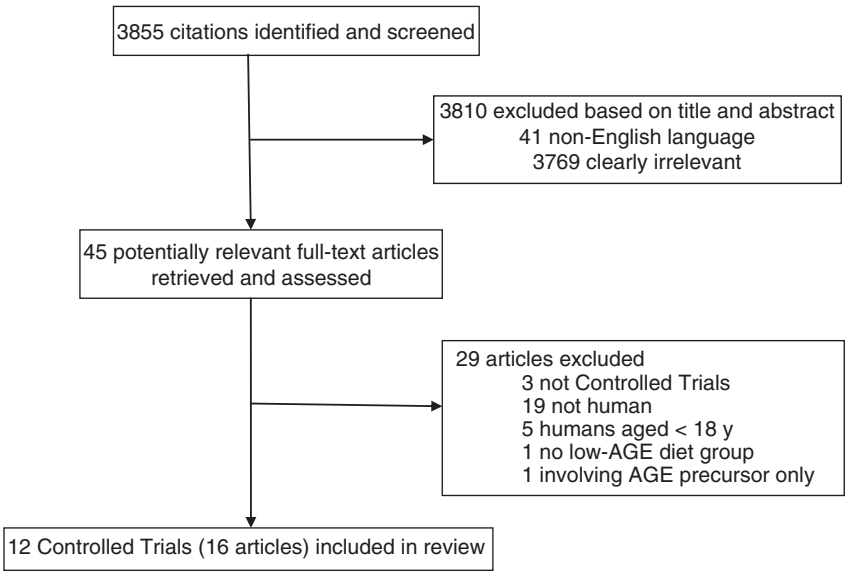


Figure 1. Flowchart showing progression of trials through the selection process.

concentration,[17,26,27,30,34–36,38–40] serum markers of insulin resistance (homeostasis model of assessment, fasting insulin, adiponectin),[26,32,38] indicators of inflammatory processes (C-reactive protein, TNF-α, macrophage migration inhibitory factor)[30,37–40] and oxidative stress (thiobarbituric acid reactive substances, 8-isoprostanes, leptin, nuclear factor κB).[30,31,35,38,40] Some studies measured surrogate biochemical markers of endothelial dysfunction (E-selectin, vascular cell adhesion molecule-1, intracellular adhesion molecule-1, monocyte chemoattractant protein-1),[30,31,37,39,40] cardiovascular disease risk factors (AGE-modified low-density lipoprotein, flow-mediated dilatation, plasminogen activator inhibitor-1),[27,28,31,36,39] anti-inflammatory molecules (AGE receptor 1, vitamin C, vitamin E, ubiquinol)[26,38,40] and plasma lipid levels.[26]

Methodological quality of trials

Methodological quality ratings for each trial according to the Heyland Methodological Quality Score are presented in Table 1. Five trials (42%) were considered to be of high quality.[26,27,30,31,40] Common methodological limitations included failure to blind researchers,[17,26–29,35,37–40] inadequate randomization [17,28,29,35,39] and failure to report an intention-to-treat analysis.[17,26–31,35,36,38,39] All of the trials failed to provide sample size calculations or explain methods of allocation concealment, and few commented on the validity and reliability of their chosen outcome measures. Most studies failed to clearly distinguish primary research outcomes from secondary research outcomes.[17,26,27,30,31,36,38,39,40] Two out of 12 studies stated a clear number and reason for withdrawals,[26,36] with the remaining ten trial reports not mentioning withdrawals. Most of the trials were of limited length, with 75% of studies less than 6 weeks duration. Small sample sizes in the majority of trials resulted in outcomes with wide CIs, introducing possible uncertainties regarding the precision of the findings.

Change in serum carboxymethyl-lysine

A summary of results of this review are presented in Table 2. Ten trials measured serum carboxymethyl-lysine (CML) concentrations after individuals had received low-AGE and standard-AGE diets (Figure 2). Two short-term trials of variable quality[33,35] were unable to detect any difference in serum CML levels after a single low-AGE or standard-AGE meal, in either healthy subjects ($n = 9$) or patients with type 2 diabetes ($n = 20$). These results conflicted with another short-term trial,[17] which measured serum CML in a sample of patients with type 1 and type 2 diabetes ($n = 15$). Pooled serum samples collected for 48 h after consumption of a low-AGE meal contained substantially lower CML levels than serum collected after a high-AGE meal (SMD 1.8; 95% CI: 0.5–3.1).

The effects of intermediate-term dietary AGE restriction on serum CML levels are mixed. In one high-quality trial,[30] circulating CML levels were significantly increased after overweight but otherwise healthy volunteers ($n = 11$) had consumed an AGE-restricted diet for 2 weeks. However, another trial of lower quality[39] supported the use of a 2-week low-AGE diet in patients with diabetes to significantly reduce serum CML concentration (SMD 1.1; 95% CI: 0.2–2.0). A high-quality investigation of serum CML levels after a 4-week low-AGE intervention in 64 healthy subjects[26] demonstrated a small but nonsignificant reduction in serum CML (SMD 0.3; 95% CI: −0.05 to 0.7). Two trials measured circulating CML concentrations in adults with renal failure after a 4-week consumption of either low- or standard-AGE diets.[38,40] These trials were determined to be clinically and statistically homogeneous ($I^2 = 0\%$), and a meta-analysis was performed using a fixed-effect model ($n = 27$). The pooled SMD for CML was 0.5 (95% CI: −0.2 to 1.3), indicating a nonsignificant reduction in serum CML concentration after the low-AGE intervention.

Two trials investigated the effects of long-term (16 weeks) low-AGE diets versus standard-AGE diets on the serum CML levels of healthy volunteers.[38,40] These trials were clinically and statistically homogeneous ($I^2 = 0\%$), and thus were subjected to meta-analysis using a fixed-effect model ($n = 48$). The pooled SMD for serum CML was 1.2 (95% CI:0.5–1.8), indicating a statistically significant effect supporting long-term dietary AGE restriction for reducing circulating CML concentrations in healthy adults. In addition, meta-analysis was performed on two homogeneous trials ($I^2 = 0\%$) involving long-term (6–16 weeks) low or standard-AGE dietary interventions in adults ($n = 42$) with type 2 diabetes.[27,38] The pooled SMD for serum CML was 2.0 (95% CI: 1.2–2.8), providing low-quality evidence that long-term low-AGE diets reduce circulating CML concentrations in people with type 2 diabetes.

Healthy subjects

Two trials of variable quality compared the effects of low-AGE and high-AGE diets on serum 8-isoprostanes and TNFα in healthy adult volunteers after 16 weeks.[38,40] These trials were determined to be clinically and statistically homogeneous ($I^2 = 16\%$ and $I^2 = 0\%$, respectively), and meta-analyses were performed using a fixed-effect model ($n = 48$). The pooled SMD for serum 8-isoprostanes

Table 1. Characteristics of included studies

Trial	*Participants/blinding*	*Study design*	*Randomization*	*Heyland MQS*	*Intervention*	*ITT analysis*	*Effect of low-AGE diet compared with standard-AGE diet, SMD (95% CI)*
Birlouez-Aragon *et al.*[26]	France, $n=64$ healthy volunteers, 32M: 32F, (mean age: 19 years, mean BMI: 21.8) Blinding: not blinded	C	Y	8	4-Week low-MRP diet (2.2 ± 0.9 mg CML/day) before crossover to 4-week standard MRP diet (5.4 ± 2.3 mg CML/day) Method of AGE measurement:	U	Serum CML: 0.3 (−0.1 to 0.7) HOMA: **3.8 (3.3–4.3)** Vitamin C: **2.5 (2.1–2.9)**
Cai *et al.*[27]	USA, $n=24$ patients with well-controlled T2 DM (mean HbA1c: 7.3 ± 0.6%, mean BMI: 28.5), normal lipid profiles and renal function Blinding: not blinded	P	Y	8	(GC–MS)/MS Random assignment to 6-week low-AGE diet ($n=13$, 3670 ± 1200 kU CML/day) or 6-week high-AGE diet ($n=11$, 16 300 ± 3700 kU CML/day) Method of AGE measurement: ELISA (in-house)	U	Serum CML: **2.0 (1.0–3.0)** AGE-modified LDL: **2.7 (1.6–3.9)**
Dittrich *et al.*[28]	Germany, $n=8$ healthy volunteers, 5M: 3F (mean age: 33 years). BMI not reported Blinding: not blinded	C	N	6	1-Week consumption of diet containing minimal quantities of thermally treated food (M^-) before crossover to 1 week consumption of diet supplemented with large quantities of thermally treated food (M^+), followed by return to 1-week M^- diet. Dietary AGE content not quantified Method of AGE measurement: AGEs not measured	U	Resistance of LDL to oxidation: **−4.4 (−7.1** to **−1.9)**
Foerster and Henle[29]	Germany, $n=7$ healthy volunteers, 4M: 3F (age range: 28–64 years). BMI not reported Blinding: not blinded	C	N	5	1 Day no dietary restrictions before crossover to 3 days avoidance of foods containing MRPs, followed by return to 1 day no dietary restrictions. Dietary AGE content not quantified Method of AGE measurement: HPLC	U	Urinary AGE (pyrraline) excretion was complete and in direct proportion to dietary pyrraline intake
Harcourt *et al.*[30]	Australia, $n=11$ healthy overweight or obese men (mean age: 30 years, mean BMI: 31.8) Blinding: outcome assessors blinded	C	Y	9	2-Week low-AGE diet (3302 kU CML/day) before crossover to 2-week high-AGE diet (14 090 kU CML/day) Method of AGE measurement: ELISA (in-house)	U	Serum CML: **−4.8 (−6.9** to **−2.7)** Urine 8-isoprostanes: 0.5 (−0.4 to 1.3)
Koschinsky *et al.*[17]	USA, $n=42$ (38 patients with T1 or T2 DM, 5 volunteers without DM). Age range: 16–84 years, mean HbA1c in patients with DM: 9.6%, mean BMI not reported Blinding: not blinded	P	N	7	Assignment to either single low-AGE egg-white meal (7 U CML/mg protein) or single high-AGE egg-white meal cooked with fructose (1617 U CML/mg protein). Measurements at 0–48 h post-prandial Method of AGE measurement: ELISA (in-house)	U	Serum CML: **1.8 (0.5–3.1)**
Negrean *et al.*[31] Stirban *et al.*[32] Stirban *et al.*[33] Stirban *et al.*[34]	Germany, $n=20$ patients with T2 DM: 14M: 6F (mean age: 55.9 years, mean BMI: 29.5 ± 0.8, mean HbA1c: 8.9 ± 0.4%, mean DM duration: 8.7 ± 1.7 years) Blinding: outcome assessors blinded	C	Y	8	Single low-AGE meal (2750 kU CML) before crossover to single high-AGE meal (15 100 kU CML). Measurement at 4 h post-prandial Method of AGE measurement: ELISA (in-house)	N	Serum CML: 0.1 (−0.5 to 0.7) VCAM-1: 0.4 (−0.2 to 1.0)
Schiekofer *et al.*[35]	Germany, $n=9$ healthy nonsmoking male volunteers (mean age: 32 years, mean BMI: 24) Blinding: not blinded	C	N	6	Single low-AGE meal (250 g minimal AGE-modified casein, 301.7 ± 49.2 ng CML/mg casein) before crossover to single high-AGE meal (250 g high AGE-modified casein, 104.2 ± 23.3 ng CML/mg casein). Measurements at 2 h post-prandial Method of AGE measurement: ELISA (commercially available)	U	Serum CML: 0.1 (−0.8 to 1.0)
Uribarri *et al.*[36] Peppa *et al.*[37]	USA, $n=18$ patients with nondiabetic ESRF on peritoneal dialysis (mean age and BMI not reported) Blinding: not blinded	P	Y	7	Random assignment to either 4-week low-AGE diet (5500 kU CML/day) or 4-week high-AGE diet (17 000 kU CML/day) Method of AGE measurement: ELISA (in-house)	N	Serum CML: 0.5 (−0.4 to 1.5) AGE-modified LDL: **1.5 (0.4–2.6)** VCAM-1: 0.9 (−0.1 to 1.8)

Table 1. (Continued)

Trial	Participants/blinding	Study design	Randomization	Heyland MQS	Intervention	ITT analysis	Effect of low-AGE diet compared with standard-AGE diet, SMD (95% CI)
Uribarri *et al.*[38]	USA, $n=36$ (18 subjects with T2 DM, 18 subjects without DM). Mean age: 64 years, mean BMI: 29.8, HbA1c in patients with DM not reported Blinding: not blinded	P	Y	6	Random assignment to either 16-week standard diet (>20 000 kU CML/day) or 16-week AGE-restricted diet (<10 000 kU CML/day) Method of AGE measurement: ELISA (in-house)	U	Serum CML (T2 DM, $n=18$): **1.9 (0.7–3.1)** Serum CML (healthy, $n=18$): **1.5 (0.4–2.6)** 8-Isoprostanes (T2 DM, $n=18$): **1.4 (0.3–2.5)** 8-Isoprostanes (healthy, $n=18$): **1.4 (0.3–2.4)** HOMA (T2 DM, $n=18$): **1.4 (0.3–2.6)** HOMA (healthy, $n=18$): −0.7 (−1.6 to 0.3) TNFα (T2 DM, $n=18$): **1.7 (0.6–3.0)** TNFα (healthy, $n=18$): **1.3 (0.2–2.3)**
Vlassara *et al.*[39]	USA, $n=11$ (two subjects with T1 DM, eight subjects with T2 DM (mean age: 52 years, mean HbA1c: 7.8 ± 0.7%, mean BMI: 28) Blinding: not blinded	C	U	7	2-Week low-AGE diet, $n=11$ (3670 kU CML/day) before crossover to 2-week high-AGE diet (16 300 kU CML/day) Method of AGE measurement: ELISA (in-house)	U	Serum CML: **1.1 (0.2–2.0)** AGE-modified LDL: 0.8 (0.0–1.7) VCAM-1: **1.0 (0.1–1.9)** TNFα: 0.3 (−0.6 to 1.1)
Vlassara *et al.*[40]	USA, $n=30$ healthy subjects and nine patients with CKD Blinding: not blinded	P	Y	8	Healthy subjects: random assignment to either 16-week regular diet (>13 000 kU CML/day) or 16-week low-AGE diet (<5500 kU CML/day) CKD patients: random assignment to groups as above but duration of intervention 4 weeks Method of AGE measurement: ELISA (in-house)	Y	Serum CML (CKD, $n=9$): 0.5 (−0.9 to 1.9) Serum CML (healthy, $n=30$): **1.0 (0.2–1.8)** 8-Isoprostanes (CKD, $n=9$): 0.2 (−1.1 to 1.6) 8-Isoprostanes (healthy, $n=30$): 0.6 (−0.1 to 1.4) VCAM-1 (CKD, $n=9$): 1.0 (−0.5 to 2.5) VCAM-1 (healthy, $n=30$): **0.9 (0.2–1.7)** TNFα (CKD, $n=9$): (−1.3 to 1.3) TNFα (healthy, $n=30$): **1.3 (0.5–2.0)**

Abbreviations: AGE, advanced glycation end-product; BMI, body mass index; C, crossover; CI, confidence interval; CKD, chronic kidney disease; CML, carboxymethyl-lysine; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; ESRF, end-stage renal failure; F, female; GC, gas chromatography; HOMA, homeostasis model assessment; HPLC, high pressure liquid chromatography; ITT, intention to treat; LDL, low-density lipoprotein; M, male; MS, mass spectrometry; MQS, Methodological Quality Score; MRP, Maillard reaction product; N, no; P, parallel; SMD, standardized mean difference; T1 and T2 DM, type 1 and type 2 diabetes mellitus; TNFα, tumor necrosis factor-α; U, unclear; VCAM-1, vascular cell adhesion molecule-1; Y, yes. Methodological quality of trials measured by the Heyland MQS, where trials scoring ⩾8 out of 14 points are considered of high methodological quality. Results in bold represent statistically significant comparisons based on the 95% CI of the SMD.

was 0.9 (95% CI:0.3–1.5), indicating a statistically significant effect favoring long-term low-AGE dietary intake over standard AGE intake. Similarly, the pooled SMD for TNFα was 1.3 (95% CI: 0.6–1.9), indicating large reductions in this inflammatory cytokine after the low-AGE intervention (Figure 3). One high-quality trial[30] involving an intermediate-term low-AGE diet in overweight/obese individuals who were otherwise healthy (2 weeks, $n=11$) found a small reduction in urinary 8-isoprostane excretion after the AGE-restricted diet when compared with the standard-AGE diet (SMD 0.5; 95% CI: −0.4 to 1.3), but the difference was not statistically significant.

Low-quality evidence in one high-quality trial[40] supported the use of a long-term (16 week) AGE-restricted diet in healthy volunteers ($n=30$) to significantly reduce the level of VCAM-1 (a metabolic marker of endothelial dysfunction), with an SMD of 0.9 (95% CI: 0.2–1.7).

Conflicting evidence was found on the intermediate-term (4 weeks, $n=64$) and long-term (16 weeks, $n=18$) effects of low-AGE diets on insulin resistance (measured by HOMA) in healthy individuals. Findings were inconsistent among two trials, with one high-quality trial finding increased insulin sensitivity after 4 weeks, with an SMD of 3.8 (95% CI: 3.3–4.3),[26] and the other lower-quality trial finding no statistically significant effect on insulin sensitivity after 16 weeks, with an SMD of 0.7 (95% CI: −1.6 to 0.3).[38]

Patients with diabetes

All included trials investigating the effect of dietary AGE restriction on patients with type 1 and/or type 2 diabetes were clinically heterogeneous, and thus meta-analyses could not be performed (Figure 3). The results of one low-quality trial provided very low-quality evidence that a long-term (16 weeks, $n=18$) low-AGE diet reduced insulin resistance (measured by HOMA) in individuals with type 2 diabetes, with an SMD of 1.4 (95% CI: 0.3–2.6).[38]

In one high-quality trial,[31] short-term (2 h post-prandial, $n=20$) comparison of low-AGE and standard-AGE meals in patients with type 2 diabetes failed to show a statistically significant difference in VCAM-1 levels, with an SMD of 0.4 (95% CI: −0.2 to 1.0) (data not shown in Forest Plot). However, one low-quality study[39] demonstrated a large reduction in VCAM-1 after an intermediate-term (2 weeks, $n=11$) low-AGE diet in patients with type 1 and type 2 diabetes, with an SMD of 1.0 (95% CI: 0.1–1.9).

Two lower-quality trials[38,39] assessed the intermediate- and long-term effects of dietary AGE restriction on TNFα in patients with diabetes. The intermediate-term intervention (2 weeks, $n=11$) did not show a statistically significant result (SMD 0.3; 95% CI: −0.6 to 1.1), but the long-term intervention (16 weeks, $n=18$) significantly reduced serum TNFα levels (SMD 1.7; 95% CI: 0.6–2.9). The same long-term intervention significantly reduced serum 8-isoprostane levels (SMD 1.4; 95% CI: 0.3–2.5).

Table 2. Summary of results according to the GRADE criteria

Low-AGE versus standard-AGE diet study reference(s)	*Patient type*	*GRADE quality of evidence*	*Serum CML*	*HOMA*	*8-Isoprostanes*	*TNFα*	*VCAM-1*	*AGE-modified LDL*
26, 33, (37, 40), 40	Healthy	Low	= (S,I), + (L)	+ (I)	+ (L)	+ (L)	+ (L)	
38	Healthy	Very low		= (L)				
30	Overweight	Low	− (I)		= (I)			
27, 31, (27, 38)	Type 2 DM	Low	+ (L)				= (S)	+ (L)
35, 38	Type 2 DM	Very low	= (S)		+ (L)	+ (L)		
17, 39	T1 and T2 DM	Very low	+ (S,I)	+ (L)		= (I)	+ (I)	= (I)
(37,40) 40	Renal failure	Low	= (S)		= (I)	= (I)	+ (I)	
37	Renal failure	Very low						+ (I)

Abbreviations: AGE, advanced glycation end-product; CML, carboxymethyl-lysine; GRADE, Grades of Recommendation, Assessment, Development & Evaluation; HOMA, homeostasis model assessment; I, intermediate-term follow-up; L, long-term follow-up; LDL, low-density lipoprotein; S, short-term follow-up; T1 and T2 DM, type 1 and 2 diabetes mellitus; TNFα, tumor necrosis factor-α. '+' Indicates an effect in favor of the low-AGE dietary intervention over the standard-AGE comparison; '—' indicates an effect in favor of the standard-AGE diet; '=' indicates no significant difference between low-AGE and standard-AGE diets; study reference numbers in parentheses indicate trials subjected to meta-analyses.

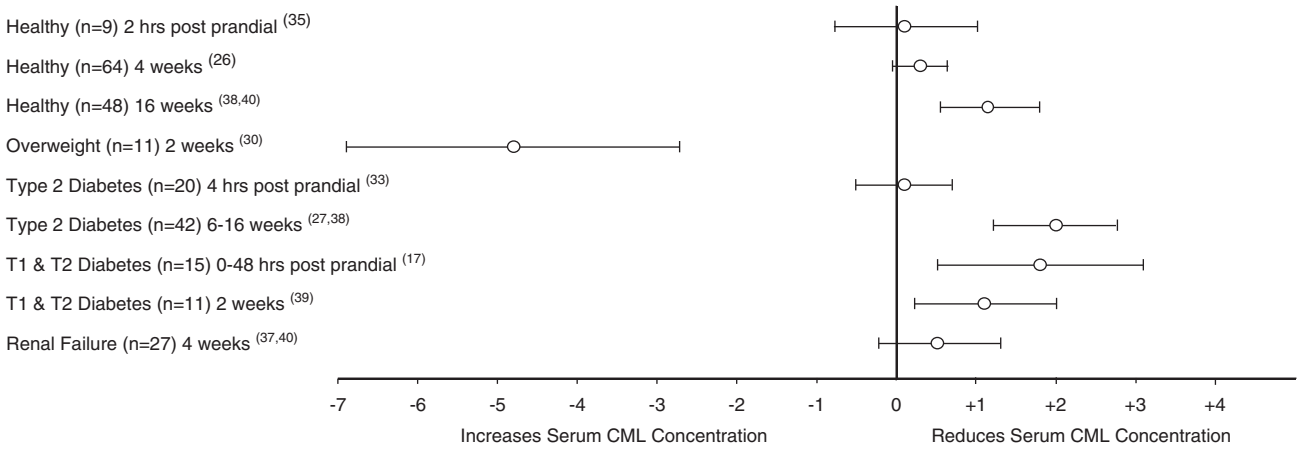


Figure 2. Effects of low-AGE diets on circulating CML concentration. Forest plot of standardised mean differences (95% CI) for individual and homogeneous pooled trials.

Similarly, AGE-modified LDL (a serum marker associated with cardiovascular disease risk) was moderately reduced by an intermediate-term AGE-restricted diet (2 weeks, $n = 11$) in people with diabetes, but the result was not statistically significant, with an SMD of 0.8 (95% CI: −0.04 to 1.7).[39] After long-term dietary AGE restriction (6 weeks, $n = 24$), however, the reduction in AGE-modified LDL was highly significant in a high-quality trial, with an SMD of 2.7 (95% CI: 1.6–3.9).[27]

Patients with renal failure

Two trials of variable quality investigated the effects of intermediate-term (4 weeks $n = 27$) low-AGE diets versus standard-AGE diets on VCAM-1 and TNFα in patients with renal failure.[37,40] These trials were clinically and statistically homogeneous ($I^2 = 0\%$ for both variables), and thus were subjected to meta-analyses using a fixed-effect model. The pooled SMD for serum VCAM-1 was 0.9 (95% CI: 0.1–1.7), indicating a statistically significant effect favoring an intermediate-term low-AGE dietary intake on endothelial function (Figure 3). However, the pooled SMD for TNFα after 4 weeks of a low-AGE diet was not statistically significant, 0.5 (95% CI: −0.3 to 1.3).

A 4-week low-AGE dietary intervention[40] in nine patients with renal failure was unable to demonstrate a statistically significant reduction in serum 8-isoprostanes when compared with those receiving the standard-AGE diet: SMD, 0.2 (95% CI: −1.1 to 1.6). Another intermediate-term (4 weeks, $n = 18$) trial provided very low-level evidence that a 4-week low-AGE diet in people with renal failure was long enough to significantly reduce AGE-modified LDL levels, with an SMD of 1.5 (95% CI: 0.4–2.6).[37]

Long-term low-AGE dietary intervention trials have not been performed in patients with renal failure.

Adverse effects of low-age diets

Some AGEs (melanoidins, aminoreductones and heterocyclic compounds) demonstrate antioxidant activity, and thus their reduction in the diet may be deleterious in the longer term. One low-quality trial reported a negative consequence of an intermediate-term low-AGE diet in healthy volunteers (1 week, $n = 8$).[28] This trial found that consumption of a diet rich in MRPs significantly increased the resistance of plasma LDL to oxidation *in vitro* when compared with consumption of a diet low in MRPs. Results demonstrated a negative effect of low-AGE diets on the oxidative resistance of LDL, with an SMD of −4.4 (95% CI: −7.1 to −1.9). No other adverse effects of low-AGE diets were reported by the trials in this review. In contrast, one high-quality trial[26] found higher concentrations of a number of serum antioxidants after a 4-week low-AGE diet compared with a standard-AGE diet in 64

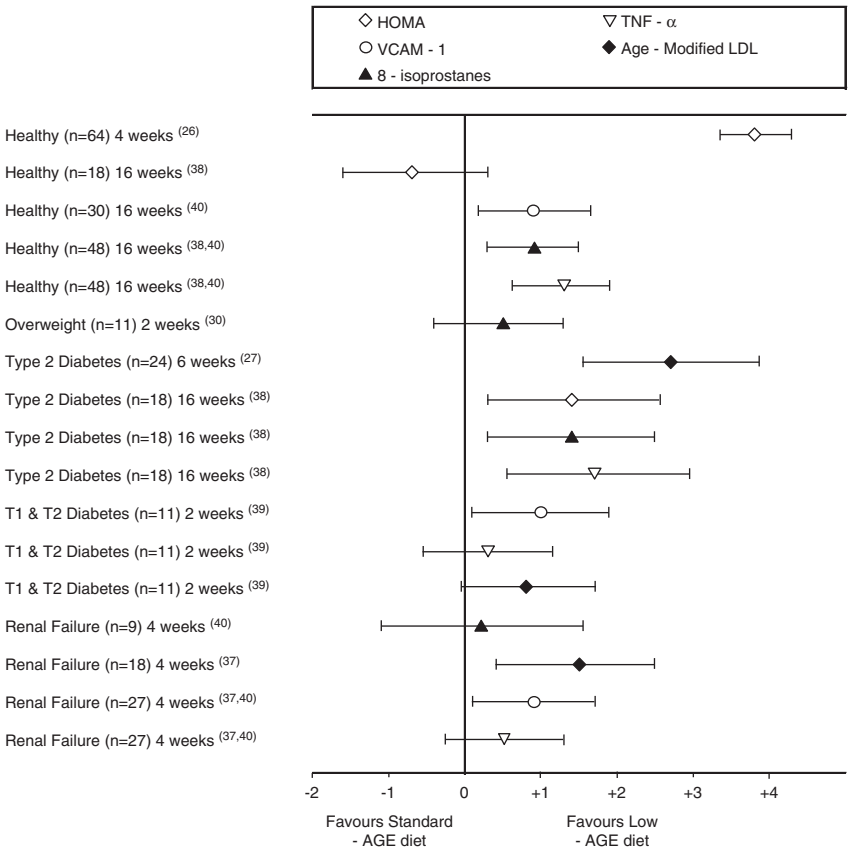


Figure 3. Effects of low-AGE diets on circulating biomarkers. Forest plot of standardised mean differences (95% CI) for individual and homogeneous pooled trials.

healthy individuals. The SMD for post-intervention vitamin C was 2.5 (95% CI: 2.1–2.9).

DISCUSSION

Efficacy of dietary age restriction

This review provides some preliminary evidence suggesting that utilization of an AGE-restricted diet might be a successful long-term intervention to reduce the body's total AGE concentration in both healthy individuals and people with type 2 diabetes. However, caution is required in the interpretation of these results. All of the individual and pooled trials showing a positive effect of low-AGE diets on reducing systemic CML levels were performed by the same research team, and while a proportion of their trials were of high quality according to the Heyland Methodological Quality Score, these studies need to be replicated by other research groups to strengthen the evidence. There has been no direct determination of the proportion of exogenous dietary AGEs or their precursors which contribute to serum CML levels, and what proportion is a result of endogenous glycation. Some studies have found that energy-restricted[41] and antioxidant-supplemented[42] (rather than AGE-restricted) diets also reduce serum CML concentrations, indicating that other factors such as body fat, circulating triglycerides and antioxidant capacity may have additional effects on serum CML levels. The rate of endogenous protein degradation and turnover is also likely to influence circulating CML concentrations. Publication bias may be a factor to consider in this particular area of research, as published cross-sectional studies exist that fail to detect any correlation between dietary AGE consumption and circulating CML levels.[43–45] Other studies with similar negative findings may remain unpublished. Finally, CML is only one of many AGEs, and further study is required to determine the effects of a low-AGE diet on other circulating AGEs and what their specific functions are.

This review found no evidence that an AGE-restricted diet reduces serum CML in individuals with renal failure. However, it is well established that kidney dysfunction impairs AGE excretion, increasing the level of circulating AGEs.[46]

This review found low-quality evidence supporting adherence to a long-term low-AGE diet for the reduction of 8-isoprostanes and TNFα (biomarkers of oxidative stress and inflammation, respectively) in healthy individuals. Very low-quality evidence supports long-term dietary AGE restriction for the reduction of 8-isoprostanes in people with type 2 diabetes. These findings are consistent with large cross-sectional studies conducted in healthy individuals[10] and patients with type 2 diabetes.[12]

Contradictory evidence was found in this review regarding the effect of a low-AGE diet on markers of insulin resistance (HOMA) in healthy individuals. This may be because people with a healthy body weight are generally insulin sensitive to begin with, and interventions to reduce insulin resistance are therefore of limited benefit. The evidence favoring a long-term low-AGE diet for the attenuation of insulin resistance in people with type 2 diabetes was of very low quality.

Low-quality evidence in this review supported an intermediate-term AGE-restricted diet for the reduction of VCAM-1 in patients with renal failure. The effects of dietary AGE restriction on VCAM-1 levels in people with diabetes were contradictory. A long-term AGE-restricted diet reduced VCAM-1 levels in healthy individuals compared with a standard AGE diet. This review also found low-quality evidence in favor of long-term dietary AGE restriction for the reduction of AGE-modified LDL in patients with type 2 diabetes. Very low-quality evidence supported an

intermediate-term low-AGE diet for the reduction of AGE-modified LDL in patients with renal failure. Cross-sectional studies support an association between Western-style dietary patterns and the pathogenesis of cardiovascular disease;[47,48] however, AGEs are likely to be only one of multiple dietary components involved.

Methodological limitations of included trials

Many of the trials included in this study contained major methodological flaws, with less than half receiving high-quality scores based on the Heyland Methodological Quality criteria. Few studies described the method of randomization used, and the method of allocation concealment was not mentioned in any of the trials. Most of the trials contained very small numbers of participants, and were likely to be underpowered. Primary research outcomes were not clearly defined in most of the trials, making it difficult to determine whether sample sizes were adequate.

Eight of the 12 trials included in this review estimated the AGE content of their test diets based on a database of the AGE content of common foods initially published by Goldberg *et al.*,[49] and later updated by Uribarri *et al.*[50] This research team utilized a nonvalidated, enzyme-linked immunosorbent assay-based method for the measurement of CML in foodstuffs. Immunological methods of AGE measurement have been associated with some limitations, as the assays are capable of detecting additional contaminants that are unrelated to the ligand of interest.[51] An additional disadvantage of this indirect, enzyme-linked immunosorbent assay method is that it only allows the AGE concentration in foods to be expressed in arbitrary units (kilounits AGE), making comparisons with other analytical techniques impossible. There is a need for all researchers in this field to use standardized, validated measurement tools for the assessment of the AGE concentration in foods. Liquid chromatography–mass spectrometry methods appear to be highly sensitive techniques for AGE quantification,[52,53] and would enable comparisons of results to be made between different laboratories.

Apart from AGEs, multiple other RAGE ligands exist and opinion is divided over the binding affinity of dietary AGEs for RAGE *in vivo*.[54] Only highly glycated proteins appear to successfully bind to and activate RAGE *in vitro*,[55] with the low-molecular-weight AGEs absorbed into the circulation after digestion of a high-AGE meal unlikely to interact with RAGE. Although post-absorptive dietary AGE precursors may modify endogenous proteins in order to form high-molecular-weight compounds capable of RAGE binding, this has not yet been determined. Elevations in postprandial hyperglycemia appear to be sufficient to increase the expression of nuclear factor κB[56] independent of the AGE content of the meal.[35]

The majority of trials in this review measured serum CML as an indicator of circulating AGEs; however, the body's total AGE concentration is currently unknown. CML is only one of many different AGEs, most of which have not yet been characterized. Although some AGEs may have a role in cellular toxicity, others may confer beneficial antioxidant effects. Individual AGEs are also likely to have different rates of absorption and excretion. A large proportion of systemic AGEs may simply be a by-product of oxidative stress and inflammation rather than a causative factor. High-quality studies are required to provide answers to the many uncertainties surrounding the function and kinetics of AGEs.

Limitations of this review

A wide variety of outcome measures were utilized by the studies included in this review, in patients with a number of different health conditions and a length of follow-up ranging from 2 h to 16 weeks. The heterogeneity among studies made it difficult to collate the findings of more than two trials for any particular outcome. Most of the evidence presented in this review was therefore derived from individual studies. More than half of the trials included in this review were determined to be of low methodological quality, with a Heyland Methodological Quality Score <8.

Because of the absence of research investigating the effects of dietary AGE restriction on patient-important primary outcomes, such as diabetes diagnosis, microvascular and macrovascular complications and quality of life, surrogate biochemical measurements (secondary end points) were evaluated in this review. Although the biochemical indices assessed in this review are considered valid metabolic markers of insulin resistance, inflammation, oxidative stress, endothelial dysfunction and cardiovascular disease risk,[57] the GRADE criteria automatically downgrades the strength of evidence favoring a particular intervention from high to medium when secondary rather than primary end points are summarized.[58] The strength of evidence from studies included in this review were further downgraded from medium to low quality by the GRADE criteria owing to their small sample sizes and wide CIs, indicating possible imprecision of results. Evidence from studies with Heyland Methodological Quality Scores <8 was also downgraded from low to very low quality because of methodological limitations. Future high-quality trials involving modulation of dietary AGE levels, which include the assessment of at least one primary outcome and the use of *a priori* sample size calculations, will enhance the strength of the current evidence base.

Seven of the 12 trials included in this review were undertaken by the same research group, possibly introducing similar methodological constraints into the majority of studies conducted on this topic.

Comparison with other reviews

No other systematic reviews have addressed the potential health benefits of AGE-restricted diets. A review of the effects of dietary factors on low-grade inflammation in overweight individuals[59] concluded that despite many gaps in the research investigating the effects of dietary AGE consumption on chronic inflammation, 'it might be prudent to advise renal failure patients to decrease their intake of 'highly heated' food'. However, as the effects of long-term dietary AGE restriction in people with renal failure are currently unknown, further dietary restrictions may contribute to a greater and unnecessary burden in this patient group.

The evidence summarized in this review suggests that patients with diabetes could potentially reduce their level of insulin resistance, systemic inflammation and oxidative stress and their risk of cardiovascular events by adhering to a long-term low-AGE diet. The minimization of MRP formation during food preparation and cooking is a simple, inexpensive and noninvasive intervention that could potentially alleviate significant diabetes-related morbidity. Preventing excessive heat treatment of food is also in line with current anticancer recommendations for restricting the consumption of heterocyclic amines and polycyclic aromatic hydrocarbons.[60] At present, however, it cannot be ruled out that the health benefits associated with reduced consumption of highly heated food may simply be a result of an increased intake of antioxidants (which would have otherwise been destroyed during the cooking process), or avoidance of deleterious compounds other than AGEs, which are generated during thermal processing such as acrylamide or heterocyclic amines. There is a need for well-designed controlled feeding trials that evaluate effects of the consumption of dietary AGEs and their precursors in purified forms, in order to rule out contributions made by other macronutrients and micronutrients in whole foods.

Reviewers' conclusions

Current evidence supporting the efficacy of medium- to long-term dietary AGE restriction for alleviating the proinflammatory milieu

in healthy individuals and patients with diabetes or renal failure is of low quality. Additional long-term high-quality RCTs with larger sample sizes measuring patient-important primary end points are required to strengthen the evidence supporting the effects of low-AGE diets. Further studies utilizing standardized methods of AGE measurement are needed to elucidate the role of specific AGEs in cellular dysfunction, and explore possible adverse effects of low-AGE diets in the long term.

Although it is likely that future research findings will support the use of dietary AGE restriction as a therapeutic strategy to assist in the management of adults with prediabetes, diabetes and renal impairment, there is currently insufficient evidence to encourage the use of AGE-restricted diets in mainstream nutrition practice. More information is required regarding the digestion, absorption, function and elimination of dietary AGEs in their pure form, along with standardized, validated tools for the measurement of deleterious AGEs in foods.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The first author is the recipient of a National Health and Medical Research Council (NHMRC) Postgraduate Public Health Scholarship APP1039709. NJK thanks Mr Brendan Kellow for IT assistance and Dr Andrew Hahne (Latrobe University) for statistical advice. We thank Dr Naiyana Wattanapenpaiboon (Monash University) for providing comments on the manuscript. NJK is supported by a NHMRC Postgraduate Public Health Scholarship APP1039709.

REFERENCES

1 Barlovic DP, Thomas MC, Jandeleit-Dahm K. Cardiovascular disease: what's all the AGE/RAGE about? *Cardiovasc Hematol Disord Drug Targets* 2010; **10**: 7–15.
2 Yamagishi S, Ueda S, Okuda S. Food-derived advanced glycation end products (AGEs): a novel therapeutic target for various disorders. *Curr Pharm Des* 2007; **13**: 2832–2836.
3 Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B *et al.* Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med* 2005; **83**: 876–886.
4 Tahara N, Yamagishi S, Matsui T, Takeuchi M, Nitta Y, Kodama N *et al.* Serum levels of advanced glycation endproducts (AGEs) are independent correlates of insulin resistance in nondiabetic subjects. *Cardiovasc Ther* 2012; **30**: 42–48.
5 Tessier FJ, Birlouez-Aragon I. Health effects of dietary Maillard reaction products: the results of ICARE and other studies. *Amino Acids* 2012; **42**: 1119–1131.
6 Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H. Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann NY Acad Sci* 2005; **1043**: 461–466.
7 Faist V, Erbersdobler HF. Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Ann Nutr Metab* 2001; **45**: 1–12.
8 Delgado-Andrade C, Tessier FJ, Niquet-Leridon C, Seiquer I, Pilar Navarro M. Study of the urinary and faecal excretion of N (epsilon)-carboxymethyllysine in young human volunteers. *Amino Acids* 2012; **43**: 595–602.
9 Kankova K, Sebekova K. Genetic variability in the RAGE gene: possible implications for nutrigenetics, nutrigenomics, and understanding the susceptibility to diabetic complications. *Mol Nutr Food Res* 2005; **49**: 700–709.
10 Uribarri J, Cai W, Peppa M, Goodman S, Ferrucci L, Striker G *et al.* Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol A Biol Sci Med Sci* 2007; **62**: 427–433.
11 Uribarri J, Peppa M, Cai W, Goldberg T, Lu M, Baliga S *et al.* Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. *Am J Kidney Dis* 2003; **42**: 532–538.
12 Chao PC, Huang CN, Hsu CC, Yin MC, Guo YR. Association of dietary AGEs with circulating AGEs, glycated LDL, IL-1alpha and MCP-1 levels in type 2 diabetic patients. *Eur J Nutr* 2010; **49**: 429–434.
13 Ramasamy R, Yan SF, Schmidt AM. Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications. *Ann N Y Acad Sci* 2011; **1243**: 88–102.
14 Coughlan MT, Yap FY, Tong DC, Andrikopoulos S, Gasser A, Thallas-Bonke V *et al.* Advanced glycation end products are direct modulators of beta-cell function. *Diabetes* 2011; **60**: 2523–2532.
15 Fiory F, Lombardi A, Miele C, Giudicelli J, Beguinot F, Van Obberghen E. Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia* 2011; **54**: 2941–2952.
16 Vlassara H, Striker GE. AGE restriction in diabetes mellitus: a paradigm shift. *Nat Rev Endocrinol* 2011; **7**: 526–539.
17 Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C *et al.* Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci USA* 1997; **94**: 6474–6479.
18 Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. *JAMA* 2001; **286**: 944–953.
19 Hedges I, Olkin I. *Statistical Methods for Meta-analysis.* 1st edn. Academic Press: Orlando, FL, USA, 1985.
20 White IR, Thomas J. Standardized mean differences in individually-randomized and cluster-randomized trials, with applications to meta-analysis. *Clin Trials* 2005; **2**: 141–151.
21 The Cochrane Collaboration. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)* Higgins JPT GS (ed.) The Cochrane Collaboration: Copenhagen, 2011.
22 Zar JH. *Biostatistical Analysis*. 2nd edn. Englewoods CliffsNew Jersey, USA: Prentice-Hall Inc, 1964.
23 Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557–560.
24 The Cochrane Collaboration. *Review Manager (RevMan)*. The Cochrane Collaboration: Copenhagen, 2011.
25 Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S *et al.* Grading quality of evidence and strength of recommendations. *BMJ* 2004; **328**: 1490.
26 Birlouez-Aragon I, Saavedra G, Tessier FJ, Galinier A, Ait-Ameur L, Lacoste F *et al.* A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *Am J Clin Nutr* 2010; **91**: 1220–1226.
27 Cai W, He JC, Zhu L, Peppa M, Lu C, Uribarri J *et al.* High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. *Circulation* 2004; **110**: 285–291.
28 Dittrich R, Dragonas C, Kannenkeril D, Hoffmann I, Mueller A, Beckmann MW *et al.* A diet rich in Maillard reaction products protects LDL against copper induced oxidation ex vivo, a human intervention trial. *Food Res Int* 2009; **42**: 1315–1322.
29 Foerster A, Henle T. Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline. *Biochem Soc Trans* 2003; **31**: 1383–1385.
30 Harcourt BE, Sourris KC, Coughlan MT, Walker KZ, Dougherty SL, Andrikopoulos S *et al.* Targeted reduction of advanced glycation improves renal function in obesity. *Kidney Int* 2011; **80**: 190–198.
31 Negrean M, Stirban A, Stratmann B, Gawlowski T, Horstmann T, Götting C *et al.* Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. *Am J Clin Nutr* 2007; **85**: 1236–1243.
32 Stirban A, Negrean M, Stratmann B, Götting C, Salomon J, Kleesiek K *et al.* Adiponectin decreases postprandially following a heat-processed meal in individuals with type 2 diabetes: an effect prevented by benfotiamine and cooking method. *Diabetes Care* 2007; **30**: 2514–2516.
33 Stirban A, Negrean M, Götting C, Uribarri J, Gawlowski T, Stratmann B *et al.* Dietary advanced glycation endproducts and oxidative stress: in vivo effects on endothelial function and adipokines. *Ann N Y Acad Sci* 2008; **1126**: 276–279.
34 Stirban A, Negrean M, Götting C, Stratmann B, Gawlowski T, Mueller-Roesel M *et al.* Leptin decreases postprandially in people with type 2 diabetes, an effect reduced by the cooking method. *Horm Metab Res* 2008; **40**: 896–900.
35 Schiekofer S, Franke S, Andrassy M, Chen J, Rudofsky G, Schneider JG *et al.* Postprandial mononuclear NF-kappaB activation is independent of the AGE-content of a single meal. *Exp Clin Endocrinol Diabetes* 2006; **114**: 160–167.
36 Uribarri J, Peppa M, Cai W, Goldberg T, Lu M, He C *et al.* Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. *J Am Soc Nephrol* 2003; **14**: 728–731.
37 Peppa M, Uribarri J, Cai W, Lu M, Vlassara H *et al.* Glycoxidation and inflammation in renal failure patients. *Am J Kidney Dis* 2004; **43**: 690–695.
38 Uribarri J, Cai W, Ramdas M, Goodman S, Pyzik R, Chen X *et al.* Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. *Diabetes Care* 2011; **34**: 1610–1616.
39 Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V *et al.* Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci USA* 2002; **99**: 15596–15601.

40 Vlassara H, Cai W, Goodman S, Pyzik R, Yong A, Chen X *et al.* Protection against loss of innate defenses in adulthood by low advanced glycation end products (AGE) intake: role of the antiinflammatory AGE receptor-1. *J Clin Endocrinol Metab* 2009; **94**: 4483–4491.
41 Gugliucci A, Kotani K, Taing J, Matsuoka Y, Sano Y, Yoshimura M *et al.* Short-term low calorie diet intervention reduces serum advanced glycation end products in healthy overweight or obese adults. *Ann Nutr Metab* 2009; **54**: 197–201.
42 Pashikanti S, de Alba DR, Boissonneault GA, Cervantes-Laurean D *et al.* Rutin metabolites: novel inhibitors of nonoxidative advanced glycation end products. *Free Radic Biol Med* 2010; **48**: 656–663.
43 De La Maza MP, Leiva BA, Gattas V, Petermann M, Garrido F, Hirsch S *et al.* Fluorescent serum and urinary advanced glycoxidation end-products in non-diabetic subjects. *Biol Res* 2007; **40**: 2003–2012.
44 Piroddi M, Palazzetti I, Quintaliani G, Pilolli F, Montaldi M, Valentina V *et al.* Circulating levels and dietary intake of the advanced glycation end-product marker carboxymethyl lysine in chronic kidney disease patients on conservative predialysis therapy: a pilot study. *J Ren Nutr* 2011; **21**: 329–339.
45 Semba RD, Ang A, Talegawkar S, Crasto C, Dalal M, Jardack P *et al.* Dietary intake associated with serum versus urinary carboxymethyl-lysine, a major advanced glycation endproduct, in adults: the Energetics Study. *Eur J Clin Nutr* 2012; **66**: 3–9.
46 Sourris KC, Forbes JM. Interactions between advanced glycation end-products (AGE) and their receptors in the development and progression of diabetic nephropathy - are these receptors valid therapeutic targets. *Curr Drug Targets* 2009; **10**: 42–50.
47 Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 2001; **73**: 673–686.
48 Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE *et al.* Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004; **80**: 1029–1035.
49 Goldberg T, Cai W, Peppa M, Dardaine V, Baliga BS, Uribarri J *et al.* Advanced glycoxidation end products in commonly consumed foods. *J Am Diet Assoc* 2004; **104**: 1287–1291.
50 Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R *et al.* Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc* 2010; **110**: 911–916.
51 Tessier FJ. The Maillard reaction in the human body. The main discoveries and factors that affect glycation. *Pathol Biol* 2010; **58**: 214–219.
52 Ames JM. Determination of N epsilon-(carboxymethyl)lysine in foods and related systems. *Ann N Y Acad Sci* 2008; **1126**: 20–24.
53 Assar SH, Moloney C, Lima M, Magee R, Ames JM. Determination of Nepsilon-(carboxymethyl)lysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids* 2009; **36**: 317–326.
54 Thornalley PJ. Dietary AGEs and ALEs and risk to human health by their interaction with the receptor for advanced glycation endproducts (RAGE)--an introduction. *Mol Nutr Food Res* 2007; **51**: 1107–1110.
55 Heizmann CW. The mechanism by which dietary AGEs are a risk to human health is via their interaction with RAGE: arguing against the motion. *Mol Nutr Food Res* 2007; **51**: 1116–1119.
56 El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG *et al.* Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 2008; **205**: 2409–2417.
57 Caballero AE. Metabolic and vascular abnormalities in subjects at risk for type 2 diabetes: the early start of a dangerous situation. *Arch Med Res* 2005; **36**: 241–249.
58 Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J *et al.* GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol* 2011; **64**: 401–406.
59 Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K *et al.* Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br J Nutr* 2011; **106**(Suppl 3): S5–S78.
60 Rohrmann S, Zoller D, Hermann S, Linseisen J. Intake of heterocyclic aromatic amines from meat in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. *Br J Nutr* 2007; **98**: 1112–1115.

