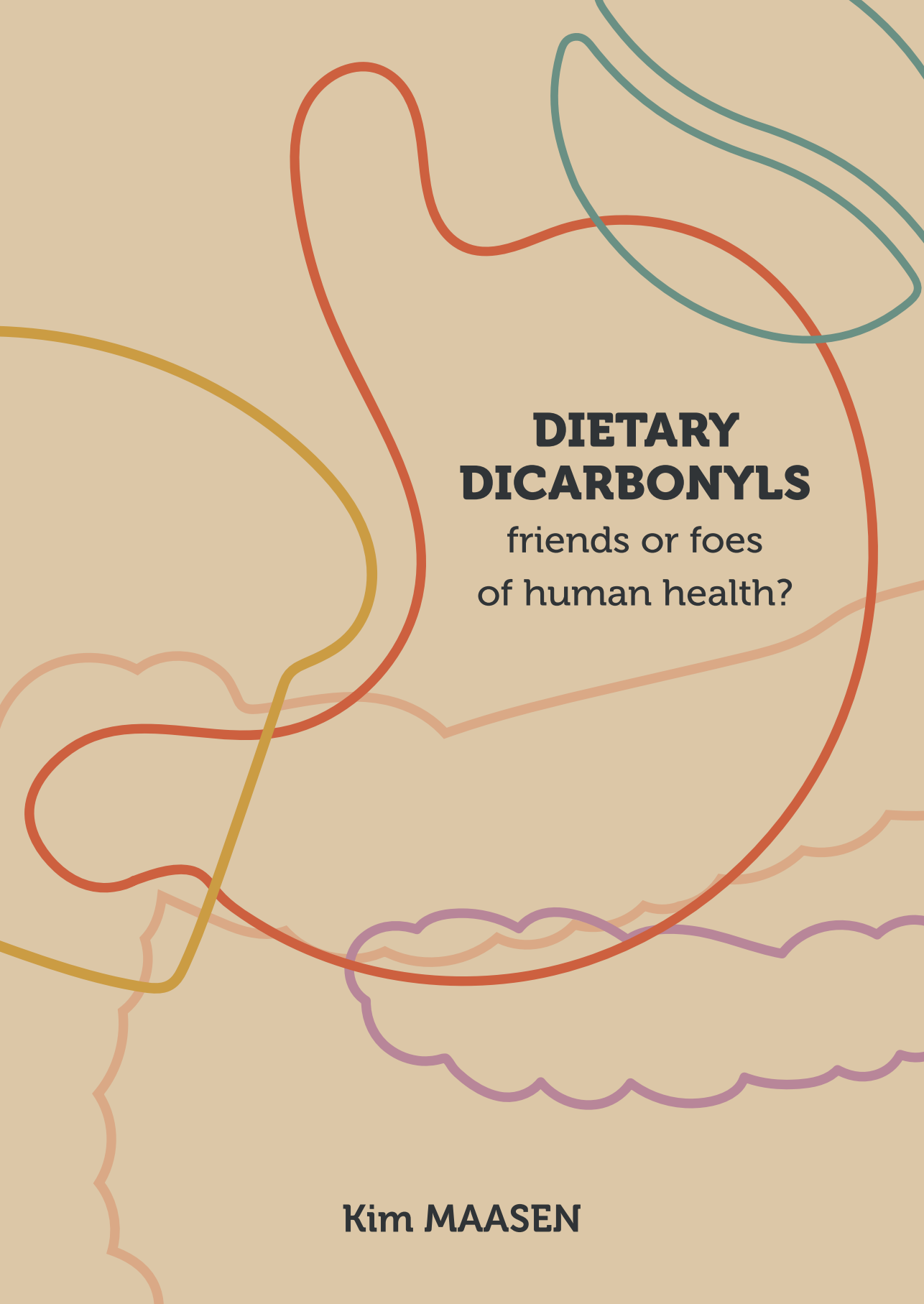
Dietary dicarbonyls



DIETARY DICARBONYLS

friends or foes
of human health?

Kim MAASEN

Dietary dicarbonyls: friends or foes of human health?

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Dietary dicarbonyls: friends or foes of human health?

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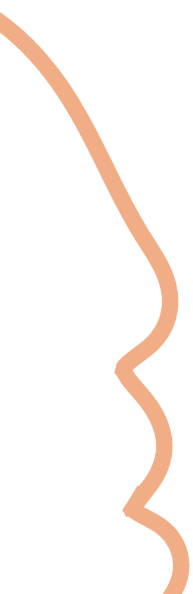
Chapter 1

General introduction



1. Dicarbonyl stress in age-related diseases

1.1 Dicarbonyl stress and advanced glycation endproducts (AGEs)

Dicarbonyls are a heterogenous group of highly reactive compounds that can modify amino acids in proteins and in DNA, leading to the formation of advanced glycation endproducts (AGEs). Elevated dicarbonyls and AGEs play a role in the development of age-related diseases, such as diabetes and cardiovascular diseases[1,2]. The dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) are major precursors in the formation of AGEs (Figure 1.1). MGO, the most reactive dicarbonyl, has a 50,000 fold higher glycation potency than glucose.

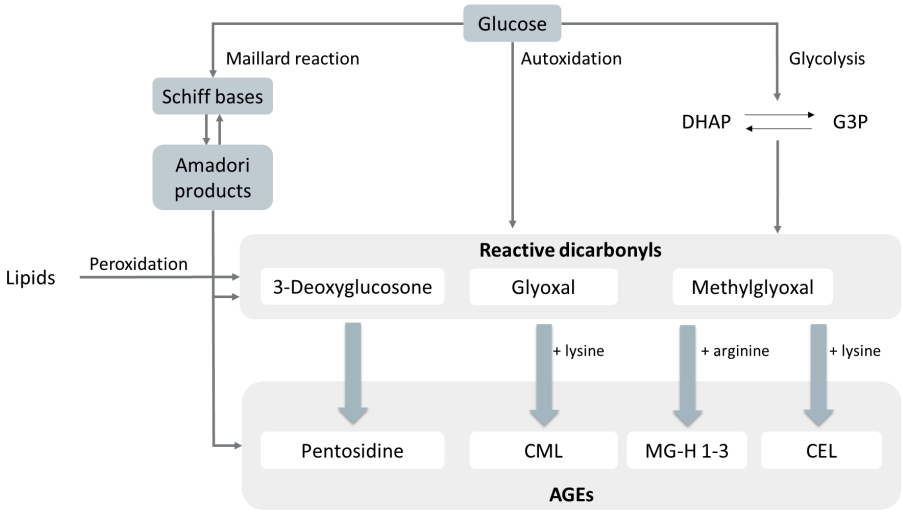


Figure 1.1 The formation of dicarbonyls and subsequent advanced glycation endproducts via glucose. Dicarbonyls and advanced glycation endproducts (AGEs) are formed via the classical pathway of the Maillard reaction (indicated in dark grey boxes). Dicarbonyls are also formed by autoxidation of glucose and by glycolysis via the triose phosphates dihydroxyacetone-phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P). Dicarbonyls can, to a lesser extent, also be formed by peroxidation of lipids, which predominantly results in formation of glyoxal (GO). Methylglyoxal (MGO) reacts with arginine to form methylglyoxal-derived hydroimidazolones MG-H 1-3, which are three isomeric structures. MGO reacts with lysine to form N^{ε}-(1-carboxyethyl)lysine (CEL). GO reacts with lysine to form N^{ε}-(carboxymethyl)lysine (CML). 3-Deoxyglucosone (3-DG) reacts to form pentosidine. Note: this figure includes dicarbonyls and AGEs that are discussed in this thesis, but is not exhaustive.