Supplementary Information accompanies the paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)

Chapter 4:

Are dietary and lifestyle behaviours associated with tissue AGE accumulation?

Ongoing debate continues regarding the usefulness of serum AGE concentrations as markers of long-term glycation-related dysfunction in humans. Indeed, circulating AGE levels may simply be an indicator of the body's capacity to detoxify and excrete AGEs in the short term, and may have no impact on long-term deposition of AGEs in tissue, which is where AGEs are thought to exert the majority of their detrimental effects. Moreover, there is contradictory evidence regarding AGE concentrations in the bloodstream during the development of obesity and the metabolic syndrome, with one group finding an increase in plasma AGEs [50], another group finding no increase in plasma AGEs [140] and other researchers finding a decrease in plasma AGEs, thought to be due to RAGE-mediated trapping of AGEs in adipose tissue [141].

The measurement of AGEs in skin tissue provides an estimate of AGE accumulation over many years, providing more information regarding the body's AGE burden and degree of vascular disease risk than a single plasma AGE value. The quantification of AGE-modified extracellular proteins with a slow rate of turnover, such as skin collagen, which has a half-life of 15 years [142], signifies cumulative metabolic and oxidative stress over an extended time period. AGE deposition in skin tissue can be measured in skin biopsies [143], however this is an invasive and time consuming procedure with limited application in large cross-sectional studies. The fluorescent nature of many AGEs at specific ultra-violet wavelengths allow for their detection in skin rapidly and non-invasively by a machine called an AGE Reader (*Diagnoptics Technologies,* Groningen, The Netherlands) (Figure 3). The AGE Reader measures skin autoflourescence (SAF) by shining a UV light source with a wavelength of 300-420 nm

Figure 3. The AGE Reader (picture courtesy of Diagnoptics, The Netherlands)

(peak intensity at 370 nm) on a 4 cm² area of skin surface on the forearm, 10 cm below the elbow. The light emitted and reflected back from the skin is detected and measured by an internal spectrometer in the range 300-600 nm. SAF is calculated by dividing the average emitted light intensity per nanometer in the range of 420-600 nm by the average excitation light intensity per nanometer in the range 300-420 nm and multiplied by 100. SAF levels are expressed in arbitrary units (AU) [144]. SAF provides an estimate of tissue AGE accumulation, with readings from the AGE Reader validated against AGE content (both fluorescent and non-fluorescent) of skin biopsies [145]. Reference values of SAF have been developed for specific age groups in Dutch [146], Slovak [147] and Chinese [148] populations. SAF values typically correlate positively with chronological age, and are significantly higher in cigarette smokers and people with diabetes compared to non-smokers and those with normal glucose tolerance [145]. The AGE Reader accurately measures SAF in people with a wide range of skin pigmentations. However, the use of skin creams, tanning lotions and sunscreen influence the results of the AGE Reader, and effects may persist for several days [149].

In cross-sectional studies, associations have been identified between SAF measured by the AGE Reader and microvascular complications including nephropathy and neuropathy in people with type 1 diabetes [150, 151]. Positive correlations were also found between SAF and the severity of both micro and macrovascular complications in adults with type 2 diabetes [152, 153]. Relationships have been identified between SAF and the extent of peripheral and autonomic nerve abnormalities in patients with diabetes, even before symptoms become clinically apparent [154], which suggests the AGE Reader may have some utility in the clinical setting as a tool to identify high risk patients. Moreover, SAF in individuals with type 2 diabetes is associated not only with HbA1c, vascular complications and age, but also increases with the number of diagnostic characteristics of the metabolic syndrome [155]. SAF predicts mortality in haemodialysis [156] and peritoneal dialysis [157] patients, which is likely due to chronic poor glucose control, inflammation and cardiovascular risk factors. SAF may also be partially related to the inability of people with impaired renal function to excrete dietary AGEs in urine.

Prospective studies provide an indication of the long-term progression of AGE deposition in tissue, making them more useful than cross-sectional results collected at one time point. While it is unknown whether skin AGE concentrations can be reduced, prevention of their continued accumulation in skin may indicate the effectiveness of anti-AGE treatments. In patients with type 2 diabetes, SAF predicted the development of microvascular complications [158] and cardiovascular events [159]. SAF was also a prognostic indicator of cardiac mortality during a follow-up period of five years in adults with type 1 and type 2 diabetes [160].

Lifestyle behaviours other than cigarette smoking may influence SAF values. The effect of cumulative long-term exposure to exogenous AGEs from the diet on SAF is unknown. Associations identified between SAF and specific lifestyle factors may provide information about behavioural strategies which could minimise AGE accumulation. A small number of studies have investigated relationships between SAF, physical activity and dietary factors (Table 4).

The following cross-sectional study aimed to examine the relationship between SAF, long-term dietary intake and physical activity in 250 healthy adults. Participants completed a general health questionnaire in order to determine their current health status, medication use and smoking habits (Appendix 3). A validated 350-item food frequency questionnaire [161] was used in order to assess habitual dietary intake habits (Appendix 4). This is the most comprehensive collection of dietary information performed in a cross-sectional SAF study. The focus of this study was to explore relationships between SAF-Skin Autofluorescence (measured using a validated technique, the AGE Reader) and dietary intake (measured using a validated technique, the EPIC food frequency questionnaire). While it would have been ideal to also estimate research participant's dietary intake of AGEs and carbonyl compounds, the published food AGE databases are of unknown validity [27]. The published AGE content of foods differ between separate research groups, even when similar measurement techniques have been utilised [23]. Physical activity and sitting times were estimated using the International Physical Activity Questionnaire – Short Form [162] (Appendices 5 & 6). This questionnaire was selected because it has been validated in a large number of countries and can be completed by research

Table 4. Cross-sectional studies exploring the relationship between SAF and lifestyle behaviours

Study	Subjects	Results	Strengths/Limitations
Hansen et al, *Diabetes Care* (2013) [163]	1531 adults without diabetes.	No association between SAF and physical activity energy expenditure, PAEE (kJ/kg/day).	Strengths: Large sample size. Limitations: PAEE not measured directly.
Jochemsen et al, *J Food Nutr Res* (2009) [164]	147 adults aged between 58-68 y (47% male), with at least three vascular risk factors	In a subset of 73 adults, no association found between SAF and dietary AGE intake. Lower mean SAF in wine drinkers and those with higher protein intake.	Strengths: Detailed dietary intake information collected. Limitations: Dietary intake was assessed using 3-day food diaries, which may not reflect long-term food habits.
Nongnuch & Davenport, *Br J Nutr* (2015) [165]	332 adults on haemodialysis, (64% male)	Mean SAF lower in vegetarians (2.71 U) versus omnivores (3.31 U). Results corrected for age, dialysis vintage, diabetes, use of phosphate binders, male sex, ethnicity.	Strengths: Haemodialysis patients only, large sample size. Limitations: Meat consumption was the only component of dietary intake assessed. Vegetarians may have lower BMI or engage in health behaviours which reduce SAF compared to omnivores.
Simon Klenovics et al, *Diab Med* (2014) [147]	63 infants ≤ 6 months of age	Mean SAF lower in breastfed infants (0.52 U) versus formula-fed infants (0.67 U).	Strengths: Nutritional intake limited to breast milk (low AGE) or infant formula (high AGE) only. Limitations: Short term tissue AGE accumulation only (≤ 6 months)
Simon Klenovics et al, *Diab Med* (2014) [147]	226 healthy non-smoking adults	Mean SAF lower in subjects exercising ≥ 3 times/wk (1.24 U) than in those exercising 1–2 times/wk (1.36 U) and in subjects who did not exercise (1.38 U).	Strengths: Large sample size. Limitations: People who exercise frequently may engage in other health behaviours which reduce SAF compared to sedentary individuals.
van Waateringe et al, *Eur J Clin Invest* (2016) [166]	9009 adults (3.5% with T2DM)	SAF increased with age, BMI, HbA1c, diabetes, creatinine clearance, current smoking, pack-years of smoking and coffee consumption. No association between SAF and coffee intake in people with T2DM.	Strengths: Large sample size. Limitations: No dietary information (other than coffee consumption) collected. Caffeine content of coffee not determined.

participants within a few minutes. However it only assesses physical activity behaviour over the previous seven days, and while the assumption is made that exercise carried out during the past week correlates with longer term exercise patterns, there is no certainty this was correct.

The results of this study identified positive associations between SAF and chronological age, cigarette smoking, waist circumference and dietary consumption of meat & meat products. There was no relationship between SAF and physical activity or long-term consumption of any micronutrients in food. This study suggests that a proportion of dietary AGEs may be at least partially absorbed and may be capable of accumulating in the skin of healthy individuals, but prospective studies and randomised controlled trials will be required to confirm this. The findings of this study also question the hypothesis that tissue AGE deposition from exogenous sources is dependent on kidney function and may only be evident or relevant in people with renal failure [167]. Effective reduction of advanced glycation in individuals at risk of chronic disease is likely to involve both the consumption of a low AGE diet to limit exogenous AGE exposure, and the reduction of endogenous AGE formation through healthy lifestyle changes which improve glucose tolerance, low-grade inflammation and dyslipidemia.

Article: Kellow NJ, Coughlan MT, Reid CM.

Association between habitual dietary and lifestyle behaviours and skin autofluorescence (SAF), a marker of tissue accumulation of advanced glycation endproducts (AGEs), in healthy adults.

Manuscript submitted, 2016

Association between habitual dietary and lifestyle behaviours and skin autofluorescence (SAF), a marker of tissue accumulation of advanced glycation endproducts (AGEs), in healthy adults.

Nicole J Kellow[1,2,3], Melinda T Coughlan[1,2], Christopher M Reid[2]

[1] Glycation, Nutrition & Metabolism Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Australia.

[2] Department of Epidemiology & Preventive Medicine, School of Public Health & Preventive Medicine, Monash University, Melbourne, Australia.

[3] Be Active Sleep & Eat (BASE) Facility, Department of Nutrition, Dietetics & Food, Monash University, Notting Hill, Australia.

Corresponding author: Nicole J Kellow, Department of Nutrition & Dietetics, Monash University, Level 1, 264 Ferntree Gully Road, Notting Hill, Victoria 3168, Australia.

Telephone: +61 3 9902 4270

Email: nicole.kellow@monash.edu

Funding Diclosure: Nicole Kellow holds a National Health & Medical Research Council (NHMRC) postgraduate scholarship grant number 1039709. The NHMRC had no role in the design, analysis or writing of this article.

Abstract:

Purpose: Advanced Glycation Endproducts (AGEs) are produced endogenously and also enter the body during the consumption of AGEs present in heat-processed food. It is unknown whether AGEs of dietary origin accumulate within the body of healthy individuals. AGEs can deposit within skin tissue long-term by crosslinking extracellular matrix proteins. The fluorescent nature of many AGEs enables their detection within the skin by non-invasively measuring skin autofluorescence (SAF). This study aimed to identify habitual dietary and lifestyle behaviours cross-sectionally associated with SAF in an adult population sample.

Methods: 251 healthy adult volunteers completed validated food frequency and physical activity questionnaires. Waist circumference, BMI, blood pressure and blood glucose was also measured. SAF was measured using an AGE Reader.

Results: Significant positive correlations were found between SAF and chronological age ($r=0.63$, $P<0.001$), waist circumference ($r=0.28$, $P<0.01$), body weight ($r=0.24$, $P<0.05$), BMI ($r=0.23$, $P<0.05$) and consumption of meat and meat products ($r=0.22$, $P<0.05$). A negative correlation was found between SAF and cereal consumption ($r=-0.21$, $P<0.05$). Cigarette smokers also had a significantly higher SAF than non-smokers (2.4 U vs 2.0 U, $P<0.05$). Regression analysis identified age, cigarette smoking, waist circumference and intake of meat products as significant predictors of SAF. The regression model explained 48% of the variation in SAF.

Conclusions: Age, cigarette smoking, waist circumference and dietary consumption of meat/meat products were positively associated with SAF in this sample. Prospective and intervention trials are now required to determine whether frequent consumption of

foods containing large quantities of dietary AGEs contribute to pathological disease processes in healthy individuals.

Key words: Advanced Glycation Endproducts (AGEs), Maillard reaction, diet, skin autofluorescence, healthy adults

Introduction:

Advanced glycation endproducts (AGEs) are formed by the non-enzymatic reaction between proteins and reactive carbonyl compounds. When generated in large quantities, AGEs exert diabetogenic and proinflammatory effects which contribute to negative metabolic and vascular health outcomes [1, 2].

Key pathways leading to increased AGE accumulation within the body include hyperglycemia, hyperlipidemia, excessive oxidative or carbonyl stress and inflammation [3]. Increased levels of AGEs have also been found in the tissue and circulation of non-diabetic individuals with insulin resistance and inflammation, indicating that elevated blood glucose levels are not essential to enhance the rate of glycation [4]. Additionally, up to 21% of patients with newly diagnosed type 2 diabetes already have early stage diabetic retinopathy [5], suggesting that the pathological vascular processes associated with increased AGE formation occur prior to the development of overt hyperglycemia. These findings have led researchers to hypothesise that excessive AGE accumulation may play a role in the pathogenesis of diabetes and cardiovascular disease.

In addition to endogenous formation, AGEs and their dicarbonyl precursors enter the body from the external environment during cigarette smoking and consumption of processed foods exposed to high temperatures. Heating of proteins and reducing sugars using methods such as frying, roasting, grilling or baking food at high temperatures stimulates AGE formation by the Maillard reaction [6]. Foods widely considered to contain large quantities of AGEs include high protein foods which have been exposed to dry heat such as meat, chicken, fish and powdered milk [7]. In some clinical trials, consumption of low-AGE diets have reduced circulating levels of AGEs and markers of AGE-related pathology [8, 9], while other trials have failed to show any such association [10, 11]. Due to variable rates of protein degradation and clearance, the length of time particular AGE moieties persist in the circulation is difficult to quantify. However, measurement of AGE-modified extracellular proteins with a slow rate of turnover, such as skin collagen, which has a half-life of 15 years [12], signifies cumulative metabolic and oxidative stress over an extended time period.

The fluorescent nature of many AGEs enables detection by absorption of UV light at specific wavelengths. Skin autofluorescence (SAF) provides an estimate of cumulative tissue AGE deposition, and can be measured rapidly and non-invasively using a desktop device known as an AGE Reader. Data produced by the AGE Reader has been validated against AGE fluorescence in skin biopsies in both healthy subjects and patients with diabetes [13]. SAF also correlates with serum levels of the well-known fluorescent AGE pentosidine, and the non-fluorescent AGEs carboxymethyllysine (CML) and carboxyethyllysine (CEL) [14]. SAF is an independent predictor of the development of microvascular complications and cardiovascular events in individuals with type 2 diabetes [15]. In the Diabetes Control and Complications Trial (DCCT),

skin AGE levels measured by the AGE Reader were a better predictor of vascular complications than HbA_{1c} and diabetes duration [16]. SAF increases with chronological age and cigarette smoking, and may inversely correlate with physical activity levels in people without diabetes [17, 18].

It is currently unknown whether AGE accumulation in skin collagen is influenced by long-term consumption of foods containing large quantities of AGEs. Identification of consistent dietary and lifestyle factors which influence tissue AGE levels may provide information about simple lifestyle modifications which could potentially prevent or slow the development of type 2 diabetes or other chronic disease in susceptible individuals. This study aimed to identify whether habitual dietary and lifestyle behaviours are associated with SAF in a cross-sectional sample of healthy adults.

Methods:

A convenience sample of 256 adults (aged 18-80 years) attending shopping centres, medical clinics and a university campus volunteered to complete a validated physical activity and food frequency questionnaire, and a general health questionnaire. All data were collected on-site from each participant within a single 30-40 minute time period. Participant body weight (UM-051 body fat/hydration scale, Tanita, Australia), height (Portstad HM200P Portable Stadiometer, Charder, Taichung City, Taiwan), waist circumference, sitting blood pressure (HEM907 Professional Blood Pressure Monitor, Omron, Australia) and random blood glucose (Accu-Chek Performa blood glucose monitor, Roche Diagnostics, Australia) were measured. SAF was assessed on the ventral site of the forearm by the AGE Reader (Diagnoptics BV, Groningen, The Netherlands). The AGE Reader is a device which uses the characteristic fluorescent

properties of certain AGEs to estimate the level of AGE accumulation in the skin [13]. SAF is calculated by dividing the mean value of the emitted light intensity per nm between 420 and 600 nm by the mean value of the excitation light intensity per nm between 300 and 420 nm, expressed in arbitrary units (AU). The intra-individual percent Bland-Altman error is <5.0% on a single day and 5.9% for seasonal changes [19,20].

Information collected in participant surveys included age, gender, cigarette smoking status, exposure to passive cigarette smoke, medications (prescribed and over-the-counter), medical history and alcohol intake. Validated surveys were used to estimate physical activity level (International Physical Activity Questionnaire-Short Form, IPAQ-SF) [21] and dietary intake (European Prospective Investigation into Cancer and Nutrition Food Frequency Questionnaire, EPIC FFQ) [22]. The EPIC FFQ provides an estimate of dietary consumption of total energy, all macronutrients (protein, total carbohydrate, sugars, total fat, saturated fat, alcohol, dietary fibre), 24 micronutrients and 13 food groups (meat & meat products, cereals & cereal products, eggs & egg dishes, fats & oils, fish & fish products, fruit, milk & milk products, non-alcoholic beverages, nuts & seeds, potatoes, vegetables, soups & sauces and sugars, preserves & snacks) consumed over the previous 12 months. A food frequency questionnaire has been developed specifically for the estimation of dietary AGE consumption [23], but it was not used in this study as it was validated against a food AGE database generated using an ELISA technique of unknown specificity [24]. Ultra-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) methods are currently considered to be the most accurate techniques for AGE quantification [24]. However, a LC-MS/MS-based validated questionnaire for determining dietary

AGE intake does not currently exist, and the published AGE content of foods differ between separate research groups, even when similar measurement techniques have been utilised [25, 26].

The minimum sample size required to detect a Pearson Correlation Coefficient of 0.2 with 80% power (2-sided), was 250 individuals. Variables were assessed for normality using the Shapiro-Wilk test. Correlation analyses were performed using the Pearson Correlation Coefficient, and unpaired t-tests were used to determine differences in SAF between two categories. Participant variables found to significantly correlate with SAF were entered into a backwards stepwise multiple linear regression model, in order to determine subject characteristics which influence SAF. All p-values reported were 2-sided, and p-values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 21.0.

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Monash University Human Research Ethics Committee. All subjects provided written informed consent prior to their participation in the study. Exclusion criteria were age <18 years, recent major changes to habitual dietary intake, renal impairment, neurological or endocrine disease or inability to complete written surveys.

Results:

Characteristics of the study sample are shown in Table 1. In total, 256 individuals participated in the study. Every participant who volunteered to attend a data collection session completed all questionnaires and clinical measurements. Results for 5

participants with FFQs indicating total energy intakes less than 1000 kcal/day were excluded from analysis due to likely under-reporting. Collectively, participants were overweight (mean BMI 26 ± 6 kg/m^2) with a mean SAF of 2.1 ± 0.6 AU (Arbitrary Units). There were no significant differences between variables when data were analysed separately for males and females, with the exception of body weight, BMI and waist circumference (which were all significantly greater in males).

Participant variables that significantly correlated with SAF are shown in Table 2. Initial correlation analyses (unadjusted for covariates) indicated no association between SAF and any dietary or lifestyle variables. However, as skin AGE deposition is known to increase with chronological age and cigarette smoking [27], variables were adjusted for both age and cigarette smoking. After controlling for chronological age, cigarette smoking, gender, total energy intake, protein intake, fat intake, carbohydrate intake, body weight and BMI, correlations between SAF and waist circumference, BMI, body weight, “intake of meat & meat products” and “intake of cereals & cereal products” were statistically significant. No association was found between SAF and systolic blood pressure (mmHg), diastolic blood pressure (mmHg), physical activity level (MET-min/week), sitting hours (hrs/day), carbohydrate intake (g/day), sucrose intake (g/day), fructose intake (g/day), total fat intake (g/day), saturated fat intake (g/day), polyunsaturated fat intake (g/day), total alcohol intake (g/day), protein intake (g/day), dietary fibre intake (g/day), intake of eggs & egg dishes (g/day), intake of fats & oils (g/day), intake of fish & fish products (g/day), fruit intake (g/day), intake of milk & milk products (g/day), intake of non-alcoholic beverages (g/day), consumption of nuts & seeds (g/day), potato consumption (g/day), vegetable intake (g/day), intake of soups & sauces (g/day), intake of sugars, preserves & snacks (g/day) and exposure to

passive cigarette smoke. No association was found between SAF and intake of any micronutrients (vitamins and minerals). However, a positive correlation between SAF and sitting time (hrs/day) approached significance (P= 0.07). (Supplementary Table 1).

In addition to known predictors of SAF (age, cigarette smoking), variables found to correlate significantly with SAF were entered into a backwards stepwise multiple linear regression model. The regression model identified age, cigarette smoking, waist circumference and consumption of meat & meat products as independent predictors of SAF (Table 3). These variables combined explained 48% of the variation in SAF (Adjusted R-squared = 0.481).

Discussion:

While there is a well-established correlation between tissue accumulation of AGEs and metabolic abnormalities such as obesity, diabetes, renal impairment and cardiovascular disease [28, 29], the association between AGE deposition in skin collagen and long-term dietary patterns is unknown. Measurement of SAF enables the accretion of AGE-modified skin proteins to be estimated in large numbers of individuals. This study identified a positive association between tissue AGE deposition and dietary intake of meat & meat products, and a negative association between tissue AGE concentration and cereal consumption (although this was not retained in the final regression model). This may be related to the higher AGE content typically found in high fat, high protein foods such as meat and powdered milk, compared to the same weight of high carbohydrate foods such as bread, breakfast cereals, pasta and rice [3]. In addition, individuals with higher carbohydrate intakes tend to consume greater

quantities of dietary fibre, which is protective against abdominal weight gain [30]. The AGE and dicarbonyl content of a large number of foods has been measured using different quantitative and semi-quantitative techniques [26, 31]. Until more comprehensive food AGE databases are developed using quantitative AGE measurement techniques, estimating the concentration of different AGE compounds in food will remain a challenge.

The quantity of Maillard Reaction Products (MRPs) in a food is dependent not only on the food composition, but also on its method of processing. Factors which enhance AGE formation in foods include high lipid and protein content, low water content during cooking, elevated pH and the application of high temperature over a short time period [32]. Greater AGEs are generated in foods exposed to dry heat (frying, baking, barbecuing and roasting) than foods cooked at lower temperatures for longer time periods in the presence of water (boiling, steaming, poaching, stewing, microwaving or slow cooking) [24]. It has been estimated that humans consume up to 1200mg AGE precursors and up to 75mg AGEs in food and fluids daily [33]. Foods thought to contribute large quantities of AGEs and dicarbonyl compounds to the diet include powdered milk and cheese, meats, fish and chicken cooked by dry heat [31], heat processed or alkaline-treated cereal-based products (bread, biscuits, bakery products, extruded breakfast cereals) [34], sweet sauces [35] and carbonated soft drinks containing high fructose corn syrup [36].

Few studies have attempted to determine whether dietary intake influences SAF. Klenovics *et al* [37] found breastfed infants to have a lower SAF than formula-fed infants in a small cross-sectional study. While the study controlled for variability in

dietary intake by studying subjects who were exclusively breast milk (low-AGE) or infant formula (high-AGE) fed, the infants were only 3-6 months of age, limiting the time for significant AGE accumulation to occur. In a study of haemodialysis patients, vegetarian individuals had a lower SAF than their meat-eating counterparts [38]. However, meat consumption was the only dietary information collected from these patients, suggesting that other dietary and lifestyle behaviours practiced by vegetarians might prevent collagen-linked AGE deposition. The current study also found a positive correlation between SAF and consumption of meat and meat products. An analysis of 147 elderly individuals with cardiovascular risk factors showed a positive correlation between SAF and butter and margarine consumption, and a negative correlation between SAF and wine intake [39]. To enable the authors of the study [39] to estimate AGE intake from food, half of the 147 participants provided dietary intake information in the form of 3-day food diaries, which may not have reflected habitual nutritional consumption. Van Waateringe *et al* (2016) found a correlation between SAF and coffee consumption in a large sample of healthy adults, but this association disappeared in people with diabetes [40]. Dietary intake of other foods and fluids was not assessed in the study and no distinction was made between consumption of caffeinated versus non-caffeinated coffee. The present study attempted to more accurately assess usual diet by using a food frequency questionnaire in a relatively healthy adult sample. While relationships between SAF and chronological age, cigarette smoking and waist circumference existed which supports the findings of previous studies [27], this study is the first to report an association between habitual dietary intake of meat & meat products and tissue AGE deposition in healthy adults. These findings suggest that in addition to increased endogenous AGE generation resulting from metabolic abnormalities, AGEs from

dietary meat sources may also contribute to the body's total AGE load. However, patterns of excessive red and processed meat consumption are associated with increased rates of obesity and metabolic dysfunction [41], which may influence SAF to a greater extent than AGE content alone. There is widespread debate regarding the metabolic fate of ingested AGEs and their precursors. Low molecular weight AGEs and carbonyl compounds are readily absorbed from the diet, but have been reported to be efficiently excreted in urine by individuals with normal renal function [42]. The results of the current study, obtained from a group of healthy adults, suggest that some dietary AGEs may not be completely excreted by the kidneys and a significant proportion could remain in the body. Further research is required to determine whether AGEs of dietary origin accumulate in tissues and have the capacity to exert toxic health effects.

Theoretically, regular physical activity might reduce AGE accumulation in skin by decreasing fasting and post-prandial blood glucose. Exercise also upregulates the antioxidant response element-nuclear respiratory factor (ARE-Nrf) pathway, increasing glutathione biosynthesis [43]. Glutathione is a co-factor of the glyoxalase system, which is the key mechanism for detoxification of methylglyoxal. In a Slovakian cross-sectional sample of healthy individuals, regular physical activity was inversely associated with SAF [37], however no such relationship existed in a large epidemiological study of an elderly population [44]. The current study was unable to identify any association between SAF and physical activity or sitting hours per day using the IPAQ-SF questionnaire, although a positive correlation between SAF and sitting hours per day approached significance (P= 0.07). As the IPAQ-SF estimates

physical activity and sedentary behaviour based on each respondent's exercise over the previous seven days, this may not adequately reflect chronic exercise habits.

Mean SAF in this Australian adult sample (2.1 AU) was higher than that found in Chinese adults (1.9 AU) [45]. However, age-dependent increases in SAF were lower in this study (y= 0.020 A + 0.29, where A= subject age in years) compared to Dutch (y= 0.023 A + 0.83) [46] and Slovak (y= 0.022 A + 0.76) [18] populations. The reasons for this are unclear, but may be related to the greater percentage of smokers in the Dutch study. Koetsier *et al* [21] reported an absolute increase in SAF due to cigarette smoking of 0.16 AU, which was considerably lower than the smoking-related increase in SAF of 0.36 AU found in the current study.

Excess body weight, particularly abdominal obesity was associated with SAF in this study. This supports the findings of investigators who have reported positive correlations between features of the metabolic syndrome and circulating AGE concentrations [4, 47]. In comparison to healthy controls, individuals with excess abdominal adipose tissue demonstrate elevated levels of inflammatory markers, oxidative stress, dyslipidemia and proinflammatory cytokine production [48], all of which are thought to contribute to increased AGE production and accumulation.

Limitations of this study include its cross-sectional design, which only enables the identification of relationships between variables rather than causative factors. While there exist many AGE moieties with a diverse range of characteristics, the AGE Reader is only able to detect those AGEs which absorb UV light. However, the quantity of fluorescent AGEs in skin tends to positively correlate with total skin AGE

concentration [17]. The use of questionnaires can be subject to recall bias when respondents are asked to report their food and fluid intake retrospectively [49]. Mean nutrient consumption values in this adult sample tended to be low in comparison to Australian nutrient consumption data [50], suggesting that food consumption may have been under-reported, particularly by the males in our sample. However, this study did not focus on absolute nutrient intake values, but instead explored trends and correlations with other variables. As many dietary AGEs are excreted via the kidneys, it would have been ideal to assess participant renal function during this study. As measurement of serum creatinine would have represented an additional participant inconvenience, an attempt was made to limit participation to subjects with normal kidney function by excluding those with known renal impairment and/or diabetes from participating in the study. The findings of this study suggest that a proportion of dietary AGEs may be absorbed and retained by the body, despite apparently normal renal function. Moreover, food-derived AGEs which are not absorbed in the small intestine may also exert pathological effects elsewhere in the gastrointestinal tract [51].

In conclusion, tissue AGE accumulation was positively influenced by a combination of chronological age, cigarette smoking, waist circumference and intake of meat & meat products in this group of healthy adults. Further investigation is required to determine whether the contribution made by dietary AGEs to the body's total AGE burden is amplified in individuals with existing chronic disease or renal impairment.

Acknowledgements:

We are grateful to the EPIC-Norfolk Study team for the use of the EPICFFQ Software. EPIC-Norfolk is supported by programme grants from the Medical Research Council UK (G9502233, G0300128) and Cancer Research UK (C865/A2883).

We wish to thank Lisa McGlashan, Liz Jenkins (Baker IDI Healthy Hearts Clinic) and Gayle Savige for assistance with data collection and the management teams at Stockland Traralgon, Mid-Valley Shopping Centre, Leongatha Amcal Pharmacy, The Alfred Centre and Baker IDI Heart & Diabetes Institute for providing permission to collect data from patrons. The authors also wish to thank all of the individuals who volunteered to participate in this study.

N.J.K. designed the research, conducted the research, analysed the data, wrote the paper and had primary responsibility for the final content. C.M.R. provided the AGE Reader and provided comments on the manuscript. M.T.C. provided comments on the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

References:

1. Tessier FJ (2010) The Maillard reaction in the human body. The main discoveries and factors that affect glycation. Pathol Biol 58:214-9.

2. Kellow NJ, Coughlan MT (2015) Effect of diet-derived advanced glycation end products on inflammation. Nutr Rev 73:737-59.

3. Tessier FJ, Birlouez-Aragon I (2012) Health effects of dietary Maillard reaction products: the results of ICARE and other studies. Amino acids 42:1119-31.

4. Tahara N, Yamagishi S, Matsui T, Takeuchi M, Nitta Y, Kodama N, et al (2012) Serum levels of advanced glycation end products (AGEs) are independent correlates of insulin resistance in nondiabetic subjects. Cardiovasc Ther 30:42-8.

5. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, et al (2004) Retinopathy in diabetes. Diabetes Care 27 Suppl 1:S84-7.

6. Li M, Zeng M, He Z, Zheng Z, Qin F, Tao G, et al (2015) Effects of Long-Term Exposure to Free Nepsilon-(Carboxymethyl)lysine on Rats Fed a High-Fat Diet. J Agric Food Chem 63:10995-1001.

7. Kellow NJ, Savage GS (2013) Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review. Eur J Clin Nutr 67: 239-248.

8. Birlouez-Aragon I, Saavedra G, Tessier FJ, Galinier A, Ait-Ameur L, Lacoste F, et al (2010) A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. Am J Clin Nutr 91:1220-6.

9. de Courten B, de Courten MP, Soldatos G, Dougherty SL, Straznicky N, Schlaich M, et al (2016) Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial. Am J Clin Nutr 103:1426-33.

10. Semba RD, Gebauer SK, Baer DJ, Sun K, Turner R, Silber HA, et al (2014) Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. J Nutr 144:1037-42.

11. Piroddi M, Palazzetti I, Quintaliani G, Pilolli F, Montaldi M, Valentina V, et al (2011) Circulating levels and dietary intake of the advanced glycation end-product marker carboxymethyl lysine in chronic kidney disease patients on conservative predialysis therapy: a pilot study. J Renal Nutr 21:329-39.

12. Dunn JA, McCance DR, Thorpe SR, Lyons TJ, Baynes JW (1991) Age-dependent accumulation of N epsilon-(carboxymethyl)lysine and N epsilon-(carboxymethyl)hydroxylysine in human skin collagen. Biochemistry 30:1205-10.

13. Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, et al (2004) Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia 47:1324-30.

14. Smit AJ, Gerrits EG (2010) Skin autofluorescence as a measure of advanced glycation endproduct deposition: a novel risk marker in chronic kidney disease. Curr Opin Nephrol Hyperten 19:527-33.

15. Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ, et al (2008) Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. Diabetes Care 31:517-21.

16. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, et al (1999) Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes 48:870-80.

17. Meerwaldt R, Links T, Graaff R, Thorpe SR, Baynes JW, Hartog J, et al (2005) Simple noninvasive measurement of skin autofluorescence. Ann N Y Acad Sci 1043:290-8.

18. Simon Klenovics K, Kollarova R, Hodosy J, Celec P, Sebekova K (2014) Reference values of skin autofluorescence as an estimation of tissue accumulation of advanced glycation end products in a general Slovak population. Diabetic Medicine 31:581-5.

19. Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans RO, et al (2007) Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. Diabetes Care 30:107-12.

20. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 327(8476): 307-10.

21. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exer 35:1381-95.

22. Mulligan AA, Luben RN, Bhaniani A, Parry-Smith DJ, O'Connor L, Khawaja AP, et al (2014) A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. BMJ Open 4(3):e004503.

23. Luevano-Contreras C, Durkin T, Pauls M, Chapman-Novakofski K (2013) Development, relative validity, and reliability of a food frequency questionnaire for a case-control study on dietary advanced glycation end products and diabetes complications. Int J Food Sci Nutr 64:1030-5.

24. Poulsen MW, Hedegaard RV, Andersen JM, de Courten B, Bugel S, Nielsen J, et al (2013) Advanced glycation endproducts in food and their effects on health. Food Chem Toxicol 60:10-37.

25. G.L.J. Hull JVW, J.M. Ames, G.J. Cuskelly (2012) Nepsilon-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet. Food Chem 131:170-4.

26. Scheijen JL, Clevers E, Engelen L, Dagnelie PC, Brouns F, Stehouwer CD, et al (2016) Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: Presentation of a dietary AGE database. Food Chem 190:1145-50.

27. Corstjens H, Dicanio D, Muizzuddin N, Neven A, Sparacio R, Declercq L, et al (2008) Glycation associated skin autofluorescence and skin elasticity are related to chronological age and body mass index of healthy subjects. Exp Gerontol 43:663-7.

28. Brownlee M, Cerami A, Vlassara H (1988) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. New Eng J Med 318:1315-21.

29. Harcourt BE, Penfold SA, Forbes JM (2013) Coming full circle in diabetes mellitus: from complications to initiation. Nat Rev Endocrinol 9:113-23.

30. Albertson AM, Reicks M, Joshi N, Gugger CK. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001-2012 (2016) Nutr J 15:8.

31. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet (2010) JADA 110:911-16 e12.

32. Vistoli G, De Maddis D, Cipak A, Zarkovic N, Carini M, Aldini G (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radical Res 47 Suppl 1:3-27.

33. Henle T (2003) AGEs in foods: do they play a role in uremia? Kidney Int Suppl 84:S145-7.

34. Henle T (2005) Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. Amino Acids 29:313-22.

35. Bengmark S (2007) Advanced glycation and lipoxidation end products--amplifiers of inflammation: the role of food. JPEN J Parenter Enteral Nutr 31:430-40.

36. Tan D, Wang Y, Lo CY, Sang S, Ho CT (2008) Methylglyoxal: its presence in beverages and potential scavengers. Ann N Y Acad Sci 1126:72-5.

37. Klenovics KS, Boor P, Somoza V, Celec P, Fogliano V, Sebekova K (2013) Advanced glycation end products in infant formulas do not contribute to insulin resistance associated with their consumption. PloS One 8(1):e53056.

38. Nongnuch A, Davenport A (2015) The effect of vegetarian diet on skin autofluorescence measurements in haemodialysis patients. Brit J Nutr 113:1040-3.

39. Jochemsen BM MD, Van Doormaal JJ, Mulder G, Volmer M, Graaf R, Smit AJ (2009) Relation between food and drinking habits, and skin autofluorescence and intima media thickness in subjects at high cardiovascular risk. J Food Nutr Res 48:51-8.

40. van Waateringe RP, Slagter SN, van der Klauw MM, van Vliet-Ostaptchouk JV, Graaff R, Paterson AD, et al (2016). Lifestyle and clinical determinants of skin autofluorescence in a population-based cohort study. Eur J Clin Invest 46:481-90.

41. Kim Y, Keogh J, Clifton P (2015) A review of potential metabolic etiologies of the observed association between red meat consumption and development of type 2 diabetes mellitus. Metabolism 64(7): 768-79.

42. Delgado-Andrade C, Seiquer I, Garcia MM, Galdo G, Navarro MP (2011) Increased Maillard reaction products intake reduces phosphorus digestibility in male adolescents. Nutrition 27:86-91.

43. Dieter BP, Vella CA (2013) A proposed mechanism for exercise attenuated methylglyoxal accumulation: activation of the ARE-Nrf pathway and increased glutathione biosynthesis. Medical Hypoth 81:813-5.

44. Hansen AL, Carstensen B, Helge JW, Johansen NB, Gram B, Christiansen JS, et al (2013) Combined heart rate- and accelerometer-assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes: the ADDITION-PRO study. Diabetes Care 36:3062-9.

45. Yue X, Hu H, Koetsier M, Graaff R, Han C (2011) Reference values for the Chinese population of skin autofluorescence as a marker of advanced glycation end products accumulated in tissue. Diabetic Medicine 28:818-23.

46. Koetsier M, Lutgers HL, de Jonge C, Links TP, Smit AJ, Graaff R (2010) Reference values of skin autofluorescence. Diabetes Technol Ther 12:399-403.

47. Uribarri J, Cai W, Woodward M, Tripp E, Goldberg L, Pyzik R, et al (2015) Elevated serum advanced glycation endproducts in obese indicate risk for the metabolic syndrome: a link between healthy and unhealthy obesity? J Clin Endocrinol Metab 100:1957-66.

48. Kim SH, Chung JH, Song SW, Jung WS, Lee YA, Kim HN (2016) Relationship between deep subcutaneous abdominal adipose tissue and metabolic syndrome: a case control study. Diabetol Metabol Syn 8:10.

49. Stice E, Palmrose CA, Burger KS (2015). Elevated BMI and Male Sex Are Associated with Greater Underreporting of Caloric Intake as Assessed by Doubly Labeled Water. J Nutr 145:2412-8.

50. Australian Bureau of Statistics (2014). Australian Health Survey: Nutrition First Results - Food and Nutrients, 2011-12. Canberra, Australia: ABS; 2014 [accessed 2015 December 1st]; Available from: www.ausstats.abs.gov.au.

51. Seiquer I, Rubio LA, Peinado MJ, Delgado-Andrade C, Navarro MP (2014) Maillard reaction products modulate gut microbiota composition in adolescents. Mol Nutr Food Res 58:1552-60.

Table 1: Characteristics of the study sample (n=256). Values reported as mean (standard deviation) or number (percentage) of subjects.

Variable	Mean (SD)
Age (years)	46.9 (16.1)
Male, n (%)	112 (44)
Smokers, n (%)	28 (11)
Skin Autofluorescence (SAF) (AU)	2.1 (0.6)
Body weight (kg)	All: 73.6 (18), M: 84.3 (16), F: 62.8 (15)**
Waist Circumference (cm)	All: 87.3 (16), M: 95.2 (16), F: 82.6 (14)**
Body Mass Index (BMI) (kg/m^2)	All: 26 (6), M: 28 (6), F: 25 (6)*
Random blood glucose (mmol/L)	5.8 (1.2)
Total energy intake (MJ per day)	6.8 (2.1) MJ/day, 1 614 (506) kcal/day
Total fat intake (g/day)	67.4 (25.3)
Saturated fat intake (g/day)	25.5 (10.8)
Total carbohydrate intake (g/day)	176.9 (65.5)
Sugar intake (g/day)	92.9 (40.4)
Protein intake (g/day)	78.0 (24.1)
Intake of meat & meat products (g/day)	92.8 (58.3)
Intake of cereals & cereal products (g/day)	182.2 (114.1)
Dietary fibre - NSP (g/day)	15.5 (6.6)
Alcohol (standard drinks/week)	3.0 (3.8)
IPAQ score (MET-min/week)	3 191 (2 830)
Sitting time (hrs/day)	6.7 (2.8)

*P<0.05, **P<0.001 between M = Males, F = Females. AU = Arbitrary Units; NSP = Non-starch polysaccharide; IPAQ = International Physical Activity Questionnaire; MET = metabolic equivalents

Table 2: Pearson Correlation Coefficient (r) between Skin Autofluorescence (SAF) and measured variables.

Variable	Correlation Coefficient (r) (unadjusted)	Correlation Coefficient (r) (adjusted for age and smoking status)	Correlation Coefficient (r) (adjusted for age, gender, smoking status, body weight, BMI, total energy, fat, protein and carbohydrate intakes)
Age (yrs)	0.63$^{\#}$	-	0.63$^{\#}$
Waist circumference (cm)	0.39$^{\#}$	0.27**	0.28**
BMI (kg/m^2)	0.34**	0.24*	0.23*
Body weight (kg)	0.30**	0.24*	0.24*
Random blood glucose (mmol/L)	0.21*	0.09 (ns)	0.09 (ns)
Intake of meat & meat products (g/day)	0.08 (ns)	0.21*	0.22*
Intake of cereals & cereal products (g/day)	-0.09 (ns)	-0.20*	-0.21*

*P<0.05, **P<0.01, $^{\#}$P<0.001, ns= not significant
Cigarette smokers (n=19) also had a significantly higher SAF than non-smokers (2.4 AU vs 2.0 AU respectively, P<0.05) assessed by independent samples t-test.

Table 3: Independent predictors of Skin Autofluorescence (SAF)

Variable	B (unstandardised)	Beta (standardised)	P	95% CI
Age (yrs)	0.020	0.578	<0.001	0.016-0.027
Cigarette Smoking (Y/N)	0.36	0.203	0.006	0.106-0.604
Waist Circumference (cm)	0.007	0.204	0.009	0.002-0.013
Intake of meat & meat products (g/day)	0.001	0.150	0.041	0.000-0.003

Variables initially entered into backwards stepwise multiple linear regression model: Age, gender, smoking status, body weight, BMI, waist circumference, intake of meat & meat products, intake of cereals & cereal products.

Variables excluded from the model: weight, BMI, gender, intake of cereals & cereal products.

Dependent variable: SAF.

Supplementary Table 1: Pearson Correlation Coefficient (r) between Skin Autofluorescence (SAF) and measured variables (all non-significant, except where indicated * P<0.01).

Variable	Correlation Coefficient (r) (unadjusted)	Correlation Coefficient (r) (adjusted for age and smoking status)
systolic blood pressure (mmHg)	0.20*	0.14
diastolic blood pressure (mmHg)	0.10	0.01
physical activity level (MET-min/week)	0.09	-0.04
sitting time (hrs/day)	-0.10	0.17
carbohydrate intake (g/day)	-0.05	-0.07
sucrose intake (g/day)	-0.05	-0.02
fructose intake (g/day)	-0.04	-0.11
total fat intake (g/day)	0.00	0.06
saturated fat intake (g/day)	0.04	0.09
polyunsaturated fat intake (g/day)	-0.07	-0.05
total alcohol intake (g/day)	-0.13	0.02
protein intake (g/day)	0.02	0.12
dietary fibre intake (g/day)	-0.00	-0.06
intake of eggs & egg dishes (g/day)	0.04	0.08
intake of fats & oils (g/day)	0.21	0.15
intake of fish & fish products (g/day)	0.04	-0.01
fruit intake (g/day)	-0.09	-0.14
intake of milk & milk products (g/day)	-0.09	-0.05
intake of non-alcoholic beverages (g/day)	0.27*	0.01
consumption of nuts & seeds (g/day)	-0.11	-0.04
potato consumption (g/day)	0.12	0.06
vegetable intake (g/day)	0.05	-0.00
intake of soups & sauces (g/day)	0.05	-0.07
intake of sugars, preserves & snacks (g/day)	-0.05	-0.05

Chapter 5:

What is currently known about the impact of dietary prebiotic supplementation on cardiometabolic health?

While genetics play a role in the development of obesity and metabolic abnormalities, environmental factors are thought to be responsible for the recent increase in the prevalence of chronic diseases. The estimated 10^{14} micro-organisms residing in the human gastrointestinal tract significantly influence host metabolism [168]. The human intestine becomes colonised by bacteria shortly after birth, with the mode of delivery affecting the microbial species which inhabit the gut [169]. Infants who are born by vaginal delivery become colonised by bacteria similar to those found in the mother's vagina. However, infants born by caesarian section become colonised predominantly by micro-organisms found on the mother's skin [170]. Breast feeding and bottle feeding also encourage the growth of different bacterial species, but the composition of the microbiota changes again and increases in complexity with the introduction of solid foods. Antibiotic use during the early years of life may also have a detrimental effect of the diversity of microbes inhabiting the colon [171]. By the age of 2-3 years it is thought that the composition of the gut microbiota becomes established and remains relatively stable, but diet, illness and ongoing use of antibiotics can influence the type and diversity of bacterial growth throughout life [172]. The first few years of infancy are of vital importance in the maturation of the human immune system, and the gut microbiota appear to play a major role in immune system development [173]. Gastrointestinal micro-organisms are also involved in the extraction of energy from the host's diet, modulation of inflammatory processes, fermentation of dietary fibres to produce short-chain fatty acids (SCFAs), alteration of human gene expression, regulation of intestinal permeability, production of some vitamins and promotion of mineral absorption by the host [174].

The human gut microbiota is dominated by four bacterial phyla: the Gram-negative *Bacteroidetes* (which includes the genus *Bacteroides*), Gram-negative Proteobacteria

(includes genera such as *Enterobacter*, *Escherichia*), Gram-positive *Actinobacteria* (including *Bifidobacteria*), and Gram-positive *Firmicutes* (including *Lactobacillus*, *Clostridium*). The adult gastrointestinal tract contains over 1000 different bacterial species [175], and alterations in their composition (known as 'dysbiosis') appear to be involved in the early low-grade inflammation and insulin resistance associated with many chronic metabolic diseases such as obesity, the metabolic syndrome, type 2 diabetes, cardiovascular disease and non-alcoholic hepatic steatosis.

Microbial degradation of food components which humans lack the enzymes to digest enables increased extraction of nutrients from the diet. Increased capacity for bacterial fermentation of indigestible dietary carbohydrates in obese individuals enhances the production of SCFAs (including acetate, butyrate and proprionate), which can subsequently be used as energy substrates [176]. Indeed, SCFAs contribute 5-10% to the daily energy intake of healthy individuals [175]. Early cross-sectional studies investigating the association between gut bacteria and obesity found an increased ratio of *Fermicutes* to *Bacteroides* in obese versus normal weight individuals [177]. These perturbations in gut microbial composition were reversed by surgically-induced or diet-induced weight loss [178]. However, larger studies have failed to confirm an association between obesity and the intestinal proportions of specific bacterial phyla, but have found reduced colonic concentrations of butyrate-producing microbes such as *Roseburia intestinalis* and *Faecalibacterium prauznitzii* in people with type 2 diabetes [179, 180].