'Dicarbonyl stress' is defined as the accumulation of dicarbonyls, potentially leading to increased modifications of proteins and DNA, which contributes to cell and tissue dysfunction, complications, and disease[3]. This accumulation is the result of a disbalance between on the one hand the endogenous formation and exogenous exposure to dicarbonyls via the diet, and on the other hand detoxification of dicarbonyls in our body (Figure 1.2). Endogenous formation of dicarbonyls occurs mainly during glycolysis by the nonenzymatic degradation of the triose phosphates glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone-phosphate (DHAP) (Figure 1.1)[4,5]. Besides endogenous formation, we are also exposed to dicarbonyls through exogenous sources, mainly via our diet. Dicarbonyls are formed during food processing, predominantly during heat-treatment as part of the Maillard reaction or caramelization, but also during fermentation. To date, it is unknown to what extent the intake of dicarbonyls from the diet contributes to the endogenous pool of dicarbonyls and AGEs. Moreover, to the best of our knowledge, the health consequences of intake of these dietary dicarbonyls have not been studied in humans. Therefore, in this thesis we aimed to examine the associations of dietary dicarbonyl intake with the endogenous pool of dicarbonyls and AGEs, and with several health outcomes, i.e. low-grade inflammation, microvascular function, insulin sensitivity, pancreatic β-cell function, and presence of prediabetes or type 2 diabetes.

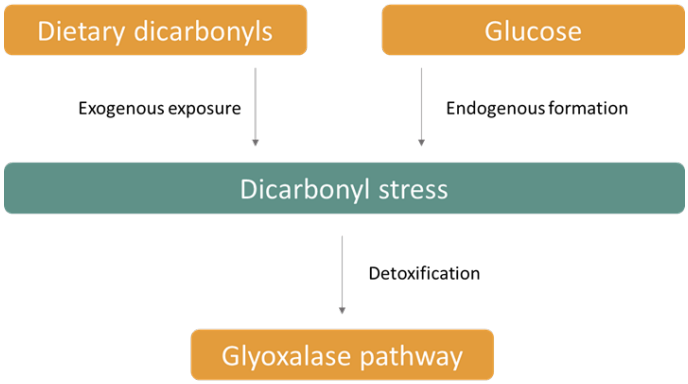


Figure 1.2 **Main drivers of dicarbonyl stress.** Dicarbonyl stress is the disbalance between elevated sources of dicarbonyls and impaired detoxification of dicarbonyls. Drivers of dicarbonyl stress are the exogenous exposure to dicarbonyls from the diet (upper left), the endogenous formation of dicarbonyls, mainly from glucose (upper right), and impaired detoxification of dicarbonyls, for example via the glyoxalase pathway for methylglyoxal (MGO) (bottom). MGO is detoxified by the glyoxalase pathway into D-lactate, involving the enzymes glyoxalase-1 and -2 (glo1 and glo2) and using reduced glutathione (GSH) as substrate. Figure is not exhaustive and other drivers of dicarbonyl stress exist, such as lipid peroxidation or impaired clearance via the kidney.

1.2 Biological consequences of dicarbonyl stress

Elevated dicarbonyl stress plays a role in the development of age-related diseases, such as type 2 diabetes, cardiovascular diseases and cancer[1]. In hyperglycemic conditions, the rate of glycolysis is increased, leading to enhanced production of these glycolytic byproducts. As such, dicarbonyls have been described as one of the mechanisms that link hyperglycemia with macrovascular and microvascular complications (Figure 1.3)[2]. In addition, a recent study showed that circulating dicarbonyls such as MGO are not only a consequence of hyperglycemia, but may itself also induce insulin resistance and β-cell dysfunction, and thus also play a role in the onset of type 2 diabetes[6]. The etiology of type 2 diabetes and cardiovascular diseases and the potential role of dicarbonyls therein will be briefly discussed below.