Alterations in the gut microbiota are believed to contribute to the development of insulin resistance and type 2 diabetes through a number of mechanisms. Bacterial

lipopolysaccharide (LPS), a structural component of the outer cell membrane of Gram negative bacteria, can be absorbed into the bloodstream in chylomicrons during high-fat feeding, inducing metabolic endotoxemia, immune-cell secretion of pro-inflammatory cytokines and chronic low-grade inflammation [181]. Compositional changes in the gut microbiota influence glucose and lipid metabolism through modulation of the integrity of the gastrointestinal lining, possibly through a GLP-2 (glucagon-like peptide-2) dependent mechanism [182]. Increased gut permeability enables the absorption of larger molecules from the intestine into the circulation, activating the innate immune system and predisposing the host to systemic inflammation and insulin resistance. Gut bacteria are also involved in the transformation of primary bile acids into secondary bile acids, which are capable of binding to the G-protein coupled receptor 19 (also known as TGR5). GPR19 induces the intestinal secretion of GLP-1 (glucagon-like peptide-1), which protects against diet-induced obesity by down-regulation of inappropriate macrophage-induced inflammation [183].

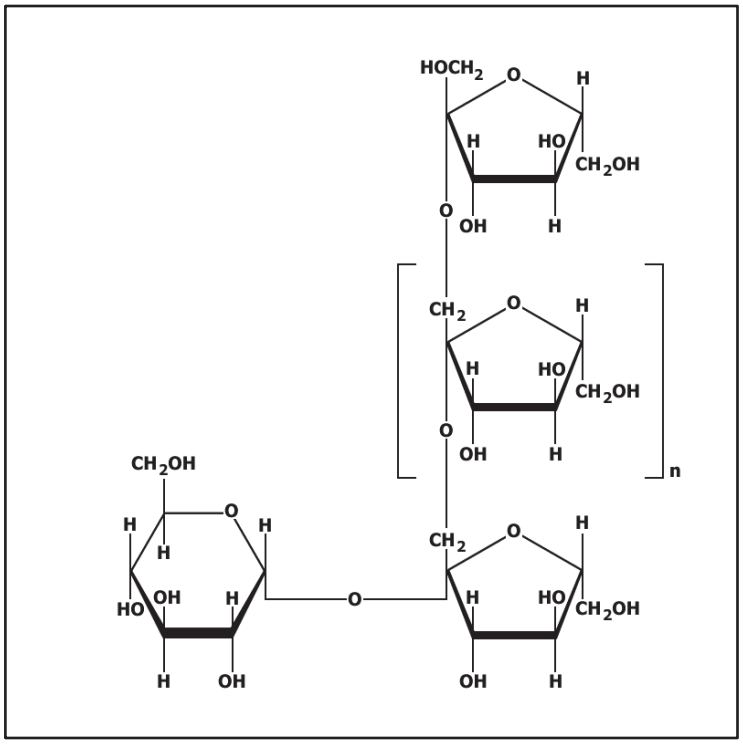
Research regarding the role of the gut microbiota in the development of the host phenotype is still in its infancy. While murine studies have shown causal relationships between alterations in intestinal bacteria and the pathogenesis of obesity, type 2 diabetes and other metabolic abnormalities, most human research performed to date has been cross-sectional so prospective cohort studies and randomised intervention trials using specific bacterial strains are now required. While murine gastrointestinal physiology and anatomy is considered to be a suitable basic model for human comparison, studies in mice are complicated by the fact that mice consume their own faeces and the faecal material of others they are caged with. In addition,

gastrointestinal contents are predominantly collected from the cecum of mice, whereas human studies are limited to the analysis of stool samples. Human studies are complicated by the heterogeneity of host genotypes, in conjunction with numerous confounders such as age, gender, dietary intake, physical activity, smoking, alcohol, pharmacological agents (including antibiotics and metformin) and underlying medical conditions. A small RCT involving faecal microbiota transplantation from healthy donors to males with the metabolic syndrome resulted in a temporary improvement in peripheral insulin sensitivity and increased intestinal microbial diversity in the recipients [184], however the interplay between human gut microbes, host genetics and environmental factors requires further elucidation.

Dietary changes are able to induce rapid and significant alterations in the composition of the intestinal microbiome. Regular consumption of prebiotic dietary fibres promotes the growth of beneficial gut bacteria capable of improving the metabolic health of the host. Prebiotics are defined as non-digestible plant-derived carbohydrates which confer health benefits to the host by acting as a fermentation substrate in the colon, stimulating the preferential growth and activity of a limited number of beneficial microbial species [185]. Non-digestible plant polysaccharides currently recognised as prebiotics include inulin, oligofructose – also known as fructo-oligosaccharides (FOS) and galacto-oligosaccharides [186]. Other non-digestible polysaccharides with potential prebiotic activity include arabinoxylan, beta-glucan and resistant starch [187]. Prebiotics escape digestion in the human stomach and small intestine, undergoing fermentation by colonic bacteria who possess the enzymes necessary to hydrolyse the bonds between carbohydrate units.

Prebiotic fructans such as inulin and oligofructose are plant storage carbohydrates found in a number of plants including leek, onion, garlic, asparagus, Jerusalem artichoke and chicory root. It has been estimated that Americans consume

Figure 4. Chemical structure of inulin and oligofructose [188]



approximately 1-4 grams of inulin-type fructans per day and Europeans consume an average of 3-10 grams [189]. Inulin consists of a chain of fructose units each linked by β(2-1) bonds, with each fructose chain connected to a terminal glucose unit by an α(1-2) linkage (Figure 4). Inulin has an average degree of polymerisation (chain length, n) of 25 units (range 11-60 units). Oligofructose is the product of inulin hydrolysis, which produces shorter fructose chains ranging from 2-10 units in length (average degree of polymerisation of 4 units) [190]. Dietary consumption of prebiotic inulin-type fructans alter the bacterial composition of the host large intestine by favouring the selective proliferation of beneficial SCFA-producing species such as *Bifidobacteria* and

Lactobacilli. SCFAs including acetate, butyrate and proprionate produced by these bacteria as a by-product of prebiotic fermentation are involved in maintaining the health and integrity of the gastrointestinal epithelial barrier, activation of intestinal gluconeogenesis, down-regulation of inflammatory signals, delayed gastric emptying and improved insulin sensitivity [(191)]. Prebiotic-stimulated increases in intestinal *Bifidobacterium* species have been shown to attenuate the production of ROS and markers of inflammation in both animals [(192)] and humans [(193)] consuming high fat diets.

The following systematic review explored evidence from high quality RCTs which investigated the effects of prebiotic supplementation on cardiometabolic parameters in humans. Meta-analyses found statistically and clinically significant reductions in appetite, post-prandial glucose and post-prandial insulin levels after dietary prebiotic supplementation in either healthy adults, overweight adults or individuals with type 2 diabetes. A major limitation of these studies was the failure to collect participant stool samples, so the metabolic improvements observed could not be directly linked to changes in the composition of the intestinal microbiota. We hypothesised that prebiotic-induced metabolic improvements may have beneficial effects on endogenous AGE production, by potentially reducing the concentrations of substrates and reactants required for AGE formation such as glucose, lipids, ROS and inflammatory molecules.

Article: Kellow NJ, Coughlan MT, Reid CM.

Metabolic benefits of dietary prebiotics in human subjects:

a systematic review of randomised controlled trials.

Br J Nutr 2014; 111: 1147-1161.

British Journal of Nutrition (2014), **111**, 1147–1161
doi:10.1017/S0007114513003607

Systematic Review

Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials

Nicole J. Kellow[1]*, Melinda T. Coughlan[2,3] and Christopher M. Reid[1]

[1]*Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria 3004, Australia*
[2]*Glycation, Nutrition and Metabolism Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria 8008, Australia*
[3]*Department of Medicine, Central Clinical School, Monash University, Alfred Medical Research and Education Precinct, Melbourne, Victoria 3004, Australia*

(Submitted 30 July 2013 – Final revision received 7 October 2013 – Accepted 8 October 2013 – First published online 13 November 2013)

Abstract

Complex relationships exist between the gut microflora and their human hosts. Emerging evidence suggests that bacterial dysbiosis within the colon may be involved in the pathogenesis of the metabolic syndrome, type 2 diabetes and CVD. The use of dietary prebiotic supplements to restore an optimal balance of intestinal flora may positively affect host metabolism, representing a potential treatment strategy for individuals with cardiometabolic disorders. The present review aimed to examine the current evidence supporting that dietary prebiotic supplementation in adults has beneficial effects on biochemical parameters associated with the development of metabolic abnormalities including obesity, glucose intolerance, dyslipidaemia, hepatic steatosis and low-grade chronic inflammation. Between January 2000 and September 2013, eight computer databases were searched for randomised controlled trials published in English. Human trials were included if at least one group received a dietary prebiotic intervention. In the present review, twenty-six randomised controlled trials involving 831 participants were included. Evidence indicated that dietary prebiotic supplementation increased self-reported feelings of satiety in healthy adults (standardised mean difference −0·57, 95 % CI −1·13, −0·01). Prebiotic supplementation also significantly reduced postprandial glucose (−0·76, 95 % CI −1·41, −0·12) and insulin (−0·77, 95 % CI −1·50, −0·04) concentrations. The effects of dietary prebiotics on total energy intake, body weight, peptide YY and glucagon-like peptide-1 concentrations, gastric emptying times, insulin sensitivity, lipids, inflammatory markers and immune function were contradictory. Dietary prebiotic consumption was found to be associated with subjective improvements in satiety and reductions in postprandial glucose and insulin concentrations. Additional evidence is required before recommending prebiotic supplements to individuals with metabolic abnormalities. Large-scale trials of longer duration evaluating gut microbial growth and activity are required.

Key words: **Prebiotics: Gut microflora: Human subjects: Metabolic disorders**

The composition and possible health effects of human gut micro-organisms have been the focus of renewed interest since the development of metagenomic techniques enabling the identification and characterisation of intestinal bacteria that cannot be cultured. In addition, the discovery of differences in gut microbial composition between lean and obese individuals[1] and people with and without type 2 diabetes[2,3] has highlighted the potential role played by the colonic microflora and their fermentation products in the pathogenesis of host metabolic health and disease.

Although the number and diversity of bacterial species within an individual's gastrointestinal tract remain relatively constant throughout life, it is possible to stimulate the proliferation of specific micro-organisms known to have beneficial health effects by manipulating the host diet. Prebiotics are defined as non-digestible plant-derived carbohydrates that

Abbreviations: GLP, glucagon-like peptide; GPR, G protein-coupled receptor; HOMA-IR, homeostasis model assessment for insulin resistance; LPS, lipopolysaccharide; NASH, non-alcoholic steatohepatitis; SMD, standardised mean difference.

* **Corresponding author:** N. J. Kellow, fax +61 3 990 30556, email nicolekellow@live.com.au

British Journal of Nutrition

act as a fermentation substrate within the colon, stimulating the preferential growth and activity of a limited number of microbial species that confer health benefits on the host[4]. Carbohydrates with an established prebiotic effect include inulin-type fructans (inulin, oligofructose and fructo-oligosaccharides) and galactans (galacto-oligosaccharides)[5], known to promote the proliferation of beneficial lactic acid-producing species such as bifidobacteria and lactobacilli[6].

Gut bacteria play an important role in the development of the host immune system[7] and modulation of inflammatory processes[8], extraction of energy from the host diet[9], fermentation of dietary fibres to produce SCFA[10], alteration of human glucose and fatty acid metabolism[11], regulation of intestinal permeability[12], production of vitamins[13] and promotion of mineral absorption by the host[14]. They may also be involved in the modification of the secretion of gut hormones to enhance satiety and improve gastrointestinal function[15]. Dietary prebiotic supplements capable of favourably altering the composition of the intestinal microflora might represent a potential therapeutic strategy for the prevention and treatment of metabolic abnormalities widespread in modern society.

The present review aimed to examine the current evidence supporting dietary prebiotic supplementation in adults on biochemical parameters associated with the development of metabolic abnormalities such as obesity, glucose intolerance, dyslipidaemia, non-alcoholic fatty liver disease and low-grade chronic inflammation.

Methods

A computer search of databases such as MEDLINE, CINAHL, Embase, Current Contents, PubMed, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and AMED was undertaken for the period between 1 January 2000 and 30 September 2013. Databases were not searched before 2000, to exclude studies utilising non-molecular, culture-dependent techniques for the characterisation of intestinal bacteria. Reference lists of all identified studies were hand-searched for relevant trials. The following search terms were used: (1) (prebiotic* OR fructan* OR oligofructose OR inulin OR fructooligosaccharide* OR galactooligosaccharide*) and (gut OR obes* OR diabet* OR lipid* OR hepat* OR immune* OR metaboli*); (2) limit 1 to year = '2000–2003'; (3) limit 2 to humans. Trials were included if they were published in English and involved human participants aged ≥18 years and at least one group of participants were randomised to receive a dietary prebiotic intervention. For the purposes of the present review, a prebiotic intervention was defined as one that contained inulin, oligofructose, fructo-oligosaccharides or galacto-oligosaccharides. Additional plant-derived carbohydrates such as arabinoxylan and β-glucan were excluded from the search, as, although demonstrated to have prebiotic effects[16,17], these compounds require further research before being formally classified as prebiotics.

Dietary prebiotic intervention studies of less than 24 h duration were excluded from the present review, as the growth of colonic microflora is unlikely to be affected in this brief time period[18]. Nutritional intervention studies involving the administration of probiotics (beneficial live micro-organisms) or synbiotics (a combination of pre- and probiotics) were also excluded. Trials involving prebiotic supplementation in people with disease conditions such as HIV and inflammatory bowel disease were considered to be outside the scope of the present review and were therefore excluded. The methodological quality of all the included trials was assessed by two authors independently using the Heyland Methodological Quality Score[19] (Table 1). This checklist rates primary research based on the use of allocation concealment during randomisation, intention-to-treat analysis, double-blinding, patient selection with minimal risk of bias, comparability of intervention and control groups at baseline, 100 % participant follow-up, clearly described treatment protocol and well-defined outcome measurements. Trials scoring ≥8 out of a possible 14 points were considered to be of high methodological quality. Disagreements between authors in assigning methodological quality scores were resolved by discussion until consensus was reached.

Trials measuring similar outcomes were subjected to a random-effects model meta-analysis using RevMan 5.1 (The Cochrane Collaboration, Copenhagen 2011). Treatment effects and 95 % CI were calculated using the Hedges (adjusted-g) standardised mean difference (SMD), to enable the comparison of effect sizes between trials using different

Table 1. Heyland Methodological Quality Score[19]

	Score		
Criterion	0	1	2
Randomisation	Not applicable	Not concealed or not sure	Concealed randomisation
Analysis	Other	Not applicable	Intention to treat
Blinding	Not blinded	Single blind	Double blind
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	Not applicable
Comparability of groups at baseline	No or not sure	Yes	Not applicable
Extent of follow-up	Less than 100 %	100 %	Not applicable
Treatment protocol	Poorly described	Reproducibly described	Not applicable
Co-interventions applied equally across groups	Not described	Described but not equal or not sure	Well described and all equal
Outcomes	Not described	Partially described	Objectively defined

The Heyland Methodological Quality Score for individual studies is based on nine quality criteria. The maximum possible score is 14 with studies scoring ≥8 considered to be of high methodological quality.

outcome measures. SMD values of 0·2, 0·5 and 0·8 were considered to represent small, moderate and large effect sizes, respectively[20]. Limited numbers of studies investigating comparable outcomes, small sample sizes and heterogeneity among trial subjects, disease conditions, prebiotic supplements, intervention duration and outcome measures limited the majority of data synthesis to a narrative analysis.

Results

Description of the selected trials

A total of 1130 citations were originally identified at the time of the initial database search and were selected to be included in the review based on the predefined inclusion criteria (Fig. 1). In the present review, twenty-nine articles reporting on twenty-six randomised controlled trials involving 831 participants were ultimately included[21–49]. The characteristics of the included trials are outlined in Table 2. Of the twenty-six trials included in the present review, thirteen trials included only healthy participants, five trials included only overweight or obese participants, one trial included only overweight participants with the metabolic syndrome, two trials included only participants with type 2 diabetes, two trials included only participants with hypercholesterolaemia, one trial included only participants with non-alcoholic steatohepatitis (NASH), one trial included only participants with gastro-oesophageal reflux disease and one trial included only elderly participants diagnosed with mild malnutrition or at risk of becoming malnourished. The duration of intervention ranged from 2 d to 28 weeks and the participants were aged 19–99 years. A variety of post-intervention outcome measures were reported including self-reported hunger and satiety ratings, total body weight, BMI, waist circumference, energy intake, gastric emptying times, concentrations of appetite-regulating hormones (ghrelin, cholecystokinin, peptide YY and glucagon-like peptide (GLP)-1), concentrations of lipids (total cholesterol, LDL, HDL, TAG, Lp(a) and NEFA), indicators of glucose homeostasis (glucose, insulin, glucagon, homeostasis model assessment for insulin resistance (HOMA-IR), HbA1c and fructosamine), inflammatory markers (TNF-α, C-reactive protein and IL), indices of immune function (natural killer cell activity and T-cell activation), and parameters associated with oxidative stress (total radical-trapping antioxidant parameter (TRAP), photosensitive chemiluminescence, total antioxidant capacity, superoxide dismutase and malondialdehyde) and liver function (aspartate aminotransferase). All the trials were of high methodological quality as assessed by the Heyland Methodological Quality Score. Methodological strengths of the trials included double-blinding utilised in the majority of the studies and random allocation of participants to intervention and control groups or treatment sequence. Methodological limitations of most of the trials included small sample sizes and short study duration. Some cross-over studies did not have a washout period or did not stipulate the duration of their washout period.

Outcomes associated with body weight

Of the five trials investigating the effect of dietary prebiotic supplementation on self-reported quantitative ratings of satiety, three demonstrated improvements in subjective satiety measurements in healthy participants consuming prebiotics in comparison with controls[22,23,49]. After the meta-analysis (n 52), the pooled SMD for satiety was −0·57 (95 % CI −1·13, −0·01; $P<0·05$), indicating a statistically significant effect favouring prebiotic supplementation over placebo (Fig. 2). Inclusion of two trials finding no change in satiety

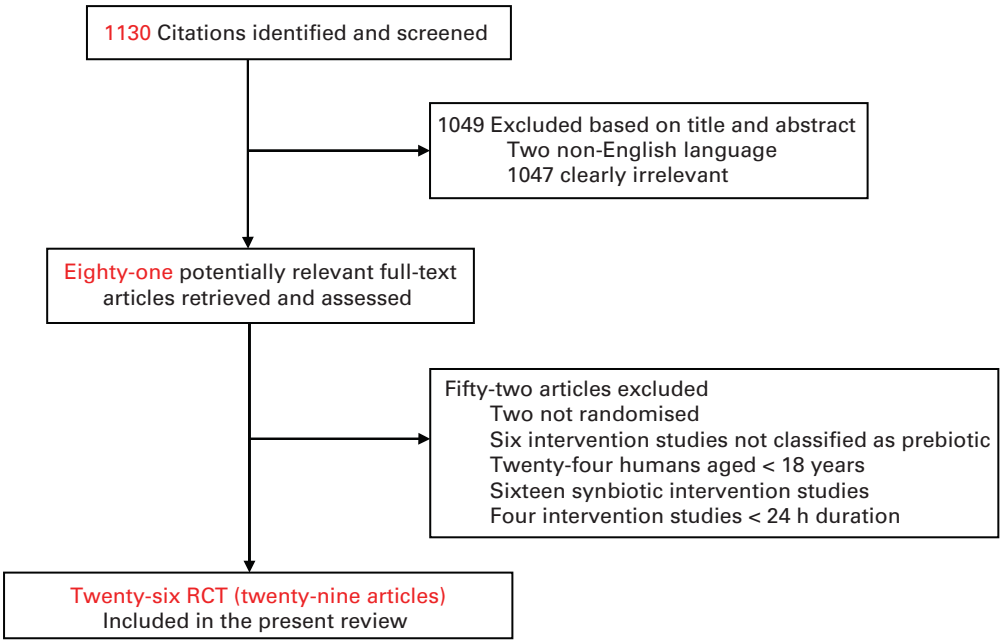


Fig. 1. Flow chart showing the progression of trials through each stage of the selection process. RCT, randomised controlled trial. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn).

Table 2. Summary of published human intervention randomised controlled trials examining the relationship between dietary prebiotic intake and metabolic health

Study	Participants	Study design/blinding	Dietary prebiotic intervention	Effect of dietary prebiotic supplements on metabolic outcomes
Bunout *et al.* (2002)[21]	Chile, *n* 43 normal-weight and overweight elderly adults: sex not stated (mean age 75·5 years; mean BMI 27 kg/m^2)	Parallel RCT double-blinded HMQS: 11	Random assignment to either a 28-week prebiotic-supplemented diet (6 g FOS/d) or a 28-week placebo-supplemented diet (6 g maltodextrin/d)	↔ IL-4 and interferon-gamma ↔ Secretory IgA
Cani *et al.* (2006)[22]	Belgium, *n* 10 healthy adults: five males and five females (mean age 27 years; mean BMI 22·3 kg/m^2)	Cross-over RCT subjects blinded HMQS: 9	Random assignment to either a 2-week prebiotic-supplemented diet (16 g oligofructose/d) or a 2-week placebo-supplemented diet (16 g maltodextrin/d) before cross-over. Washout period: 2 weeks	↑ Satiety after breakfast and dinner ↓ Hunger after dinner ↓ Energy intake after dinner ↓ Total energy intake
Cani *et al.* (2009)[23]	Belgium, *n* 10 healthy adults: five males and five females (mean age 26 years; mean BMI 21·6 kg/m^2)	Parallel RCT double-blinded HMQS: 10	Random assignment to either a 2-week prebiotic-supplemented diet (16 g chicory-derived fructan/d) or a 2-week placebo-supplemented diet (16 g maltodextrin/d)	↑ Plasma GLP-1 ↑ Plasma peptide YY ↓ Postprandial plasma glucose ↓ Hunger ↔ Satiety
Causey *et al.* (2000)[24]	USA, *n* 12 adult males with mild hypercholesterolaemia (age range 27–49 years)	Cross-over RCT double-blinded HMQS: 8	Random assignment to either a 3-week prebiotic-supplemented diet (20 g inulin/d in low-fat ice cream) or a 3-week placebo-supplemented diet (regular low-fat ice cream containing sucrose). Washout period: nil	↓ TAG ↑ Postprandial plasma glucagons
Daubioul *et al.* (2005)[25]	Belgium, *n* 7 adult males with NASH (mean age 55 years; mean BMI 29·1 kg/m^2)	Cross-over RCT double-blinded HMQS: 8	Random assignment to either an 8-week prebiotic-supplemented diet (16 g oligofructose/d) or an 8-week placebo-supplemented diet (16 g maltodextrin/d) before cross-over. Washout period: 5 weeks	↓ Serum AST ↔ Serum insulin ↔ Serum TAG
Dehghan *et al.* (2013)[26] Pourghassem Gargari *et al.* (2013)[27]	Iran, *n* 49 women with type 2 diabetes (mean age 48·3 years; mean BMI 30·8 kg/m^2; time after DM diagnosis >6 months; mean HbA1c levels 8·3 %)	Parallel RCT double-blinded HMQS: 9	Random assignment to either an 8-week prebiotic-supplemented diet (10 g inulin/d) or an 8-week placebo-supplemented diet (10 g maltodextrin/d)	↓ Fasting glucose ↓ Energy intake and HbA1c ↓ CRP and TNF-α ↓ Lipopolysaccharide ↓ Malondialdehyde ↑ TAC and ↑ SOD activity ↔ Fasting insulin and HOMA-IR ↔ Glutathione peroxidase activity
De Luis *et al.* (2011)[28]	Spain, *n* 30 obese adults: twelve males and eighteen females (mean age 51 years; mean BMI 39·2 kg/m^2)	Parallel RCT double-blinded HMQS: 13	Random assignment to either 4-week prebiotic + ALA-supplemented cookies (2 cookies/d containing 2 g inulin + 3·1 g FOS + 3·2 g ALA) or 4-week placebo cookies (2 control cookies/d)	↓ Total cholesterol, LDL and CRP (only males) ↔ Total body weight, HOMA, TAG and HDL
Dewulf *et al.* (2013)[29]	Belgium, *n* 30 obese women (mean age 47·5 years; mean BMI 35·9 kg/m^2)	Parallel RCT double-blinded HMQS: 12	Random assignment to either a 3-month prebiotic-supplemented diet (16 g/d of inulin/oligofructose 50:50 mix) or a 3-month placebo-supplemented diet (16 g maltodextrin/d). All the subjects received dietary advice for weight reduction	↓ Glucose after OGTT ↔ Insulin after OGTT ↔ Fasting glucose and fasting insulin ↔ HbA1c and HOMA ↔ Lipid levels ↔ CRP
Genta *et al.* (2009)[30]	Argentina, *n* 35 obese women (mean age 40·5 years; mean BMI 33·5 kg/m^2)	Parallel RCT double-blinded HMQS: 10	Random assignment to either 17-week consumption of Yacon syrup (approximately 12·5 g FOS/d), *n* 20, or 17-week consumption of placebo syrup, *n* 15	↓ Total body weight, ↓ BMI ↓ Waist circumference ↓ Fasting serum insulin ↓ HOMA and serum LDL ↑ Satiety (only qualitative) ↔ Fasting serum glucose ↔ Serum total cholesterol, HDL and TAG

Table 2. *Continued*

Study	Participants	Study design/blinding	Dietary prebiotic intervention	Effect of dietary prebiotic supplements on metabolic outcomes
Giacco *et al.* (2004)[31]	Italy, *n* 30 adults with plasma cholesterol concentrations between 5·17 and 7·76 mmol/l: twenty males and ten females (mean age 45·5 years; mean BMI 26·6 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 2-month prebiotic-supplemented diet (10·6 g short-chain FOS/d) or a 2-month placebo-supplemented diet (7·5 g maltodextrin/d) before cross-over. Washout period: not stated	↑ Fasting plasma Lp(a) ↓ Postprandial serum insulin ↔ Fasting plasma cholesterol and TAG ↔ Postprandial glucose, NEFA and TAG
Lecerf *et al.* (2012)[32]	France, *n* 59 healthy adults: twenty-six males and thirty-three females (mean age 20 years; mean BMI 21 kg/m^2)	Parallel RCT double-blinded HMQS: 10	Random assignment to a 4-week xylo-oligosaccharide supplement (5 g XOS/d) or a 4-week xylo-oligosaccharide + inulin supplement (1 g XOS + 3 g inulin/d) or 4-week placebo (4 g maltodextrin/d)	↓ Lipopolysaccharide ↓ IL-1β and TNF-α expression ↑ IL-13 and IL-10 expression
Letexier *et al.* (2003)[33]	France, *n* 8 healthy adults: four males and four females (age range 23–32 years; BMI range 19–25 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 3-week prebiotic-supplemented diet (10 g inulin/d) or a 3-week placebo-supplemented diet (10 g maltodextrin/d) before cross-over. Washout period: 4 months	↓ Hepatic lipogenesis ↓ Plasma TAG ↔ Total cholesterol, LDL and HDL ↔ Glucose, NEFA, insulin and glucagon ↔ Total body weight
Lomax *et al.* (2012)[34]	UK, *n* 43 normal-weight and overweight adults: eleven males and thirty-two females (mean age 55 years; mean BMI 25 kg/m^2)	Parallel RCT double-blinded HMQS: 11	Random assignment to either a 4-week prebiotic-supplemented diet (8 g fructans/d) or a 4-week placebo-supplemented diet (8 g maltodextrin/d)	↔ T-cell activation ↔ NK cell activity ↔ Cytokine production ↔ Monocyte respiratory burst ↔ Basal hepatic glucose production
Luo *et al.* (2000)[35]	France, *n* 10 adults with type 2 diabetes: six males and 4 females (mean age 57 years; mean BMI 28 kg/m^2; mean time after DM diagnosis 11 years; mean HbA1c levels 7·7 %)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either 4-week prebiotic-supplemented cookies (20 g FOS/d) or 4-week control-supplemented cookies (sucrose) before cross-over. Washout period: nil	↔ Fasting plasma glucose and insulin ↔ Fasting lipids, Lp(a) and NEFA ↔ ApoA1 and apoB
Parnell *et al.* (2009)[36]	Canada, *n* 39 overweight and obese adults with BMI > 25 kg/m^2: seven males and thirty-two females (mean age 40 years; mean BMI 30 kg/m^2)	Parallel RCT double-blinded HMQS: 10	Random assignment to either a 12-week prebiotic-supplemented diet (21 g oligofructose/d) or a 12-week placebo-supplemented diet (7·9 g maltodextrin/d)	↓ Body weight and fat mass ↓ Energy intake ↓ Postprandial ghrelin ↓ Postprandial insulin ↔ Postprandial glucose ↔ Postprandial peptide YY and GLP-1
Peters *et al.* (2009)[37]	The Netherlands, *n* 21 normal-weight and overweight adults: five males and sixteen females (mean age 53 years; mean BMI 25·9 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to 2 d consumption of breakfast prebiotic meal-replacement bar (8 g FOS) or breakfast prebiotic + barley meal-replacement bar (8 g FOS + 8 g barley) or breakfast barley meal-replacement bar (8 g barley) or breakfast control meal-replacement bar (oats)	↔ Appetite ↔ Satiety ↔ Energy intake
Piche *et al.* (2003)[38]	France, *n* 9 adults with gastro-oesophageal reflux disease: five males and four females (mean age 52 years).	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 1-week prebiotic-supplemented low-residue diet (19·8 g FOS/d) or a 1-week placebo-supplemented low-residue diet (sucrose) before cross-over. Washout period: minimum 3 weeks	↑ Postprandial plasma GLP-1 ↔ Postprandial peptide YY ↔ Postprandial cholecystokinin
Russo *et al.* (2008)[39] Russo *et al.* (2010)[40]	Italy, *n* 15 healthy males (mean age 19 years; mean BMI 22·8 kg/m^2)	Cross-over RCT double-blinded HMQS: 9	Random assignment to either a 5-week prebiotic-supplemented diet (11 % inulin-enriched pasta) or a 5-week placebo-supplemented diet (control wheat pasta) before cross-over. Washout period: 8 weeks	↓ Lp(a) and TAG ↑ HDL ↓ Total cholesterol:HDL ratio ↓ Fasting glucose and fructosamine ↓ HbA1c and HOMA-IR ↓ Gastric emptying

Table 2. *Continued*

Study	Participants	Study design/blinding	Dietary prebiotic intervention	Effect of dietary prebiotic supplements on metabolic outcomes
Russo *et al.* (2011)[41] Russo *et al.* (2012)[42]	Italy, *n* 20 healthy males (mean age 19 years; mean BMI 22·8 kg/m^2)	Cross-over RCT double-blinded HMQS: 9	Random assignment to either a 5-week prebiotic-supplemented diet (11 % inulin-enriched pasta) or a 5-week placebo-supplemented diet (control wheat pasta) before cross-over. Washout period: 8 weeks	↑ Neurotensin and somatostatin ↑ Plasma GLP-2 ↓ Gastric emptying ↓ Small-intestinal permeability ↓ Serum zonulin ↔ Corticotropin-releasing factor
Schiffrin *et al.* (2007)[43]	Switzerland, *n* 74 elderly adults with mild malnutrition: eighteen males and fifty-six females (mean age 84 years; mean BMI 25 kg/m^2)	Parallel RCT double-blinded HMQS: 12	Random assignment to either a 12-week prebiotic-supplemented drink (2–4 g FOS/d) or a 12-week identical drink (without FOS)	↓ *IL-6* mRNA and *TNF-α* mRNA ↓ Soluble CD14
Seidel *et al.* (2007)[44]	Germany, *n* 38 males: twenty smokers and eighteen non-smokers (mean age 27 years; mean BMI 23·2 kg/m^2)	Parallel RCT double-blinded HMQS: 9	Subjects participated in a pre-randomisation run-in period (consumed at least 200 g wheat-rye bread/d for 5 weeks), followed by randomisation to an intervention period (nineteen participants consumed at least 200 g prebiotic bread/d for 5 weeks and nineteen participants consumed at least 200 g prebiotic + antioxidant bread/d), followed by a post-intervention period (all thirty-eight participants received a standard diet for 1 week). Intervention breads contained 4 g inulin/100 g	↑ TRAP ↑ PCL ↑ CD19 ↓ ICAM-1 ↓ CD3 + NK + ↑ CD3 + HLA-DR + (activated T cells) ↔ Body weight
Tovar *et al.* (2012)[45]	Mexico, *n* 110 overweight and obese women (age range 18–50 years; BMI ≥25 kg/m^2)	Parallel RCT not blinded HMQS: 8	Random assignment to 12-week partial meal replacement or 12-week partial meal replacement + prebiotic (10 g inulin/d) or 12-week prebiotic (10 g inulin/d) or 12-week control (no meal replacement or inulin). All the subjects received a low-energy diet	↓ TAG ↔ Total cholesterol, HDL and glucose
Verhoef *et al.* (2011)[46]	The Netherlands, *n* 29 normal-weight and overweight adults: nine males and twenty females (mean age 28 years; mean BMI 24·8 kg/m^2)	Cross-over RCT double-blinded HMQS: 9	Random assignment to either a 13 d prebiotic-supplemented diet (10 g FOS/d or 16 g FOS/d) or a 13 d placebo-supplemented diet (16 g maltodextrin) before cross-over. Washout period: 2 weeks	Outcomes for 16 g FOS/d intervention *v.* placebo: ↔ Appetite ↔ Satiety ↔ Energy intake ↔ Plasma GLP-1 ↑ Plasma peptide YY
Vulevic *et al.* (2008)[47]	UK, *n* 44 normal-weight and overweight elderly adults: sixteen males and twenty-eight females (age range 64–79 years; BMI range 22–31 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 10-week prebiotic-supplemented diet (5·5 g GOS/d) or a 10-week placebo-supplemented diet (5·5 g maltodextrin) before cross-over. Washout period: 4 weeks	↑ NK cell activity ↑ IL-10 production ↓ IL-6, IL-1β and TNF-α production ↔ Total cholesterol and HDL
Vulevic *et al.* (2013)[48]	UK, *n* 45 overweight and obese adults with the metabolic syndrome: sixteen males and twenty-nine females (mean age 45 years; mean BMI 31 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 12-week prebiotic-supplemented diet (5·5 g GOS/d) or a 12-week placebo-supplemented diet (5·5 g maltodextrin) before cross-over. Washout period: 4 weeks	↓ Plasma CRP and insulin ↓ Total cholesterol and TAG ↓ TC:HDL ratio ↔ IL-6, IL-10, IL-8 and TNF-α
Whelan *et al.* (2006)[49]	UK, *n* 11 healthy adults: five males and six females (mean age 28 years; mean BMI 23·5 kg/m^2)	Cross-over RCT double-blinded HMQS: 10	Random assignment to either a 2-week prebiotic-supplemented liquid enteral formula (approximately 18 g pea fibre + 10 g FOS/d) or a 2-week placebo liquid enteral formula (standard formula) before cross-over. Washout period: 4 weeks	↑ Mean fullness and minimum fullness ↑ Minimum satiety

RCT, randomised controlled trial; HMQS, Heyland Methodological Quality Score, where trials scoring ≥8 out of 14 points are considered to be of high methodological quality; FOS, fructo-oligosaccharide; GLP, glucagon-like peptide; NASH, non-alcoholic steatohepatitis; AST, aspartate aminotransferase; DM, diabetes mellitus; CRP, C-reactive protein; TAC, total antioxidant capacity; SOD, superoxide dismutase; HOMA-IR, homeostasis model assessment for insulin resistance; ALA, α-linolenic acid; OGTT, oral glucose tolerance test; Lp(a), lipoprotein (a); XOS, xylo-oligosaccharide; NK, natural killer; CD, cluster of differentiation; TRAP, total radical-trapping antioxidant parameter; PCL, photosensitive chemiluminescence; ICAM-1, intracellular adhesion molecule-1; GOS, galacto-oligosaccharide; TC, total cholesterol; ↓, significantly lower than that in the comparison diet group after intervention; ↑, significantly higher than that in the comparison diet group after intervention; ↔, no significant difference between the prebiotic-supplemented diet and control diet groups after intervention; HLA-DR, human leucocyte antigen-D-related.

after prebiotic consumption[37,46] was not possible in the meta-analysis, as they did not report study data and did not provide results when contacted by the reviewers. An additional trial carried out in obese subjects was also excluded from the meta-analysis because it reported only qualitative improvements in satiety[30]. Of the five trials measuring energy intakes in normal-weight and overweight participants and those with type 2 diabetes, three found a significant reduction in total energy consumption during the prebiotic intervention when compared with placebo[22,26,36]. However, the reduction in energy intake lost statistical significance after the meta-analysis (n 208) yielded a pooled SMD of −0·51 (95% CI −1·20, 0·19; P=0·16). The duration of one trial finding no difference in energy intake between control and intervention groups was 2 d[37], and the trials finding reduced energy intake by the intervention groups lasted a minimum of 2 weeks. Available evidence supported that dietary prebiotic supplementation for at least 2 weeks' duration increases circulating peptide YY concentrations in normal-weight and overweight adults[23,36,46], but the effect was not statistically significant after the meta-analysis (n 100), with a combined SMD of −0·96 (95% CI −1·98, 0·06; P=0·07). Of four high-quality trials, two found increased GLP-1 concentrations after prebiotic supplementation in healthy and overweight subjects[23,38]. The increase in GLP-1 concentrations was not significant after the meta-analysis (n 117), with a pooled SMD of −0·32 (95% CI −0·87, 0·23; P=0·25). Each of the trials reported significant reductions in ghrelin concentrations[36] and increased GLP-2 concentrations[42] in subjects consuming dietary prebiotics. Contradictory results were reported by five trials examining the effect of prebiotic intervention on body weight. Significant reductions in body weight after prebiotic supplementation in comparison with placebo were reported by two trials[30,36], while no change in body weight was observed in three trials[26,28,44]. Trials of longer duration (12–17 weeks) were more likely to observe reductions in body weight than shorter trials lasting 4–8 weeks. The meta-analysis (n 191) indicated a non-significant reduction in body weight after prebiotic supplementation, with a pooled SMD of −0·48 (95% CI −1·19, 0·23; P=0·19).

Outcomes associated with glucose homeostasis

Of the four studies measuring the effect of prebiotic supplementation on postprandial glucose concentrations, two reported significant reductions in glycaemia in normal-weight and obese participants[23,29]. Following the meta-analysis (n 131), the pooled SMD for postprandial glucose concentrations was −0·76 (95% CI −1·41, −0·07; P<0·05), indicating a statistically significant effect supporting that prebiotic consumption results in the reduction of postprandial glucose concentrations (Fig. 3). Of three studies, two reported significant reductions in postprandial insulin concentrations following prebiotic intervention in overweight and hypercholesterolaemic subjects[31,36]. Meta-analysis of these trials (n 121) indicated a statistically significant reduction in postprandial insulin concentrations, with a combined SMD of −0·77 (95% CI −1·50, −0·04; P<0·05) (Fig. 4). Significant delays in gastric emptying times in healthy males consuming prebiotic supplements were found in two trials carried out by the same study group[40,41]. Studies investigating fasting glucose and fasting insulin concentrations and insulin resistance (HOMA-IR) reported conflicting results. Significant reductions in HbA1c levels in healthy participants after only 5 weeks of prebiotic supplementation[40] and in women with type 2 diabetes after 8 weeks[26] were found by two trials, while no change in HbA1c levels in obese women after prebiotic supplementation lasting 3 months was found by another trial[29].

Outcomes associated with cardiovascular and hepatic health

There was insufficient evidence to support that prebiotic supplementation reduces total cholesterol or LDL concentrations in healthy, obese or dyslipidaemic individuals, with the

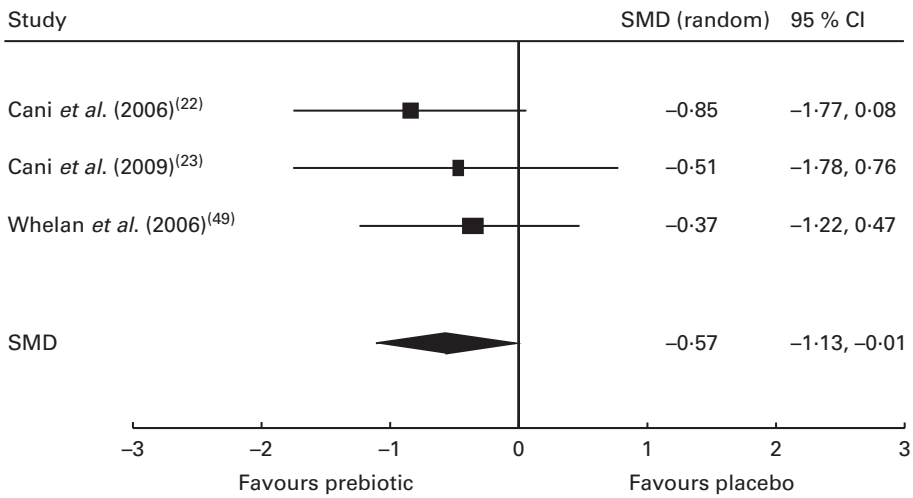


Fig. 2. Effects of dietary prebiotic supplementation on self-reported satiety. Forest plot of standardised mean differences (SMD, 95% CI) for individual and pooled trials.

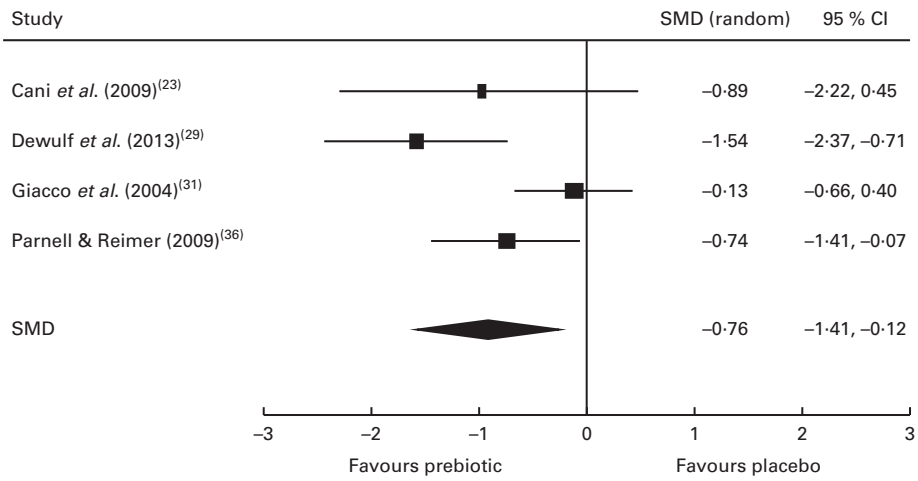


Fig. 3. Effects of dietary prebiotic supplementation on postprandial glucose concentrations. Forest plot of standardised mean differences (SMD, 95 % CI) for individual and pooled trials.

majority of studies finding no change in the concentrations of these lipids after intervention. Of the eleven trials investigating the effect of prebiotic supplementation on circulating TAG concentrations, five reported significant reductions in healthy, overweight or hypercholesterolaemic individuals compared with controls[24,33,39,45,48]. However, the remaining six trials that failed to detect changes in TAG concentrations were also carried out in healthy, overweight or hypercholesterolaemic subjects[25,28–31,35]. These trials were subjected to meta-analysis (n 402), resulting in a non-significant pooled SMD for TAG concentrations of −0·11 (95 % CI −0·31, 0·08; P=0·26) (Fig. 5). A significant reduction in serum aspartate aminotransferase concentrations was reported by one small trial carried out in people with NASH[25].

Outcomes associated with inflammation and immune function

Of the four trials investigating the impact of dietary prebiotic supplementation on circulating C-reactive protein (a biochemical marker of inflammation) concentrations, three found significant reductions in overweight and obese adults and women with type 2 diabetes in comparison with controls[26,28,48]. Meta-analysis of these trials (n 181) indicated a non-significant reduction in C-reactive protein concentrations after prebiotic supplementation, however, with a pooled SMD of −0·85 (95 % CI −2·11, 0·42; P=0·19). Studies measuring the production of pro-inflammatory cytokines (TNF-α and IL) and immune cell activity (T-cell activation and natural killer cell activation) yielded contradictory results. Significant increases in the measures of antioxidant status (total antioxidant capacity[27], total radical-trapping antioxidant parameter and photosensitive chemiluminescence[44]) were found by two studies, and a decrease in small-intestinal permeability[42] following prebiotic interventions was reported by one trial. Significant reductions in circulating lipopolysaccharide (LPS) concentrations after dietary prebiotic supplementation in healthy adults[32] and women with type 2 diabetes[26] were identified by two studies.

Discussion

Simple, safe and effective interventions are urgently needed to prevent and treat obesity and its associated co-morbidities. The human gut microbiota and its metabolites influence host

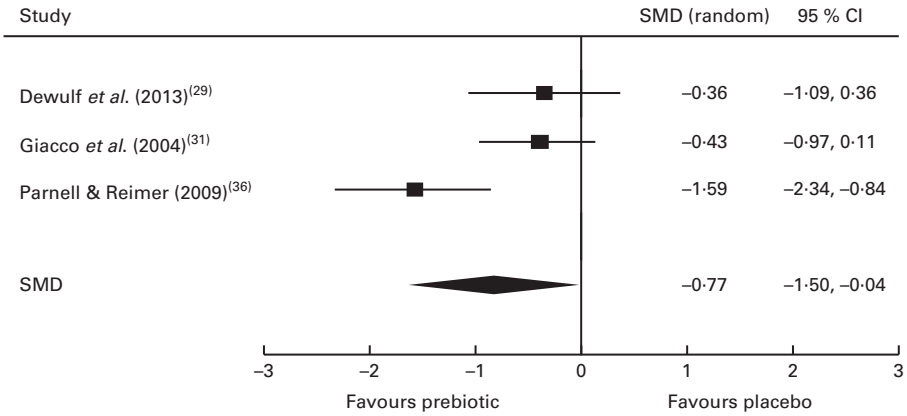


Fig. 4. Effects of dietary prebiotic supplementation on postprandial insulin concentrations. Forest plot of standardised mean differences (SMD, 95 % CI) for individual and pooled trials.

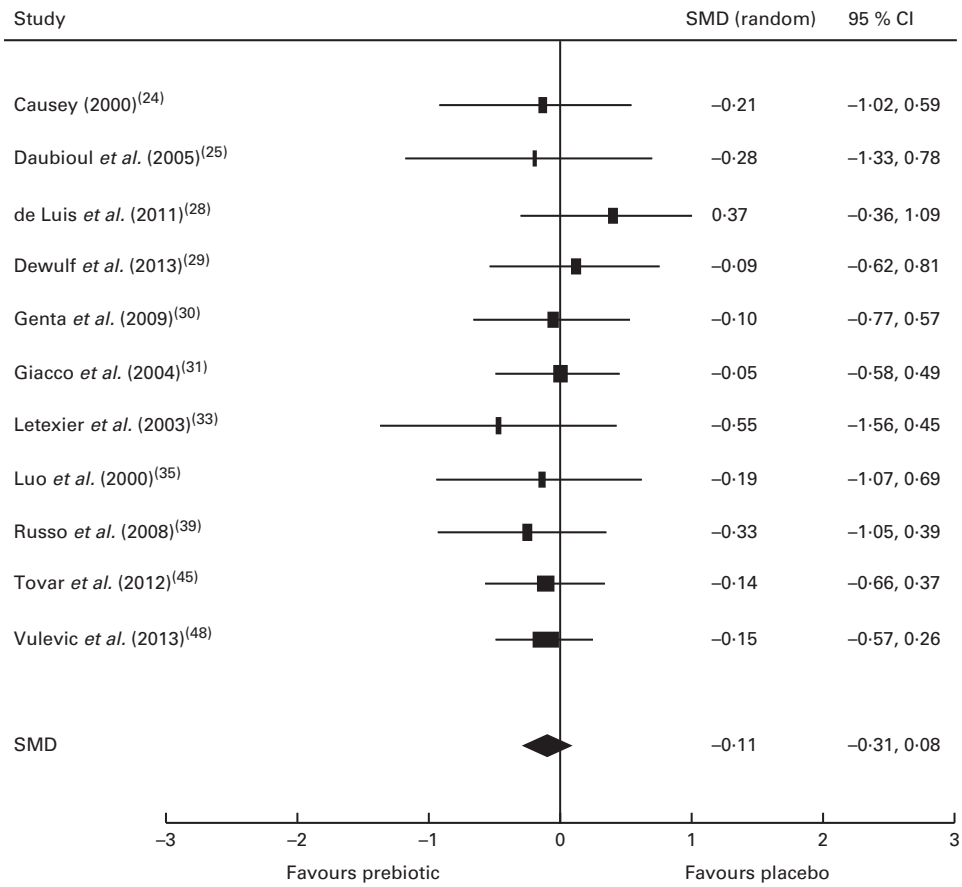


Fig. 5. Effects of dietary prebiotic supplementation on TAG concentrations. Forest plot of standardised mean differences (SMD, 95 % CI) for individual and pooled trials.

physiology, energy homeostasis, inflammatory processes and immune function both locally and within distal tissues. The use of dietary prebiotic supplements to promote the selective proliferation of beneficial intestinal microbes might represent an important nutritional strategy in the management of metabolic abnormalities and chronic disease.

Prebiotics and overweight/obesity

The SCFA acetate, proprionate and butyrate are produced as by-products of bacterial prebiotic fermentation in the colon. In addition to representing a source of energy for the host, these SCFA play a number of beneficial roles including the maintenance of human intestinal health and modulation of metabolic and immune processes. SCFA are the only known ligands for two G protein-coupled receptors, GPR41 and GPR43, which are expressed in a variety of gastrointestinal cells and stimulate the secretion of hormones involved in the regulation of energy intake and expenditure. Binding of SCFA to GPR41 increases the production of peptide YY and GLP-1, hormones that reduce appetite, delay gastric emptying and increase insulin sensitivity[50]. SCFA also promote the differentiation of intestinal L-cells, contributing to increased endogenous GLP-1 production[51].

In animal studies, dietary supplementation of the SCFA butyrate has been found to prevent diet-induced obesity and improve insulin sensitivity with a concomitant increase in energy expenditure and fatty acid oxidation and an increase in mitochondrial respiration[52]. In mice, the selective growth of certain *Lactobacillus* species in the colon has been found to reduce body fat storage through the up-regulation of *Fiaf* (fasting-induced adipose factor) gene expression and inhibition of lipoprotein lipase[53,54]. Indeed, several animal studies have demonstrated the protective effects of prebiotics on the development of obesity and insulin resistance[55,56]; however, more robust human studies are required to confirm the protective effects of prebiotics on these pathways in human physiology.

The present review found consensus among three of the five high-quality trials supporting that the daily consumption of a prebiotic supplement for a minimum of 2 weeks increases satiety cues in healthy adults. However, these findings were based on self-reports from relatively small numbers of subjects (*n* 81) and prebiotic supplementation failed to result in significant weight reduction. Weight reduction was unlikely to be observed in these trials due to the short duration of their prebiotic interventions (2 d–2 weeks). The addition of pea fibre to the prebiotic supplement confounded one trial reporting an increase in satiety after 2 weeks of prebiotic consumption, making it difficult to draw conclusions about the action of either type of fibre individually[49]. Of the two trials that did not detect any change in satiety after prebiotic supplementation,

one involved an intervention period of 2 d[37], which may have been insufficient time to modify the growth and activity of intestinal bacteria to influence changes in host physiology. Increases in breath hydrogen production in the intervention group, indicative of enhanced intestinal bacterial fermentation, were detected by one of the trials investigating the effects of prebiotic supplementation on satiety sensations[23]. However, no trials analysed the stool samples of participants for changes in microbial growth; therefore, alterations in hunger and fullness reported by the subjects in these studies may have occurred independently of any changes in gut microbial fermentation. Prebiotics are soluble fibres capable of modifying the intestinal transit of food due to their water-binding and bulking capacity[57]. Indeed, study participants consuming prebiotics within a single meal have reported increased levels of satiety, well before any changes in colonic bacterial growth could have taken place[58,59]. Trials quantitatively evaluating the effect of prebiotic consumption on satiety and gut bacterial growth in overweight and obese individuals are now required.

Of the five high-quality trials, two provided consistent evidence favouring dietary prebiotic consumption for at least 2 weeks' duration for the reduction of total energy intake in normal-weight and overweight individuals and in women with type 2 diabetes. However, the pooled reduction in energy consumption was not statistically significant after the meta-analysis. A longer prebiotic supplementation period lasting 12 weeks was required before participants' reduced energy intake resulted in significant weight reduction[36]. Where body composition was evaluated, the weight lost was predominantly fat mass rather than lean tissue or fluid[36]. Trials of extended duration are now needed to determine whether dietary prebiotic consumption is a safe and effective therapeutic option for long-term weight and body fat reduction or whether physiological adaptations by the host eventually compensate for this energy imbalance to minimise weight loss.

The majority of trials investigating the effect of dietary prebiotic supplementation on the regulation of intestinal peptide (peptide YY) and incretin (GLP-1) secretion reported significant increases in the production of these molecules after 2 weeks, but the combined changes were not significant after the meta-analysis. The unique role played by prebiotics and specific bacteria in gut hormone kinetics requires further investigation, as non-prebiotic dietary fibres have also been reported to be associated with increased SCFA, peptide YY and GLP-1 production in human feeding studies[60,61].

Prebiotics and glucose intolerance

Reduced levels of bifidobacteria and lactobacilli and increased gastrointestinal permeability are found in mice consuming a diet high in saturated fat when compared with those consuming a standard diet. The provision of dietary prebiotic supplements subsequently restores the growth of these beneficial bacterial species and improves the integrity of the gut barrier[11,62]. Animal studies have shown a causal link between the consumption of a high-fat diet and increased intestinal levels of LPS-containing bacteria, or concentrations of circulating LPS, and the development of obesity and insulin resistance[63]. LPS is the major component of the outer membrane of Gram-negative bacteria and is composed of a hydrophobic lipid (lipid A), a hydrophilic core oligosaccharide and a repeating hydrophilic polysaccharide side chain (O-antigen). In the setting of a high-fat diet, LPS is able to translocate from the intestine into the host circulation, resulting in 'metabolic endotoxaemia'[64]. LPS stimulates the overproduction of reactive oxygen species and pro-inflammatory cytokines by macrophages, resulting in subclinical systemic inflammation, weight gain and insulin resistance development[65,66]. Human subjects with type 2 diabetes have been found to possess serum endotoxin levels that are 2-fold higher than those observed in non-diabetic controls[67]. Metabolic endotoxaemia is also positively correlated with total energy intake and fasting insulin concentrations in the non-diabetic population[68]. In mice with high-fat diet-induced metabolic endotoxaemia, nutritional supplementation with prebiotics restores intestinal levels of Gram-positive bacteria, improves glucose tolerance and reduces circulating concentrations of LPS and pro-inflammatory cytokines[56].

Prebiotics and their fermentation products have been shown to reduce gastrointestinal permeability by a variety of mechanisms. The SCFA butyrate is involved in the maintenance of gut epithelial integrity by acting as the principal fuel for colonocytes and promoting the transcription of tight junction proteins between gastrointestinal cells[69]. Butyrate also reduces gastrointestinal permeability by enhancing the activation of the peroxisomal proliferator-activated receptor gamma (*PPARγ*) gene, a nuclear factor receptor involved in the attenuation of inflammation in colonic epithelial cells[70,71]. Prebiotic-induced changes in gut microbiota also increase the endogenous production of GLP-2, which enhances gut barrier function by promoting the proliferation of crypt cells[12,72].

The present review found general agreement among trials supporting that the consumption of dietary prebiotic supplements reduces postprandial glucose and insulin concentrations in healthy and overweight individuals. Pooled reductions in postprandial glucose and insulin concentrations were statistically significant after the meta-analysis. High-quality randomised controlled trials conducted in subjects with either impaired glucose tolerance[73] or type 2 diabetes[74] have also found reduced postprandial serum insulin concentrations after the consumption of arabinoxylan (a potential prebiotic fibre). Whether these results were mediated by alterations in intestinal bacterial growth or activity is unclear, as stool samples were not analysed in these trials. Significant delays in gastric emptying times after prebiotic supplementation in healthy males were found by two studies. However, these trials were conducted by the same research group, and it is unclear whether some subjects participated in both the studies. Therefore, further independent research is required before definitive conclusions can be drawn about the effects of prebiotic consumption on gastric emptying. The findings of studies investigating fasting glucose and fasting insulin concentrations and insulin resistance (HOMA-IR) after prebiotic supplementation were contradictory. Long-term prebiotic intervention studies in people with pre-diabetes

or the metabolic syndrome are now required to determine whether prebiotics confer some protection against the future development of type 2 diabetes in high-risk individuals.

Prebiotics and dyslipidaemia

The abundance of particular bacterial species in the gut has been shown to be positively correlated with serum total cholesterol and LDL-cholesterol concentrations in subjects with CVD[75]. It has also been hypothesised that bacteria found in atherosclerotic plaques may have originated from the gastrointestinal tract, as the DNA of specific micro-organisms can be found in both the colon and coronary atheroma of the same individual[76]. As the development of CVD involves multiple pro-inflammatory pathways, it is plausible that pathogenic microbes may potentiate inflammation within atherosclerotic plaques by delivering macrophages to the arterial wall and stimulating their production of reactive oxygen species and cytokines or their conversion to foam cells[77].

Proprionate, a SCFA product of prebiotic fermentation, may play a significant role in the modification of hepatic lipid metabolism. In the liver, proprionate is a possible substrate for gluconeogenesis and may contribute to the inhibition of cholesterol synthesis by altering the activity of 3-hydroxy-3-methylglutaryl-CoA reductase[5]. In addition, prebiotic supplementation might attenuate cholesterol and TAG production by stimulating the synthesis of *cis*-9, *trans*-11-conjugated linoleic acid from PUFA by beneficial bacterial species. This isoform of *cis*-9, *trans*-11-conjugated linoleic acid has been shown to reduce cholesterol and TAG concentrations in animal studies[78], but the results of human trials are less conclusive. Gut microbes are also an essential requirement for the production of secondary bile acids in the colon. These bile acids are de-conjugated and are therefore unavailable for enterohepatic recirculation. As a result, the liver is forced to produce additional bile acids from circulating cholesterol[79].

The findings of human intervention studies investigating the effect of dietary prebiotic supplementation on circulating total and LDL-cholesterol concentrations were contradictory. Of the two studies reporting significant reductions in total cholesterol concentrations, one found a reduction in only male subjects and was complicated by the use of an intervention containing both prebiotics and α-linolenic acid[28]. α-Linolenic acid may have contributed to the cholesterol-lowering effect in this instance. There is limited evidence to support that prebiotic supplementation reduces total or LDL-cholesterol concentrations in hypercholesterolaemic individuals, as the only two trials conducted in participants with hypercholesterolaemia found no significant changes in total cholesterol, LDL-cholesterol or HDL-cholesterol concentrations[24,31]. However, these trials involved short-term prebiotic intervention periods (3–8 weeks' duration) and studies of longer duration are therefore required.

The present review found conflicting evidence describing the effect of prebiotic supplementation on circulating TAG concentrations in healthy, overweight/obese and hypercholesterolaemic subjects. In addition to a prebiotic supplement, one study[45] provided a low-energy diet co-intervention to all the trial participants, making it difficult to establish whether the TAG-lowering effect was associated with prebiotic-induced intestinal microbial changes alone or whether weight reduction or a reduced fat intake together with the action of the modified microflora produced a synergistic effect. Additional systematic reviews exploring this topic have also reported equivocal conclusions. The use of prebiotics for the reduction of TAG concentrations in humans regardless of health condition was favoured by one review of trials published between 1995 and 2005[80], with the majority of trials being conducted in normolipidaemic individuals. The other meta-analysis of trials published between 1999 and 2010 supported the TAG-lowering effects of inulin in only hypercholesterolaemic subjects[75], but the reduction in TAG concentrations lost significance when results from both hyperlipidaemic and normolipidaemic subjects were combined. The present review also found a non-significant reduction in TAG concentrations after the meta-analysis of pooled trials. Future trials must simultaneously quantify lipid concentrations and gut bacterial growth and activity to determine whether prebiotic-induced modulation of the intestinal flora contributes to the reduction of serum TAG concentrations.

Prebiotics and non-alcoholic steatohepatitis

NASH is an asymptomatic disease characterised by fatty infiltration of the liver and inflammation, which can eventually lead to fibrosis, cirrhosis, portal hypertension, hepatocellular carcinoma and liver failure[81]. Obesity, dyslipidaemia, insulin resistance and diabetes have frequently been reported to be associated with the development of NASH. Increased plasma endotoxaemia, overproduction of inflammatory cytokines and excessive oxidative stress within hepatic cells are also thought to contribute to the pathogenesis of NASH. The use of dietary prebiotic supplements to restore an optimal microbial balance within the gastrointestinal tract of individuals with NASH may assist in the reduction of TAG accumulation in the liver, attenuate inflammation and promote hepatic secretion of lipoproteins such as VLDL[15]. The translocation of Gram-negative bacteria from the intestine into the circulation has been reported to be associated with an increased severity of cirrhosis[82]. By maintaining gut barrier function and reducing bacterial translocation, prebiotics may be effective in the management of liver disease complications[83].

Studies exploring the effects of dietary prebiotic consumption on metabolic parameters in individuals with NASH are scarce. In the present review, one small trial involving seven adult males was included, which found a significant reduction in serum aspartate aminotransferase concentrations after prebiotic supplementation[25]. This finding was supported by a larger randomised controlled trial (n 66), which administered a synbiotic (fructo-oligosaccharide + *Bifidobacterium longum*) to participants for 24 weeks[84]. In addition to a significant reduction in serum aspartate aminotransferase concentrations, researchers found a reduction in the concentrations of circulating cytokines (TNF-α) and markers of inflammation (C-reactive protein), reduced concentrations of serum LDL-cholesterol and

endotoxins, an improvement in insulin sensitivity (HOMA-IR) and a reduction in hepatic steatosis (determined by liver biopsy). More research is required in this potentially very promising area of study.

Prebiotics and immune cell dysfunction

Gut microbiota, innate immune function and metabolism are inextricably linked, with early pathological processes occurring at the molecular level (subclinical inflammation, immune cell activation, increased oxidative and endoplasmic reticulum stress, altered production of vascular adhesion molecules and advanced glycation end products) contributing to the eventual development of metabolic disturbances such as hyperlipidaemia, atherosclerosis, insulin resistance and weight gain. Colonic bacteria and their prebiotic fermentation products may play a key role in the modulation of immune function by both increasing host resistance to infection and down-regulating inappropriate immune responses in the case of allergic reactions or chronic inflammatory conditions[5]. By maintaining the integrity of the gastrointestinal barrier, prebiotics reduce the invasion of pathogenic intestinal bacteria and their products (including LPS) into the circulation, preventing downstream immune cell activation. Prebiotics are thought to encourage increased intestinal mucin production, protecting the intestinal wall from bacterial adherence and invasion[85]. SCFA produced as a by-product of bacterial prebiotic fermentation interact with GPR41 and GPR43 receptors on neutrophils and inhibit NF-κB activation, reducing the production of pro-inflammatory cytokines[86]. Additional bacterial fermentation products such as polysaccharide A and peptidoglycan exert anti-inflammatory effects on the host immune system[87].

There is insufficient evidence at present to recommend dietary prebiotics for the modulation of immune function to improve cardiometabolic health. There are very few human trials available, and most have reported contradictory findings. Although individual studies have found significant increases in the measures of antioxidant capacity and reductions in small-intestinal permeability and circulating LPS concentrations after prebiotic interventions, further studies are required to verify these results. Inulin exhibits antioxidant properties independent of altering gut bacterial growth and is able to scavenge a number of reactive oxygen species, which may help to reduce lipid peroxidation in the stomach[88]. Future studies must distinguish between health benefits derived solely from the consumption of soluble fibres and those associated with the growth and activity of beneficial gut microbes. Future intervention studies exploring the effect of dietary prebiotics on immune function need to be conducted in healthy individuals who are subsequently exposed to an immune challenge.

Conclusions

Although animal studies have provided convincing evidence to support the beneficial role of prebiotics in metabolic health, the results of human trials to date have been less conclusive. Research involving laboratory animals enables the provision of tightly controlled diets, whereas studies involving free-living humans are complicated by the variety of foods consumed by individuals from day to day. Some human studies have been complicated by the use of nutritional supplements containing prebiotics in combination with additional health-promoting components such as live bacteria, antioxidants and other dietary fibres, making it difficult to attribute changes in metabolism to prebiotics alone. To rule out cardiometabolic benefits associated with concomitant nutrients, prebiotic supplements in their pure form must be used in future trials.

In addition to bifidobacteria and lactobacilli, dietary prebiotics modulate the growth of numerous other gastrointestinal micro-organisms, the identity and function of which have not yet been fully characterised. Different species of bifidobacteria also have a variety of functions, which require further elucidation. Prebiotics are likely to undergo cross-fermentation by other microbial species of unknown benefit to the host. Bacterial analyses of human stool samples provide information only about the micro-organisms inhabiting the colon and are unlikely to accurately reflect the microbial composition of the proximal intestine. Responses to dietary prebiotics are variable in humans, with bifidogenic potential being affected by an individual's age, body weight, antibiotic use, dietary macronutrient intake, physical activity and baseline levels of colonic bifidobacteria[89,90]. More research is required to determine host lifestyle behaviours capable of promoting intestinal normobiosis and to establish the optimal prebiotic dose required to maximise health benefits.

In conclusion, the present review found convincing evidence from short-term high-quality human trials supporting the use of dietary prebiotics as a potential therapeutic intervention for the regulation of appetite and the reduction of circulating postprandial glucose and insulin concentrations. Further studies are needed to correlate these findings with changes in the growth and function of specific gut bacteria. There is insufficient evidence at present to recommend dietary prebiotics for reducing energy intake and body weight, increasing gastric peptide YY and GLP-1 secretion, improving insulin sensitivity, lowering lipid levels and modulating immune function. Long-term prospective trials investigating primary metabolic end points are now required.

Acknowledgements

N. J. K. was the recipient of National Health and Medical Research Council (NHMRC) Postgraduate Public Health Scholarship APP1039709. N. J. K. thanks Mr Brendan Kellow for IT assistance.

N. J. K. was supported by NHMRC Postgraduate Public Health Scholarship APP1039709. The NHMRC had no role in the design and analysis of this work or in the writing of this article.

The authors' contributions are as follows: N. J. K. designed the review, conducted the literature search, extracted and analysed the data and drafted the manuscript; M. T. C. and C. M. R. provided comments on the manuscript. All the authors read and approved the final manuscript.

None of the authors has any conflicts of interest to declare.

References

1. Diamant M, Blaak EE & de Vos WM (2011) Do nutrient–gut–microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes? *Obes Rev* **12**, 272–281.
2. Wu X, Ma C, Han L, *et al.* (2010) Molecular characterisation of the faecal microbiota in patients with type II diabetes. *Curr Microbiol* **61**, 69–78.
3. Larsen N, Vogensen FK, van den Berg FW, *et al.* (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE* **5**, e9085.
4. Gibson GR, Probert HM, Loo JV, *et al.* (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* **17**, 259–275.
5. Roberfroid M, Gibson GR, Hoyles L, *et al.* (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* **104**, Suppl. 2, S1–S63.
6. Ramirez-Farias C, Slezak K, Fuller Z, *et al.* (2009) Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br J Nutr* **101**, 541–550.
7. Chung H & Kasper DL (2010) Microbiota-stimulated immune mechanisms to maintain gut homeostasis. *Curr Opin Immunol* **22**, 455–460.
8. Ding S, Chi MM, Scull BP, *et al.* (2010) High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PLoS ONE* **5**, e12191.
9. Backhed F, Manchester JK, Semenkovich CF, *et al.* (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci U S A* **104**, 979–984.
10. Havenaar R (2011) Intestinal health functions of colonic microbial metabolites: a review. *Benef Microbes* **2**, 103–114.
11. Cani PD, Neyrinck AM, Fava F, *et al.* (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**, 2374–2383.
12. Cani PD, Possemiers S, Van de Wiele T, *et al.* (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103.
13. Hill MJ (1997) Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev* **6**, Suppl. 1, S43–S45.
14. Scholz-Ahrens KE, Ade P, Marten B, *et al.* (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J Nutr* **137**, 838S–846S.
15. Musso G, Gambino R & Cassader M (2011) Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annu Rev Med* **62**, 361–380.
16. Neyrinck AM & Delzenne NM (2010) Potential interest of gut microbial changes induced by non-digestible carbohydrates of wheat in the management of obesity and related disorders. *Curr Opin Clin Nutr Metab Care* **13**, 722–728.
17. Cloetens L, Ulmius M, Johansson-Persson A, *et al.* (2012) Role of dietary beta-glucans in the prevention of the metabolic syndrome. *Nutr Rev* **70**, 444–458.
18. Kootte RS, Vrieze A, Holleman F, *et al.* (2012) The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. *Diabetes Obes Metab* **14**, 112–120.
19. Heyland DK, Novak F, Drover JW, *et al.* (2001) Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. *JAMA* **286**, 944–953.
20. White IR & Thomas J (2005) Standardized mean differences in individually-randomized and cluster-randomized trials, with applications to meta-analysis. *Clin Trials* **2**, 141–151.
21. Bunout D, Hirsch S, Pia de la Maza M, *et al.* (2002) Effects of prebiotics on the immune response to vaccination in the elderly. *JPEN J Parenter Enteral Nutr* **26**, 372–376.
22. Cani PD, Joly E, Horsmans Y, *et al.* (2006) Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* **60**, 567–572.
23. Cani PD, Lecourt E, Dewulf EM, *et al.* (2009) Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* **90**, 1236–1243.
24. Causey JL (2000) Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutr Res* **20**, 191–201.
25. Daubioul CA, Horsmans Y, Lambert P, *et al.* (2005) Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. *Eur J Clin Nutr* **59**, 723–726.
26. Dehghan P, Gargari BP, Jafar-Abadi MA, *et al.* (2013) Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutr* (Epublication ahead of print version 24 September 2013).
27. Pourghassem Gargari B, Dehghan P, Aliasgharzadeh A, *et al.* (2013) Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes. *Diabetes Metab J* **37**, 140–148.
28. de Luis DA, de la Fuente B, Izaola O, *et al.* (2011) Double blind randomized clinical trial controlled by placebo with an alpha linoleic acid and prebiotic enriched cookie on risk cardiovascular factor in obese patients. *Nutr Hosp* **26**, 827–833.
29. Dewulf EM, Cani PD, Claus SP, *et al.* (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **62**, 1112–1121.
30. Genta S, Cabrera W, Habib N, *et al.* (2009) Yacon syrup: beneficial effects on obesity and insulin resistance in humans. *Clin Nutr* **28**, 182–187.
31. Giacco R, Clemente G, Luongo D, *et al.* (2004) Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. *Clin Nutr* **23**, 331–340.
32. Lecerf JM, Depeint F, Clerc E, *et al.* (2012) Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br J Nutr* **108**, 1847–1858.
33. Letexier D, Diraison F & Beylot M (2003) Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. *Am J Clin Nutr* **77**, 559–564.
34. Lomax AR, Cheung LV, Tuohy KM, *et al.* (2012) β2-1 Fructans have a bifidogenic effect in healthy middle-aged human subjects but do not alter immune responses examined in the absence of an *in vivo* immune challenge: results from a randomised controlled trial. *Br J Nutr* **108**, 1818–1828.
35. Luo J, Van Yperselle M, Rizkalla SW, *et al.* (2000) Chronic consumption of short-chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. *J Nutr* **130**, 1572–1577.
36. Parnell JA & Reimer RA (2009) Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* **89**, 1751–1759.

37. Peters HP, Boers HM, Haddeman E, *et al.* (2009) No effect of added beta-glucan or of fructooligosaccharide on appetite or energy intake. *Am J Clin Nutr* **89**, 58–63.
38. Piche T, des Varannes SB, Sacher-Huvelin S, *et al.* (2003) Colonic fermentation influences lower esophageal sphincter function in gastroesophageal reflux disease. *Gastroenterology* **124**, 894–902.
39. Russo F, Chimienti G, Riezzo G, *et al.* (2008) Inulin-enriched pasta affects lipid profile and Lp(a) concentrations in Italian young healthy male volunteers. *Eur J Nutr* **47**, 453–459.
40. Russo F, Riezzo G, Chiloiro M, *et al.* (2010) Metabolic effects of a diet with inulin-enriched pasta in healthy young volunteers. *Curr Pharm Des* **16**, 825–831.
41. Russo F, Clemente C, Linsalata M, *et al.* (2011) Effects of a diet with inulin-enriched pasta on gut peptides and gastric emptying rates in healthy young volunteers. *Eur J Nutr* **50**, 271–277.
42. Russo F, Linsalata M, Clemente C, *et al.* (2012) Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutr Res* **32**, 940–946.
43. Schiffrin EJ, Thomas DR, Kumar VB, *et al.* (2007) Systemic inflammatory markers in older persons: the effect of oral nutritional supplementation with prebiotics. *J Nutr Health Aging* **11**, 475–479.
44. Seidel C, Boehm V, Vogelsang H, *et al.* (2007) Influence of prebiotics and antioxidants in bread on the immune system, antioxidative status and antioxidative capacity in male smokers and non-smokers. *Br J Nutr* **97**, 349–356.
45. Tovar AR, Caamano Mdel C, Garcia-Padilla S, *et al.* (2012) The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: a randomized clinical trial in obese Mexican women. *Nutr J* **11**, 44.
46. Verhoef SP, Meyer D & Westerterp KR (2011) Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *Br J Nutr* **106**, 1757–1762.
47. Vulevic J, Drakoularakou A, Yaqoob P, *et al.* (2008) Modulation of the fecal microflora profile and immune function by a novel *trans*-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. *Am J Clin Nutr* **88**, 1438–1446.
48. Vulevic J, Juric A, Tzortzis G, *et al.* (2013) A mixture of *trans*-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. *J Nutr* **143**, 324–331.
49. Whelan K, Efthymiou L, Judd PA, *et al.* (2006) Appetite during consumption of enteral formula as a sole source of nutrition: the effect of supplementing pea-fibre and fructo-oligosaccharides. *Br J Nutr* **96**, 350–356.
50. Flint HJ, Scott KP, Louis P, *et al.* (2012) The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**, 577–589.
51. Cani PD, Hoste S, Guiot Y, *et al.* (2007) Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *Br J Nutr* **98**, 32–37.
52. Gao Z, Yin J, Zhang J, *et al.* (2009) Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**, 1509–1517.
53. Backhed F, Ding H, Wang T, *et al.* (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* **101**, 15718–15723.
54. Aronsson L, Huang Y, Parini P, *et al.* (2010) Decreased fat storage by *Lactobacillus paracasei* is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). *PLoS ONE* **5**, e13087.
55. Parnell JA & Reimer RA (2012) Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. *Gut Microbes* **3**, 29–34.
56. Everard A, Lazarevic V, Derrien M, *et al.* (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **60**, 2775–2786.
57. Nyman M (2002) Fermentation and bulking capacity of indigestible carbohydrates: the case of inulin and oligofructose. *Br J Nutr* **87**, Suppl. 2, S163–S168.
58. Perrigue MM, Monsivais P & Drewnowski A (2009) Added soluble fiber enhances the satiating power of low-energy-density liquid yogurts. *J Am Diet Assoc* **109**, 1862–1868.
59. Tarini J & Wolever TM (2010) The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl Physiol Nutr Metab* **35**, 9–16.
60. Costabile A, Klinder A, Fava F, *et al.* (2008) Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, cross-over study. *Br J Nutr* **99**, 110–120.
61. Freeland KR, Wilson C & Wolever TM (2010) Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *Br J Nutr* **103**, 82–90.
62. Serino M, Luche E, Gres S, *et al.* (2012) Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut* **61**, 543–553.
63. Cani PD, Amar J, Iglesias MA, *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772.
64. Cani PD, Osto M, Geurts L, *et al.* (2012) Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* **3**, 279–288.
65. Nakamura YK & Omaye ST (2012) Metabolic diseases and pro- and prebiotics: Mechanistic insights. *Nutr Metab (Lond)* **9**, 60.
66. Liang H, Hussey SE, Sanchez-Avila A, *et al.* (2013) Effect of lipopolysaccharide on inflammation and insulin action in human muscle. *PLOS ONE* **8**, e63983.
67. Creely SJ, McTernan PG, Kusminski CM, *et al.* (2007) Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am J Physiol Endocrinol Metab* **292**, E740–E747.
68. Amar J (2010) Energy intake is associated with endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes* **59**, 9.
69. Delzenne NM & Cani PD (2011) Interaction between obesity and the gut microbiota: relevance in nutrition. *Annu Rev Nutr* **31**, 15–31.
70. Eun CS, Han DS, Lee SH, *et al.* (2006) Attenuation of colonic inflammation by PPARgamma in intestinal epithelial cells: effect on Toll-like receptor pathway. *Dig Dis Sci* **51**, 693–697.
71. Lewis K, Lutgendorff F, Phan V, *et al.* (2010) Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. *Inflamm Bowel Dis* **16**, 1138–1148.
72. Cani PD (2012) Crosstalk between the gut microbiota and the endocannabinoid system: impact on the gut barrier function and the adipose tissue. *Clin Microbiol Infect* **18**, Suppl. 4, 50–53.
73. Garcia AL, Otto B, Reich SC, *et al.* (2007) Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance. *Eur J Clin Nutr* **61**, 334–341.

74. Lu ZX, Walker KZ, Muir JG, *et al.* (2004) Arabinoxylan fibre improves metabolic control in people with type II diabetes. *Eur J Clin Nutr* **58**, 621–628.
75. Guo Z, Liu XM, Zhang QX, *et al.* (2012) Effects of inulin on the plasma lipid profile of normolipidemic and hyperlipidemic subjects: a meta-analysis of randomized controlled trials. *Clin Lipidol* **7**, 215–222.
76. Koren O, Spor A, Felin J, *et al.* (2011) Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci U S A* **108**, Suppl. 1, 4592–4598.
77. Wang Z, Klipfell E, Bennett BJ, *et al.* (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63.
78. Baddini Feitoza A, Fernandes Pereira A, Ferreira da Costa N, *et al.* (2009) Conjugated linoleic acid (CLA): effect modulation of body composition and lipid profile. *Nutr Hosp* **24**, 422–428.
79. Fava F, Lovegrove JA, Gitau R, *et al.* (2006) The gut microbiota and lipid metabolism: implications for human health and coronary heart disease. *Curr Med Chem* **13**, 3005–3021.
80. Brighenti F (2007) Dietary fructans and serum triacylglycerols: a meta-analysis of randomized controlled trials. *J Nutr* **137**, 2552S–2556S.
81. Henao-Mejia J, Elinav E, Jin C, *et al.* (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**, 179–185.
82. Sheth AA & Garcia-Tsao G (2008) Probiotics and liver disease. *J Clin Gastroenterol* **42**, Suppl. 2, S80–S84.
83. Gratz SW, Mykkanen H & El-Nezami HS (2010) Probiotics and gut health: a special focus on liver diseases. *World J Gastroenterol* **16**, 403–410.
84. Malaguarnera M, Vacante M, Antic T, *et al.* (2012) *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci* **57**, 545–553.
85. Schley PD & Field CJ (2002) The immune-enhancing effects of dietary fibres and prebiotics. *Br J Nutr* **87**, Suppl. 2, S221–S230.
86. Macfarlane GT & Macfarlane S (2011) Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. *J Clin Gastroenterol* **45**, S120–S127.
87. Maslowski KM & Mackay CR (2011) Diet, gut microbiota and immune responses. *Nat Immunol* **12**, 5–9.
88. Stoyanova S, Geuns J, Hideg E, *et al.* (2011) The food additives inulin and stevioside counteract oxidative stress. *Int J Food Sci Nutr* **62**, 207–214.
89. Russell WR, Gratz SW, Duncan SH, *et al.* (2011) High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* **93**, 1062–1072.
90. Walton GE, van den Heuvel EG, Kosters MH, *et al.* (2012) A randomised crossover study investigating the effects of galacto-oligosaccharides on the faecal microbiota in men and women over 50 years of age. *Br J Nutr* **107**, 1466–1475.

British Journal of Nutrition

Chapter 6:

Could dietary prebiotic supplementation effect the advanced glycation pathway?

Highly cross-linked dietary AGEs are believed to escape digestion in the upper small intestine, and act as a substrate for the growth of pathogenic bacteria in the colon [41]. It is currently unknown whether prebiotic-induced changes in the composition of the human gut microbiota has any effect on the endogenous production or exogenous absorption of AGEs and their dicarbonyl precursors. Commercial dietary prebiotic supplements derived from chicory root or Jerusalem artichoke which contain a mixture of inulin and fructo-oligosaccharides are now widely available to purchase. These supplements are available in a finely powdered form, are tasteless and can be dissolved in liquids and semi-liquid foods. Proposed mechanisms whereby dietary prebiotic consumption might reduce AGE formation and absorption are outlined in Figure 5, Table 5 and Figure 6.

Production of SCFAs by gut microbial fermentation of prebiotics lowers the colonic pH. This changes the colonic environment to favour the proliferation of carbohydrate-degrading bacteria. At the same time, growth of protein-fermenting bacteria is reduced, limiting the accumulation of toxic metabolites, particularly ammonia. The SCFA acetate increases colonic blood flow while production of butyrate is critical as the principal energetic fuel used by colonocytes [208]. Butyrate also has important effects promoting epithelial barrier function (Figure 6). Firstly, it enhances the expression of nuclear receptor PPAR-γ in colonic epithelial cells which in turn has many flow-on effects, such as attenuating production of cytokines and other inflammatory factors and maintaining health of epithelial cells lining the colon to reduce gastrointestinal permeability [209]. Secondly, butyrate upregulates the mucin-associated genes responsible for generating production of the thick mucin layer that plays an important role in maintaining the integrity of the intestinal mucosal barrier [210].

Additionally, bacterial prebiotic fermentation products support gut barrier function by increasing the production of glucagon-like peptide 2 (GLP-2), which promotes the proliferation of the crypt cells from which new epithelial cells are derived [211]. GLP-2 also upregulates the transcription of tight-junction proteins, which connect the actin cytoskeletons of adjacent colonocytes [212].

Figure 5. Potential mechanisms for AGE reduction by prebiotic consumption

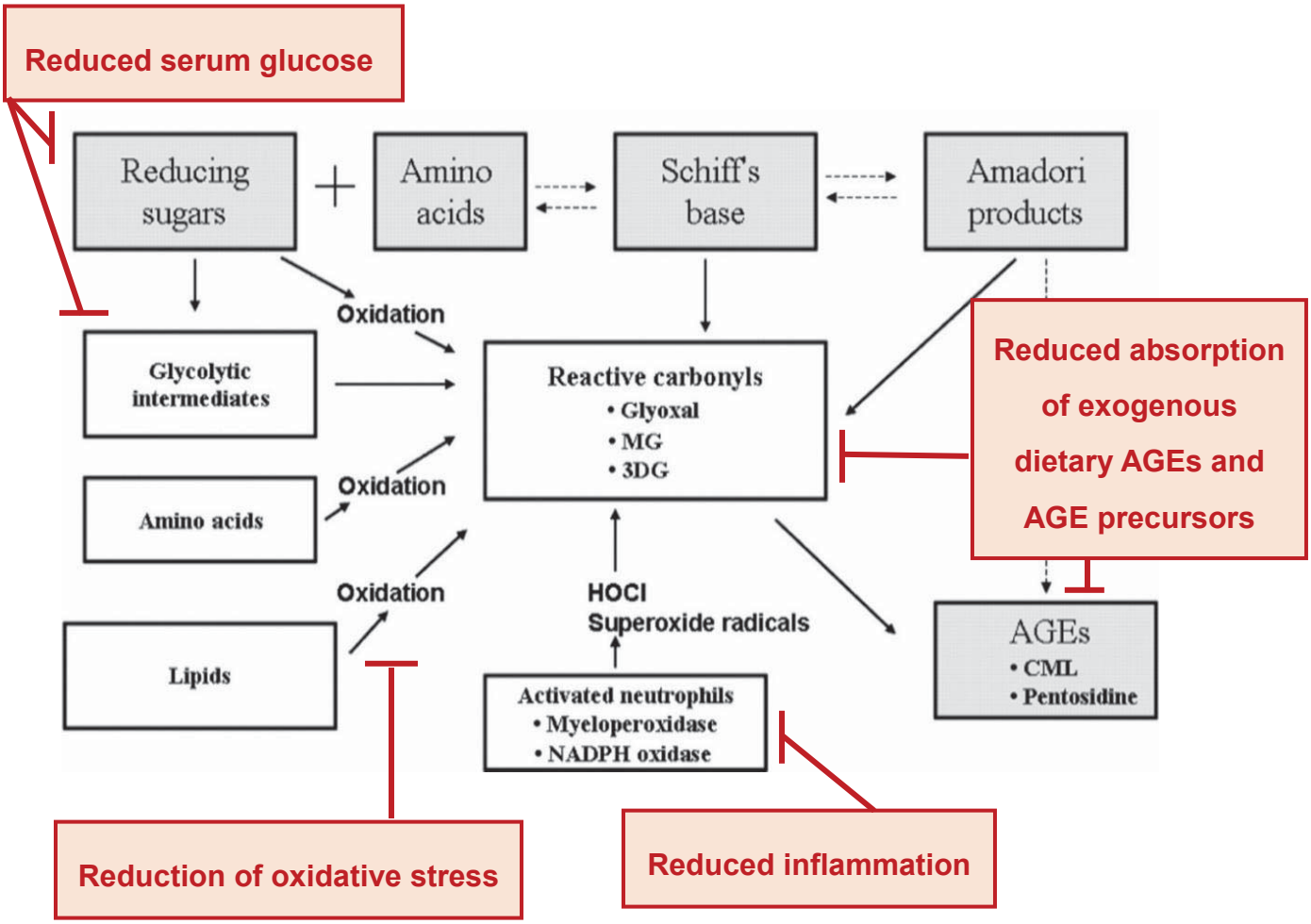
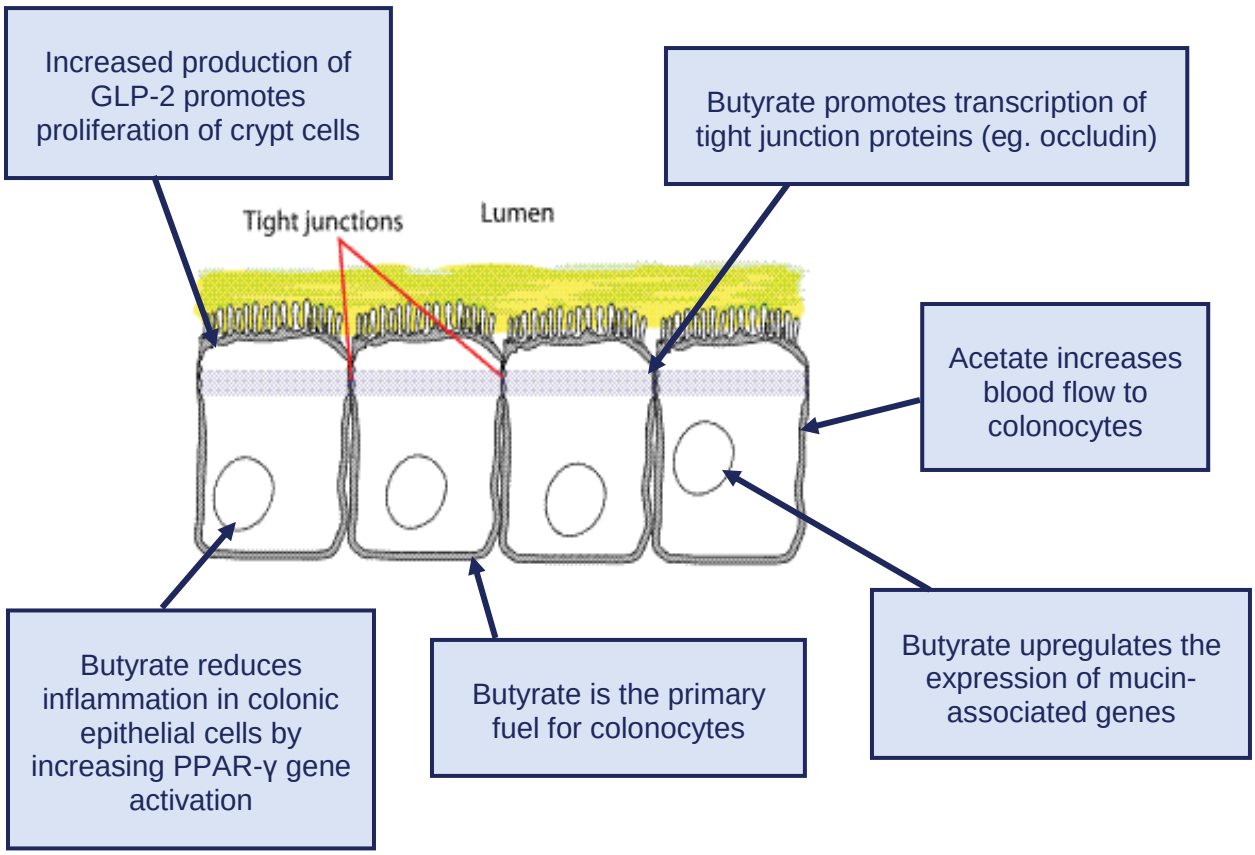


Table 5. Proposed role of prebiotic supplementation in AGE reduction

Physiological outcome	Mechanism for AGE reduction	Role of prebiotic supplement
Restoration of optimal microbial balance in the gastrointestinal tract	↑ growth of beneficial bacteria (may prevent highly cross-linked dietary AGEs which escape digestion in the small intestine acting as a colonic fermentation substrate for pathogenic microbes).	↑ growth of lactic-acid producing bacterial species; ↓ pH of GIT by lactate & SCFAs prevents growth of undesirable species [194]
Reduced gastrointestinal permeability	↓ intestinal absorption of dietary AGEs and other deleterious molecules (Figure 5, Figure 6)	↑ *Akkermansia muciniphilia* → ↑ integrity of GIT mucus layer → ↓ translocation of LPS into circulation [195]; ↑ SCFA → promotes GIT health [196]; ↑ SCFA → ↑ GLP-2, ↑ production of tight junction proteins occludin, ZO-1, clauden [197]
Improved glycemic control	↓ glucose substrate for AGE formation (Figure 5)	↑ SCFA → ↑ GIT gluconeogenesis [198]; ↑ SCFA → ↑ GLP-1 → ↑ Gastric emptying times, ↑ glucose stimulated insulin secretion [199]
Regulation of immune function and reduction of inflammation	↓ production of carbonyl compounds (Figure 5) ↓ ligands (eg. HMGB1) interacting with RAGE	↓ permeability of GIT epithelium → ↓ absorption of LPS → ↓ immune cell activation, ↓ production of pro-inflammatory cytokines [200]
Reduced oxidative stress and improved insulin sensitivity	↓ glycoxidation of glucose ↓ auto-oxidation of fatty acids resulting in ↓ production of carbonyl compounds (Figure 5)	↑ SCFA → ↑ intestinal ALP activity, ↓ GIT absorption of LPS → ↓ immune cell activation, ↓ ROS [181]; ↑ SCFA binds to GPR43 → ↑ GLP-1 release from intestinal L-cells → ↑ insulin sensitivity [201]
Appetite regulation and reduced body fat	↓ free fatty acids ↓ inflammation ↑ glucose tolerance	↑ SCFA binds to GPR41 → ↑ PYY, ↑ GLP-1, ↓ Grehlin →↓ appetite, ↑ insulin sensitivity, ↓ gut motility, ↑ intestinal transit time [202]; Microbiota →↓Fiaf →↑LPL→↓fat storage [203]; ↑ SCFA binds to GPR43 on adipocytes → ↓ insulin signalling, ↓ fat accumulation [204]
Increased production and absorption of antioxidants, carbonyl traps & metal ion chelators	↓ production of carbonyl compounds	↑ Growth of bacteria with genes involved in vitamin synthesis (eg. Thiamine) → ↑ trapping of carbonyl groups [205]
Prevention of glutathione depletion	↑ detoxification of dicarbonyls by glyoxylase system	↑ SCFA → ↑ PPAR-gamma → ↓ ROS → ↑ glutathione [206]
Lipid lowering	↓ lipid substrate for AGE formation	↑ SCFA →↓ HMG-CoA reductase → ↓ cholesterol synthesis [174]; ↑ Production of secondary bile acids from cholesterol [207]

Abbreviations: ALP: Alkaline phosphatase; Fiaf: Fasting-induced adipose factor; GIT: Gastointestinal tract; GLP: Glucagon-like peptide; GPR: G-protein coupled receptor; HMGB1: High-mobility group box 1 protein; HMG-CoA: 3-hydroxy-3-methylglutaryl-Coenzyme A; LPL: Lipoprotein lipase; SCFAs: short chain fatty acids; ROS: Reactive Oxygen Species; TG: Triglyceride; LPS: Lipopolysaccharide; PPAR-gamma: Peroxisome proliferator-activated receptor gamma; PYY: Peptide YY; ZO-1: zonula occludens-1.

Figure 6. Role of SCFAs in the maintenance of gut barrier function



The following article describes the methodology of a double-blind randomised placebo-controlled crossover trial designed to determine the effect of 12 week consumption of a prebiotic dietary supplement on the advanced glycation pathway, insulin sensitivity and chronic low-grade inflammation in adults with pre-diabetes.

Article: Kellow NJ, Coughlan MT, Savige GS, Reid CM.

Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial.

BMC Endocrine Disorders 2014; 14: 55.

Kellow et al. BMC Endocrine Disorders 2014, 14:55
http://www.biomedcentral.com/1472-6823/14/55

BMC Endocrine Disorders

STUDY PROTOCOL Open Access

Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial

Nicole J Kellow[1*], Melinda T Coughlan[2,3], Gayle S Savige[1] and Christopher M Reid[1]

Abstract

Background: Advanced glycation endproducts (AGEs) contribute to the development of vascular complications of diabetes and have been recently implicated in the pathogenesis of diabetes. Since AGEs are generated within foodstuffs upon food processing, it is increasingly recognised that the modern diet is replete with AGEs. AGEs are thought to stimulate chronic low-grade inflammation and promote oxidative stress and have been linked to the development of insulin resistance. Simple therapeutic strategies targeted at attenuating the progression of chronic low-grade inflammation and insulin resistance are urgently required to prevent or slow the development of type 2 diabetes in susceptible individuals. Dietary modulation of the human colonic microbiota has been shown to confer a number of health benefits to the host, but its effect on advanced glycation is unknown. The aim of this article is to describe the methodology of a double-blind placebo-controlled randomised crossover trial designed to determine the effect of 12 week consumption of a prebiotic dietary supplement on the advanced glycation pathway, insulin sensitivity and chronic low-grade inflammation in adults with pre-diabetes.

Methods/Design: Thirty adults with pre-diabetes (Impaired Glucose Tolerance or Impaired Fasting Glucose) aged between 40–60 years will be randomly assigned to receive either 10 grams of prebiotic (inulin/oligofructose) daily or 10 grams placebo (maltodextrin) daily for 12 weeks. After a 2-week washout period, study subjects will crossover to receive the alternative dietary treatment for 12 weeks. The primary outcome is the difference in markers of the advanced glycation pathway carboxymethyllysine (CML) and methylglyoxal (MG) between experimental and control treatments. Secondary outcomes include HbA_{1c}, insulin sensitivity, lipid levels, blood pressure, serum glutathione, adiponectin, IL-6, E-selectin, myeloperoxidase, C-reactive protein, Toll-like Receptor 4 (TLR4), soluble receptor for AGE (sRAGE), urinary 8-isoprostanes, faecal bacterial composition and short chain fatty acid profile. Anthropometric measures including BMI and waist circumference will be collected in addition to comprehensive dietary and lifestyle data.