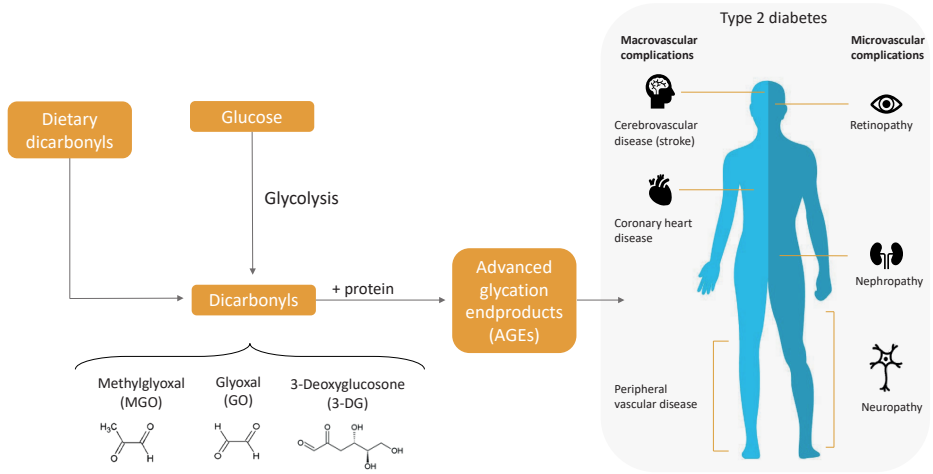


Figure 1.3 **Circulating dicarbonyls and advanced glycation endproducts (AGEs) play a role in macrovascular and microvascular complications.** Dicarbonyls and AGEs are compounds of the glycation pathway; one of four biochemical pathways linking hyperglycemia with complications of type 2 diabetes. Complications of type 2 diabetes are macrovascular complications including stroke, coronary heart disease and peripheral vascular disease and microvascular complications, including retinopathy, nephropathy, and neuropathy. Dicarbonyls are formed endogenously, mainly during glycolysis. Dicarbonyls are also present in food products and hence we are exposed to them via the diet. Adapted from Schalkwijk and Stehouwer, Physiol Rev. 2021.

1.2.1 Type 2 diabetes and cardiovascular diseases

Age-related, non-communicable chronic inflammatory diseases like type 2 diabetes and cardiovascular diseases are a major health concern of the 21st century. In 2019,

almost 1 in 10 adults worldwide (463 million) had diabetes, and the prevalence and incidence have dramatically risen over the last decades[7]. It is often seen as a disease of high-income countries, but with the shift to a Western lifestyle and diet the prevalence has been rising more rapidly in low- and middle-income countries than in high-income countries[8]. Most of the deaths due to these diseases are currently in low- and middle income countries, making it one of the largest threats to health globally[8,9]. In the Netherlands, currently 1.2 million people have diabetes, and every day 150 people are newly diagnosed with diabetes[10].

Of the various types of diabetes, type 2 diabetes is the most common, comprising around 90% of the cases. Type 2 diabetes is characterized by hyperglycemia, as a result of impaired insulin sensitivity of cells, and impaired insulin production by the β-cells in the pancreas. Type 2 diabetes is caused by an interplay of genetic, metabolic, and environmental risk factors, such as ethnicity, family history of diabetes, older age, overweight and obesity, unhealthy diet, physical inactivity and smoking[11,12].

There is a globally agreed target to halt the rise in type 2 diabetes and obesity by 2025[9], and in order to achieve this goal, it is important to not only focus on treatment, but also on prevention. Prevention of type 2 diabetes is particularly important because the progression of diabetes often evolves without clear symptoms, and thus may be diagnosed several years after onset, when complications have already arisen[12]. Although the symptoms seem mild, diabetes has severe complications. Microvascular and macrovascular complications of diabetes are the main cause of morbidity and mortality in diabetic individuals. Microvascular complications occur in small vessels in the eye, kidney and nerves, causing diabetic retinopathy, nephropathy and neuropathy, respectively[13]. Macrovascular complications are related to progression of atherosclerosis, and affect the coronary, the carotid and the peripheral arteries, thereby increasing the risk on heart attack, stroke and diabetic foot (Figure 1.3)[14].

1.2.2 The role of dicarbonyl stress in type 2 diabetes and cardiovascular diseases

The role of dicarbonyl stress in diabetes and cardiovascular diseases has been described in detail in several reviews[15-17]. In short, dicarbonyl stress plays a role in several aspects of type 2 diabetes and cardiovascular diseases, including inflammation, insulin resistance, β-cell function, and macrovascular and microvascular complications. The modification of proteins by the different dicarbonyls and subsequent formation of AGEs (as described in section 1.1), can alter the function of intracellular proteins, lead to cross-linking due to modification of extracellular matrix proteins, and activate intracellular signaling pathways via

binding of circulating proteins to the receptor for AGEs (RAGE)[2]. RAGE binding induces an inflammatory cascade, involving activation of the transcription factor nuclear factor-κB (Nf-κB) and stimulating the production of pro-inflammatory cytokines (Figure 1.4)[17].