Discussion: Prebiotics which selectively stimulate the growth of beneficial bacteria in the human colon might offer protection against AGE-related pathology in people at risk of developing type 2 diabetes.

(Continued on next page)

* Correspondence: nicole.kellow@monash.edu
[1]Department of Epidemiology & Preventive Medicine, School of Public Health & Preventive Medicine, Monash University, The Alfred Centre, Melbourne, Victoria 3004, Australia
Full list of author information is available at the end of the article

(Continued from previous page)

Trial registration: Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12613000130763.

Keywords: Advanced glycation end products, Maillard reaction, Prebiotics, Gut microbiota, Type 2 diabetes mellitus, Insulin resistance, Inflammation

Background

Advanced Glycation Endproducts (AGEs) are formed via the Maillard reaction, which consists of a complex network of non-enzymatic reactions involving the carbonyl groups of reducing sugars which react with the amino groups of proteins [1]. AGEs are generated *in vivo* as a normal consequence of metabolism, but their formation is accelerated under conditions where blood glucose is chronically elevated such as poorly controlled diabetes [2]. AGE formation is also increased in the presence of oxidative stress, which is frequently observed in individuals with the metabolic syndrome [3,4]. Overproduction of reactive oxygen species (ROS) can result in maladaptive responses including interruption of cellular glycolysis, which can generate highly reactive dicarbonyl compounds capable of rapid AGE formation [5].

Excessive AGE accumulation can lead to several pathophysiological consequences. AGE-modification of proteins results in changes in structure and/or function. For example, the AGE-modification of extracellular collagen reduces its elasticity and solubility, and results in increased stiffness, disturbed cellular adhesion and reduced turnover contributing to basement membrane thickening [6]. Intracellularly, AGE-modification of mitochondrial proteins is associated with suppression in the activity of respiratory chain enzymes and overproduction of ROS [7,8]. Indeed, glycated proteins provide stable active sites for catalysing the formation of free radicals [9]. Finally, AGEs are able to bind and activate a range of receptors, which then trigger a downstream cascade of pathogenic mediators. Interaction of AGEs with the Receptor for AGEs (RAGE) promotes activation of the transcription factor nuclear factor kappa-B (NF-κB), with subsequent upregulation of chemokines, such as MCP-1 and profibrogenic mediators such as TGFβ in addition to pro-inflammatory cytokines which are known to be involved in thrombogenesis, vascular inflammation and pathological angiogenesis. These RAGE-mediated events contribute to many of the long-term complications of diabetes [10]. AGE/RAGE ligation also promotes overproduction of ROS which can then activate NF-κB [11], a key driver of inflammation.

More recently, AGEs have been implicated in the pathogenesis of both type 1 and type 2 diabetes. Several studies have shown that AGEs are associated with insulin resistance [12,13], and can induce low-grade inflammation [14] and pancreatic beta cell dysfunction [15,16].

In contrast to endogenous AGE formation, AGEs are also absorbed by the body from exogenous sources such as cigarette smoke and through consumption of processed foods [17]. Since AGEs are generated within foodstuffs upon heating and food processing, it is increasingly recognised that the modern diet is replete with AGEs [18]. AGE-restricted diets can arrest the development of type 2 diabetes in animal models [19]. A recent study found that excess consumption of AGE-precursors in mice over several generations led to the development of insulin resistance [20]. Human trials have found that dietary AGE restriction can improve insulin sensitivity [21,22] and decrease markers of oxidative stress [23] or inflammation [24]. Further studies are required to confirm the long-term benefits of dietary AGE-restriction in humans [25]. However, simple, safe and effective interventions which prevent or minimise excessive AGE accumulation and subsequent AGE-related pathology in people with diabetes and/or in those at risk of developing the condition are warranted.

Interventions which influence the human intestinal microbiota are worthy of further investigation given that specific micro-organisms have the ability to significantly affect host metabolism. Gut bacteria play an important role in the host immune system, modulation of inflammatory processes, extraction of energy from the host's diet, fermentation of dietary fibres to produce short-chain fatty acids, alteration of human gene expression, regulation of intestinal permeability, production of some vitamins and promotion of mineral absorption by the host [26-31]. Furthermore, the total quantity and relative proportions of distinct bacterial species found in the colon differ between lean and obese individuals as well as between individuals with and without diabetes [32,33].

It is thought that certain dietary AGEs are largely undigested by human gut enzymes and eventually enter the colon, where they may act as a growth substrate for detrimental bacteria such as some Clostridium and Bacteroides species [34]. Therefore it is conceivable that individuals who consume highly processed diets (which contain large quantities of AGEs) may adversely alter their colonic microbial composition, potentially enhancing their risk for the development of metabolic diseases such as obesity and type 2 diabetes [35].

Therapeutic manipulation of the gut microbiota and restoration of normobiosis could potentially reduce circulating AGE levels and improve the metabolic health

of individuals at risk for the development of type 2 diabetes. Regular consumption of prebiotics to promote the growth of beneficial gut bacterial flora is one such avenue currently under investigation. Prebiotics are non-digestable plant-derived carbohydrates which confer health benefits to the host by acting as a fermentation substrate in the colon, stimulating the preferential growth and activity of a limited number of beneficial microbial species [36]. Supplementation of the human diet with prebiotic fructans such as inulin or fructo-oligosaccharides alters the bacterial composition of the large intestine by favouring the selective proliferation of beneficial lactic acid-producing species such as bifidobacteria and lactobacilli. Prebiotic-stimulated increases in intestinal Bifidobacterium species have been shown to attenuate the production of ROS and markers of inflammation in individuals consuming high fat diets [37].

While the complex interactions between diet, intestinal microbiota and host metabolism are still being elucidated, no studies have investigated the effect of dietary prebiotics on circulating AGE concentrations. This trial was designed to investigate the effect of a prebiotic dietary supplement on AGE accumulation and explore changes to the growth and activity of specific gut microbiota in adults diagnosed with prediabetes.

Methods/Design

Study design and setting

This is a 6.5-month randomised crossover controlled clinical trial (RCT) in which adults aged 40–60 years with diagnosed pre-diabetes will be enrolled. Potential study participants will be identified from General Practice (GP) clinics throughout South Gippsland, Victoria. The study design is presented in Figure 1.

Inclusion criteria

Individuals aged between 40–60 years and diagnosed with prediabetes (Impaired Fasting Glucose or Impaired Glucose Tolerance) within the previous 12 months. Diagnosis will have been made at each individual's local GP clinic after undertaking an Oral Glucose Tolerance Test (OGTT). Prediabetes was defined as a fasting plasma glucose concentration ≥ 6.1 and < 7.0 mmol/L followed by a 2-hour post glucose load glucose concentration < 7.8 mmol/L, or a fasting plasma glucose < 7.0 mmol/L followed by a 2-hour post glucose load glucose concentration ≥ 7.8 and < 11.1 mmol/L [38].

Exclusion criteria

Individuals previously diagnosed with type 1, type 2 diabetes or impaired renal function (eGFR <90 mL/min/1.73 m^2), individuals with known gastrointestinal pathology (coeliac disease, inflammatory bowel disease), pregnant women, smokers, individuals who have taken antibiotics, dietary prebiotic or probiotic nutritional supplements within the previous three months, individuals taking aspirin or Vitamin B, individuals who have made major dietary or lifestyle changes in the previous three months, individuals who are unwilling to provide blood, urine and stool samples or are unable to attend their local pathology collection centre.

Ethics

The trial has received ethical approval from the Monash University Human Research Ethics Committee.

Sample size calculation

The minimum difference we wish to detect is 0.4 micromol/L serum CML (20% reduction in CML), with a standard deviation of 0.4 [39] with 5% Significance and 80% Power. This calculates as a total sample size of 18 individuals, plus 12 individuals to allow for withdrawals = 30 subjects required. Epidemiological studies have demonstrated a positive correlation between serum CML and all-cause and cardiovascular mortality, cardiovascular disease, glucose intolerance, impaired insulin secretion, renal impairment and diabetic vascular complications [40,41]. A 0.4 micromol/L increase in serum CML concentration represented a 68% increased risk for all-cause mortality over seven years in a large prospective cohort study [42].

Baseline assessment

The study timeline is presented in Figure 2. Following recruitment and screening, consenting participants will undergo a baseline assessment at their local GP clinic. The baseline assessment will be undertaken by the research dietitian, who will collect demographic details, medical and social history (living situation, marital status, current occupation), physical activity questionnaire, dietary intake assessment, anthropometric measurements including Body Mass Index (BMI) and waist circumference. Participants will also be instructed to attend the local pathology centre to provide a stool sample, 24-hour urine collection, and have blood taken for analysis.

Randomisation

Randomisation of participants to intervention/placebo sequence will be completed by a local pharmacist using a permuted-block randomisation stratified by gender via the web site www.randomization.com. The pharmacist will distribute the experimental and placebo nutritional supplements to participants in the appropriate sequence. Other than provision of supplements, the pharmacist will have no contact with study participants or involvement in data collection or analysis. Dietary supplements will be provided in sealed opaque packages which will be weighed at the conclusion of the study in order to assess compliance. The supplements will be packaged

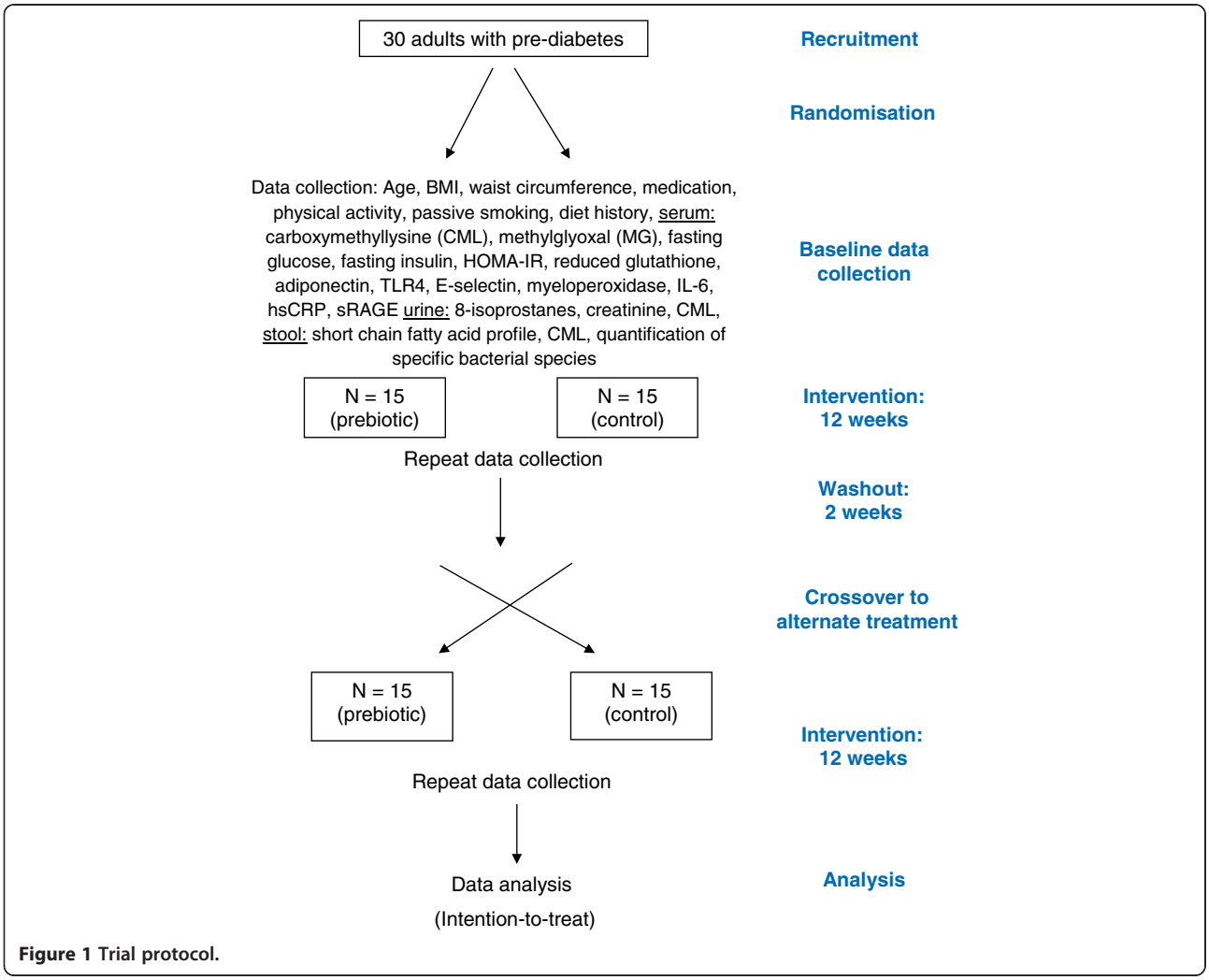


Figure 1 Trial protocol.

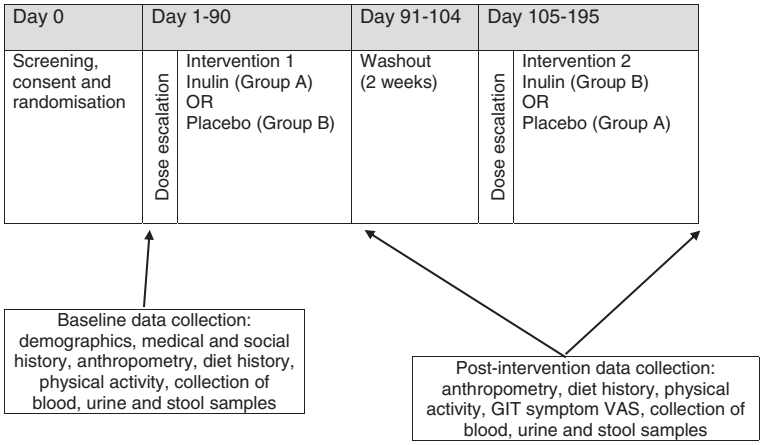


Figure 2 Study flowchart.

off-site by an external pharmaceutical packaging agency. Each package will contain a participant's study identification number and will be labelled as either Supplement 1 or Supplement 2 corresponding to the first and second intervention periods. This will ensure all investigators and participants are blinded to the treatment. Blinding will cease only after statistical analysis of the data has been completed.

Intervention

Participants will be randomly assigned to receive either 10 g of chicory-derived inulin/oligofructose powder (kindly provided by Beneo-Orafti Active Food Ingredients, Belgium) (intervention) or 10 g of maltodextrin powder (placebo) daily. Previous studies have demonstrated the bifidogenic effect of daily consumption of 5 g and 8 g dietary inulin supplements [43]. The inulin and maltodextrin powders to be consumed are both tasteless and can be mixed into hot or cold liquids or semi-solid foods. Participants will be instructed how to incorporate their supplement into their usual diet, and advised to gradually increase their dose over ten successive days until the target dose is reached. This stepped escalation in supplement dose aims to minimise gastrointestinal discomfort for participants, as a sudden increase in dietary prebiotic intake may result in increased stool frequency, abdominal bloating and flatulence until the bowel adapts to the increased fibre intake [44]. Written instructions will also be provided. Participants will be advised to consume each dietary supplement daily for 90 days, and otherwise maintain their usual dietary intake and level of physical activity. Gastrointestinal tolerance to the dietary supplements will be indicated by each study participant using a visual analogue scale as described below.

Follow up visits

Visits to the GP clinic will be scheduled for each participant at the conclusion of both Intervention 1 and Intervention 2 treatment periods, in order to provide follow-up data. Information collected will include anthropometric measurements, dietary intake assessment and physical activity assessment. Each participant will complete a self-administered questionnaire designed to assess gastrointestinal tolerance to the dietary supplement. Completed questionnaires will be placed into sealed opaque envelopes in order to maintain blinding of the researcher collecting data during the follow-up visits. Participants will also be instructed to attend the local pathology centre to provide urine and stool samples, and have blood taken for analysis.

Safety considerations

All adverse events will be documented.

Outcome measurements

The primary outcome of interest is the difference in serum AGE and AGE-precursor concentration (measured as CML and MG respectively) between experimental and control treatments. Secondary outcomes include HbA1c, insulin resistance (measured indirectly by homeostasis model assessment), antioxidant capacity (reduced glutathione), markers of oxidative stress on lipid molecules (urinary 8-isoprostanes), inflammatory biomarkers (serum IL-6, high sensitivity C-reactive protein, MCP-1, sRAGE), adhesion molecules (E-selectin), gut barrier integrity (TLR4), 24-hour urine and faecal CML and MG concentrations, faecal bacterial composition (*Bifidobacterium* spp., *Lactobacillus* spp., *Roseburia* spp., *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*) and faecal short chain fatty acid concentrations. Lipid levels (serum total cholesterol, LDL, HDL, TG) and blood pressure will also be measured. Anthropometric measurements including BMI and waist circumference will be collected, in addition to comprehensive dietary and lifestyle data. Gastrointestinal side-effects will be assessed using a visual analogue scale adapted from Lecerf *et al.* [45]. The scale rates nine items including flatulence, bloating, rumbling, abdominal cramps, a global digestive tolerance score calculated from the five previous items, stool consistency, stool frequency and general well-being through disturbances in usual and professional activities as well as disturbances in personal and social activities. Items are scored on a 10 cm linear scale.

Data collection

Dietary intake

An experienced research dietitian will obtain a comprehensive dietary history from each study participant at baseline and at the completion of each intervention period. A dietary history is a structured interview method consisting of questions regarding habitual food intake. It involves a 24-hour diet recall followed by a food frequency questionnaire to clarify information about usual consumption over the previous month. Usual portion sizes will be obtained in household measures and with the use of photographic aids. The dietitian will review the dietary history with each participant, probing for details on portion sizes and cooking methods in order to improve data accuracy. Dietary AGE content will be estimated from an open source database which lists the AGE concentration of foods using validated analytical techniques [46]. Dietary macro and micronutrient intakes will be estimated using the Foodworks nutrient software program (Xyris Software, NSW, Australia). Each participant will also be randomly contacted by telephone during the study and asked to provide a 24-hour diet recall, and changes in urinary urea excretion will be monitored in order to validate the dietary history data collected [47].

Physical activity will be estimated by asking participants to complete the International Physical Activity Questionnaire (Short Form) prior to and at the completion of experimental and placebo intervention periods [48].

Anthropometry

Body weight will be measured in participants wearing light clothes without shoes using a digital scale (Seca, Germany) to the nearest 0.1 kg. Height will be measured using a portable stadiometer (Seca, Germany) to the nearest 0.1 cm. BMI is calculated by dividing weight (kg) per height (m) square. Waist circumference will be measured at the midpoint of the lowest rib and iliac crest using a measuring tape to the nearest 0.1 cm. Body composition (total body water, fat mass, fat-free mass) will be determined using Bioelectrical Impedance Analysis (BodyStat-1500, Bodystat, Douglas, Isle of Man, United Kingdom). Ambulatory blood pressure will be measured using an electronic blood pressure machine (Omron Corporation, Kyoto, Japan), with subjects at rest in a seated position. All anthropometric measurements will be conducted in duplicate, with the mean measurement recorded.

Laboratory investigations

Biological samples will be collected at baseline and at completion of each three-month supplement intervention period and stored at −80°C immediately after collection. Twenty ml of fasting venous blood will be collected from each participant by phlebotomy into a sodium fluoride EDTA tube, a heparin-lined vacuum tube and a clean glass test tube. Twenty-four hour urine collections and morning stool samples will be collected in sterile containers.

Serum, urinary and faecal CML will be measured using a competitive ELISA (AGE-CML ELISA, Microcoat, Penzberg, Germany) [49]. This assay has been validated [50], is specific, and shows no cross-reactivity with other compounds [49]. The within assay and between-assay coefficient of variation are both less than 5%, respectively. Methylglyoxal will be measured by HPLC. Serum total cholesterol and triglyceride concentrations will be determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol will be determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL-cholesterol will be calculated using the Friedewald formula. Plasma glucose levels will be determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin will be measured by enzymatic colorimetry (WAKO Pure-Chemical Industries, Osaka, Japan). Insulin Resistance (IR) will be estimated by the homeostasis model assessment (HOMA) index as [FI × (fasting glucose/22.5)], where FI is insulin in microunits per millilitre and fasting glucose is in millimoles per litre [51,52]. HbA1c will be measured by autoanalyser (Roche Diagnostics, Mannheim, Germany). Plasma IL-6, MCP-1, E-selectin, hsCRP, TLR4, glutathione (GSH), and myeloperoxidase will be measured by commercial ELISA kits (Biosource International, Camarillo, CA, USA). Urine 8-isoprostanes will be measured by ELISA (Oxford Biomedical Research, MI, USA).

Stool samples will be homogenised in a blender and stored at −20°C for SCFA analysis. Samples will be thawed and 5 g aliquots placed in Centriprep fluid concentrators, MWCO 30,000 kDa (Amicon Inc., Beverly, MA, USA). Samples will be centrifuged for 30 minutes at 1000 × g, room temperature and supernatants placed in 15 ml polypropylene tubes. 0.3 ml of 25% m-phosphoric acid will be added to each tube, samples will be vortexed and incubated at room temperature for 25 minutes. Samples will be centrifuged at 5000 × g for 15 minutes at room temperature. Supernatants will be decanted and frozen overnight. The following day, samples will be thawed and the pH of each sample adjusted to 6.5 using 4 N KOH. Oxalic acid will be added at a final concentration of 0.03% and SCFA concentrations determined by gas chromatography with use of a Hewlett-Packard 5880A gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) containing an 80/120 Carbopack B-DA/4% Carbowax 20 M column (Supelco Inc., Bellefonte, PA, USA).

Quantitative Real-time PCR will be used to determine faecal concentrations of *Bifidobacterium* spp., *Lactobacillus* spp., *Roseburia* spp., *Faecalbacterium prausnitzii* and *Akkermansia muciniphila*. The primers used will be based on the following 16S rRNA gene sequences: *Bifidobacterium* spp: F-CTCCTGGAAACGGGTGG and R-GGTGTTCTTCCCGATATCTACA [53], *Lactobacillus* spp: F-AGCAGTAGGGAATCTTCCA and R-CACCGCT ACACATGGAG [54], *Roseburia* spp: F-CGKACTAGAG TGTCGGAGG and R-GTCATCTAGAGTGTCGGAGG [55], *Faecalbacterium prausnitzii*: F-GGAGGAAGAAGG TCTTCGG and R-AATTCCGCCTACCTCTGCACT [56], and *Akkermansia muciniphila:* F-CAGCACGTGAA GGTGGGGAC and R-CCTTGCGGTTGGCTTCAGAT [57]. PCR amplification and detection will be achieved with an ABI 7300 Real-time PCR system (Applied Biosystems, Foster City, CA, USA) using Mighty Amp for Real-time (SYBR Plus) and Rox Reference Dye (Invitrogen, Carlsbad, CA, USA). Each assay will be performed in duplicate in the same run. The cycle threshold of each sample will then be compared with a standard curve (performed in duplicate) made by diluting genomic DNA (tenfold serial dilution). Prior to isolating the DNA, the cell counts will be determined in culture and expressed as "colony forming units" (CFU). Data will be expressed as log CFU/g of faeces.

Statistical analysis

Outcome analyses will be undertaken on an intention-to-treat basis. Data will be presented as means ± SD. The Kolmogorov-Smirnov goodness-of-fit test will be used to test for normal distribution, and data not normally distributed will be log-transformed. Correlation analyses will be performed using the Pearson correlation coefficient. Significance of changes during the study will be assessed by comparing change of means between placebo and prebiotic treatment periods by paired sample t-tests. Trial data will be analysed using a linear mixed model design based on repeated measures to account for fixed factors such as treatment sequence (inulin – placebo vs placebo - inulin) and treatment period (intervention 1 vs intervention 2) in addition to participants as a random factor. Significant differences will be defined as a value of $P < 0.05$ based on two-sided tests. Any differences in physical activity levels, anthropometry, energy or nutrient intake during the course of the trial will be identified using ANOVA. Gastrointestinal symptom data obtained by visual analogue scale will be analysed using the Wilcoxon signed rank test. Effect sizes including 95% confidence intervals will be calculated for all significant outcomes. Data analysis will be performed using SPSS 20.0 software (SPSS, Chicago, IL).

Discussion

AGEs are derived from both exogenous and endogenous sources, and the rate at which AGEs accumulate in the body is dependent to a large extent on the chronological age, lifestyle and metabolic health of an individual. Smoking cigarettes and consuming foods containing high concentrations of AGEs (and their precursors) increases the accumulation of AGEs from exogenous sources. Endogenous AGE formation is accelerated under conditions of hyperglycaemia, dyslipidaemia and increased oxidative stress, conditions that are common in individuals with diabetes and in those at risk of developing type 2 diabetes (such as those with prediabetes and the metabolic syndrome) [58]. Moreover, in individuals with impaired renal function, urinary AGE excretion may be diminished resulting in a greater accumulation of AGEs in the body [59].

Risk factors for the development of type 2 diabetes include obesity, hypertension and cardiovascular disease; conditions that are commonly associated with unhealthy lifestyles including poor food habits. Restricting the intake of foods high in AGEs might potentially reduce AGE accumulation, but adherence to such diets can be challenging given that foods high in AGEs are very palatable due to their enhanced flavour, colour and aroma [60].

Supplementation of the diet with bifidogenic prebiotic fibres (such as inulin) may reduce or retard the accumulation of AGEs in individuals at risk of developing type 2 diabetes. Prebiotics have been shown to improve and restore optimal microbial balance within the gastrointestinal tract, potentially reducing AGE absorption and/or production by the human host. Preliminary investigations indicate that consuming a high-AGE diet is sufficient to favour the proliferation of potentially pathogenic colonic bacteria over more beneficial species. Consumption of glycated proteins [34], fried meats [61] and toasted wheat flakes [62] encouraged the preferential growth of greater numbers of detrimental gram negative and sulphate-reducing colonic micro-organisms when compared to control diets. Short Chain Fatty Acids (SCFAs) produced as a bacterial by-product of prebiotic fermentation act to lower the intestinal pH, inhibiting the growth of protein-degrading micro-organisms capable of producing potentially toxic metabolites. SCFAs also stimulate colonic smooth muscle contractions, speeding intestinal transit and limiting the time available for protein fermentation and putrefaction to occur in the gut [63]. Therapeutic manipulation of the gut microbiota with prebiotics may restore gut normobiosis and reduce AGE accumulation in humans at risk for type 2 diabetes development by the following mechanisms:

Maintenance of gut barrier function

Kinetic studies have estimated that up to 30% of dietary AGEs consumed are intestinally absorbed [64]. Under circumstances of increased intestinal permeability, it is likely that greater quantities of dietary AGEs and their reactive dicarbonyl precursors may be able to gain entry into the systemic circulation. Elevated levels of circulating proinflammatory cytokines and ROS frequently observed in individuals with prediabetes are known to compromise tight junctions between cells, disrupting the integrity of the intestinal barrier and enabling the absorption of larger, potentially deleterious compounds [65]. Prebiotic fermentation products may reduce gastrointestinal permeability and as a result limit the absorption of exogenous AGEs.

The SCFAs acetate and butyrate are intricately involved in the maintenance of gut epithelial integrity. Acetate increases colonic blood flow and butyrate is the principal fuel for colonocytes, assisting to optimise epithelial cell health [66]. Butyrate reduces gastrointestinal permeability by enhancing the activation of the peroxisomal proliferator-activated receptor gamma (PPARgamma) gene, a nuclear receptor involved in the attenuation of inflammation in colonic epithelial cells [67]. Butyrate also upregulates the expression of mucin-associated genes important in maintaining the integrity of the intestinal mucosal barrier [68]. Oligofructose supplementation in mice has been shown to increase the expression of zonula and occludin, proteins important in the maintenance of tight junctions between gastrointestinal cells [69], and a dietary inulin intervention reduced markers of intestinal permeability in healthy adult males [70]. Butyrate is a histone deacetylase inhibitor and is likely to promote the transcription of these tight junction

proteins. Prebiotic-induced changes in gut microbiota also increase endogenous production of Glucagon-like peptide 2 (GLP-2), which enhances gut barrier function by promoting the proliferation of crypt cells [71,72].

Reduction of oxidative stress, inflammation and insulin resistance

Increased production of ROS stimulate endogenous AGE formation by oxidising glucose and unsaturated fatty acids to generate reactive dicarbonyls. Experimental drug treatments which attenuate oxidative stress have previously demonstrated reductions in serum AGE levels [73]. Cytokine production at sites of inflammation stimulate immune cell activation of NADPH oxidase (NOX) and production of myeloperoxidase, enzymes involved in the oxidation of amino acids to form AGE precursors [74]. Activated immune cells can also secrete the high-mobility group box 1 (HMGB-1) protein which is capable of binding to RAGE, thereby inducing further inflammation [75].

A high fat diet (independent of the level of obesity in the host) is associated with negative changes in bacterial communities within the colon [76]. In response to a high fat meal, bacterial lipopolysaccharide (LPS) translocates from the intestine into the host circulation, resulting in 'metabolic endotoxemia' [77,78]. LPS is a major component of the outer membrane of gram negative bacteria, and is a potent activator of the mammalian immune system. LPS interaction with immune cells stimulates macrophage over-production of ROS, enhances the secretion of pro-inflammatory cytokines, and contributes to weight gain and development of insulin resistance [79,80]. Individuals with type 2 diabetes have been found to possess endotoxemia levels 2-fold higher than people without diabetes [81]. Metabolic endotoxemia also positively correlates with total energy intake and fasting insulin levels in the general population [82]. In mice with high fat diet-induced metabolic endotoxemia, nutritional supplementation of the diet with prebiotics restored intestinal levels of gram positive bacteria (particularly Bifidobacterium species) and subsequently improved glucose tolerance and reduced circulating concentrations of LPS and pro-inflammatory cytokines [83]. Human trials involving dietary prebiotic supplementation have successfully reduced serum LPS levels [45] and markers of lipid peroxidation (a process which generates AGE precursors), possibly through the reduction of ROS production or the direct antioxidant ability of some Bifidobacterium and Lactobacillus bacterial species [84,85].

SCFAs produced as a bacterial by-product of prebiotic fermentation are absorbed into the host circulation, effecting the expression of a wide range of genes in distal tissues associated with cell proliferation, differentiation and apoptosis. SCFAs are ligands for the G-protein coupled receptors (GPRs) GPR41 and GPR43 [86] on immune cells. These receptors are involved in down-regulating inappropriate immune cell production of pro-inflammatory cytokines, chemokines and ROS [87]. The SCFAs acetate, proprionate and butyrate exhibit a variety of anti-inflammatory actions through inhibition of NFκB activation, prevention of LPS-stimulated TNFα production in neutrophils and suppression of cytokine production [31,88-90].

Numerous other immune modulating effects have been observed secondary to gut bacterial activity including the production of anti-inflammatory compounds such as polysaccharide A [91], peptidoglycan [92] and conjugated linoleic acid [93], and the induction of T-regulatory cells [26].

Promotion of weight reduction

An energy-restricted diet resulting in weight loss has been shown to reduce serum AGE levels in overweight and obese individuals [94]. The consumption of prebiotics in human clinical trials has promoted self-reported satiety [95], weight reduction, reduced production of the orexigenic hormone grehlin and stimulated expression of the appetite-reducing hormone peptide YY (PYY) [96]. Interaction with GPR41 by the SCFAs proprionate and butyrate increases satiety [97], upregulates PYY production and modulates the expression of leptin, a hormone important in controlling energy intake and expenditure [31].

In mice, the selective growth of certain lactobacillus species in the colon reduced body fat storage through the up-regulation of fiaf (fasting induced adipose factor) gene expression and inhibition of lipoprotein lipase (LPL) [98-100]. These findings may have the potential to affect weight reduction in humans and subsequently reduce circulating AGE levels.

Enhanced antioxidant capacity

Inulin enhances the proliferation of lactic acid producing bacteria capable of synthesising B-group vitamins, some of which have an antioxidant capacity [101]. These vitamins can be utilised by the human host to neutralise ROS. Vitamins B1 and B6 trap the carbonyl groups of highly reactive AGE precursors before they can react with proteins [102]. Some Lactobacillus and Bifidobacterium species are efficient scavengers of the lipid peroxidation product malandialdehyde, protecting the host from excessive accumulation of this toxic AGE precursor [84]. Inulin also exhibits antioxidant properties independent of altering gut bacterial growth and is able to scavenge a number of ROS, which may help to reduce lipid peroxidation in the stomach [103].

The SCFA butyrate, produced as a bacterial by-product of inulin fermentation, has been shown to increase colonic glutathione production [104]. Glutathione is an antioxidant co-factor required for glyoxalase I activity, an enzyme which degrades the AGE precursor methylglyoxyl. Increased production of ROS is also thought to deplete

glutathione levels. Through the reduction of oxidative stress, prebiotics may assist in the maintainance or up-regulation of the glyoxylase pathway.

Reduction of hyperglycemia

Both transient and chronic elevations in blood glucose increase endogenous AGE generation. Activation of GPR43 in adipocytes by proprionate inhibits lipolysis and lowers glycemia in healthy individuals [105]. Butyrate has been shown to reverse diet-induced insulin resistance in animal studies [106], possibly by enhancing PPARgamma expression which increases fatty acid oxidation in muscle.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to consumption of carbohydrates and fats. GLP-1 potentiates glucose-induced insulin secretion, reducing post-prandial blood glucose levels. GLP-1 also enhances satiety and slows gastric emptying. Prebiotic feeding in rats promoted L-cell differentiation in the colon and increased GLP-1 production [107,108], probably through an increase in bacterial production of butyrate.

The consumption of inulin as a dietary supplement may also influence circulating AGE concentrations independently of its prebiotic function. High glycemic index (GI) diets and their resultant hyperglycemic effect have been shown to enhance AGE formation in healthy individuals [109]. Inulin is a soluble fibre which when consumed daily may play a role in the reduction of the GI of the diet. Many soluble fibres are known for their ability to delay gastric emptying and slow the rate of intestinal nutrient absorption, reducing the GI of the carbohydrates in the meal. Regular consumption of prebiotic soluble fibre reduces fasting and post-prandial serum glucose levels in people with impaired glucose tolerance [110] and type 2 diabetes [111].

Study strengths

- To our knowledge, this is the first trial to investigate the potential effects of gut bacterial modulation on advanced glycation.
- Random allocation of participants to treatment sequence and intention to treat analysis will ensure study bias is minimised.
- Double-blind crossover placebo-controlled trial.
- Dietary data will be obtained to determine dietary patterns as well as estimates of total energy intake, fat and AGE consumption.
- CML in this study will be measured using a validated ELISA method.
- Dietary AGE consumption will be estimated from an open-source food AGE database, containing AGE values obtained using validated measurement techniques.

Study limitations

Type 2 diabetes is a chronic condition which often develops over decades, making it difficult to conduct intervention studies using the presence or absence of diabetes as the primary biological end-point. This study will measure surrogate biochemical markers of early type 2 diabetes pathogenesis, which could be considered a limitation of the trial. Long-term studies will need to be conducted in order to confirm the results of this research.

This study will measure serum CML as an indicator of AGE concentration in blood samples. Multiple other forms of AGEs exist, many of which have not yet been characterised, so the findings of this trial cannot be applied to all members of the AGE family. However, serum CML concentration shows a moderate to high correlation with other known circulating AGEs [39].

Applicability of research findings

In Australia, conservative estimates predict that at least 2 million adults will have been diagnosed with type 2 diabetes by 2025 [112]. The burden of disease associated with diabetes has a substantial impact on costs associated directly with health care as well as loss of productivity and decreased quality of life. The widespread consumption of high fat, heat processed foods and the increasing prevalence of obesity in Australia warrant simple interventions including those that prevent AGE-mediated damage. If dietary treatments aimed at altering the gut microbiota prove to be effective strategies for preventing or slowing the development of type 2 diabetes, they could become mainstream therapies for individuals with diabetes risk factors.

Abbreviations

AGE: Advanced glycation endproduct; sRAGE: Soluble receptor for advanced glycation endproducts; HOMA: Homeostasis model assessment; TAC: Total antioxidant capacity; TNFα: Tumour necrosis factor alpha; IL-6: Interleukin 6; VCAM-1: Vascular cell adhesion molecule 1; RAGE: Receptor for advanced glycation endproducts; CML: Carboxymethyl-lysine; MG: Methylglyoxal; LPS: Lipopolysaccharide; SCFA: Short chain fatty acid; GPR: G-protein coupled receptor; PYY: Peptide YY; GLP-2: Glucagon-like peptide 2; ACE: Angiotensin converting enzyme; DPP-4: Dipeptidyl peptidase 4; Fiaf: Fasting-induced adipocyte factor; ELISA: Enzyme linked immunosorbent assay; NFκB: Nuclear Factor kappa-B; TLR4: Toll-like receptor 4.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NJK and MTC designed the study, NJK drafted the manuscript with assistance from MTC, GSS and CMR. All authors read and approved the final manuscript.

Acknowledgements

The authors thank Associate Professor Rory Wolfe (SPHPM, Monash University) for advice regarding biostatistical analyses. NJ Kellow is the recipient of a National Health and Medical Research Council (NHMRC) Postgraduate Public Health Scholarship APP1039709. MT Coughlan is supported by an Australian and New Zealand Society of Nephrology Early Career Fellowship.

Author details
[1]Department of Epidemiology & Preventive Medicine, School of Public Health & Preventive Medicine, Monash University, The Alfred Centre, Melbourne, Victoria 3004, Australia. [2]Glycation, Nutrition & Metabolism Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria 8008, Australia. [3]Department of Medicine, Central Clinical School, Monash University, Alfred Medical Research & Education Precinct, Melbourne, Victoria 3004, Australia.

Received: 24 September 2013 Accepted: 30 June 2014
Published: 10 July 2014

References

1. Henle T: **Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods.** *Amino Acids* 2005, **29**(4):313–322.
2. Brownlee M: **Biochemistry and molecular cell biology of diabetic complications.** *Nature* 2001, **414**(6865):813–820.
3. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, Perego C, Muscogiuri G: **The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: avenues for a mechanistic-based therapeutic approach.** *Curr Diabetes Rev* 2011, **7**(5):313–324.
4. Kotani K, Sakane N: **C-reactive protein and reactive oxygen metabolites in subjects with metabolic syndrome.** *J Int Med Res* 2012, **40**(3):1074–1081.
5. Thornalley PJ: **Dicarbonyl intermediates in the maillard reaction.** *Ann N Y Acad Sci* 2005, **1043:**111–117.
6. Avery NC, Bailey AJ: **The effects of the Maillard reaction on the physical properties and cell interactions of collagen.** *Pathol Biol* 2006, **54**(7):387–395.
7. Rosca MG, Mustata TG, Kinter MT, Ozdemir AM, Kern TS, Szweda LI, Brownlee M, Monnier VM, Weiss MF: **Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation.** *Am J Physiol Renal Physiol* 2005, **289**(2):F420–F430.
8. Coughlan MT, Thorburn DR, Penfold SA, Laskowski A, Harcourt BE, Sourris KC, Tan AL, Fukami K, Thallas-Bonke V, Nawroth PP, Brownlee M, Bierhaus A, Cooper ME, Forbes JM: **RAGE-induced cytosolic ROS promote mitochondrial superoxide generation in diabetes.** *J Am Soc Nephrol* 2009, **20**(4):742–752.
9. Yim MB, Yim HS, Lee C, Kang SO, Chock PB: **Protein glycation: creation of catalytic sites for free radical generation.** *Ann N Y Acad Sci* 2001, **928:**48–53.
10. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D: **Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice: A potential mechanism for the accelerated vasculopathy of diabetes.** *J Clin Invest* 1995, **96**(3):1395–1403.
11. Morita M, Yano S, Yamaguchi T, Sugimoto T: **Advanced glycation end products-induced reactive oxygen species generation is partly through NF-kappa B activation in human aortic endothelial cells.** *J Diabetes Complications* 2013, **27**(1):11–15.
12. Tahara N, Yamagishi S, Matsui T, Takeuchi M, Nitta Y, Kodama N, Mizoguchi M, Imaizumi T: **Serum levels of advanced glycation end products (AGEs) are independent correlates of insulin resistance in nondiabetic subjects.** *Cardiovasc Ther* 2012, **30**(1):42–48.
13. Tan KC, Shiu SW, Wong Y, Tam X: **Serum advanced glycation end products (AGEs) are associated with insulin resistance.** *Diabetes Metab Res Rev* 2011, **27**(5):488–492.
14. Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H: **Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects.** *Ann N Y Acad Sci* 2005, **1043:**461–466.
15. Fiory F, Lombardi A, Miele C, Giudicelli J, Beguinot F, Van Obberghen E: **Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E.** *Diabetologia* 2011, **54**(11):2941–2952.
16. Coughlan MT, Yap FY, Tong DC, Andrikopoulos S, Gasser A, Thallas-Bonke V, Webster DE, Miyazaki J, Kay TW, Slattery RM, Kaye DM, Drew BG, Kingwell BA, Fourlanos S, Groop PH, Harrison LC, Knip M, Forbes JM: **Advanced glycation end products are direct modulators of beta-cell function.** *Diabetes* 2011, **60**(10):2523–2532.
17. Birlouez-Aragon I, Saavedra G, Tessier FJ, Galinier A, Ait-Ameur L, Lacoste F, Niamba CN, Alt N, Somoza V, Lecerf JM: **A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases.** *Am J Clin Nutr* 2010, **91**(5):1220–1226.
18. Tessier FJ, Birlouez-Aragon I: **Health effects of dietary Maillard reaction products: the results of ICARE and other studies.** *Amino Acids* 2012, **42**(4):1119–1131.
19. Sandu O, Song K, Cai W, Zheng F, Uribarri J, Vlassara H: **Insulin resistance and type 2 diabetes in high-fat-fed mice are linked to high glycotoxin intake.** *Diabetes* 2005, **54**(8):2314–2319.
20. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H: **Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1.** *Proc Natl Acad Sci U S A* 2012, **109**(39):15888–15893.
21. Uribarri J, Cai W, Ramdas M, Goodman S, Pyzik R, Chen X, Zhu L, Striker GE, Vlassara H: **Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1.** *Diabetes Care* 2011, **34**(7):1610–1616.
22. Mark AB, Poulsen MW, Andersen S, Andersen JM, Bak MJ, Ritz C, Holst JJ, Nielsen J, de Courten B, Dragsted LO, Bugel S: **Consumption of a diet low in advanced glycation endproducts for 4 weeks improves insulin sensitivity in overweight women.** *Diabetes Care* 2014, **37**(1):88–95.
23. Luevano-Contreras C, Garay-Sevilla ME, Wrobel K, Malacara JM: **Dietary advanced glycation end products restriction diminishes inflammation markers and oxidative stress in patients with type 2 diabetes mellitus.** *J Clin Biochem Nutr* 2013, **52**(1):22–26.
24. Harcourt BE, Sourris KC, Coughlan MT, Walker KZ, Dougherty SL, Andrikopoulos S, Morley AL, Thallas-Bonke V, Chand V, Penfold SA, de Courten MP, Thomas MC, Kingwell BA, Bierhaus A, Cooper ME, Courten B, Forbes JM: **Targeted reduction of advanced glycation improves renal function in obesity.** *Kidney Int* 2011, **80**(2):190–198.
25. Kellow NJ, Savige GS: **Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review.** *Eur J Clin Nutr* 2013, **67**(3):239–248.
26. Round JL, Mazmanian SK: **The gut microbiota shapes intestinal immune responses during health and disease.** *Nat Rev Immunol* 2009, **9**(5):313–323.
27. Ding S, Chi MM, Scull BP, Rigby R, Schwerbrock NM, Magness S, Jobin C, Lund PK: **High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse.** *PLoS One* 2010, **5**(8):e12191.
28. Diamant M, Blaak EE, de Vos WM: **Do nutrient-gut-microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes?** *Obes Rev* 2011, **12**(4):272–281.
29. Backhed F, Manchester JK, Semenkovich CF, Gordon JI: **Mechanisms underlying the resistance to diet-induced obesity in germ-free mice.** *Proc Natl Acad Sci U S A* 2007, **104**(3):979–984.
30. Hill MJ: **Intestinal flora and endogenous vitamin synthesis.** *Eur J Cancer Prev* 1997, **6**(Suppl 1):S43–S45.
31. Macfarlane GT, Macfarlane S: **Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics.** *J Clin Gastroenterol* 2011, **45**(Suppl):S120–S127.
32. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, Yu P, Zhao C, Li L, Zhou A, Wang J, Moore JE, Millar BC, Xu J: **Molecular characterisation of the faecal microbiota in patients with type II diabetes.** *Curr Microbiol* 2010, **61**(1):69–78.
33. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sorensen SJ, Hansen LH, Jakobsen M: **Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults.** *PLoS One* 2010, **5**(2):e9085.
34. Mills DJ, Tuohy KM, Booth J, Buck M, Crabbe MJ, Gibson GR, Ames JM: **Dietary glycated protein modulates the colonic microbiota towards a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis subjects.** *J Appl Microbiol* 2008, **105**(3):706–714.
35. Nakamura YK, Omaye ST: **Metabolic diseases and pro- and prebiotics: mechanistic insights.** *Nutr Metab (Lond)* 2012, **9**(1):60.
36. Quigley EM: **Therapies aimed at the gut microbiota and inflammation: antibiotics, prebiotics, probiotics, synbiotics, anti-inflammatory therapies.** *Gastroenterol Clin North Am* 2011, **40**(1):207–222.
37. Delzenne NM, Neyrinck AM, Backhed F, Cani PD: **Targeting gut microbiota in obesity: effects of prebiotics and probiotics.** *Nat Rev Endocrinol* 2011, **7**(11):639–646.
38. Colagiuri SDD, Girgis S, Colagiuri R: *National evidence based guideline for case detection and diagnosis of type 2 diabetes.* Canberra, Australia: Diabetes Australia and the National Health and Medical Research Council; 2009.
39. Semba RD, Arab L, Sun K, Nicklett EJ, Ferrucci L: **Fat mass is inversely associated with serum carboxymethyl-lysine, an advanced glycation end product, in adults.** *J Nutr* 2011, **141**(9):1726–1730.

40. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, Laakso M: **Increased serum levels of methylglyoxal-derived hydroimidazolone-AGE are associated with increased cardiovascular disease mortality in nondiabetic women.** *Atherosclerosis* 2009, **205**(2):590–594.
41. Lapolla A, Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, Baccarin L, Laverda B, Fedele D: **Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease.** *Diabetes Care* 2007, **30**(3):670–676.
42. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L: **Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults.** *J Am Geriatr Soc* 2009, **57**(10):1874–1880.
43. Kolida S, Meyer D, Gibson GR: **A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans.** *Eur J Clin Nutr* 2007, **61**(10):1189–1195.
44. Causey JL: **Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men.** *Nutr Res* 2000, **20**(2):191–201.
45. Lecerf JM, Depeint F, Clerc E, Dugenet Y, Niamba CN, Rhazi L, Cayzeele A, Abdelnour G, Jaruga A, Younes H, Jacobs H, Lambrey G, Abdelnour AM, Pouillart PR: **Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties.** *Br J Nutr* 2012, **108**(10):1847–1858.
46. Hull GLJ, Ames JM, Cuskelly GJ: **NEpsilon-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet.** *Food Chem* 2012, **131:**170–174.
47. Bihuniak JD, Simpson CA, Sullivan RR, Caseria DM, Kerstetter JE, Insogna KL: **Dietary protein-induced increases in urinary calcium are accompanied by similar increases in urinary nitrogen and urinary urea: a controlled clinical trial.** *J Acad Nutr Diet* 2013, **113**(3):447–451.
48. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P: **International physical activity questionnaire: 12-country reliability and validity.** *Med Sci Sports Exerc* 2003, **35**(8):1381–1395.
49. Boehm BO, Schilling S, Rosinger S, Lang GE, Lang GK, Kientsch-Engel R, Stahl P: **Elevated serum levels of N(epsilon)-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema.** *Diabetologia* 2004, **47**(8):1376–1379.
50. Zhang X, Frischmann M, Kientsch-Engel R, Steinmann K, Stopper H, Niwa T, Pischetsrieder M: **Two immunochemical assays to measure advanced glycation end-products in serum from dialysis patients.** *Clin Chem Lab Med* 2005, **43**(5):503–511.
51. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: **Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.** *Diabetologia* 1985, **28**(7):412–419.
52. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M: **Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity.** *Diabetes Care* 2000, **23**(1):57–63.
53. Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu H, Tanaka R: **Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces.** *Appl Environ Microbiol* 2002, **68**(11):5445–5451.
54. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM: **Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women.** *Gut* 2013, **62**(8):1112–1121.
55. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P: **Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii.** *Br J Nutr* 2009, **101**(4):541–550.
56. Wang RF, Cao WW, Cerniglia CE: **PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples.** *Appl Environ Microbiol* 1996, **62**(4):1242–1247.
57. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD: **Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity.** *Proc Natl Acad Sci U S A* 2013, **110**(22):9066–9071.
58. Sebekova K, Boor P, Valachovicova M, Blazicek P, Parrak V, Babinska K, Heidland A, Krajcovicova-Kudlackova M: **Association of metabolic syndrome risk factors with selected markers of oxidative status and microinflammation in healthy omnivores and vegetarians.** *Mol Nutr Food Res* 2006, **50**(9):858–868.
59. Vlassara H, Uribarri J, Cai W, Striker G: **Advanced glycation end product homeostasis: exogenous oxidants and innate defenses.** *Ann N Y Acad Sci* 2008, **1126:**46–52.
60. Goldberg T, Cai W, Peppa M, Dardaine V, Baliga BS, Uribarri J, Vlassara H: **Advanced glycoxidation end products in commonly consumed foods.** *J Am Diet Assoc* 2004, **104**(8):1287–1291.
61. Shen Q, Chen YA, Tuohy KM: **A comparative in vitro investigation into the effects of cooked meats on the human faecal microbiota.** *Anaerobe* 2010, **16**(6):572–577.
62. Connolly ML, Lovegrove JA, Tuohy KM: **In vitro fermentation characteristics of whole grain wheat flakes and the effect of toasting on prebiotic potential.** *J Med Food* 2012, **15**(1):33–43.
63. De Preter V, Hamer HM, Windey K, Verbeke K: **The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health?** *Mol Nutr Food Res* 2011, **55**(1):46–57.
64. Faist V, Erbersdobler HF: **Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction.** *Ann Nutr Metab* 2001, **45**(1):1–12.
65. Rapin JR, Wiernsperger N: **Possible links between intestinal permeability and food processing: A potential therapeutic niche for glutamine.** *Clinics (Sao Paulo)* 2010, **65**(6):635–643.
66. Scheppach W: **Effects of short chain fatty acids on gut morphology and function.** *Gut* 1994, **35**(1 Suppl):S35–S38.
67. Eun CS, Han DS, Lee SH, Paik CH, Chung YW, Lee J, Hahm JS: **Attenuation of colonic inflammation by PPARgamma in intestinal epithelial cells: effect on Toll-like receptor pathway.** *Dig Dis Sci* 2006, **51**(4):693–697.
68. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A: **Potential beneficial effects of butyrate in intestinal and extraintestinal diseases.** *World J Gastroenterol* 2011, **17**(12):1519–1528.
69. Delzenne NM, Cani PD: **Interaction between obesity and the gut microbiota: relevance in nutrition.** *Annu Rev Nutr* 2011, **31:**15–31.
70. Russo F, Linsalata M, Clemente C, Chiloiro M, Orlando A, Marconi E, Chimienti G, Riezzo G: **Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers.** *Nutr Res* 2012, **32**(12):940–946.
71. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM: **Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability.** *Gut* 2009, **58**(8):1091–1103.
72. Cani PD: **Crosstalk between the gut microbiota and the endocannabinoid system: impact on the gut barrier function and the adipose tissue.** *Clin Microbiol Infect* 2012, **18**(Suppl 4):50–53.
73. Schalkwijk CG, Miyata T: **Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics.** *Amino Acids* 2012, **42**(4):1193–1204.
74. Anderson MM, Heinecke JW: **Production of N(epsilon)-(carboxymethyl) lysine is impaired in mice deficient in NADPH oxidase: a role for phagocyte-derived oxidants in the formation of advanced glycation end products during inflammation.** *Diabetes* 2003, **52**(8):2137–2143.
75. Huebschmann AG, Regensteiner JG, Vlassara H, Reusch JE: **Diabetes and advanced glycoxidation end products.** *Diabetes Care* 2006, **29**(6):1420–1432.
76. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD: **High-fat diet determines the composition of the murine gut microbiome independently of obesity.** *Gastroenterology* 2009, **137**(5):1716–1724. e1711-1712.
77. Vrieze A, Holleman F, Zoetendal EG, de Vos WM, Hoekstra JB, Nieuwdorp M: **The environment within: how gut microbiota may influence metabolism and body composition.** *Diabetologia* 2010, **53**(4):606–613.
78. Esteve E, Ricart W, Fernandez-Real JM: **Gut microbiota interactions with obesity, insulin resistance and type 2 diabetes: did gut microbiote co-evolve with insulin resistance?** *Curr Opin Clin Nutr Metab Care* 2011, **14**(5):483–490.
79. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R: **Metabolic endotoxemia initiates obesity and insulin resistance.** *Diabetes* 2007, **56**(7):1761–1772.

80. Priebe MG, Wang H, Weening D, Schepers M, Preston T, Vonk RJ: **Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation.** *Am J Clin Nutr* 2010, **91**(1):90–97.
81. Creely SJ, McTernan PG, Kusminski CM, Fisher FM, Da Silva NF, Khanolkar M, Evans M, Harte AL, Kumar S: **Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes.** *Am J Physiol Endocrinol Metab* 2007, **292**(3):E740–E747.
82. Amar J: **Energy intake is associated with endotoxemia induces adipose inflammation and insulin resistance in humans.** *Diabetes* 2010, **59**:9.
83. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM: **Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia.** *Diabetologia* 2007, **50**(11):2374–2383.
84. Lin MY, Yen CL: **Inhibition of lipid peroxidation by Lactobacillus acidophilus and Bifidobacterium longum.** *J Agric Food Chem* 1999, **47**(9):3661–3664.
85. Yen CH, Kuo YW, Tseng YH, Lee MC, Chen HL: **Beneficial effects of fructo-oligosaccharides supplementation on fecal bifidobacteria and index of peroxidation status in constipated nursing-home residents–a placebo-controlled, diet-controlled trial.** *Nutrition* 2011, **27**(3):323–328.
86. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR: **Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43.** *Nature* 2009, **461**(7268):1282–1286.
87. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R: **Regulation of inflammation by short chain fatty acids.** *Nutrients* 2011, **3**(10):858–876.
88. Lewis K, Lutgendorff F, Phan V, Soderholm JD, Sherman PM, McKay DM: **Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate.** *Inflamm Bowel Dis* 2010, **16**(7):1138–1148.
89. Erridge C, Attina T, Spickett CM, Webb DJ: **A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation.** *Am J Clin Nutr* 2007, **86**(5):1286–1292.
90. Krogh-Madsen R, Plomgaard P, Akerstrom T, Moller K, Schmitz O, Pedersen BK: **Effect of short-term intralipid infusion on the immune response during low-dose endotoxemia in humans.** *Am J Physiol Endocrinol Metab* 2008, **294**(2):E371–E379.
91. Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, Vieira AT, Kranich J, Mackay CR: **Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases.** *Immunol Rev* 2012, **245**(1):164–176.
92. Maslowski KM, Mackay CR: **Diet, gut microbiota and immune responses.** *Nat Immunol* 2011, **12**(1):5–9.
93. Musso G, Gambino R, Cassader M: **Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes.** *Annu Rev Med* 2011, **62**:361–380.
94. Gugliucci A, Kotani K, Taing J, Matsuoka Y, Sano Y, Yoshimura M, Egawa K, Horikawa C, Kitagawa Y, Kiso Y, Kimura S, Sakane N: **Short-term low calorie diet intervention reduces serum advanced glycation end products in healthy overweight or obese adults.** *Ann Nutr Metab* 2009, **54**(3):197–201.
95. Cani PD, Joly E, Horsmans Y, Delzenne NM: **Oligofructose promotes satiety in healthy human: a pilot study.** *Eur J Clin Nutr* 2006, **60**(5):567–572.
96. Parnell JA, Reimer RA: **Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults.** *Am J Clin Nutr* 2009, **89**(6):1751–1759.
97. Ruijschop RBA, te Giffela MC: **Satiety effects of a dairy beverage fermented with propionic acid bacteria.** *Int Dairy J* 2008, **18**:5.
98. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI: **The gut microbiota as an environmental factor that regulates fat storage.** *Proc Natl Acad Sci U S A* 2004, **101**(44):15718–15723.
99. Aronsson L, Huang Y, Parini P, Korach-Andre M, Hakansson J, Gustafsson JA, Pettersson S, Arulampalam V, Rafter J: **Decreased fat storage by Lactobacillus paracasei is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4).** *PLoS One* 2010, **5**(9). doi:10.1371/journal.pone.0013087.
100. Dewulf EM, Cani PD, Neyrinck AM, Possemiers S, Van Holle A, Muccioli GG, Deldicque L, Bindels LB, Pachikian BD, Sohet FM, Mignolet E, Francaux M, Larondelle Y, Delzenne NM: **Inulin-type fructans with prebiotic properties counteract GPR43 overexpression and PPARgamma-related adipogenesis in the white adipose tissue of high-fat diet-fed mice.** *J Nutr Biochem* 2011, **22**(8):712–722.
101. Vyas U, Ranganathan N: **Probiotics, prebiotics, and synbiotics: gut and beyond.** *Gastroenterology Res Pract* 2012, **2012**:872716.
102. Mehta R, Shangari N, O'Brien PJ: **Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents.** *Mol Nutr Food Res* 2008, **52**(3):379–385.
103. Stoyanova S, Geuns J, Hideg E, Van Den Ende W: **The food additives inulin and stevioside counteract oxidative stress.** *Int J Food Sci Nutr* 2011, **62**(3):207–214.
104. Wu WT, Chen HL: **Effects of konjac glucomannan on putative risk factors for colon carcinogenesis in rats fed a high-fat diet.** *J Agric Food Chem* 2011, **59**(3):989–994.
105. Todesco T, Rao AV, Bosello O, Jenkins DJ: **Propionate lowers blood glucose and alters lipid metabolism in healthy subjects.** *Am J Clin Nutr* 1991, **54**(5):860–865.
106. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J: **Butyrate improves insulin sensitivity and increases energy expenditure in mice.** *Diabetes* 2009, **58**(7):1509–1517.
107. Cani PD, Hoste S, Guiot Y, Delzenne NM: **Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats.** *Br J Nutr* 2007, **98**(1):32–37.
108. Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R: **Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor.** *Diabetes* 2006, **55**(5):1484–1490.
109. Uchiki T, Weikel KA, Jiao W, Shang F, Caceres A, Pawlak D, Handa JT, Brownlee M, Nagaraj R, Taylor A: **Glycation-altered proteolysis as a pathobiologic mechanism that links dietary glycemic index, aging, and age-related disease (in nondiabetics).** *Aging Cell* 2012, **11**(1):1–13.
110. Garcia AL, Otto B, Reich SC, Weickert MO, Steiniger J, Machowetz A, Rudovich NN, Mohlig M, Katz N, Speth M, Meuser F, Doerfer J, Zunft HJ, Pfeiffer AH, Koebnick C: **Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance.** *Eur J Clin Nutr* 2007, **61**(3):334–341.
111. Lu ZX, Walker KZ, Muir JG, O'Dea K: **Arabinoxylan fibre improves metabolic control in people with Type II diabetes.** *Eur J Clin Nutr* 2004, **58**(4):621–628.
112. Magliano DJ, Peeters A, Vos T, Sicree R, Shaw J, Sindall C, Haby M, Begg SJ, Zimmet PZ: **Projecting the burden of diabetes in Australia–what is the size of the matter?** *Aust N Z J Public Health* 2009, **33**(6):540–543.

doi:10.1186/1472-6823-14-55
Cite this article as: Kellow *et al.*: Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial. *BMC Endocrine Disorders* 2014 **14**:55.

Chapter 7:

Can prebiotic-induced changes in the gut microbiota reduce AGEs and biomarkers of metabolic health in people with prediabetes?

Article: Kellow NJ, Sourris KC, Reid CM, Coughlan MT.
Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes. A randomised placebo-controlled crossover trial.
Manuscript submitted, 2016

The following article reports on the preliminary findings of the randomised placebo-controlled crossover trial described in Chapter 6 of this thesis. Variables which have been measured and are presented in the following chapter include patient anthropometry, blood pressure, some biochemical variables (including fasting blood glucose, HbA1c, HOMA-IR, lipid studies, urea & electrolytes, liver function tests, inflammatory markers, urine albumin), plasma and urinary CML concentrations (measured by ELISA), dietary intake data and gastrointestinal symptoms.

Some variables are still to be measured, and will not be presented in this thesis. Stool samples were collected from all trial participants, and funding applications have been submitted to enable analysis of the bacterial composition and the SCFA concentration of these samples to be undertaken in future. Additional serum and urine samples were also collected and frozen in preparation for transport to an overseas laboratory for AGE analysis by tandem mass spectrometry – high performance liquid chromatography (LC-MS/MS), which is considered the most accurate method of AGE quantification currently available. Methylglyoxal (an AGE precursor) concentrations will also be measured in serum and urine samples, by ELISA and LC-MS/MS.

Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes. A randomised placebo-controlled crossover trial.

Kellow NJ[1,2,3], Sourris KC[1], Reid CM[2], Coughlan MT[1,2,4]

[1] Glycation, Nutrition & Metabolism Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Australia
[2] Department of Epidemiology & Preventive Medicine, School of Public Health & Preventive Medicine, Monash University, Melbourne, Australia
[3] Be Active Sleep & Eat (BASE) Facility, Department of Nutrition & Dietetics, Monash University, Notting Hill, Australia
[4] Department of Medicine, Central Clinical School, Monash University, Melbourne, Australia

Abstract:

Excessive Advanced Glycation Endproducts (AGEs) promote chronic low-grade inflammation, oxidative stress and insulin resistance, and may contribute to the development of type 2 diabetes. While AGEs are generated endogenously, they also enter the body from external sources such as cigarette smoke and the consumption of heat-processed foods. Modulation of the human colonic microbiota by dietary consumption of prebiotics has been shown to confer a number of metabolic health benefits to the host, but its effect on advanced glycation is unknown. A double-blind randomised placebo-controlled trial was conducted to determine the effect of 12-week consumption of a prebiotic dietary supplement on serum AGEs, insulin sensitivity and chronic low-grade inflammation in adults with pre-diabetes.

Twenty-seven adults with pre-diabetes aged between 40-60 years were randomly assigned to receive either 10 grams of prebiotic supplement (inulin-enriched oligofructose) or 10 grams placebo (maltodextrin) daily for 12 weeks. After a 2-week washout, study subjects crossed over to receive the alternative dietary treatment for

12 weeks. The primary outcome was the difference in serum and urinary concentrations of the AGE carboxymethyllysine (CML) between experimental and control treatments.

Intention-to-treat unadjusted analyses indicated a statistically significant reduction in waist circumference ($P<0.05$) and an increase in HDL-cholesterol ($P<0.05$) following prebiotic treatment. There were no significant differences between prebiotic and placebo treatments for serum CML, urine CML, hsCRP or HOMA-IR. Linear mixed-effects model analysis (adjusted for baseline data) showed a significant reduction in urinary albumin excretion between groups following prebiotic treatment ($P<0.05$). Prebiotic consumption was associated with an increase in gastrointestinal side effects including borborygmi ($P=0.01$), frequency of bowel actions ($P=0.001$) and flatulence ($P=0.002$).

Dietary prebiotic consumption was not associated with reductions in serum or urinary CML concentration in adults with prediabetes, however, measurement of other AGEs using validated methods is required to rule out any effects on the advanced glycation pathway. Prebiotic consumption led to a decrease in waist circumference and urinary albumin, and an increase in plasma HDL, indicating that prebiotics confer protective metabolic effects in people with prediabetes.

Trial Registration: Australia and New Zealand Clinical Trials Register (ANZCTR): ACTRN12613000130763.

Funding Source: NJK holds a National Health & Medical Research Council (NHMRC) postgraduate scholarship grant number 1039709. The NHMRC had no role in the design, analysis or writing of this article. MTC is supported by a Career Development Award from the JDRF.

Introduction:

Advanced Glycation Endproducts (AGEs) are formed during a complex series of non-enzymatic reactions between proteins and reactive aldehydes, initiated by the Maillard reaction (1). The accumulation of AGEs within the body increases with ageing and the metabolic perturbations associated with diabetes. Excessive endogenous AGE production contributes to inflammation, oxidative stress and endothelial dysfunction, and correlates with long-term vascular complications (2). Modern methods of food preparation and processing also generate large quantities of AGEs, which are believed to enter the body during consumption of highly processed foods (3).

Excessive consumption of dietary AGEs and their dicarbonyl precursors induce diabetogenic and atherogenic effects in animal studies (4, 5) and are hypothesised to promote similar cardiometabolic dysfunction in humans (6, 7). Low AGE diets have effectively increased insulin sensitivity in short-term randomised controlled human feeding trials in both healthy and overweight individuals (8-10). However, as dietary AGEs contribute to the characteristic flavour, colour and aroma of heat-treated foods, long-term adherence to a low-AGE diet may be difficult to achieve.

The progression from normal glucose tolerance through to the development of type 2 diabetes is associated with specific changes in the bacterial composition of the gastrointestinal tract. Stool samples collected from individuals with prediabetes (Impaired Glucose Tolerance or Impaired Fasting Glucose) contain fewer butyrate-producing microbial species such as *Akkermansia muciniphilia* and *Faecalibacterium prausnitzii* compared to those with normal glucose metabolism (11). Prebiotics are non-digestible plant-derived carbohydrates which encourage the selective proliferation

of specific beneficial bacterial species residing within the host gastrointestinal tract, conferring health benefits to the host (12). Regular consumption of prebiotics such as inulin, fructo-oligosaccharides and galacto-oligosaccharides stimulate the growth of colonic lactic acid bacteria, which in turn improve host gastrointestinal function, maintain the integrity of the gut barrier, reduce inflammation and enhance glucose tolerance. These benefits may subsequently reduce the endogenous production of AGEs, or reduce their gastrointestinal absorption from dietary sources. As prediabetes increases an individual's risk for the development of type 2 diabetes and cardiovascular disease, this may represent an ideal window of opportunity to implement simple interventions in order to delay or prevent the incidence of these chronic diseases.

Dietary inulin supplementation in women with type 2 diabetes was shown to reduce fasting blood glucose and levels of circulating malondialdehyde (a product of lipid peroxidation and marker of oxidative stress) after 8 weeks (13). AGE deposition in renal tissue was significantly reduced in diabetic rats fed a prebiotic supplement for 6 weeks (14), but the prebiotic contributed 10% by weight to the total diet, which is a much greater quantity than would typically be consumed by humans.

This study aimed to explore whether dietary consumption of prebiotic fructans (10 grams/day) could attenuate the formation or absorption of advanced glycation endproducts and their pathological effects in individuals with prediabetes.

Aim:

To determine the effect of 12 week consumption of a prebiotic dietary supplement on serum and urinary CML, insulin sensitivity and chronic low-grade inflammation in adults with prediabetes.

Methods:

This study was a 6.5-month double-blind randomised controlled crossover clinical trial involving adults aged between 40-60 years and diagnosed with prediabetes (Impaired Glucose Tolerance or Impaired Fasting Glucose) within the previous 12 months. The study protocol has been published in detail (15). Briefly, participants were randomised to consume either 10 grams of chicory-derived inulin-enriched oligofructose powder (kindly provided by Beneo-Orafti Active Food Ingredients, Belgium) (intervention) or 10 grams powdered maltodextrin (placebo) daily for 3 months. This was followed by a 2-week washout, after which participants crossed over to receive the alternative treatment for a further 3 months. Participants were advised to otherwise maintain their usual dietary intake and level of physical activity (Appendices 7 & 8). Data and samples (including blood, urine and stool samples) were collected at baseline, after the first treatment period and after the second treatment period.

The primary outcomes of interest were the differences in the serum and urinary concentrations of the advanced glycation endproduct CML (carboxymethllysine) following treatment with the prebiotic and placebo. Secondary outcomes included fasting serum glucose and insulin concentrations, HOMA-IR (Homeostasis Model Assessment – Insulin Resistance), serum U&Es (urea and electrolytes), serum LFTs (Liver Function Tests), lipid studies, hsCRP (high sensitivity C-Reactive Protein,

HbA_{1c}, BMI (Body Mass Index), waist circumference, blood pressure, urinary albumin and urinary creatinine. Gastrointestinal tolerance to the prebiotic supplement was assessed using a visual analogue scale (VAS) questionnaire completed by participants to report symptoms such as stool frequency, abdominal cramps and flatulence (Appendix 9). Dietary intake information was collected from participants on five occasions during the trial (baseline, random collection during treatment 1, end of treatment 1, random collection during treatment 2 and end of treatment 2) and nutritional analyses were performed using Foodworks nutrient software program version 7.0 (Xyris Software, NSW, Australia). Dietary CML intake was estimated using a published food AGE database (16).

Data were analysed as intention-to-treat using SPSS version 22.0 software (IBM Corp, Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilk test, and non-parametric tests were used to analyse non-normally distributed data. Significance of changes during the study were assessed by comparing changes of means between prebiotic and placebo treatment periods using paired samples t-tests. Outcome data were analysed using a repeated-measures linear mixed-effects model in order to account for fixed factors such as treatment order and covariates such as baseline data. Gastrointestinal symptom data obtained by the visual analogue scale were analysed using the Wilcoxon signed rank test. Significant differences were defined as a P value of <0.05 based on two-sided tests.

Results:

Thirty-four individuals volunteered to participate in the study, with 27 meeting the inclusion criteria (Figure 1). Seven individuals did not complete the study for medical

or personal reasons unrelated to the trial. Four participants withdrew from the trial while receiving the prebiotic, and three discontinued while receiving the placebo. There were no significant differences in baseline characteristics between trial completers and non-completers. Characteristics of the study participants at baseline are shown in Table 1. There were no significant differences between Group A (prebiotic first) or Group B (placebo first) for any variables at baseline. Collectively, the individuals participating in the study were predominantly female (78%), middle-aged (mean age = 52 years) and obese (mean BMI = 33 kg/m^2). Most participants exhibited multiple characteristics of the metabolic syndrome, including increased waist circumference (mean = 106 cm), dyslipidemia (mean LDL cholesterol = 3.7 mmol/L), glucose intolerance (mean HbA1c = 5.7%) and high-normal BP (mean BP = 135/86 mmHg). Treatment order did not influence the study outcomes, as independent samples t-tests (or Mann-Whitney tests for non-normally distributed variables) indicated no significant differences between changes in variables observed during the first and second prebiotic period and the first and second placebo period.

All randomised individuals were included in the analysis, regardless of compliance to the protocol or withdrawal from the trial. Weighing of returned sachets indicated 94% consumption of the dietary supplements (range: 82 – 100%). No statistically significant changes in dietary intake (total energy, macronutrients or estimated dietary CML intake) occurred throughout the trial, with the exception of dietary fibre intake which increased by a median of 5 grams per day (IQR: 3.9 - 7.6 grams) during prebiotic supplementation.

Paired-samples t-tests (unadjusted for baseline measurements) revealed a significant within-subjects reduction following prebiotic versus placebo treatment for waist circumference ($P< 0.05$) and a significant increase in serum HDL-cholesterol ($P< 0.05$). Reductions in body weight and BMI approached significance (both $P=0.07$) following prebiotic intervention (Table 2). There were no significant differences for any other variables. Repeated measures linear mixed-effects model analysis (adjusted for baseline data) showed a significant reduction in urine albumin between groups following prebiotic treatment ($P< 0.05$), in addition to a significant reduction in serum ALT between groups ($P< 0.001$) (Table 3).

A gastrointestinal symptom questionnaire was completed by each participant during prebiotic and placebo treatment periods (Table 4). Significant increases in side-effects such as borborygmi ($P= 0.01$), flatulence ($P= 0.002$) and increased frequency of bowel actions ($P= 0.001$) were reported during the prebiotic intervention (Figure 2). However, no participants withdrew from the trial based on side-effects.

Discussion:

This randomised placebo-controlled crossover trial investigated the acceptability and efficacy of a dietary prebiotic supplement to reduce the concentration of a circulating Advanced Glycation Endproduct (carboxymethyllysine) in individuals with prediabetes. Prebiotic consumption had no significant effect on serum or urinary CML concentrations, insulin resistance (measured by HOMA-IR) or the serum inflammatory marker high sensitivity C-reactive protein. A significant within-subjects increase in fasting plasma HDL and reduction in waist circumference was observed following the prebiotic treatment compared to the placebo treatment. Low plasma HDL

concentration (<1.0 mmol/L) is a significant risk factor for cardiovascular disease, with a 0.1 mmol/L increase in HDL associated with a 10% reduction in coronary heart disease risk (17). The HDL particle performs a broad range of functions besides reverse cholesterol transport. HDL plays a protective anti-inflammatory role in the development of chronic diseases such as obesity and diabetes, it affects the innate immune system response to endotoxin and promotes endothelial cell differentiation and wound healing (18). In the current trial, the mean difference in participant HDL concentration following prebiotic and placebo treatment was 0.06 mmol/L ($P<0.05$). A similar result was found in a prebiotic intervention trial undertaken in healthy adults, where dietary supplementation with 8 grams of xylo-oligosaccharide for 21 days increased mean HDL concentration by 0.07 mmol/L (19). However, dietary inulin supplementation of 15 grams per day had no significant impact on plasma lipid levels in a two-week trial of 10 overweight men (20), perhaps due to the brevity of the intervention period.

Potential mechanisms whereby prebiotic-induced gut microbial fermentation might positively influence host lipid metabolism include the production of secondary bile acids from cholesterol (21), and the downregulation of HMG-CoA reductase activity in the liver by short chain fatty acids (SCFAs), reducing hepatic cholesterol synthesis (12). Moreover, inulin exhibits significant antioxidant activity (22) and prebiotics and other soluble fibres are able to bind to dietary cholesterol, reducing its intestinal absorption (23).

While there was no change in body weight ($P=0.07$), total energy intake ($P=0.10$) or self-reported decrease in appetite ($P=0.09$) during this study, trial participants

demonstrated a small but significant reduction in waist circumference (-1.0 cm, $P<0.05$) after receiving the prebiotic supplement when compared to the placebo. This might be related to the ability of the prebiotic to increase microbial production of SCFAs, which are ligands for the G-protein coupled receptor 43 (GPR43). Engagement of SCFAs with GPR43 on adipocytes suppresses insulin signalling in adipose tissue and prevents fat accumulation (24). Interaction of SCFAs with other G-protein coupled receptors (GPR41 and GPR19) stimulates the gastrointestinal expression of the peptide YY and glucagon-like peptide-1 (GLP-1), hormones which slow gastric emptying, improve insulin sensitivity and reduce appetite (25, 26). Other human trials involving prebiotic supplementation have demonstrated reductions in body weight, particularly in body fat (27, 28).

After adjustment for baseline measurements, a significant reduction in urine albumin was detected following prebiotic supplementation, however all urine albumin measurements remained within normal range (<20 mg/L) for the duration of the study. Associations have been observed between altered gut microbial composition and chronic kidney disease (29), and high fibre diets encouraging the growth of beneficial colonic bacteria have reduced serum urea and creatinine concentrations in clinical trials (30, 31). Animal studies indicate a relationship between SCFAs and improved blood pressure control as well as reduced acute kidney injury (32), however more research is required to confirm these observations.

During prebiotic consumption, trial participants reported increased gastrointestinal side-effects including borborygmi, flatulence and increased bowel action frequency. These symptoms are well-known consequences of increased fermentable

carbohydrate intake due to an osmotic effect and enhanced microbial gas production. In this study, both intervention and placebo treatments were commenced at 5 grams per day and gradually increased to the maximum dose of 10 grams per day over a period of one week. In spite of this, a small number of individuals continued to experience gastrointestinal side-effects, however none were considered severe enough to withdraw from the trial.

Limitations of this study include its small sample size and lack of bacterial analysis of participant stool samples. We have assumed that prebiotic-induced changes in the gut microbiota contributed to the metabolic improvements observed in the study participants, however without undertaking faecal bacterial analyses we cannot be certain this is correct. Stool samples were collected from all trial participants, but funding limitations have prevented their immediate analysis. Serum and urinary CML was measured in this study using ELISA, which is not considered the most accurate method of AGE quantification currently available. Tandem mass spectrometry – high performance liquid chromatography (LC-MS/MS) is not currently available in Australia, but additional serum and urine samples were collected and frozen in preparation for future transport to an overseas laboratory for LC-MS/MS analysis. Additionally, it would be of interest to measure the methylglyoxal concentrations in the serum and urine samples, as methylglyoxal is a highly reactive AGE precursor which is independently capable of inducing early metabolic dysfunction in animal studies (33). While CML is one of the most commonly studied AGEs, many other AGE moieties exist which were not measured during this trial, so the effect of prebiotic supplementation on other AGEs is unknown. Due to increased gastrointestinal side effects experienced by some individuals participating in this trial, a small number of

participants were able to correctly assume they were receiving the prebiotic treatment, which may have resulted in unintentional changes to lifestyle behaviours which could have potentially affected the trial outcomes. However, analysis of dietary intake data revealed no significant changes in dietary energy or macronutrient consumption throughout the course of the trial.

In conclusion, dietary prebiotic consumption did not affect serum or urinary concentrations of the advanced glycation endproduct CML, or metabolic markers of inflammation or insulin resistance in adults with prediabetes, but was associated with improvements in plasma HDL cholesterol, waist circumference and urine albumin. Longer term intervention studies are required to determine whether these changes are sufficient to prevent or slow the development type 2 diabetes.

Figure 1. CONSORT Flow Diagram

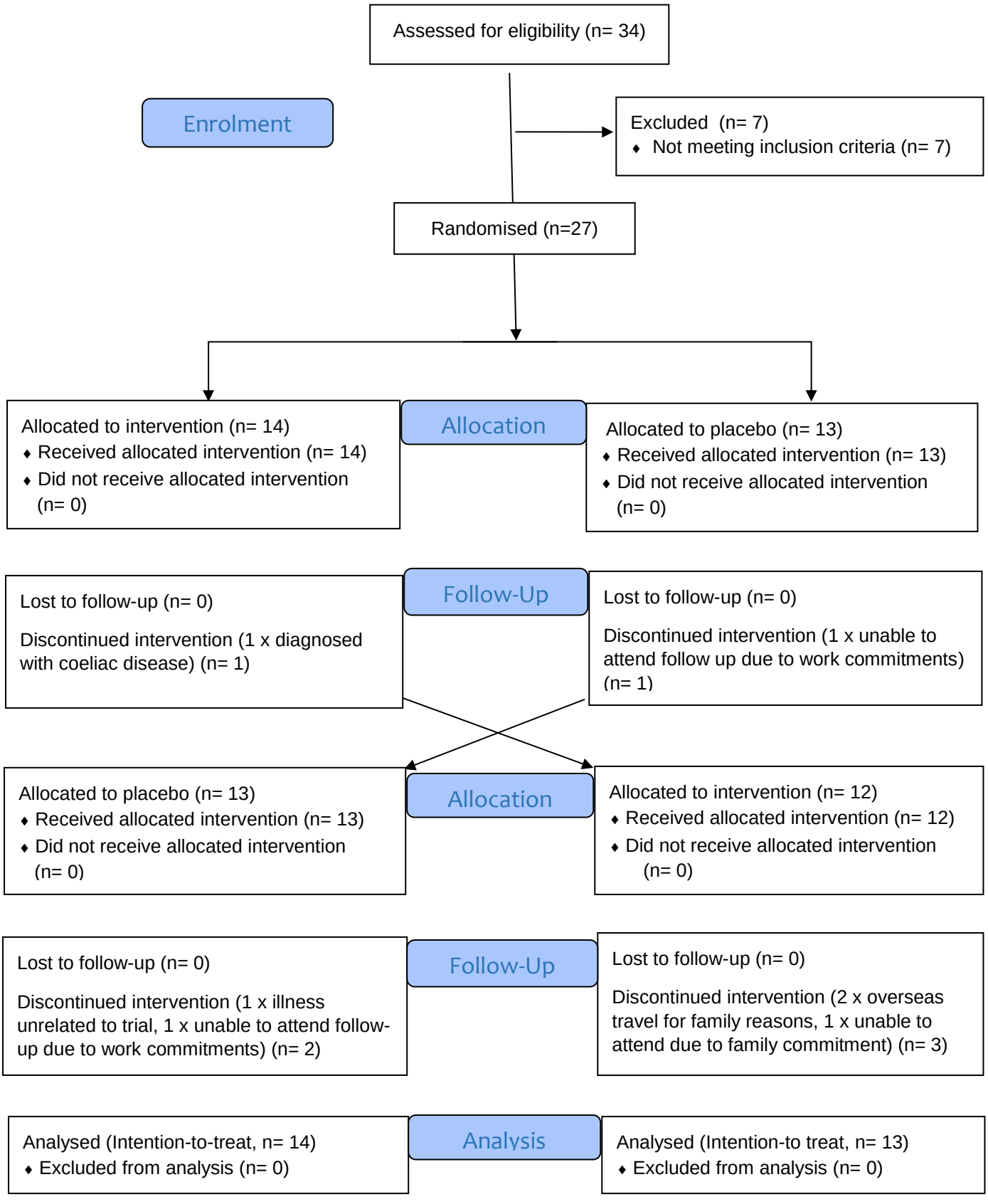


Table 1. Baseline characteristics of study participants (n = 27)

Variable	Mean	Standard Deviation
Age (years)	52.3	6.8
Gender n female (% female)	21 (78)	
Weight (kg)	93.1	17.7
BMI (kg/m²)	33	5.2
Waist Circumference (cm)	106.0	14.1
Fasting glucose (mmol/L)	4.9	0.4
HbA1c (%)	5.7	0.3
Fasting insulin	9.4	4.4
HOMA-IR	2.1	1.1
Total Cholesterol (mmol/L)	5.8	0.9
LDL Cholesterol (mmol/L)	3.7	0.8
HDL Cholesterol (mmol/L)	1.5	0.5
Triglyceride (mmol/L)*	1.2	(0.8, 1.6)
BP Systolic (mmHg)	135.1	16.3
BP Diastolic (mmHg)	86.2	12.1
eGFR (mL/min/1.73m²)*	90	(89, 90)
Serum Creatinine (µmol/L)	67.2	9.4
High sensitivity CRP (mg/L)	3.7	2.9
ALT (units/L)	25.9	11.1
GGT (units/L)	32.7	22.2
ALP (units/L)	75.0	17.3
Urine albumin (mg/L)*	5.0	(5.0, 5.0)
Urine Creatinine (mmol/L)*	5.9	(4.5, 7.8)
Serum CML (µg/ml)	1.2	0.48
Urine CML (µg)	5014.2	3494.0
Urine CML (µg/mmol Creatinine)	415.1	256.5
Total energy intake (kJ/day)	9560.7	1335.9
Total fat intake (g/day)	94.2	21.5
Saturated fat intake (g/day)	34.9	9.7
Carbohydrate intake (g/day)	196.9	68.4
Sugar intake (g/day)*	88.0	(58.0, 119.0)
Protein intake (g/day)	135.1	30.3
Alcohol intake (g/day)*	8.0	(0, 12.5)
Dietary fibre intake (g/day)*	21.0	(18.5, 27.5)
Sodium intake (mg/day)*	1990.7	(1630.3, 2686.3)
Estimated CML intake (mg/day)	12.89	6.71

*Serum eGFR, Serum triglyceride, Urine albumin, Urine creatinine, Sugar intake, Alcohol intake, Dietary fibre intake and Sodium intake values were not normally distributed, so median (25^{th},75^{th} percentile) is reported.

Table 2. Unadjusted mean (SD) clinical values following prebiotic and placebo consumption (n= 27)

Variable	Prebiotic	Placebo	P
Weight (kg)	91.5 (19.4)	92.4 (19.6)	0.07
BMI (kg/m²)	32.3 (5.5)	32.7 (5.6)	0.07
Waist Circumference (cm)	103.4 (14.7)	104.5 (14.6)	0.03*
Fasting glucose (mmol/L)	4.87 (0.36)	4.98 (0.51)	0.30
HbA1c (%)	5.57 (0.28)	5.60 (0.34)	0.40
Fasting insulin (mU/L)	8.8 (3.4)	11.5 (12.4)	0.31
HOMA-IR	1.96 (0.8)	2.78 (3.5)	0.31
Total Cholesterol (mmol/L)	5.91 (0.9)	5.94 (1.1)	0.50
LDL Cholesterol (mmol/L)	3.79 (0.83)	3.85 (0.98)	0.39
HDL Cholesterol (mmol/L)	1.53 (0.50)	1.47 (0.51)	0.02*
TG (mmol/L)[#]	1.1 (0.7, 1.5)	1.2 (0.9, 2.0)	0.41
BP Systolic (mmHg)	131.3 (14.4)	131.3 (16.2)	0.99
BP Diastolic (mmHg)	85.4 (7.7)	83.4 (11.2)	0.44
eGFR (mL/min/1.73m²)[#]	90.0 (86, 90)	90.0 (86, 90)	0.37
High sensitivity CRP (mg/L)	4.62 (3.4)	4.59 (3.7)	0.93
ALT (units/L)	27.4 (13.3)	28.6 (15.6)	0.64
GGT (units/L)	35.6 (22.4)	36.2 (32.4)	0.89
ALP (units/L)	74.1 (18.5)	74.7 (17.9)	0.73
Urine albumin (mg/L)[#]	5.0 (5.0, 5.0)	5.0 (5.0, 5.3)	0.10
Urine creatinine (mmol/L)[#]	5.1 (4.1, 8.0)	5.8 (4.0, 7.4)	0.84
Serum CML (µg/ml)	1.17 (0.63)	1.05 (0.37)	0.50
Urine CML (µg)	7499.2 (7610)	6061.7 (5986)	0.54
Urine CML (µg/mmol Cr)	628.2 (534.6)	475.9 (416.7)	0.37

*P< 0.05 using paired samples t-tests
[#]Data not normally distributed so median (25^{th}, 75^{th} percentile) is reported and Wilcoxon matched pairs test was used

Table 3. Estimated marginal means and 95% CI (adjusted for baseline data) between groups following prebiotic and placebo consumption (n=27).

Variable	Prebiotic	Standard Error	Placebo	Standard Error	P
Waist Circumference (cm)	105.1 (97.8-112.3)	3.53	103.7 (95.8-111.5)	3.81	0.79
HbA1c (%)	5.64 (5.50-5.78)	0.07	5.54 (5.39-5.69)	0.07	0.33
HDL Cholesterol (mmol/L)	1.54 (1.27-1.81)	0.13	1.51 (1.22-1.78)	0.14	0.88
High sensitivity CRP (mg/L)	4.47 (2.82-6.11)	0.80	3.57 (1.79-5.35)	0.87	0.45
HOMA-IR	2.06 (1.56-2.52)	0.22	1.77 (1.27-2.27)	0.24	0.40
Serum CML (µg/ml)	1.17 (0.98-1.38)	23.7	1.17 (0.95-1.38)	21.7	0.97
Urine CML (µg)	2855.1 (1037.7-4672.5)	867.2	4791.7 (2562.0-7021.5)	1068.4	0.18
Urine CML (µg/mmol Cr)	288.4 (144.0-432.7)	69.4	495.3 (319.9-670.5)	84.7	0.07
ALT (units/L)	29.71 (24.5-34.9)	2.54	18.75 (13.1-24.4)	2.74	0.007**
Urine albumin (mg/L)	5.07 (4.73-5.41)	0.17	5.59 (5.23-5.96)	0.18	0.041*

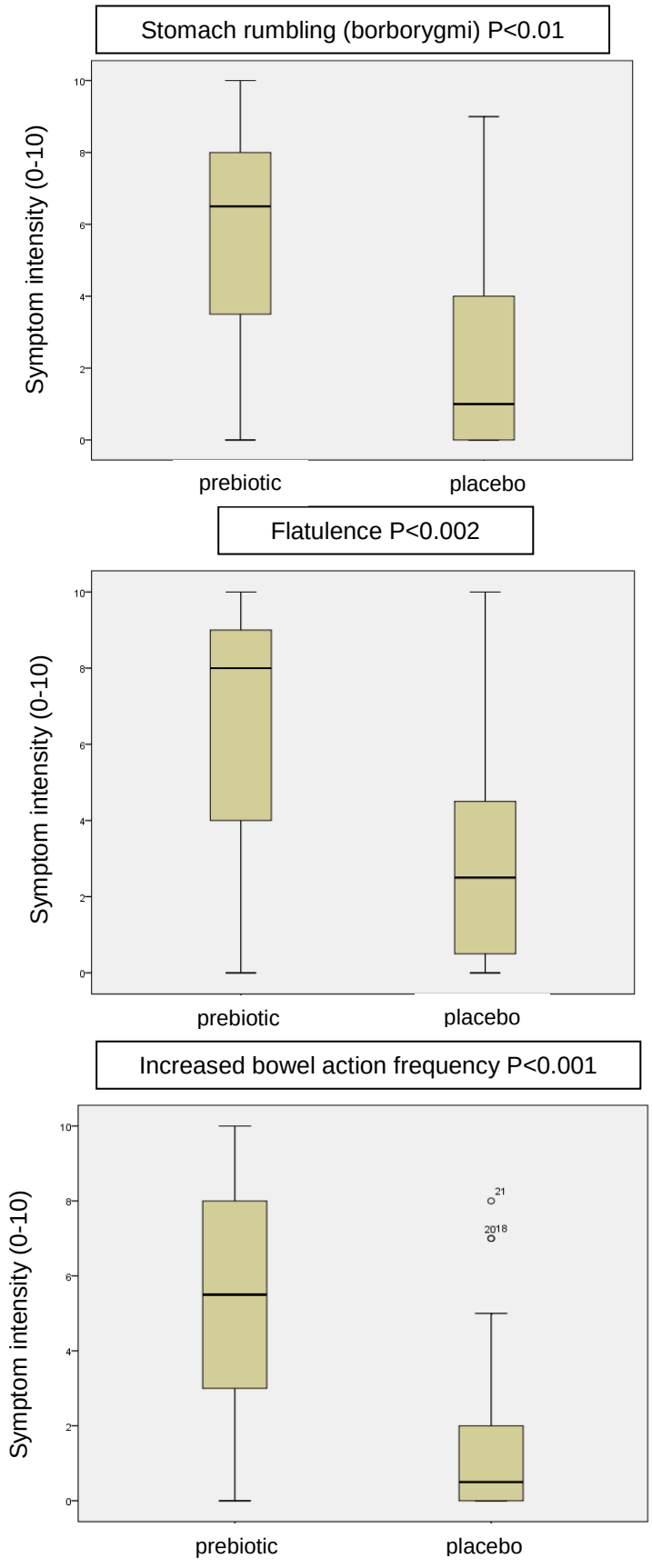
*P< 0.05 or **P< 0.01 using repeated measures linear mixed-effects model analysis

Table 4. Gastrointestinal side-effects (Median, 25^{th},75^{th} percentile) during prebiotic and placebo consumption (n = 23). Participants rated each symptom on a 10-point Likert scale where 0= not experienced and 10= experienced frequently.

Symptom	Prebiotic	Placebo	P
Constipation	1.0 (0, 3.0)	0 (0, 2.0)	0.918
Diarrhoea	2.0 (0, 5.0)	1.0 (0, 3.0)	0.189
Reduced appetite	3.0 (1.0, 6.0)	0 (0, 5.0)	0.092
Increased appetite	1.0 (0, 3.0)	0 (0, 2.0)	0.800
Abdominal bloating	3.0 (2.0, 7.0)	1.0 (0, 2.0)	0.054
Abdominal pain/cramping	1.0 (0, 5.0)	0 (0, 3.0)	0.729
Stomach rumbling/gurgling	6.0 (3.0, 8.0)	1.0 (0, 2.0)	0.010*
Flatulence	8.0 (5.0, 9.0)	2.0 (0, 4.0)	0.002**
Increase frequency of bowel actions	5.0 (2.0, 8.0)	1.0 (0, 2.0)	0.001**

*P<0.05, **P<0.01 using Wilcoxon signed ranks test.

Figure 2. Median (IQR) gastrointestinal side-effects during prebiotic and placebo consumption (n=23)



References:

1. Tessier FJ. The Maillard reaction in the human body. The main discoveries and factors that affect glycation. Pathologie-biologie. 2010;58(3):214-9. Epub 2009/11/10.
2. Schalkwijk CG, Miyata T. Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. Amino acids. 2012;42(4):1193-204. Epub 2010/10/21.
3. Poulsen MW, Hedegaard RV, Andersen JM, de Courten B, Bugel S, Nielsen J, et al. Advanced glycation endproducts in food and their effects on health. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2013;60:10-37. Epub 2013/07/23.
4. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(39):15888-93. Epub 2012/08/22.
5. Sena CM, Matafome P, Crisostomo J, Rodrigues L, Fernandes R, Pereira P, et al. Methylglyoxal promotes oxidative stress and endothelial dysfunction. Pharmacological research. 2012;65(5):497-506. Epub 2012/03/20.
6. Uribarri J, Cai W, Pyzik R, Goodman S, Chen X, Zhu L, et al. Suppression of native defense mechanisms, SIRT1 and PPARgamma, by dietary glycoxidants precedes disease in adult humans; relevance to lifestyle-engendered chronic diseases. Amino acids. 2014;46(2):301-9. Epub 2013/05/03.
7. Vlassara H, Striker GE. AGE restriction in diabetes mellitus: a paradigm shift. Nature reviews Endocrinology. 2011;7(9):526-39. Epub 2011/05/26.
8. Birlouez-Aragon I, Saavedra G, Tessier FJ, Galinier A, Ait-Ameur L, Lacoste F, et al. A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. The American journal of clinical nutrition. 2010;91(5):1220-6. Epub 2010/03/26.
9. Mark AB, Poulsen MW, Andersen S, Andersen JM, Bak MJ, Ritz C, et al. Consumption of a diet low in advanced glycation end products for 4 weeks improves insulin sensitivity in overweight women. Diabetes care. 2014;37(1):88-95. Epub 2013/08/21.
10. de Courten B, de Courten MP, Soldatos G, Dougherty SL, Straznicky N, Schlaich M, et al. Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial. The American journal of clinical nutrition. 2016;103(6):1426-33. Epub 2016/04/01.
11. Zhang X, Shen D, Fang Z, Jie Z, Qiu X, Zhang C, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PloS one. 2013;8(8):e71108. Epub 2013/09/10.
12. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. The British journal of nutrition. 2010;104 Suppl 2:S1-63. Epub 2010/10/06.
13. Pourghassem Gargari B, Dehghan P, Aliasgharzadeh A, Asghari Jafar-Abadi M. Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes. Diabetes & metabolism journal. 2013;37(2):140-8. Epub 2013/05/04.
14. Gobinath D, Madhu AN, Prashant G, Srinivasan K, Prapulla SG. Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. The British journal of nutrition. 2010;104(1):40-7. Epub 2010/03/02.
15. Kellow NJ, Coughlan MT, Savige GS, Reid CM. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial. BMC endocrine disorders. 2014;14:55. Epub 2014/07/12.
16. Scheijen JLJM CE, Engelen L, Dgnelie PC, Brouns F, Stehouwer CDA, Schalkwijk CG. Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: presentation of a dietary AGE database. Food chemistry. 2016;190:1145-50. Epub 2015, Dec 18.

17. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79(1):8-15. Epub 1989/01/01.
18. Simha V, Kudva YC. HDL Cholesterol Story Is Dead: Long Live HDL! Diabetes. 2016;65(10):2826-8. Epub 2016/09/24.
19. Childs CE, Roytio H, Alhoniemi E, Fekete AA, Forssten SD, Hudjec N, et al. Xylo-oligosaccharides alone or in synbiotic combination with Bifidobacterium animalis subsp. lactis induce bifidogenesis and modulate markers of immune function in healthy adults: a double-blind, placebo-controlled, randomised, factorial cross-over study. The British journal of nutrition. 2014;111(11):1945-56. Epub 2014/03/26.
20. Tripkovic L, Muirhead NC, Hart KH, Frost GS, Lodge JK. The effects of a diet rich in inulin or wheat fibre on markers of cardiovascular disease in overweight male subjects. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2015;28(5):476-85. Epub 2014/06/13.
21. De Preter V, Hamer HM, Windey K, Verbeke K. The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health? Molecular nutrition & food research. 2011;55(1):46-57. Epub 2011/01/06.
22. Pasqualetti V, Altomare A, Guarino MP, Locato V, Cocca S, Cimini S, et al. Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. PloS one. 2014;9(5):e98031. Epub 2014/05/20.
23. Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. The American journal of clinical nutrition. 1999;69(1):30-42. Epub 1999/01/30.
24. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nature communications. 2013;4:1829. Epub 2013/05/09.
25. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012;61(2):364-71. Epub 2011/12/23.
26. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(43):16767-72. Epub 2008/10/22.
27. Genta S, Cabrera W, Habib N, Pons J, Carillo IM, Grau A, et al. Yacon syrup: beneficial effects on obesity and insulin resistance in humans. Clin Nutr. 2009;28(2):182-7. Epub 2009/03/04.
28. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. The American journal of clinical nutrition. 2009;89(6):1751-9. Epub 2009/04/24.
29. Ramezani A, Raj DS. The gut microbiome, kidney disease, and targeted interventions. Journal of the American Society of Nephrology : JASN. 2014;25(4):657-70. Epub 2013/11/16.
30. Vaziri ND, Liu SM, Lau WL, Khazaeli M, Nazertehrani S, Farzaneh SH, et al. High amylose resistant starch diet ameliorates oxidative stress, inflammation, and progression of chronic kidney disease. PloS one. 2014;9(12):e114881. Epub 2014/12/10.
31. Kieffer DA, Piccolo BD, Vaziri ND, Liu S, Lau WL, Khazaeli M, et al. Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. American journal of physiology Renal physiology. 2016;310(9):F857-71. Epub 2016/02/05.
32. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJ, de Almeida DC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. Journal of the American Society of Nephrology : JASN. 2015;26(8):1877-88. Epub 2015/01/16.
33. Tikellis C, Pickering RJ, Tsorotes D, Huet O, Cooper ME, Jandeleit-Dahm K, et al. Dicarbonyl stress in the absence of hyperglycemia increases endothelial inflammation and atherogenesis similar to that observed in diabetes. Diabetes. 2014;63(11):3915-25. Epub 2014/05/09.

Chapter 8:

Conclusions and avenues for further study

The papers and chapters presented in this thesis aimed to address the following questions: **What is the effect of diet on advanced glycation in humans?** and **Can dietary consumption of a prebiotic supplement improve biomarkers of metabolic health and reduce circulating AGE levels in adults with pre-diabetes?**

This was approached by undertaking the following activities:

1) Reviewing the current evidence examining the role of dietary AGEs in low-grade chronic inflammatory processes in both animal and human studies.
2) Exploring the use of pharmacological agents and nutritional supplements which may exhibit anti-glycation properties.
3) Investigating the utility of dietary AGE restriction in humans for the amelioration of insulin resistance, endothelial dysfunction or oxidative stress.
4) Determining whether long-term dietary patterns and lifestyle behaviours effect tissue AGE accumulation in healthy adults.
5) Reviewing the effects of dietary prebiotic consumption on cardiometabolic health in humans, and
6) Assessing the efficacy of dietary prebiotic supplementation for the reduction of AGEs and biomarkers of metabolic health in adults with risk factors for the development of type 2 diabetes.

What is the effect of dietary AGEs on inflammation? (Chapter 2)

The majority of dietary AGE feeding trials conducted in rodents have contributed to a large body of evidence supporting the role of dietary AGEs and their carbonyl precursors in the upregulation of pro-inflammatory pathways involved in both the development and progression of cardiometabolic disease. While excessive AGEs in food are likely to induce inflammation by a variety of mechanisms, the primary method

whereby AGEs are thought to amplify inflammatory cytokine production is by ligation to RAGE. The binding of AGEs to RAGE *in vitro* is sufficient to activate NF-κB for up to 24 hours. However *in vivo*, AGE-RAGE interaction stimulates the sustained activation of NF-κB for at least one week, promoting oxidative stress and potentiating further AGE production. Dietary AGEs are thought to be only partially absorbed, as highly cross-linked moieties are resistant to degradation by human gastrointestinal enzymes. In contrast, low molecular weight carbonyl compounds are likely to be well absorbed but are less efficient at interacting with RAGE. Post-absorptive carbonyl compounds would be required to react further with amino groups to form AGEs before being capable of activating RAGE. Our current knowledge of the degree of digestion and absorption different food-derived AGEs undergo is limited, which prevents dietary AGEs from being considered a major threat to human health. Longer-term rodent feeding trials using defined synthetic AGEs and AGE-precursors are required in order to fully investigate their metabolic fate and function.

Human dietary AGE restriction trials have produced contradictory results. Inconsistencies in results might be explained by the different AGE quantification and outcome measurement techniques utilised, the heterogeneity of patient populations studied, diversity of the test diets administered and variations in study quality between trials. Standardised AGE measurement techniques are clearly needed to improve our knowledge of high AGE food and fluids, and the efficacy of interventions aimed at reducing the AGE content of foodstuffs. Dietary interventions used in clinical trials must contain the same macro and micronutrient content as the control or standard diet, differing only in AGE content. All meals and snacks should be provided to participants for the duration of dietary AGE clinical trials, as suggested food items and

cooking instructions can be interpreted differently between individuals. Additionally, trials should focus on specific patient types as dietary AGEs may have minimal effects on healthy individuals with the capacity to detoxify or excrete them, and may only exert pathological effects in people with pre-existing metabolic dysfunction or renal impairment. Hundreds of different AGEs exist, and it is unlikely that all are harmful to human health. More research is required to identify the primary diet-derived AGEs and their food sources which exert pathogenic effects in the human body.

A variety of vitamins, antioxidants and polyphenols derived from natural food products exhibit anti-glycation activity *in vitro* and may represent a future therapeutic option for individuals with metabolic risk factors. The ideal treatment would combine a number of different beneficial compounds, each targeting a specific stage of the glycation pathway. Human trials are now required to determine the effectiveness of these compounds, their interactions with other nutrients and whether the dosage required is well tolerated with minimal side effects.

Is there currently sufficient evidence to recommend therapeutic AGE-restricted diets in healthy or overweight individuals, people with diabetes or those with renal impairment for the prevention or attenuation of insulin resistance, the improvement of endothelial function and the reduction of biomarkers of inflammation and oxidative stress? (Chapter 3)

A systematic review was conducted to determine whether there was sufficient evidence from human randomised controlled trials to recommend dietary AGE restriction as a dietary intervention for the reduction of circulating AGEs, insulin resistance, and markers of oxidative stress and endothelial dysfunction. Half of the

trials were of low methodological quality, short duration, involved small sample sizes, and the heterogeneity of trial participants and outcome measures made it difficult to pool studies of similar design. In addition, most of the trials measured AGEs in food and body fluids using semi-quantitative immunological techniques, providing limited evidence to support low-AGE diets as therapeutic interventions until further research using tandem mass spectrometry-high performance liquid chromatography (LC-MS/MS) AGE measurement techniques is undertaken.

Based on these trials, there is insufficient evidence at present to suggest that healthy people who follow an AGE-restricted diet will gain any health benefits. There is also inadequate evidence to recommend individuals with renal failure to reduce the AGE content of their diet, as this will place an additional dietary restriction (which is not yet supported by high quality RCTs) on people who may already be required to limit their protein, potassium, phosphorus and fluid intake. Two high quality feeding trials recently conducted in overweight individuals with type 2 diabetes risk factors demonstrated an improvement in insulin sensitivity following 2-4 weeks consumption of a low-AGE diet. Both trials measured AGEs using LC-MS/MS techniques, and provided isoenergetic diets differing only in AGE content to intervention and control groups. While these studies were of short duration and involved small numbers of participants, there is now a sufficient evidence-base to support dietary AGE restriction trials of longer duration in greater numbers of both overweight adults and people with type 2 diabetes in future.

Insulin sensitivity can be improved by a number of lifestyle interventions such as reduction of total energy, saturated fat and/or carbohydrate intake in order to achieve

weight loss, initiation of a strength/resistance exercise training program and use of pharmacological insulin-sensitising agents. Dietary AGE restriction may provide an alternative avenue for increasing insulin sensitivity which is preferable to taking medication and is easier to maintain than weight reduction diets or regular exercise programs.

Do habitual dietary and lifestyle factors influence long-term tissue AGE accumulation in a large sample of healthy adults? (Chapter 4)

Quantifying AGEs within the human body is challenging. In the past, AGEs have been measured in serum, plasma, urine, stool, tears, saliva and tissue samples. There is a clear requirement for AGE researchers to determine the ideal location within the human body from which to measure AGE levels, in order to provide an accurate reflection of the total body AGE burden. Indeed, sampling from not one but multiple sites may be required. Dietary AGEs from a single meal may be distributed throughout a variety of body organs, including the kidney, liver and vascular system. Undigested dietary AGEs may be excreted in urine or act as a source of nutrients for bacteria in the colon. Measurement of glycated proteins in plasma is thought to be unreliable in overweight and obese individuals, due to accumulation of AGEs in adipose tissue and increased total body interstitial fluid volume in overweight people. Albumin more readily undergoes transcapillary movement from plasma to interstitial fluid in obese individuals, thus spending less time in plasma available for glycation [213].

The measurement of AGEs attached to long-lived proteins in skin tissue provides an estimate of AGE accumulation over many years, providing more information regarding the body's AGE burden and degree of vascular disease risk than a single plasma AGE

value. Whether AGEs from dietary sources influence AGE deposition in skin is unknown, and a cross-sectional study was designed to investigate whether habitual lifestyle behaviours are associated with skin autofluorescence (SAF) in healthy adults.

After adjustment for age and total energy intake, the study identified positive correlations between SAF and chronological age, cigarette smoking, waist circumference and dietary intake of meat & meat products. There was no relationship between SAF and physical activity or long-term consumption of any macro- or micronutrients in food. Limitations of the study were its cross-sectional design and lack of participant renal function assessment. The findings of this study cannot be applied to individuals with obesity, type 2 diabetes or renal failure, as these conditions are associated with variations in oxidative stress, glucose tolerance, inflammation and AGE excretion which are not observed in healthy individuals. It is possible that dietary AGEs make an even greater contribution to tissue AGE concentrations in individuals with chronic disease, and this requires further investigation. This study is also unable to exclude food-derived AGE accumulation at sites other than skin tissue.

The findings of this study suggest that some dietary AGEs may be at least partially absorbed and could accumulate within body tissues. Even in healthy adults with normal renal function, dietary AGEs may not be completely detoxified and/or excreted, and could contribute to tissue AGE concentrations. Future prospective and randomised controlled feeding studies are required to determine the degree of digestion and absorption of dietary AGEs and their precursors by administering synthetic AGEs whose metabolic endpoints can be traced. The existing scientific literature provides contradictory information about the degree of absorption of dietary

AGEs and dicarbonyls [32, 214], and these discrepancies require further clarification in order to identify the AGEs in food which pose the greatest risk to human health. As both exogenous and endogenously generated AGEs are likely to make significant contributions to total body AGE concentrations, dietary interventions targeting both reduced oral intake of AGEs and the attenuation of metabolic dysfunction are likely to provide the greatest benefit to individuals at risk of chronic disease.

What is currently known about the effects of dietary prebiotic supplementation on human cardiometabolic health? (Chapter 5)

The development of new analytical technologies such as high throughput metagenomic sequencing and genome-wide association studies have rapidly expanded our knowledge of the bacterial inhabitants of the human gastrointestinal tract, and how they might influence host metabolism and disease. Nevertheless, there is still much to learn about how changes in the quantity and diversity of human gut microbiota effect body weight, immune function, inflammation and intestinal health. The role of specific colonic microbes in people with type 2 diabetes has been difficult to study, as the condition is often associated with alterations in gastrointestinal motility, bacterial overgrowth in the small intestine, and the use of microbiota-modulating medications such as Metformin.

A systematic review explored evidence from high quality RCTs investigating the effects of prebiotic supplementation on cardiometabolic parameters in humans. Meta-analyses found statistically and clinically significant reductions in appetite, post-prandial glucose and post-prandial insulin levels after dietary prebiotic supplementation in either healthy adults, overweight adults or individuals with type 2

diabetes. Individual trials also showed significant reductions in markers of inflammation, pro-inflammatory cytokines and immune cell activity, and improvements in insulin sensitivity, antioxidant capacity and markers of hepatic damage following prebiotic supplementation. A small number of trials also demonstrated reductions in gastrointestinal permeability and circulating LPS concentrations following prebiotic consumption. Although one trial identified an increase in participant breath hydrogen production (an indicator of bacterial fermentation) in the experimental group, no studies collected stool samples for microbial analyses, so it was not possible to demonstrate whether metabolic improvements were associated with alterations in the gut microbiota. Future studies will be required to collect participant stool samples in order to identify the effect of prebiotic interventions on both bacterial growth and the identification of microbial genes involved in promoting human health.

Different strains of bacteria are now frequently being identified in the gastrointestinal tract and hypothesised to play a beneficial role in the wellbeing of the host. Ongoing work must continue to identify beneficial micro-organisms and determine their function by conducting intervention trials using single bacterial strains. Bacteria which utilise prebiotics as a food source require further characterisation, as the most beneficial micro-organisms may not be direct prebiotic metabolisers, but instead could be cross-feeding on the by-products of prebiotic digestion produced by other bacterial genera. Indeed, future studies will need to determine if consumption of dietary prebiotics is necessary to promote the growth of SCFA-producing species in the colon, or whether it is sufficient to simply administer oral butyrate in order to achieve the same metabolic benefits [(215)]. The ideal diet for the promotion of optimal microbial composition is yet to be determined - it may contain prebiotics or synbiotics (a combination of prebiotics

and probiotics) but the optimal dose and types of bacteria required is currently unknown. Dietary supplements of the future may need to be specifically tailored to each individual's unique phenotype, genotype and family history of disease. This is not difficult to imagine, given the recent interest in faecal microbial transplants and 'vaginal seeding' of new-born babies delivered by caesarean section.

What is the effect of 12-week consumption of a prebiotic dietary supplement on circulating AGE levels, insulin resistance and inflammatory biomarkers in adults diagnosed with pre-diabetes, in comparison to a placebo dietary supplement? (Chapters 6 and 7)

Prebiotic-induced stimulation of beneficial gut microbial growth was hypothesised to contribute to a reduction in endogenous AGE production, by potentially reducing the concentrations of substrates and reactants required for AGE formation such as glucose, lipids, reactive oxygen species and inflammatory molecules. Short chain fatty acid production by the microbiota might also limit exogenous AGE absorption by reducing gut permeability and promoting the health and integrity of the gastrointestinal tract.

A randomised placebo-controlled crossover trial was performed in 27 adults with prediabetes in order to determine the metabolic effects of consuming 10 grams of prebiotic supplement powder per day for 3 months versus 10 grams of a placebo maltodextrin powder for 3 months. Maltodextrin is entirely digested and absorbed in the small intestine, and therefore does not influence bacterial growth in the colon.

There were no significant changes in serum and urinary CML concentrations, fasting blood glucose, insulin resistance (measured by HOMA-IR) and the inflammatory

marker high sensitivity CRP following the prebiotic intervention. However, significant reductions in waist circumference, urine albumin and increased concentrations of HDL-cholesterol were observed after prebiotic supplementation. Gastrointestinal side effects including borborygmi, flatulence and increased frequency of bowel actions were reported more frequently during the prebiotic intervention, but were not severe enough to result in any participant withdrawals from the trial.

Limitations of this study include its small sample size and lack of bacterial analysis of participant stool samples. Stool samples were collected from all trial participants, and funding applications have been submitted to enable analysis of the bacterial composition and the SCFA concentration of these samples to be undertaken in future. Additional serum and urine samples were also collected and frozen in preparation for transport to an overseas laboratory for AGE analysis by tandem mass spectrometry – high performance liquid chromatography (LC-MS/MS), which is considered the most accurate method of AGE quantification currently available. It would be of interest to measure the methylglyoxal concentrations in the serum, urine and stool samples, as methylglyoxal is an AGE precursor which is independently capable of inducing early metabolic dysfunction in animal studies. While CML is one of the most commonly studied AGEs, many other AGE moieties exist which were not measured during this trial, so the effect of prebiotic supplementation on other AGEs is unknown. Due to increased gastrointestinal side effects experienced by some individuals participating in this trial, a small number of participants were able to correctly assume they were receiving the prebiotic treatment, which may have resulted in unintentional changes to lifestyle behaviours which could have potentially affected the trial outcomes. However, analysis of dietary intake data revealed no significant changes in dietary energy or

macronutrient consumption throughout the course of the trial. Further research should also be conducted in order to clarify the role of poorly digested dietary AGEs on the growth of the gut microbiota, and the metabolites produced as a result of bacterial AGE fermentation.

In conclusion, dietary prebiotic consumption did not affect serum or urinary concentrations of the advanced glycation endproduct CML, or metabolic markers of inflammation or insulin resistance in adults with prediabetes, but was associated with improvements in plasma HDL cholesterol, waist circumference and urine albumin. Longer term intervention studies are required to determine whether these changes are sufficient to prevent or slow the development type 2 diabetes.

References:

1. Vistoli G, De Maddis D, Cipak A *et al.* (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. *Free Radic Res* **47 Suppl 1**, 3-27.

2. Schalkwijk CG, Miyata T (2012) Early- and advanced non-enzymatic glycation in diabetic vascular complications: the search for therapeutics. *Amino Acids* **42**, 1193-1204.

3. Henle T (2005) Protein-bound advanced glycation endproducts (AGEs) as bioactive amino acid derivatives in foods. *Amino Acids* **29**, 313-322.

4. Poulsen MW, Hedegaard RV, Andersen JM *et al.* (2013) Advanced glycation endproducts in food and their effects on health. *Food Chem Toxicol* **60**, 10-37.

5. Gautieri A, Passini FS, Silvan U *et al.* (2016) Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biol*, doi: 10.1016/j.matbio.2016.09.001. [Epub ahead of print].

6. Thomas MC (2011) Advanced glycation end products. *Contrib Nephrol* **170**, 66-74.

7. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813-820.

8. Kilhovd BK, Giardino I, Torjesen PA *et al.* (2003) Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes. *Metabolism* **52**, 163-167.

9. Uribarri J, Tuttle KR (2006) Advanced glycation end products and nephrotoxicity of high-protein diets. *Clin J Am Soc Nephrol* **1**, 1293-1299.

10. Ahmed N (2005) Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract* **67**, 3-21.

11. Haucke E, Navarrete-Santos A, Simm A *et al.* (2014) Glycation of extracellular matrix proteins impairs migration of immune cells. *Wound Repair Regen* **22**, 239-245.

12. Singh R, Barden A, Mori T *et al.* (2001) Advanced glycation end-products: a review. *Diabetologia* **44**, 129-146.

13. Soro-Paavonen A, Zhang WZ, Venardos K *et al.* (2010) Advanced glycation end-products induce vascular dysfunction via resistance to nitric oxide and suppression of endothelial nitric oxide synthase. *J Hypertens* **28**, 780-788.

14. Akhter F, Salman Khan M, Shahab U *et al.* (2013) Bio-physical characterization of ribose induced glycation: a mechanistic study on DNA perturbations. *Int J Biol Macromol* **58**, 206-210.

15. Angelo MF, Aguirre A, Aviles Reyes RX *et al.* (2014) The proinflammatory RAGE/NF-kappaB pathway is involved in neuronal damage and reactive gliosis in a model of sleep apnea by intermittent hypoxia. *PLoS One* **9**, e107901.

16. Ramasamy R, Yan SF, Schmidt AM (2012) Advanced glycation endproducts: from precursors to RAGE: round and round we go. *Amino Acids* **42**, 1151-1161.

17. Yim MB, Yim HS, Lee C *et al.* (2001) Protein glycation: creation of catalytic sites for free radical generation. *Ann N Y Acad Sci* **928**, 48-53.

18. Prasad K, Dhar I & Caspar-Bell G (2015) Role of Advanced Glycation End Products and Its Receptors in the Pathogenesis of Cigarette Smoke-Induced Cardiovascular Disease. *Int J Angiol* **24**, 75-80.

19. Tessier FJ, Birlouez-Aragon I (2012) Health effects of dietary Maillard reaction products: the results of ICARE and other studies. *Amino Acids* **42**, 1119-1131.

20. Goldberg T, Cai W, Peppa M *et al.* (2004) Advanced glycoxidation end products in commonly consumed foods. *J Am Diet Assoc* **104**, 1287-1291.

21. Uribarri J, Woodruff S, Goodman S *et al.* (2010) Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc* **110**, 911-916 e912.

22. Sharma C, Kaur A, Thind SS *et al.* (2015) Advanced glycation End-products (AGEs): an emerging concern for processed food industries. *J Food Sci Technol* **52**, 7561-7576.

23. Scheijen JL, Clevers E, Engelen L *et al.* (2016) Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: Presentation of a dietary AGE database. *Food Chem* **190**, 1145-1150.

24. Bengmark S (2007) Advanced glycation and lipoxidation end products--amplifiers of inflammation: the role of food. *JPEN J Parenter Enteral Nutr* **31**, 430-440.

25. Degen J, Hellwig M, Henle T (2012) 1,2-dicarbonyl compounds in commonly consumed foods. *J Agric Food Chem* **60**, 7071-7079.

26. Tan D, Wang Y, Lo CY *et al.* (2008) Methylglyoxal: its presence in beverages and potential scavengers. *Ann N Y Acad Sci* **1126**, 72-75.

27. Hull GLJ, Woodside, J.V., Ames, J.M., Cuskelly, G.J. (2012) N-epsilon-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet. *Food Chem* **131**, 170-174.

28. Ahmed N, Mirshekar-Syahkal B, Kennish L *et al.* (2005) Assay of advanced glycation endproducts in selected beverages and food by liquid chromatography with tandem mass spectrometric detection. *Mol Nutr Food Res* **49**, 691-699.

29. Hellwig M, Geissler S, Matthes R *et al.* (2011) Transport of free and peptide-bound glycated amino acids: synthesis, transepithelial flux at Caco-2 cell monolayers, and interaction with apical membrane transport proteins. *Chembiochem* **12**, 1270-1279.

30. Seiquer I, Diaz-Alguacil J, Delgado-Andrade C *et al.* (2006) Diets rich in Maillard reaction products affect protein digestibility in adolescent males aged 11-14 y. *Am J Clin Nutr* **83**, 1082-1088.

31. Faist V, Erbersdobler HF (2001) Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Ann Nutr Metab* **45**, 1-12.

32. Hellwig M, Matthes R, Peto A *et al.* (2014) N-epsilon-fructosyllysine and N-epsilon-carboxymethyllysine, but not lysinoalanine, are available for absorption after simulated gastrointestinal digestion. *Amino Acids* **46**, 289-299.

33. Foerster A, Henle T (2003) Glycation in food and metabolic transit of dietary AGEs (advanced glycation end-products): studies on the urinary excretion of pyrraline. *Biochem Soc Trans* **31**, 1383-1385.

34. Somoza V, Wenzel E, Weiss C *et al.* (2006) Dose-dependent utilisation of casein-linked lysinoalanine, N(epsilon)-fructoselysine and N(epsilon)-carboxymethyllysine in rats. *Mol Nutr Food Res* **50**, 833-841.

35. Alamir I, Niquet-Leridon C, Jacolot P *et al.* (2013) Digestibility of extruded proteins and metabolic transit of N epsilon -carboxymethyllysine in rats. *Amino Acids* **44**, 1441-1449.

36. Semba RD, Ang A, Talegawkar S *et al.* (2012) Dietary intake associated with serum versus urinary carboxymethyl-lysine, a major advanced glycation end product, in adults: the Energetics Study. *Eur J Clin Nutr* **66**, 3-9.

37. Uribarri J, Cai W, Peppa M *et al.* (2007) Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol A Biol Sci Med Sci* **62**, 427-433.

38. Uribarri J, Peppa M, Cai W *et al.* (2003) Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. *Am J Kidney Dis* **42**, 532-538.

39. Hellwig M, Bunzel D, Huch M *et al.* (2015) Stability of Individual Maillard Reaction Products in the Presence of the Human Colonic Microbiota. *J Agric Food Chem* **63**, 6723-6730.

40. Mills DJ, Tuohy KM, Booth J *et al.* (2008) Dietary glycated protein modulates the colonic microbiota towards a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis subjects. *J Appl Microbiol* **105**, 706-714.

41. Seiquer I, Rubio LA, Peinado MJ *et al.* (2014) Maillard reaction products modulate gut microbiota composition in adolescents. *Mol Nutr Food Res* **58**, 1552-1560.

42. Marungruang N, Fak F & Tareke E (2016) Heat-treated high-fat diet modifies gut microbiota and metabolic markers in apoe-/- mice. *Nutr Metab (Lond)* **13**, 22.

43. Fong DS, Aiello LP, Ferris FL, 3rd *et al.* (2004) Diabetic retinopathy. *Diabetes Care* **27**, 2540-2553.

44. UKPDS Study Group (2003) Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* **290**, 2159-2167.

45. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circ Res* **107**, 1058-1070.

46. Tikellis C, Pickering RJ, Tsorotes D *et al.* (2014) Dicarbonyl stress in the absence of hyperglycemia increases endothelial inflammation and atherogenesis similar to that observed in diabetes. *Diabetes* **63**, 3915-3925.

47. Tahara N, Yamagishi S, Matsui T *et al.* (2012) Serum levels of advanced glycation end products (AGEs) are independent correlates of insulin resistance in nondiabetic subjects. *Cardiovasc Ther* **30**, 42-48.

48. Tan KC, Shiu SW, Wong Y *et al.* (2011) Serum advanced glycation end products (AGEs) are associated with insulin resistance. *Diabetes Metab Res Rev* **27**, 488-492.

49. Forbes JM, Sourris KC, de Courten MP *et al.* (2014) Advanced glycation end products (AGEs) are cross-sectionally associated with insulin secretion in healthy subjects. *Amino Acids* **46**, 321-326.

50. Uribarri J, Cai W, Woodward M *et al.* (2015) Elevated serum advanced glycation endproducts in obese indicate risk for the metabolic syndrome: a link between healthy and unhealthy obesity? *J Clin Endocrinol Metab* **100**, 1957-1966.

51. Nin JW, Jorsal A, Ferreira I *et al.* (2011) Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. *Diabetes Care* **34**, 442-447.

52. Luft VC, Duncan BB, Schmidt MI *et al.* (2015) Carboxymethyl lysine, an advanced glycation end product, and incident diabetes: a case-cohort analysis of the ARIC Study. *Diabet Med* **33**, 1392-8.

53. Hanssen NM, Engelen L, Ferreira I *et al.* (2013) Plasma levels of advanced glycation endproducts Nepsilon-(carboxymethyl)lysine, Nepsilon-(carboxyethyl)lysine, and pentosidine are not independently associated with cardiovascular disease in individuals with or without type 2 diabetes: the Hoorn and CODAM studies. *J Clin Endocrinol Metab* **98**, E1369-1373.

54. Coughlan MT, Yap FY, Tong DC *et al.* (2011) Advanced glycation end products are direct modulators of beta-cell function. *Diabetes* **60**, 2523-2532.

55. Mark AB, Poulsen MW, Andersen S *et al.* (2014) Consumption of a diet low in advanced glycation end products for 4 weeks improves insulin sensitivity in overweight women. *Diabetes Care* **37**, 88-95.

56. Peppa M, Uribarri J, Cai W *et al.* (2004) Glycoxidation and inflammation in renal failure patients. *Am J Kidney Dis* **43**, 690-695.

57. Cai W, Uribarri J, Zhu L *et al.* (2014) Oral glycotoxins are a modifiable cause of dementia and the metabolic syndrome in mice and humans. *Proc Natl Acad Sci U S A* **111**, 4940-4945.

58. Stenman LK, Burcelin R, Lahtinen S (2015) Establishing a causal link between gut microbes, body weight gain and glucose metabolism in humans - towards treatment with probiotics. *Benef Microbes*, 1-12.

59. Larsen N, Vogensen FK, van den Berg FW *et al.* (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* **5**, e9085.

60. Macfarlane GT, Macfarlane S (2011) Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. *J Clin Gastroenterol* **45 Suppl**, S120-127.

61. Geurts L, Neyrinck AM, Delzenne NM *et al.* (2014) Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics. *Benef Microbes* **5**, 3-17.

62. Leung C, Herath CB, Jia Z *et al.* (2014) Dietary glycotoxins exacerbate progression of experimental fatty liver disease. *J Hepatol* **60**, 832-838.

63. Kandaraki E, Chatzigeorgiou A, Piperi C *et al.* (2012) Reduced ovarian glyoxalase-I activity by dietary glycotoxins and androgen excess: a causative link to polycystic ovarian syndrome. *Mol Med* **18**, 1183-1189.

64. Lin RY, Choudhury RP, Cai W *et al.* (2003) Dietary glycotoxins promote diabetic atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* **168**, 213-220.

65. Sandu O, Song K, Cai W *et al.* (2005) Insulin resistance and type 2 diabetes in high-fat-fed mice are linked to high glycotoxin intake. *Diabetes* **54**, 2314-2319.

66. Sebekova K, Klenovics KS, Boor P *et al.* (2012) Behaviour and hormonal status in healthy rats on a diet rich in Maillard reaction products with or without solvent extractable aroma compounds. *Physiol Behav* **105**, 693-701.

67. Matafome P, Santos-Silva D, Crisostomo J *et al.* (2012) Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity. *Arch Physiol Biochem* **118**, 58-68.

68. Shangari N, Depeint F, Furrer R *et al.* (2007) A thermolyzed diet increases oxidative stress, plasma alpha-aldehydes and colonic inflammation in the rat. *Chem Biol Interact* **169**, 100-109.

69. Poulsen MW, Andersen JM, Hedegaard RV *et al.* (2016) Short-term effects of dietary advanced glycation end products in rats. *Br J Nutr* **115**, 629-636.

70. Anton PM, Craus A, Niquet-Leridon C *et al.* (2012) Highly heated food rich in Maillard reaction products limit an experimental colitis in mice. *Food Funct* **3**, 941-949.

71. Birlouez-Aragon I, Saavedra G, Tessier FJ *et al.* (2010) A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *Am J Clin Nutr* **91**, 1220-1226.

72. de Courten B, de Courten MP, Soldatos G *et al.* (2016) Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial. *Am J Clin Nutr* **103**, 1426-33.

73. Uribarri J, Cai W, Ramdas M *et al.* (2011) Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. *Diabetes Care* **34**, 1610-1616.

74. Vlassara H, Cai W, Goodman S *et al.* (2009) Protection against loss of innate defenses in adulthood by low advanced glycation end products (AGE) intake: role of the antiinflammatory AGE receptor-1. *J Clin Endocrinol Metab* **94**, 4483-4491.

75. Luevano-Contreras C, Garay-Sevilla ME, Wrobel K *et al.* (2013) Dietary advanced glycation end products restriction diminishes inflammation markers and oxidative stress in patients with type 2 diabetes mellitus. *J Clin Biochem Nutr* **52**, 22-26.

76. Semba RD, Gebauer SK, Baer DJ *et al.* (2014) Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. *J Nutr* **144**, 1037-1042.

77. Vlassara H, Cai W, Crandall J *et al.* (2002) Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci U S A* **99**, 15596-15601.

78. Harcourt BE, Sourris KC, Coughlan MT *et al.* (2011) Targeted reduction of advanced glycation improves renal function in obesity. *Kidney Int* **80**, 190-198.

79. Negrean M, Stirban A, Stratmann B *et al.* (2007) Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. *Am J Clin Nutr* **85**, 1236-1243.

80. Kellow NJ, Savige GS (2013) Dietary advanced glycation end-product restriction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review. *Eur J Clin Nutr* **67**, 239-248.

81. Sakharova OV, Lleva RR, Dziura JD *et al.* (2012) Effects on post-prandial glucose and AGE precursors from two initial insulin strategies in patients with type 2 diabetes uncontrolled by oral agents. *J Diabetes Complications* **26**, 333-338.

82. Marx N, Walcher D, Ivanova N *et al.* (2004) Thiazolidinediones reduce endothelial expression of receptors for advanced glycation end products. *Diabetes* **53**, 2662-2668.

83. Beisswenger P, Ruggiero-Lopez D (2003) Metformin inhibition of glycation processes. *Diabetes Metab* **29**, 6S95-103.

84. Forslund K, Hildebrand F, Nielsen T *et al.* (2015) Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262-266.

85. Augustyniak A, Bartosz G, Cipak A *et al.* (2010) Natural and synthetic antioxidants: an updated overview. *Free Radic Res* **44**, 1216-1262.

86. Stefek M, Gajdosik A, Tribulova N *et al.* (2002) The pyridoindole antioxidant stobadine attenuates albuminuria, enzymuria, kidney lipid peroxidation and matrix collagen cross-linking in streptozotocin-induced diabetic rats. *Methods Find Exp Clin Pharmacol* **24**, 565-571.

87. Ansari NH, Bhatnagar A, Fulep E *et al.* (1994) Trolox protects hyperglycemia-induced cataractogenesis in cultured rat lens. *Res Commun Chem Pathol Pharmacol* **84**, 93-104.

88. Srivastava SK, Ansari NH (1988) Prevention of sugar-induced cataractogenesis in rats by butylated hydroxytoluene. *Diabetes* **37**, 1505-1508.

89. Huang M, Que Y & Shen X (2015) Correlation of the plasma levels of soluble RAGE and endogenous secretory RAGE with oxidative stress in pre-diabetic patients. *J Diabetes Complications* **29**, 422-426.

90. Silvan JM, Srey C, Ames JM *et al.* (2014) Glycation is regulated by isoflavones. *Food Funct* **5**, 2036-2042.

91. Nascimento MM, Suliman ME, Murayama Y *et al.* (2006) Effect of high-dose thiamine and pyridoxine on advanced glycation end products and other oxidative stress markers in hemodialysis patients: a randomized placebo-controlled study. *J Ren Nutr* **16**, 119-124.

92. Erbersdobler H, Gunsser I & Weber G (1970) [Degradation of fructoselysine by the intestinal flora]. *Zentralbl Veterinarmed A* **17**, 573-575.

93. Brownlee M, Vlassara H, Kooney A *et al.* (1986) Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* **232**, 1629-1632.

94. Freedman BI, Wuerth JP, Cartwright K *et al.* (1999) Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control Clin Trials* **20**, 493-510.

95. Karachalias N, Babaei-Jadidi R, Kupich C *et al.* (2005) High-dose thiamine therapy counters dyslipidemia and advanced glycation of plasma protein in streptozotocin-induced diabetic rats. *Ann N Y Acad Sci* **1043**, 777-783.

96. Stirban A, Negrean M, Stratmann B *et al.* (2006) Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. *Diabetes Care* **29**, 2064-2071.

97. Berrone E, Beltramo E, Solimine C *et al.* (2006) Regulation of intracellular glucose and polyol pathway by thiamine and benfotiamine in vascular cells cultured in high glucose. *J Biol Chem* **281**, 9307-9313.

98. Srey C, Hull GL, Connolly L *et al.* (2010) Effect of inhibitor compounds on Nepsilon-(carboxymethyl)lysine (CML) and Nepsilon-(carboxyethyl)lysine (CEL) formation in model foods. *J Agric Food Chem* **58**, 12036-12041.

99. Sourris KC, Harcourt BE, Forbes JM (2009) A new perspective on therapeutic inhibition of advanced glycation in diabetic microvascular complications: common downstream endpoints achieved through disparate therapeutic approaches? *Am J Nephrol* **30**, 323-335.

100. Baye E, Ukropcova B, Ukropec J *et al.* (2016) Physiological and therapeutic effects of carnosine on cardiometabolic risk and disease. *Amino Acids* **48**, 1131-1149.

101. de Courten B, Jakubova M, de Courten MP *et al.* (2016) Effects of carnosine supplementation on glucose metabolism: Pilot clinical trial. *Obesity (Silver Spring)* **24**, 1027-1034.

102. Nagai R, Murray DB, Metz TO *et al.* (2012) Chelation: a fundamental mechanism of action of AGE inhibitors, AGE breakers, and other inhibitors of diabetes complications. *Diabetes* **61**, 549-559.

103. Urios P, Grigorova-Borsos AM & Sternberg M (2007) Aspirin inhibits the formation of pentosidine, a cross-linking advanced glycation end product, in collagen. *Diabetes Res Clin Pract* **77**, 337-340.

104. McPherson JD, Shilton BH & Walton DJ (1988) Role of fructose in glycation and cross-linking of proteins. *Biochemistry* **27**, 1901-1907.

105. Zhang J, Slevin M, Duraisamy Y *et al.* (2006) Comparison of protective effects of aspirin, D-penicillamine and vitamin E against high glucose-mediated toxicity in cultured endothelial cells. *Biochim Biophys Acta* **1762**, 551-557.

106. Cooper GJ (2012) Selective divalent copper chelation for the treatment of diabetes mellitus. *Curr Med Chem* **19**, 2828-2860.

107. Cooper GJ (2011) Therapeutic potential of copper chelation with triethylenetetramine in managing diabetes mellitus and Alzheimer's disease. *Drugs* **71**, 1281-1320.

108. Nagai R, Nagai M, Shimasaki S *et al.* (2010) Citric acid inhibits development of cataracts, proteinuria and ketosis in streptozotocin (type 1) diabetic rats. *Biochem Biophys Res Commun* **393**, 118-122.

109. Ohtomo S, Nangaku M, Izuhara Y *et al.* (2008) Cobalt ameliorates renal injury in an obese, hypertensive type 2 diabetes rat model. *Nephrol Dial Transplant* **23**, 1166-1172.

110. Thornalley PJ (2003) Glyoxalase I--structure, function and a critical role in the enzymatic defence against glycation. *Biochem Soc Trans* **31**, 1343-1348.

111. Yao D, Brownlee M (2010) Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes* **59**, 249-255.

112. Liu YW, Zhu X, Zhang L *et al.* (2013) Up-regulation of glyoxalase 1 by mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. *Eur J Pharmacol* **721**, 355-364.

113. Ma Q, He X (2012) Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. *Pharmacol Rev* **64**, 1055-1081.

114. Thornalley PJ, Rabbani N (2012) Dietary and synthetic activators of the antistress gene response in treatment of renal disease. *J Ren Nutr* **22**, 195-202.

115. Myint KM, Yamamoto Y, Doi T *et al.* (2006) RAGE control of diabetic nephropathy in a mouse model: effects of RAGE gene disruption and administration of low-molecular weight heparin. *Diabetes* **55**, 2510-2522.

116. Wang K, Zhou Z, Zhang M *et al.* (2006) Peroxisome proliferator-activated receptor gamma down-regulates receptor for advanced glycation end products and inhibits smooth muscle cell proliferation in a diabetic and nondiabetic rat carotid artery injury model. *J Pharmacol Exp Ther* **317**, 37-43.

117. Stone JC, Furuya-Kanamori L, Barendregt JJ *et al.* (2015) Was there really any evidence that rosiglitazone increased the risk of myocardial infarction or death from cardiovascular causes? *Pharmacoepidemiol Drug Saf* **24**, 223-227.

118. Aldini G, Vistoli G, Stefek M *et al.* (2013) Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products. *Free Radic Res* **47 Suppl 1**, 93-137.

119. Ishibashi Y, Nishino Y, Matsui T *et al.* (2011) Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. *Metabolism* **60**, 1271-1277.

120. Wendt TM, Tanji N, Guo J *et al.* (2003) RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am J Pathol* **162**, 1123-1137.

121. Engelen L, Stehouwer CD, Schalkwijk CG (2013) Current therapeutic interventions in the glycation pathway: evidence from clinical studies. *Diabetes Obes Metab* **15**, 677-689.

122. Aviram M, Rosenblat M, Bisgaier CL *et al.* (1998) Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. *Atherosclerosis* **138**, 271-280.

123. Zhang X, Hu S, Chen F *et al.* (2014) Treatment of proteins with dietary polyphenols lowers the formation of AGEs and AGE-induced toxicity. *Food Funct* **5**, 2656-2661.

124. Angoorani P, Ejtahed HS, Mirmiran P *et al.* (2016) Dietary consumption of advanced glycation end products and risk of metabolic syndrome. *Int J Food Sci Nutr* **67**, 170-176.

125. Chao PC, Huang CN, Hsu CC *et al.* (2010) Association of dietary AGEs with circulating AGEs, glycated LDL, IL-1alpha and MCP-1 levels in type 2 diabetic patients. *Eur J Nutr* **49**, 429-434.

126. Piroddi M, Palazzetti I, Quintaliani G *et al.* (2011) Circulating levels and dietary intake of the advanced glycation end-product marker carboxymethyl lysine in chronic kidney disease patients on conservative predialysis therapy: a pilot study. *J Ren Nutr* **21**, 329-339.

127. Koschinsky T, He CJ, Mitsuhashi T *et al.* (1997) Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci U S A* **94**, 6474-6479.

128. Beisswenger BG, Delucia EM, Lapoint N *et al.* (2005) Ketosis leads to increased methylglyoxal production on the Atkins diet. *Ann N Y Acad Sci* **1043**, 201-210.

129. Uchiki T, Weikel KA, Jiao W *et al.* (2012) Glycation-altered proteolysis as a pathobiologic mechanism that links dietary glycemic index, aging, and age-related disease (in nondiabetics). *Aging Cell* **11**, 1-13.

130. Itsiopoulos C, Brazionis L, Kaimakamis M *et al.* (2011) Can the Mediterranean diet lower HbA1c in type 2 diabetes? Results from a randomized cross-over study. *Nutr Metab Cardiovasc Dis* **21**, 740-747.

131. Lopez-Moreno J, Quintana-Navarro GM, Delgado-Lista J *et al.* (2016) Mediterranean Diet Reduces Serum Advanced Glycation End Products and Increases Antioxidant Defenses in Elderly Adults: A Randomized Controlled Trial. *J Am Geriatr Soc* **64**, 901-904.

132. Gugliucci A, Kotani K, Taing J *et al.* (2009) Short-term low calorie diet intervention reduces serum advanced glycation end products in healthy overweight or obese adults. *Ann Nutr Metab* **54**, 197-201.

133. Rodriguez JM, Leiva Balich L, Concha MJ *et al.* (2015) Reduction of serum advanced glycation end-products with a low calorie Mediterranean diet. *Nutr Hosp* **31**, 2511-2517.

134. Iwashige K, Kouda K, Kouda M *et al.* (2004) Calorie restricted diet and urinary pentosidine in patients with rheumatoid arthritis. *J Physiol Anthropol Appl Human Sci* **23**, 19-24.

135. Davis KE, Prasad C, Vijayagopal P *et al.* (2015) Contribution of dietary advanced glycation end products (AGE) to circulating AGE: role of dietary fat. *Br J Nutr* **114**, 1797-1806.

136. Rastogi A (2013) Sevelamer revisited: pleiotropic effects on endothelial and cardiovascular risk factors in chronic kidney disease and end-stage renal disease. *Ther Adv Cardiovasc Dis* **7**, 322-342.

137. Vlassara H, Uribarri J, Cai W *et al.* (2012) Effects of sevelamer on HbA1c, inflammation, and advanced glycation end products in diabetic kidney disease. *Clin J Am Soc Nephrol* **7**, 934-942.

138. Macias-Cervantes MH, Rodriguez-Soto JM, Uribarri J *et al.* (2015) Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition* **31**, 446-451.

139. Uribarri J, Cai W, Pyzik R *et al.* (2014) Suppression of native defense mechanisms, SIRT1 and PPARgamma, by dietary glycoxidants precedes disease in adult humans; relevance to lifestyle-engendered chronic diseases. *Amino Acids* **46**, 301-309.

140. Teichert T, Hellwig A, Pessler A *et al.* (2015) Association between Advanced Glycation End Products and Impaired Fasting Glucose: Results from the SALIA Study. *PLoS One* **10**, e0128293.

141. Sebekova K, Krivosikova Z & Gajdos M (2014) Total plasma Nepsilon-(carboxymethyl)lysine and sRAGE levels are inversely associated with a number of metabolic syndrome risk factors in non-diabetic young-to-middle-aged medication-free subjects. *Clin Chem Lab Med* **52**, 139-149.

142. Jacobs K, Navarrete Santos A, Simm A *et al.* (2014) The skin autofluorescence reflects the posttranslational glycation grade of the matrix protein collagen. *Free Radic Biol Med* **75 Suppl 1**, S34.

143. Beisswenger PJ, Makita Z, Curphey TJ *et al.* (1995) Formation of immunochemical advanced glycosylation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. *Diabetes* **44**, 824-829.

144. Smit AJ, Gerrits EG (2010) Skin autofluorescence as a measure of advanced glycation endproduct deposition: a novel risk marker in chronic kidney disease. *Curr Opin Nephrol Hypertens* **19**, 527-533.

145. Meerwaldt R, Graaff R, Oomen PH *et al.* (2004) Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* **47**, 1324-1330.

146. Koetsier M, Lutgers HL, de Jonge C *et al.* (2010) Reference values of skin autofluorescence. *Diabetes Technol Ther* **12**, 399-403.

147. Simon Klenovics K, Kollarova R, Hodosy J *et al.* (2014) Reference values of skin autofluorescence as an estimation of tissue accumulation of advanced glycation end products in a general Slovak population. *Diabet Med* **31**, 581-585.

148. Yue X, Hu H, Koetsier M *et al.* (2011) Reference values for the Chinese population of skin autofluorescence as a marker of advanced glycation end products accumulated in tissue. *Diabet Med* **28**, 818-823.

149. Noordzij MJ, Lefrandt JD, Graaff R *et al.* (2011) Dermal factors influencing measurement of skin autofluorescence. *Diabetes Technol Ther* **13**, 165-170.

150. Chabroux S, Canoui-Poitrine F, Reffet S *et al.* (2010) Advanced glycation end products assessed by skin autofluorescence in type 1 diabetics are associated with nephropathy, but not retinopathy. *Diabetes Metab* **36**, 152-157.

151. Januszewski AS, Sachithanandan N, Karschimkus C *et al.* (2012) Non-invasive measures of tissue autofluorescence are increased in Type 1 diabetes complications and correlate with a non-invasive measure of vascular dysfunction. *Diabet Med* **29**, 726-733.

152. Lutgers HL, Graaff R, Links TP *et al.* (2006) Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diabetes Care* **29**, 2654-2659.

153. Tanaka K, Tani Y, Asai J *et al.* (2012) Skin autofluorescence is associated with severity of vascular complications in Japanese patients with Type 2 diabetes. *Diabet Med* **29**, 492-500.

154. Meerwaldt R, Links TP, Graaff R *et al.* (2005) Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy. *Diabetologia* **48**, 1637-1644.

155. Monami M, Lamanna C, Gori F *et al.* (2008) Skin autofluorescence in type 2 diabetes: beyond blood glucose. *Diabetes Res Clin Pract* **79**, 56-60.