Most of the research to date has focused on the most reactive dicarbonyl, MGO. Elevated MGO concentration in the circulation is associated with impaired insulin sensitivity and β-cell function, and induces type 2 diabetes in animals[18,19]. We previously showed that in humans an elevated plasma concentration of the MGO metabolite D-lactate was associated with insulin resistance, independent of other risk factors[20]. Mechanisms by which MGO can contribute to insulin resistance and β-cell dysfunction include functional modification of insulin and the insulin receptor, direct effects on β-cells, and interference in insulin signaling in β-cells (Figure 1.4)(as reviewed in [21]).

In humans, increased dicarbonyl stress is related to the development of the macrovascular complications of diabetes, including stroke[22,23], and microvascular complications of diabetes, such as nephropathy, retinopathy, and neuropathy[24-26]. Dicarbonyls may contribute to impaired microvascular function through dysregulation of endothelial NO synthase (eNOS), which is a regulator of vascular tone through the production of nitric oxide (NO) required for vasoregulation (Figure 1.4)[17,27-29]. Other mechanisms linking MGO with microvascular dysfunction could be via induction of oxidative stress or via the activation of Nf-κB, which is also linked with endothelial dysfunction[30].

Interestingly, besides these undesirable consequences, more recently also beneficial effects of (low doses of) MGO have been reported. Experimental studies in model organisms showed that low-dose MGO promotes life-span whereas high-dose reduces lifespan[6,31]. This led to the proposition of a hormetic effect of MGO, with favorable effects at low concentrations and harmful effects at high concentrations. It is thought that low doses of MGO trigger the defense system, leading to upregulation the anti-oxidant system and the glyoxalase pathway, a major pathway in the detoxification of MGO[32]. Indeed, upregulation of the glyoxalase system was reported after incubation with low dose MGO and downregulation after high doses of MGO[33]. These favorable effects are thought to be via MGO-modification of Kelch-like ECH-associated protein 1 (KEAP1) and subsequent induction of the transcription factor Nrf2 (Figure 1.4)[33,34]. Nrf2 is a key regular in oxidative stress, increasing the production of specific antioxidant enzymes and glyoxalase-1 (Glo1), both involved in cellular protection against glycation[35]. A study in mice showed that activation of the KEAP1-Nrf2 pathway suppressed the onset of type 2 diabetes[36]. Another potential pathway for the

induction of anti-oxidative effects by MGO, is the formation of methylglyoxal-derived hydroimidazolone-3 (MG-H3). This is one of the three structural isoforms that are formed in the glycation of arginine residues by MGO[37], and was found to possess antioxidant properties *in vitro*[38].

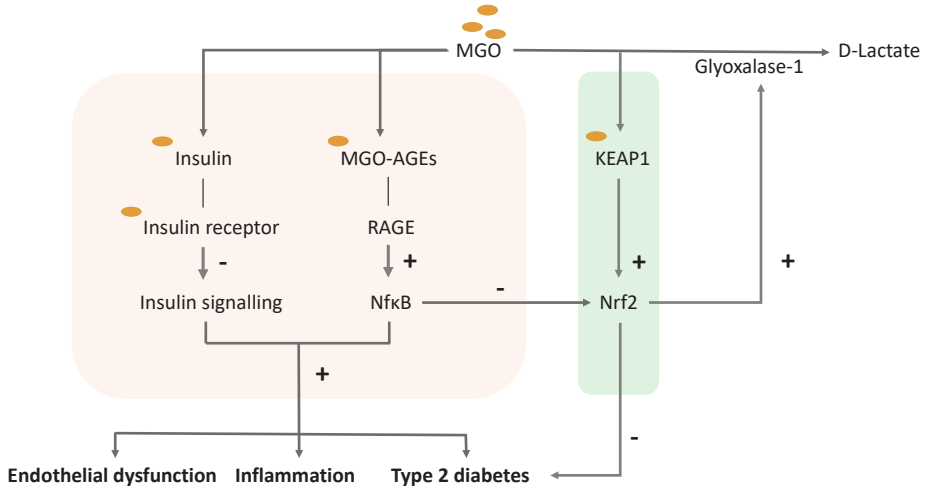


Figure 1.4 **Schematic overview of potential modifications by methylglyoxal (MGO) and its consequences.** MGO can bind to arginine residues of insulin (present in the circulation or in pancreatic cells) or to the receptor for insulin. Modification of insulin or the insulin receptor can lead to loss of function, resulting in a disrupted insulin signalling cascade and subsequent insulin resistance and type 2 diabetes. This may also lead to endothelial dysfunction, because the production of nitric oxide (NO) via PI3K/Akt and endothelial NO synthase (eNOS) is insulin dependent. Other consequences of MGO are the binding of MGO-derived advanced glycation endproducts (AGEs) to the receptor for AGEs (RAGE). AGE-RAGE binding results in activation of intracellular pathways such as the NfκB pathway, which induces an inflammation cascade. NfκB is also linked with endothelial dysfunction, and may inhibit the transcriptional activity of Nrf2, thereby reducing expression of the detoxifying enzyme for MGO, glyoxalase-1. MGO can also modify KEAP1, inducing the anti-oxidative transcription factor Nrf2, which is thought to upregulate glyoxalase-1. Presumable undesirable effects are indicated in red. Presumable beneficial effects are indicated in green. Arrows with '+' indicate upregulation and arrows with '-' indicate downregulation.

2. Contribution of the diet to dicarbonyl stress

2.1 Presence of dicarbonyls in foods and drinks

Dicarbonyls are formed during food processing and preparation, as part of non-enzymatic browning (Maillard reaction), the caramelization reaction, lipid-peroxidation, or enzymatically by microorganisms in fermented foods (as reviewed

in[39]). The presence of dicarbonyls in certain foods and drinks has been evaluated in several studies. These studies often focused on specific, sugar-rich or fermented products. For example, dicarbonyl concentrations have been reported for honey[39-44], coffee[45-47], soft drinks[48,49], dried fruits[50], and fermented products such as beer[51,52], wine[53,54], and soy sauce[46,55]. These studies include various dicarbonyls, most often MGO, GO, and 3-DG, but also 3-deoxygalactosone (3-DGal), glucosone, diacetyl, and 3,4-dideoxyglucosone-3-ene, and are performed using different analytical techniques, including liquid chromatography–mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC-MS), and high performance liquid chromatography with UV detection (HPLC-UV).

In the majority of foods and drinks, 3-DG is the most abundant dicarbonyl, with concentrations exceeding those of MGO and GO[40,48,56]. Structurally, 3-DG is a compound with a six-carbon backbone similar to glucose and fructose (Figure 1.3). MGO and GO are smaller compounds with a three and two-carbon backbone, respectively. 3-DG concentrations in foods seem highly correlated with sugar content, in particular in food items in which monosaccharides like glucose and fructose are used as a sweetener, since these are more susceptible to degradation compared to sucrose[56]. Especially glucose-fructose syrup is thought to be prone to formation of dicarbonyls[56]. MGO and GO concentrations are less strongly correlated with sugar content of food items, and these short-chain dicarbonyls are also formed during fermentation[57]. Concentrations of MGO and GO are generally lower in foods, with the exception of Manuka honey, which is known for its high MGO concentrations[58].

The most extensive study to date quantified MGO, 3-DG, and 3-DGal in 173 foods and drinks[56]. Highest dicarbonyl concentrations were reported in balsamic vinegar, honey and candy, and particularly food items that contained caramel or molasses (both rich in sugar and exposed to intense heat-treatment) had high dicarbonyl concentrations. In this study several factors that impact dicarbonyl formation in foods are described, i.e. the type of sugar used, the intensity of heat treatment and accompanying reduction of water content, food processes such as ripening, fermentation and storage, and the absence of reactants like amino acid side chains of proteins that may result in subsequent formation of AGEs and thus reduce dicarbonyl content[56]. However, this study focuses on sugar-rich foods, and does not include food groups that make up a large part of the habitual diet such as meat, fish, vegetables, fruits, ready-made meals, breakfast cereals, dairy, and tea. This research gap will be addressed in this thesis with the composition of a dietary dicarbonyl database, including MGO, GO, and 3-DG concentrations in a wide range of foods and

drinks commonly consumed in the Western diet, measured with ultra high-performance liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS).