156. Meerwaldt R, Hartog JW, Graaff R *et al.* (2005) Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* **16**, 3687-3693.

157. Macsai E, Benke A, Kiss I (2015) Skin Autofluorescence and Mortality in Patients on Peritoneal Dialysis. *Medicine (Baltimore)* **94**, e1933.

158. Gerrits EG, Lutgers HL, Kleefstra N *et al.* (2008) Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. *Diabetes Care* **31**, 517-521.

159. Lutgers HL, Gerrits EG, Graaff R *et al.* (2009) Skin autofluorescence provides additional information to the UK Prospective Diabetes Study (UKPDS) risk score for the estimation of cardiovascular prognosis in type 2 diabetes mellitus. *Diabetologia* **52**, 789-797.

160. Meerwaldt R, Lutgers HL, Links TP *et al.* (2007) Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* **30**, 107-112.

161. Mulligan AA, Luben RN, Bhaniani A *et al.* (2014) A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open* **4**, e004503.

162. Craig CL, Marshall AL, Sjostrom M *et al.* (2003) International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* **35**, 1381-1395.

163. Hansen AL, Carstensen B, Helge JW *et al.* (2013) Combined heart rate- and accelerometer-assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes: the ADDITION-PRO study. *Diabetes Care* **36**, 3062-3069.

164. Jochemsen BM MD, Van Doormaal JJ, Mulder G, Volmer M, Graaff R, Smit AJ (2009) Relation between food and drinking habits, and skin autofluorescence and intima media thickness in subjects at high cardiovascular risk. *Journal of Food and Nutrition Research* **48**, 51-58.

165. Nongnuch A, Davenport A (2015) The effect of vegetarian diet on skin autofluorescence measurements in haemodialysis patients. *Br J Nutr* **113**, 1040-1043.

166. van Waateringe RP, Slagter SN, van der Klauw MM *et al.* (2016) Lifestyle and clinical determinants of skin autofluorescence in a population-based cohort study. *Eur J Clin Invest* **46**, 481-490.

167. Miyata T, Wada Y, Cai Z *et al.* (1997) Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* **51**, 1170-1181.

168. Qin J, Li R, Raes J *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59-65.

169. Koren O, Goodrich JK, Cullender TC *et al.* (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* **150**, 470-480.

170. Dominguez-Bello MG, Costello EK, Contreras M *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* **107**, 11971-11975.

171. Zeissig S, Blumberg RS (2014) Life at the beginning: perturbation of the microbiota by antibiotics in early life and its role in health and disease. *Nat Immunol* **15**, 307-310.

172. David LA, Maurice CF, Carmody RN *et al.* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563.

173. Martin R, Nauta AJ, Ben Amor K *et al.* (2010) Early life: gut microbiota and immune development in infancy. *Benef Microbes* **1**, 367-382.

174. Roberfroid M, Gibson GR, Hoyles L *et al.* (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* **104 Suppl 2**, S1-63.

175. Tilg H, Moschen AR (2014) Microbiota and diabetes: an evolving relationship. *Gut* **63**, 1513-1521.

176. Schwiertz A, Taras D, Schafer K *et al.* (2010) Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* **18**, 190-195.

177. Ley RE, Turnbaugh PJ, Klein S *et al.* (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022-1023.

178. Duncan SH, Lobley GE, Holtrop G *et al.* (2008) Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond)* **32**, 1720-1724.

179. Qin J, Li Y, Cai Z *et al.* (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55-60.

180. Karlsson FH, Tremaroli V, Nookaew I *et al.* (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99-103.

181. Cani PD, Amar J, Iglesias MA *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761-1772.

182. Cani PD, Possemiers S, Van de Wiele T *et al.* (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091-1103.

183. Thomas C, Gioiello A, Noriega L *et al.* (2009) TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* **10**, 167-177.

184. Vrieze A, Van Nood E, Holleman F *et al.* (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913-916 e917.

185. Roberfroid MB (1998) Prebiotics and synbiotics: concepts and nutritional properties. *Br J Nutr* **80**, S197-202.

186. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* **125**, 1401-1412.

187. Lafiandra D, Riccardi G & Shewry PR (2014) Improving cereal grain carbohydrates for diet and health. *J Cereal Sci* **59**, 312-326.

188. Bosscher D, Breynaert A, Pieters L *et al.* (2009) Food-based strategies to modulate the composition of the intestinal microbiota and their associated health effects. *J Physiol Pharmacol* **60 Suppl 6**, 5-11.

189. van Loo J, Coussement P, de Leenheer L *et al.* (1995) On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit Rev Food Sci Nutr* **35**, 525-552.

190. Niness KR (1999) Inulin and oligofructose: what are they? *J Nutr* **129**, 1402S-1406S.

191. Musso G, Gambino R & Cassader M (2011) Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. *Annu Rev Med* **62**, 361-380.

192. Cani PD, Neyrinck AM, Fava F *et al.* (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**, 2374-2383.

193. Dehghan P, Gargari BP, Jafar-Abadi MA *et al.* (2014) Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutr* **65**, 117-123.

194. Roberfroid M (1993) Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Crit Rev Food Sci Nutr* **33**, 103-148.

195. Everard A, Belzer C, Geurts L *et al.* (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* **110**, 9066-9071.

196. Roberfroid MB (2007) Inulin-type fructans: functional food ingredients. *J Nutr* **137**, 2493S-2502S.

197. Everard A, Cani PD (2013) Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* **27**, 73-83.

198. De Vadder F, Kovatcheva-Datchary P, Goncalves D *et al.* (2014) Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* **156**, 84-96.

199. Chambers ES, Morrison DJ, Frost G (2015) Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proc Nutr Soc* **74**, 328-336.

200. Esteve E, Ricart W & Fernandez-Real JM (2011) Gut microbiota interactions with obesity, insulin resistance and type 2 diabetes: did gut microbiote co-evolve with insulin resistance? *Curr Opin Clin Nutr Metab Care* **14**, 483-490.

201. Tolhurst G, Heffron H, Lam YS *et al.* (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**, 364-371.

202. Samuel BS, Shaito A, Motoike T *et al.* (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U S A* **105**, 16767-16772.

203. Backhed F, Crawford PA, O'Donnell D *et al.* (2007) Postnatal lymphatic partitioning from the blood vasculature in the small intestine requires fasting-induced adipose factor. *Proc Natl Acad Sci U S A* **104**, 606-611.

204. Kimura I, Ozawa K, Inoue D *et al.* (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* **4**, 1829.

205. Polizzi FC, Andican G, Cetin E *et al.* (2012) Increased DNA-glycation in type 2 diabetic patients: the effect of thiamine and pyridoxine therapy. *Exp Clin Endocrinol Diabetes* **120**, 329-334.

206. Johnson AR, Wilkerson MD, Sampey BP *et al.* (2016) Cafeteria diet-induced obesity causes oxidative damage in white adipose. *Biochem Biophys Res Commun* **473**, 545-550.

207. De Preter V, Hamer HM, Windey K *et al.* (2011) The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health? *Mol Nutr Food Res* **55**, 46-57.

208. Scheppach W (1994) Effects of short chain fatty acids on gut morphology and function. *Gut* **35**, S35-38.

209. Eun CS, Han DS, Lee SH *et al.* (2006) Attenuation of colonic inflammation by PPARgamma in intestinal epithelial cells: effect on Toll-like receptor pathway. *Dig Dis Sci* **51**, 693-697.

210. Canani RB, Costanzo MD, Leone L *et al.* (2011) Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J Gastroenterol* **17**, 1519-1528.

211. Cani PD, Osto M, Geurts L *et al.* (2012) Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* **3**, 279-288.

212. Bischoff SC, Barbara G, Buurman W *et al.* (2014) Intestinal permeability--a new target for disease prevention and therapy. *BMC Gastroenterol* **14**, 189.

213. Masania J, Malczewska-Malec M, Razny U *et al.* (2016) Dicarbonyl stress in clinical obesity. *Glycoconj J*, doi:10.1007/s10719-016-9692-0.

214. Degen J, Vogel M, Richter D *et al.* (2013) Metabolic transit of dietary methylglyoxal. *J Agric Food Chem* **61**, 10253-10260.

215. Jin CJ, Sellmann C, Engstler AJ *et al.* (2015) Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH). *Br J Nutr* **114**, 1745-1755.

Appendix 1:
Publications, conference presentations and research activities
Feb 2012 – Nov 2016

Publications in peer reviewed journals included in PhD thesis:

1. Kellow NJ, Savige GS. Dietary advanced glycation end-product (AGE) reduction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review. *European Journal of Clinical Nutrition* 2013; 67: 239-248.

2. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in humans: a systematic review of randomised controlled trials. *British Journal of Nutrition* 2014; 111: 1147-1161.

3. Kellow NJ, Coughlan MT, Reid CM, Savige GS. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial. *BMC Endocrine Disorders* 2014; 14: 55.

4. Kellow NJ, Coughlan MT. Effect of diet-derived advanced glycation end products on inflammation. *Nutrition Reviews* 2015; 73: 737-759.

5. Kellow NJ, Coughlan MT, Reid CM. Habitual dietary intake of Advanced Glycation Endproducts (AGEs) is an independent predictor of Skin Autofluorescence (SAF) in healthy adults. Manuscript submitted, Sept 2016.

6. Kellow NJ, Sourris K, Reid CM, Coughlan MT. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes. Manuscript submitted, Nov 2016.

Oral Conference Presentations:

1. “Dietary advanced glycation end-product (AGE) reduction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review.” **International Congress of Dietetics, Sydney (September 2012).** (Highly Commended New International Conference Presenter Award).

2. “Is there sufficient evidence to recommend AGE-restricted diets to our patients? A systematic review of controlled feeding trials”. **Monash University Research in Focus Conference, Gippsland (September 2012).**

3. “PhD topic”. **Three minute thesis competition, Monash University Department of Rural and Indigenous Health HDR Conference (May 2013).** (Second Place Award).

4. “Increasing beneficial gut bacteria: a recipe for the AGEs?” **Short talks on Nutrition, Cardiovascular Disease, Diabetes and Metabolism, Baker IDI (October 2013).**

5. “Association between habitual dietary intake of Advanced Glycation Endproducts (AGEs) and Skin Autofluorescence (SAF) in healthy adults”. **International Maillard Reaction Society (IMARS) Conference, Tokyo, Japan (August 2015).**

6. “Association between habitual dietary intake of Advanced Glycation Endproducts (AGEs) and Skin Autofluorescence (SAF) in healthy adults”. **Dietitians Association of Australia National Conference, Melbourne, Australia (May, 2016).** (Research in Practice Highly Commended Award).

7. Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes. **Nutrition Society of Australia National Conference, Melbourne, Australia (November, 2016).**

Poster Conference Presentations:

1. “Dietary advanced glycation end-product (AGE) reduction for the attenuation of insulin resistance, oxidative stress and endothelial dysfunction: a systematic review.” **Australian Diabetes Society (ADS) National Conference, Queensland (August, 2012).**

2. “Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a randomised controlled clinical trial”. **Monash University Research in Focus Conference, Gippsland (September, 2012).**

3. “Metabolic benefits of dietary prebiotics in humans: a systematic review of randomised controlled trials”. **ADS National Diabetes Conference, Melbourne (August 2014).**

4. “Association between habitual dietary intake of Advanced Glycation Endproducts (AGEs) and Skin Autofluorescence (SAF) in healthy adults”. **ADS National Diabetes Conference, Adelaide (August 2015).**

Speaking invitations:

1.”Carbohydrate debate”. **Invited speaker, ADS/ADEA National Diabetes Conference, Melbourne (August 2014).**

2. “Benefits of dietary inulin-type fructans for blood glucose management”. **Invited speaker, Asia Pacific Conference on Clinical Nutrition (APCCN), Kuala Lumpur, Malaysia (January 2015).**

3. “Gut microbiota in obesity and diabetes”. **Invited speaker: Gut microbiota: from research to practice. Dietitians Association of Australia (Victorian Branch) symposium, Melbourne (October, 2016).**

Publications not related to thesis (2012-2016):

1. Kellow N, Savige G. Type 2 diabetes diagnosis using HbA1c: will it delay opportunities for early intervention? *Australian Diabetes Educator* 2012; 15(1): 18-23.

2. Kellow N, Khalil H. A review of the pharmacological management of type 2 diabetes in a rural Australian primary care cohort. *Int J Pharm Pract* 2013; 21: 297-304.

3. Kellow NJ, Walker KZ. Chapter 12: Authorised EU Health Claim for Arabinoxylan (book chapter), in ‘Foods, Nutrients and Food Ingredients with Authorised EU Health Claims, Volume 3’, Woodhead Publishing 2016 (in press).

Other Activities:

1. Member of Australian Diabetes Educators Association of Australia (ADEA) Nutrition Policy Working Party (February 2012 – February 2013).

2. Conducted peer reviews of articles submitted to the *Australian Diabetes Educator* (2012), *Journal of Adolescent Health* (2012, 2013), *Cell Biochemistry & Function* (2013), *Pharmacological Research* (2013), *Metabolism* (2014), *Nutrients* (2014), *British Journal of Nutrition* (2015, 2016), *Annals of Nutrition & Metabolism* (2015), *Diabetes Care* (2015), *Contemporary Clinical Trials* (2015), *Diabetologia* (2016).

3. Conducted peer reviews of funding applications submitted to NHMRC (2013, 2015) and Diabetes Australia Research Trust (2014, 2016).

4. Assessment and scoring of abstracts submitted to the Australian Diabetes Educators Association of Australia (ADEA) Victorian Conference (March 2012).

5. Attended one-day workshop at Melbourne University General Practice and Primary Health Care Academic Centre: “Cluster Randomised Controlled Trials in Diabetes – Complex Interventions” (June 2012).

6. Nursing, Medical and Dietetics student teaching (Monash University Gippsland, East Gippsland Medical School & Monash Clayton), (2012: 12 hours, 2013: 22 hours, 2014: 8 hours, 2015: 20 hours).

7. Attended Ethics & Good Research Practice Short Course, Monash University School of Public Health & Preventive Medicine (November 2013).

8. Attended one-day Diabetes Epidemiology Training Course, Baker IDI Heart and Diabetes Institute (November, 2013).

9. Attended the International Diabetes Congress, Melbourne (December 2013).

10. Completed Monash University Master of Public Health coursework units: Introductory Epidemiology, Semester 1 (2014, Grade: Distinction) and Introductory Biostatistics, Semester 1 (2014, Grade: Distinction).

11. Completed STATA one day training course, Sept 2014 (Monash University School of Public Health & Preventive Medicine).

12. Member of ADEA Program organising committee and ADS/ADEA Local Organising Committee for the ADS/ADEA National Diabetes Conference, 2014.

13. Co-chaired “Nutrition and Diabetes” scientific session. ADS/ADEA National Diabetes Conference, Melbourne (August 2014).

14. Co-author of article for “The Conversation”. Coughlan MT & Kellow NJ. Food additives and chronic disease risk: what role do emulsifiers play? (April, 2015).

15. Research in Progress (RIP) presentation, Baker IDI Heart & Diabetes Institute (October 2015).

16. Member of Nutrition Society Australia 2016 Conference Organising Committee (Nov 2015 – Dec 2016).

17. Assessment and scoring of submitted conference workshop proposals for ADEA National Diabetes Conference (Jan, 2016).

18. Employed full-time as a lecturer at the department of Nutrition & Dietetics, Monash University (Feb 2016 – present).

<u>Research funding received:</u>

2013: NHMRC Public Health Post-graduate Scholarship 2013-2015 ($103,000)

2017: ADEA Diabetes Research Foundation 2016-2017 ($59,900)

Appendix 2:
Nicole Kellow NHMRC PhD Scholarship (Public Health) Application 2012

Title of Proposed Study (maximum number of characters is 112 including spaces):
Does dietary modulation of the colonic microflora attenuate the effects of advanced glycation endproducts?

Lay Description (maximum of 100 words):
Advanced glycation endproducts (AGEs) occur naturally in the human body, and also enter the body from external sources such as cigarettes and certain foods. Too many AGEs in the body can contribute to the development of diabetes. Changing the types of bacteria which colonise the human bowel may prevent or slow diabetes development. This project will determine whether dietary supplements aimed at increasing beneficial bacteria in the human bowel can reduce AGE levels and their negative effects.

Institution of Enrolment:
Monash University

Administering Institution Details:
Research Office
Faculty of Medicine, Nursing and Health Sciences
PO Box 64
Monash University
Victoria 3800
Australia

Project Outline: (Maximum 2 pages excluding references)

Title: Does dietary modulation of the colonic microflora attenuate the effects of advanced glycation endproducts (AGEs)?

Aims:

1) To review the available literature and determine the effectiveness of dietary AGE restriction to reduce AGEs and AGE-related insulin resistance in humans.
2) To identify anthropometric, dietary, lifestyle and pharmacological characteristics which predict tissue AGE levels in a large population-based human sample.
3) To determine the effectiveness of a prebiotic nutritional supplement to alter the composition of the gut microbiota and reduce insulin resistance, serum AGE levels and inflammatory markers in overweight and obese adults at risk of developing type 2 diabetes, in comparison to those receiving a placebo supplement.

Background:
Advanced glycation endproducts (AGEs) are derived from normal endogenous glucose-protein interactions, but also enter the body through external sources such as cigarette smoking and consumption of heat-processed foods (Barlovic *et al*, 2010 & Uribarri et al, 2010). AGEs (also called glycotoxins) are known to induce diabetogenic and nephrotoxic effects, induce low-grade inflammation, enhance oxidative stress and promote atherosclerosis (Sebekova & Somoza, 2007). Ingested AGEs may exert some of their negative effects at the level of the gut, where they are absorbed into the circulation and contribute to the body's total AGE pool.

Therapeutic strategies targeted at attenuating the development of inflammation and insulin resistance are urgently needed to prevent or slow type 2 diabetes development in susceptible individuals. Interventions including low-AGE diets, antioxidant therapies and pharmacological agents aimed at reducing the AGE burden in human and animal models have produced mixed results (Capellini *et al*, 2010 & Tan *et al*, 2010).

Recently the gut microflora has been identified as a significant site of immune cell activation (Kolb & Mandrup-Poulsen, 2010). Manipulation of the gut microflora for the benefit of human health could be achieved by antibiotic treatment or dietary intervention. In mice, a high fat diet and low-grade inflammation have been shown to be associated with significantly lower numbers of intestinal *Bifidobacterium* species. Subsequent supplementation of the high fat diet with oligofructose (a prebiotic fructan) completely restored bifidobacteria levels, improved glucose tolerance and reduced fasting insulin and pro-inflammatory cytokine concentrations (Cani *et al*, 2007). Prebiotics are non-digestible plant-derived carbohydrates which act as a fermentation substrate to promote the selective growth of certain beneficial gastrointestinal micro-organisms. Prebiotics which selectively stimulate the growth of bifidobacteria in the human colon might offer protection against weight gain, inflammation and insulin resistance associated with high fat and AGE intakes ubiquitous in Western diets.

Proposed Research Program:
This research will consist of a series of related projects, leading to a crossover randomised placebo-controlled trial (RCT). Projects will include:

1) A systematic literature review and meta-analysis of the effectiveness of dietary AGE restriction to reduce AGE levels in human subjects at risk of type 2 diabetes development or diagnosed with pre-diabetes will be conducted. Literature will be reviewed from RCTs, cohort studies, and case-control studies. Available RCTs will be used in the meta-analysis.

2) A population-based observational study will be conducted to identify predictors of tissue AGE levels. An age-stratified sample of 300 individuals will have their tissue AGE levels measured by an AGE-reader. The AGE-reader non-invasively measures skin autofluorescence, which has been shown to be strongly correlated to AGE accumulation in healthy subjects and patients with diabetes (Meerwaldt *et al*, 2008). Individuals will have additional information collected including age, BMI, waist circumference, blood pressure, random blood glucose level, fruit & vegetable intake, cooking methods utilised, alcohol intake, medications and nutritional supplements taken, smoking status, physical activity levels and dietary fat/fibre intake. Validated data collection tools will be used where available. Tissue AGE levels will be divided into quartiles and differences between groups identified by one-way ANOVA. Multivariate regression analysis will be used to determine subject characteristics which predict AGE levels.

3) A prospective crossover RCT will be undertaken. **Recruitment:** Adult subjects will be invited to participate if they meet one of the following eligibility criteria: elevated tissue AGE levels (determined during the observational study) or the metabolic syndrome or diagnosed pre-diabetes. Thirty participants will be recruited from the observational study, from advertisements in local media and through local GP clinics. **Randomisation:** Eligible participants will be randomised to one of two groups via random selection of sealed, opaque envelopes. Each group will contain 15 subjects. **Intervention:** Both groups will make no changes to their diet and lifestyle. One group

will consume 10 grams of a powdered prebiotic supplement (Synergy 1™) daily for 3 months (intervention) while the other group consumes 10 grams of maltodextrin powder daily for 3 months (placebo). After a 2 week washout, individuals will crossover to receive the alternative (intervention or placebo) treatment for a further 3 months. **Baseline assessment:** Research participants will be interviewed to obtain demographic, anthropometric, pharmacological and lifestyle information. Detailed diet histories will be collected from participants, and blood samples collected for assessment of glycemic control and insulin sensitivity (fasting glucose, fasting insulin, HbA1c, calculation of HOMA-IR), inflammation (high sensitivity CRP, myeloperoxidase), pro-inflammatory cytokines (interleukin-6, TNF-alpha), serum AGEs (CML and methylglyoxal) and markers of endothelial dysfunction (E-selectin). Stool samples will be collected for microbial analyses and 24-hour urine samples will be collected for AGE analyses. **Outcome assessment:** Baseline assessments will be repeated after the first 3-month treatment and the second 3-month treatment. **Data analysis:** The trial will conform to the CONSORT guidelines and intention-to-treat analyses will be conducted. Between-groups ANOVA will be conducted for all outcome measures at baseline and after each intervention. Effect sizes and 95% confidence intervals will be calculated for each outcome.

Proposal Significance:

In Australia, conservative estimates predict that at least 2 million adults will have been diagnosed with type 2 diabetes by 2025 (Magliano *et al*, 2009). The burden of disease associated with diabetes has a substantial impact on costs to direct health care as well as loss of productivity and decreased quality of life. The widespread consumption of high fat, heat processed foods and the increasing prevalence of obesity in Australia highlight the need for simple interventions to prevent AGE-mediated damage. If dietary treatments aimed at altering the gut microflora prove to be effective strategies for preventing or slowing the development of type 2 diabetes, they may become mainstream therapies for individuals with diabetes risk factors.

References:

Barlovic DP, Thomas MC & Jandeleit-Dahm K. *Cardiovasc & Haematol Dis-Drug Targets.* **10**: 7-15 (2010).

Capellini VK, Celotto AC, Baldo CF *et al. Curr Vasc Pharmacol.* **8**: 526-544 (2010).

Cani PD, Neyrinck AM, Fava F et al. *Diabetologia*. **50**: 2374-2383 (2007).

Kolb H & Mandrup-Poulsen T. *Diabetologia*. **53**: 10-20 (2010).

Magliano DJ, Peeters A, Vos T *et al*. *Aust NZ J Public Health*. **33**: 540-543 (2009).

Meerwaldt R, Links T, Zeebregts C *et al*. *Cardiovasc Diabetol.* **7**: 29-37 (2008).

Sebekova K, Somoza V. *Mol Nutr Food Res.* **51**: 1079-1084 (2007).

Tan ALY, Sourris KC, Harcourt BE et al. *Am J Physiol Renal Physiol.* **298**: F763–F770 (2010).

Uribarri J, Woodruff S, Goodman S et al. *J Am Diet Assoc*. **110**: 911-916 (2010).

Appendix 3:

General Health Questionnaire

Participant ID Number_____

Date:	Your date of birth:	Your postcode:	Gender: Male ☐ Female ☐

Do you have any medical or health conditions? Please list them here:

Are there any health conditions which run in your family? Please list them here:

Do you take any prescribed medications? Please list them here (If you don't know the name of a medication, please indicate what you take it for):

Do you smoke (please tick)?

☐ Yes ☐ Never smoked ☐ Former smoker: Year quit:_____

If you smoke, how many cigarettes do you smoke each day? ☐

Do you live in a house where other people smoke inside? ☐

Do you drink alcohol? ☐ Yes ☐ No

If you drink alcohol, how many standard alcoholic drinks do you usually consume each week? ☐

(Note: One standard alcoholic drink is equal to one pot of regular beer, one stubbie of light beer, one glass of wine = 100ml/3½ oz., one small glass of fortified wine = 60ml/2 oz., one shot of spirits = 30ml/1oz.)

Thank you for completing this questionnaire.
The section below will be completed by the researcher

Weight(kg):	Height(m):	Waist Circ (cm):	Skin AF (U):	BP(mmHg):	RBG(mmol/L): Time:

Appendix 4:
EPIC Food Frequency Questionnaire

Dietary Questionnaire

Please put a cross (X) on every line

1. How often do you usually consume the following foods?

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
MEAT, CHICKEN, FISH (medium serving)									
Beef: roast, steak, mince, casserole									
Hamburger, beefburger									
Pork: roast, chops, stew, sliced									
Lamb: roast, chops or stew									
Chicken or turkey									
Bacon									
Ham									
Corned beef, luncheon meats									
Sausages									
Meat pie, steak & kidney pie, pasties, sausage rolls									
Liver, liver pate									
Fried fish in batter (as in fish & chips)									
Fish fingers, fish cakes									
Other white fish (cod, whiting, snapper, flake)									
Oily fish, fresh or canned (tuna, salmon, sardines)									
Shellfish (eg. crab, prawns, oyster)									
Fish eggs, caviar									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
BREAD AND SAVOURY BISCUITS (one slice of bread or one biscuit)									
White bread and rolls									
Wholemeal (brown) bread and rolls									
Multigrain bread and rolls									
Toast (any type of bread)									
Crispbread (Ryvita, Salada)									
Savoury biscuits (Sao, Clix)									
Do you eat the crust on bread?	Yes				No				
BREAKFAST CEREALS (one bowl)									
Cooked cereal (eg. porridge)									
Uncooked cereal (eg. corn flakes, muesli)									
POTATO, RICE & PASTA (medium serving)									
Boiled, mashed or jacket potatoes									
Hot chips									
Roast potatoes									
Potato salad									
White rice									
Brown rice									
Pasta (eg. spaghetti, macaroni, noodles)									
Wholemeal pasta									
Lasagne									
Pizza									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
DAIRY PRODUCTS & FATS									
Cream and sour cream (1 tablesp)									
Double cream (1 tablesp)									
Yogurt – low fat (125g carton)									
Yogurt – full fat or Greek yoghurt (125g carton)									
Dairy dessert (Fruche, LeRice, custard, mousse) – 125g									
Cheese (Cheddar, tasty) – 1 slice									
Low fat soft cheese (Cottage, ricotta) – 1 tablesp									
Eggs (boiled, fried, scrambled) (one)									
Quiche (medium serving)									
Salad dressing – low fat (1 tablesp)									
Regular salad dressing or mayonnaise (1 tablesp)									
French dressing (1 tablesp)									
Other salad dressing (1 tablesp)									
The following on bread or vegetables (1 teaspoon)									
Butter									
Margarine									
Polyunsaturated margarine, eg Flora									
Butter/margarine blend									
Reduced fat spread (eg. Flora Light)									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
SNACKS OR DESSERTS (medium serving)									
Chocolate biscuit – one biscuit									
Plain sweet biscuit – one biscuit (eg. Milk arrowroot, Nice)									
Cake (home baked) – average slice									
Cake (purchased) – average slice									
Fruit bun, scone, pancake (home baked)									
Fruit bun, scone, pancake (purchased)									
Apple pie, fruit tarts, fruit crumble (home baked)									
Apple pie, fruit tarts, fruit crumble (purchased)									
Sponge puddings (home baked)									
Sponge puddings (purchased)									
Milk puddings (custard, trifle)									
Ice cream (1 small bowl)									
Chocolate (individual chocolate or square)									
Chocolate which contains caramel (eg. Mars Bar, Snickers)									
Lollies or mints (one small handful)									
Sugar added to hot drinks or cereal (1 teasp)									
Potato chips (crisps) or pretzels									
Peanuts or other nuts – one handful									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
SOUPS, SAUCES AND SPREADS									
Vegetable soups (bowl)									
Meat soups (bowl)									
White sauce or cheese sauce (1 tbsp)									
Soy sauce or BBQ sauce (1 tbsp)									
Tomato sauce (1 tablesp)									
Pickles, chutney (1 tablesp)									
Vegemite, marmite (1 teaspoon)									
Jam, honey, marmalade (1 teaspoon)									
Peanut butter (1 teaspoon)									
DRINKS									
Tea (1 cup)									
Coffee, instant or ground (1 cup)									
Coffee, decaffeinated (1 cup)									
Coffee whitener (1 teaspoon)									
Cocoa, hot chocolate (1 cup)									
Milo, Ovaltine (1 cup)									
Wine (glass)									
Beer or cider (1 pot)									
Port, sherry, liqueurs (small glass)									
Spirits: vodka, whiskey (1 shot)									
Diet soft drink (1 glass)									
Soft drink eg. lemonade (1 glass)									
Cola-flavoured soft drink (regular or diet) – 1 glass									
100% fruit juice (1 glass)									
Fruit drink or cordial (1 glass)									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
FRUIT									
Apple (1 fruit)									
Pear (1 fruit)									
Orange, mandarin (1 fruit)									
Grapefruit (half)									
Banana (1 fruit)									
Grapes (1 handful)									
Melon (1 slice)									
Peaches, plums, apricots (1 fruit)									
Berries (1 handful), kiwifruit (2 fruits)									
Tinned fruit (1/2 cup))									
Dried fruit (1 handful)									
VEGETABLES									
Carrots									
Spinach									
Broccoli									
Brussels sprouts									
Cabbage									
Peas									
Green beans									
zucchini									
Cauliflower									
Parsnip, turnips									
Leeks									
Onions									
Garlic									
Mushrooms									

Average use in the last 12 months:	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
VEGETABLES (continued)									
Capsicum									
Bean sprouts									
Green salad, lettuce, cucumber, celery									
Tomatoes									
Sweetcorn									
Beetroot									
Coleslaw									
Avocado									
Baked beans									
Lentils, chick peas, 3 bean mix									
Tofu									
Roasted vegetables (any type)									

Over the last 12 months:

2. Are there any other foods which you ate more than once a week? Yes ☐ No ☐

If yes, please list below

Food	Usual serving size	Number of times eaten each week

3. What type of milk did you use most often?
Select one only: Full cream ☐ Reduced fat (eg. Rev) ☐

Skim (eg. Skinny) ☐ Soy ☐ None ☐

Other, please specify: ☐

4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?

None ☐ ½ a cup ☐ 1 cup ☐ 1 ½ cups ☐ 2 cups ☐ More than 2 cups ☐

5. Did you usually eat uncooked breakfast cereal (excluding porridge)? Yes ☐ No ☐

If yes, what type of cereal (including muesli), did you usually eat? **List the one or two types eaten most often:**

eg. Corn flakes

6. What kind of fat did you most often use for frying, roasting etc?

Select one only:

Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐

If you used vegetable oil, please give type eg. Canola, Sunflower ☐

7. What kind of fat did you most often use for baking cakes, biscuits etc?

Select one only:

Butter ☐
Lard/dripping ☐
Vegetable oil ☐
Solid vegetable fat ☐
Margarine ☐
None ☐

If you used margarine, please give name or type eg. Flora, Meadow Lea ☐

8. How often did you eat food that is fried at home?

Daily ☐
1-3 times a week ☐
Less than once a week ☐
4-6 times a week ☐
Never ☐

9. How often did you eat fried food away from home?

Daily ☐
1-3 times a week ☐
Less than once a week ☐
4-6 times a week ☐
Never ☐

10. What did you do with the visible fat on meat?

Ate most of the fat ☐
Ate some of the fat ☐
Ate as little fat as possible ☐
Did not eat meat ☐

11. How often did you eat grilled or roast meat? ☐ times a week

12. How well cooked did you usually have grilled or roast meat?

Well done ☐ Rare/lightly cooked ☐
Medium ☐ Do not eat meat ☐

13. How often do you add salt to food while cooking?

Always ☐ Usually ☐ Sometimes ☐ Rarely ☐ Never ☐

14. How often do you add salt to any food at the table?

Always ☐ Usually ☐ Sometimes ☐ Rarely ☐ Never ☐

15. Did you regularly use a salt substitute (eg. LoSalt)? Yes ☐ No ☐

16. During the last 12 months, on average, how many times a week did you eat the following foods?

Food Type	Times eaten per week	Portion size
Vegetables (not including potatoes)		Medium serve (1/2 cup cooked)
Salads		1 cup
Fruit (not including fruit juice)		1 fruit
Fish and fish products		Medium serve
Meat, chicken, bacon, ham		Medium serve
Roasted nuts (any type)		1 handful
Sweet pastry (eg. donut, vanilla slice, éclair)		1 serve

17. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the last 12 months?

☐ Yes ☐ No ☐ Don't know

If yes, please complete the table below. If you have taken more than 5 types of supplement please list the ones you took most often:

Vitamin Supplements	Average frequency Tick **one** box per line to show how often on average you consumed supplements									
Name and brand (if known)	**Dose** Please state number of tablets or teaspoons consumed	Never or less than once a month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ Per day

Thank you for completing this questionnaire

Appendix 5:
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don’t know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don’t know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating

Appendix 6:

Thank you for taking part in this research project

These are your results:

Body Mass Index (BMI) and Blood Pressure (BP)

Underweight: BMI less than 19
Healthy weight: BMI between 20-25
Overweight: BMI between 25-30
Obese: BMI greater than 30

Your weight: _____kg, Your height:_____m, Your BMI: _____ kg/m²

Your blood pressure:_____mmHg

A BMI less than 19 or greater than 30 has been associated with an increased risk for health problems. Normal blood pressure should be between 90/60 and 120/80mmHg. Please discuss your BMI or BP with your doctor if you are concerned about your result.

Skin Autofluorescence – measured by the AGE Reader

Age Range (years)	Skin Autofluorescence (AU)
10-20	0.91-1.31
20-30	1.23-1.83
30-40	1.31-2.15
40-50	1.45-2.17
50-60	1.73-2.45
60-70	1.89-3.03
70-80	2.18-3.28
80+	2.27-3.15

Your skin AGE level is:_____AU

Above average levels of Advanced Glycation End products (AGEs) in skin have been seen in people who smoke cigarettes and people who have diabetes. Skin AGE levels also increase as we get older. They can be associated with an increased risk for developing heart disease or diabetes complications. If you are concerned about your result, please see your doctor to have your blood glucose and lipid levels measured.

Random blood glucose (sugar)

Your random blood glucose level is:_____mmol/L at time:_____am/pm

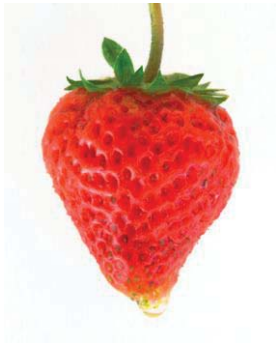
Your random blood glucose (sugar) should ideally be less than 7.8 mmol/L. If your random blood glucose is 11.1 mmol/L or greater and you have never been diagnosed with diabetes, you should see your doctor straight away. This finger-prick test result cannot be used to diagnose diabetes – a pathology blood test must be ordered by your doctor. Please see your doctor if you are concerned about your result.

Appendix 7:
RCT Recruitment Brochure

Am I eligible to participate?

You will not be able to take part in this study if you have any of the following conditions: type 1 diabetes, type 2 diabetes, kidney disease, bowel conditions such as coeliac disease, inflammatory bowel disease or fructose malabsorption. Other people who should not take part in this study include individuals already taking dietary fibre supplements, individuals who are uncomfortable with having a blood sample taken (venipuncture) on 3 occasions or are unable to attend their local pathology collection centre on 3 occasions.

Your personal details will be kept strictly confidential, and the results of this study will not contain any patient names when published.



If you would like to participate in this research project or you would like more information, please contact

Nicole Kellow
Student Researcher

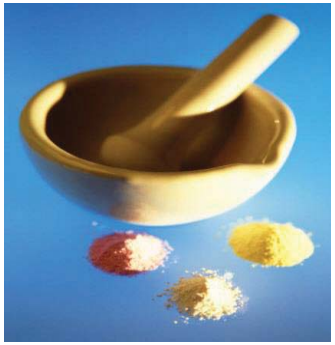
Phone: 0488 488 808

Have you recently been diagnosed with pre-diabetes?

Would you like to participate in a research project?

Name of research project:

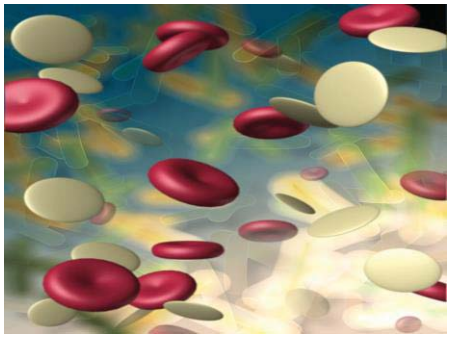
Dietary prebiotic supplementation in adults with pre-diabetes



My name is Nicole Kellow and I am conducting a research project with Professor Chris Reid (a lecturer in the School of Public Health) towards a PhD at Monash University. This means that I will be writing a thesis which is the equivalent of a short book.

You are invited to take part in this study. Please read this brochure and the attached Explanatory Statement in full before making a decision.

We are looking for people with pre-diabetes to participate in this research project. Being in this study is completely voluntary and you are under no obligation to participate.



Why are we doing this research?

We are conducting this research to find out whether people with a high risk of developing type 2 diabetes in the future can reduce their risk by consuming a fibre supplement each day for three months. There are substances in our blood stream called Advanced Glycation Endproducts (AGEs). Everybody produces AGEs, but people with diabetes have very high levels of AGEs in their blood. Consuming a particular type of dietary fibre supplement might reduce the level of AGEs in the blood of people at risk of developing type 2 diabetes.

There will be 30 individuals participating in this project. Fifteen people will receive the fibre supplement, and fifteen will receive a placebo (which will look like the fibre supplement, but will not have any effect in the body). Participants will then swap over to take the alternative supplement.

What does this study involve?

- If you decide to take part in this research project, you will need to attend 3 interviews with Nicole at your local GP clinic. The first interview will be held at the beginning of the project, and the second interview will be 3 months later, and the third will be after another 3 months. Nicole will collect information about your diet, activity and medications at these interviews. You will also be weighed at the beginning, middle and end of the research project.

- You will also be required to give three blood, urine and stool samples at your local pathology, first at the beginning of the project, again 3 months later, and then 3 months after that.

- You will be asked to consume ten grams (1/2 tablespoon) of a tasteless powder every day for six months. This powder can be mixed into any food or drink. You will not know which order you have taken the fibre supplement and the placebo until the end of the study.

Appendix 8:

Supplement guide for research participants

Thank you for participating in this research project. Thirty individuals are involved in this project, and all will be including a powdered supplement in their diet each day for six months. Fifteen people will receive powdered inulin (a dietary fibre supplement) for 3 months and fifteen will receive powdered maltodextrin (starch) for 3 months. After a break of 2 weeks, the study participants will cross over to receive the alternative powder for a further 3 months. Neither participants nor researchers will know the order people are taking the inulin supplement or the maltodextrin supplement until the end of the project.

Both of the supplements are in a white powdered form, both are tasteless and both can be completely dissolved into liquids and soft foods (such as tea/coffee, breakfast cereals, soup, mashed potato, yoghurt). Approximately half a tablespoon of the supplement (provided in each silver sachet) contains 10 grams.

In most individuals, starch will have no side effects. However, inulin may cause some side effects, especially in people who had low levels of dietary fibre in their usual diet prior to starting this study. These side effects may include stomach bloating, stomach cramps and excessive wind. Most of these side effects can be avoided if the supplement is slowly introduced into the diet, in order to allow your digestive system to become accustomed to the change in your diet.

We recommend you introduce both supplements into your diet in small amounts at first, and slowly increase the dose towards the full sachet (10 grams) over approximately 7 days.

<u>Day 1 to Day 7:</u> Take 5 grams of the supplement each day (half of the sachet).

<u>Day 8 onwards:</u> Take 10 grams of the supplement each day (entire sachet).

If you experience unpleasant symptoms despite following the above recommendations, please contact Nicole Kellow on 0488 488 808 for further advice.

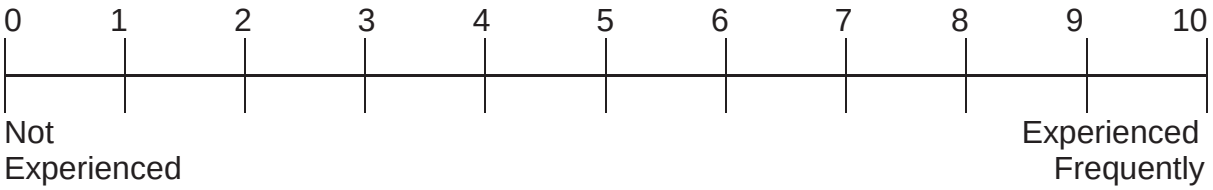
Appendix 9:
Clinical Trial Symptom Questionnaire

Participant Number: _____ Date:_____

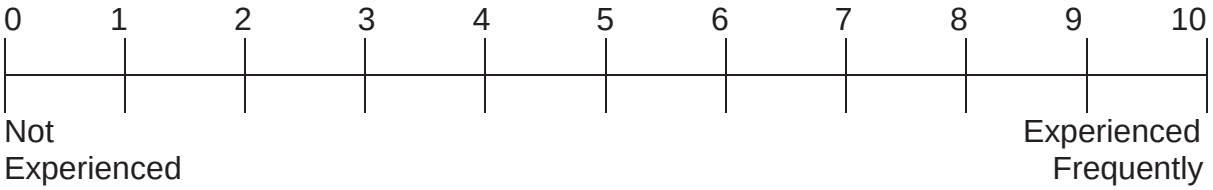
Below are a list of gastrointestinal side effects/symptoms experienced by some people taking fibre supplements. During the trial, you may experience none, some or all of these side effects.

Please rate your current symptoms below on a scale from 0 (not experienced/not detected) to 10 (experienced frequently):

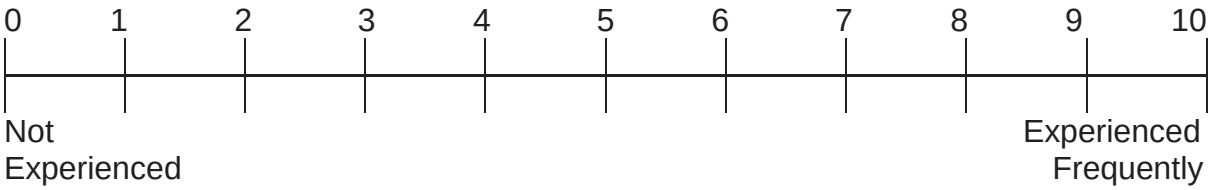
1.) Constipation (hard bowel motions that require straining to pass):



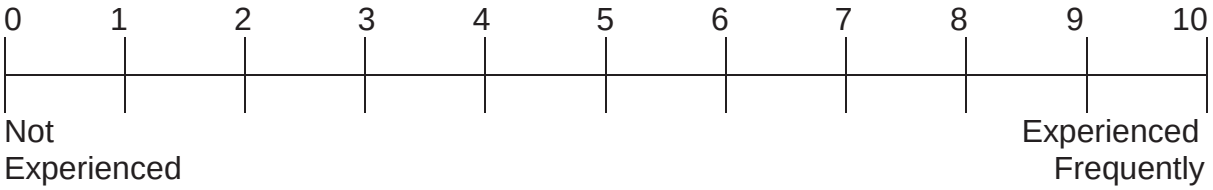
2.) Diarrhoea (loose, watery bowel motions):



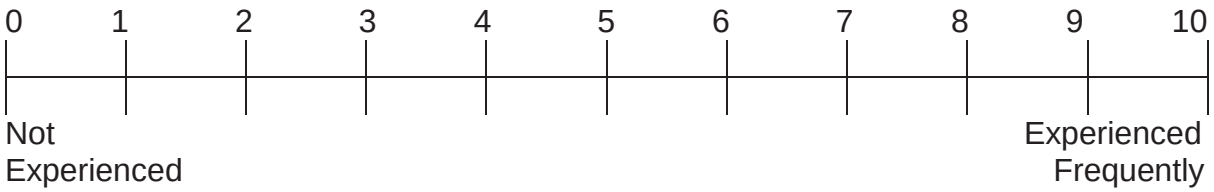
3.) Reduced appetite:



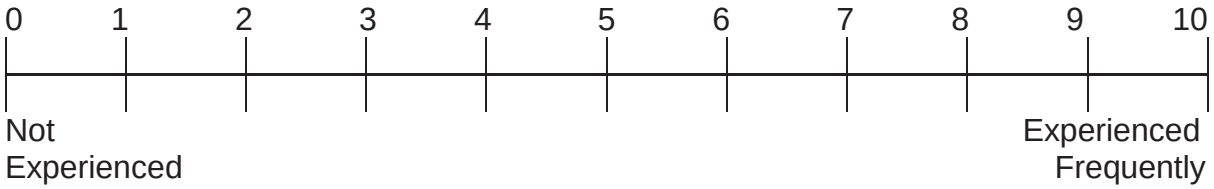
4.) Increased appetite:



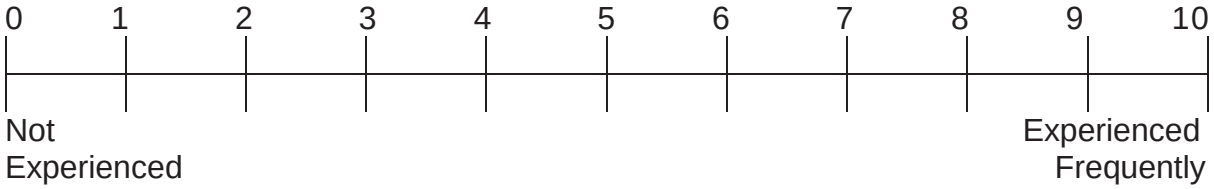
5.) Abdominal bloating:



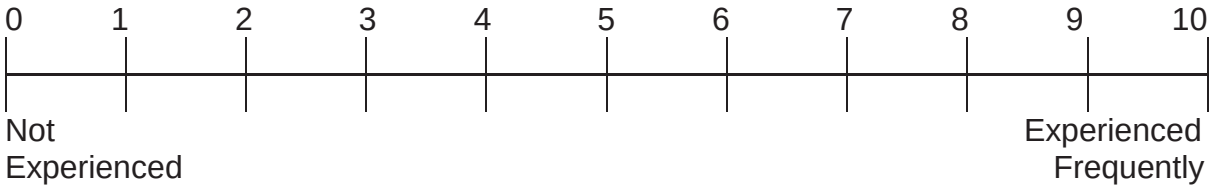
6.) Abdominal pain or cramping:



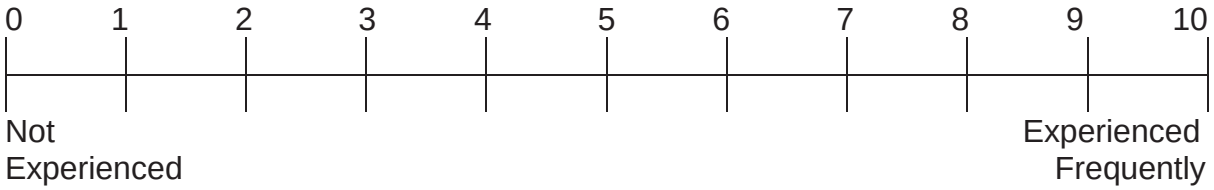
7.) Stomach rumbling/gurgling:



8.) Passing wind (flatulence):



9.) Increased frequency of bowel actions:



Thank you very much for completing this questionnaire