2.2 *Metabolism of dicarbonyls in the gastrointestinal tract*

Dicarbonyls are relatively small compounds, and it is conceivable that they are absorbed from foods in the intestine. Indeed, previous studies in rats showed increased plasma concentrations of MGO after high oral MGO administration via drinking water[59-61]. In humans, two small-scale studies have been performed, in which a single oral dose of wild honey naturally containing a high amount of MGO or 3-DG (~500 μmol) was given[62,63]. After a single dose of 3-DG, a significant increase in urinary excretion of 3-DG and its metabolite 3-deoxyfructose were reported[62], with a recovery of approximately 10-15% of the administered 3-DG in urine, suggesting dietary absorption of 3-DG. In contrast, a single dose of MGO did not lead to changes in urinary MGO excretion[63], but no data on plasma MGO or AGEs was available. Because MGO is more reactive than 3-DG, it may be that dietary MGO, after absorption, rapidly reacts with proteins and is therefore not excreted as free MGO in the urine fraction.

It is also possible that these dicarbonyl compounds from foods react with proteins before they are absorbed, for example with other proteins present in the food matrix or gastro-intestinal tract[63,64], with digestive enzymes[65], or with intestinal proteins[57]. Modification of food proteins could lead to *de novo* formation of AGEs, that can thereafter be absorbed or exert local effects in the gastro-intestinal tract. Modification of digestive proteins may lead to altered digestion, whereas modification of intestinal proteins may lead to gut inflammation, increased permeability or altered microbiome composition[39,66-68]. Besides direct absorption, the concentrations of plasma dicarbonyls could potentially be elevated more indirectly, for example by exhaustion of the glyoxalase pathway - responsible for detoxification of dicarbonyls - in the intestine after long-term exposure to dicarbonyls from the diet[69].

The reactivity of dicarbonyls during digestion has so far only been studied *in-vitro*. Previous studies in *in-vitro* human intestinal cells, reported that both MGO and GO induced an increased secretion of the pro-inflammatory cytokines interleukine-6 (IL-6) and interleukin-8 (IL-8)[70,71]. In most studies, a decrease in dicarbonyl concentration was observed during simulated gastrointestinal digestion[46,63,72]. In contrast, an increase in dicarbonyls concentration has also been reported, in soy sauce, possibly due to their release from reversibly-bound adducts[46]. This indicates that reactions during digestion may play an important role in the actual exposure to dicarbonyls throughout the gastro-intestinal tract.

Important to note here is that a reduction in dicarbonyls does not per se mean that they are harmless, since the putative consequences of the reaction-products are unknown. Furthermore, it should be emphasized that these models might not represent in vivo digestion. Studies with stable isotope labelled dicarbonyls are required in order to trace the gastrointestinal metabolism and possible absorption of orally ingested dicarbonyls in vivo.

When investigating the gastrointestinal metabolism of dicarbonyls, it is important to consider the food matrix in which dicarbonyls are present. The food matrix might affect their release, which can depend on the complexity of the food matrix, or on the presence of scavengers of dicarbonyls. Examples of such potential scavengers are creatinine in meat[73], and melanoidins in coffee, cacao, and bread, for which dicarbonyl-scavenging effects have been reported *in-vitro*[74].

Taken together, little is known about the gastrointestinal metabolism and potential absorption of dicarbonyls from the diet. From the limited studies available, it seems that only a small amount of dietary dicarbonyls are absorbed. Long-term exposure to large amounts of dicarbonyls from the diet may exert biological effects, something which has, to the best of our knowledge, never been studied in humans.

2.3 Consequences of dietary dicarbonyls

The consequences of exogeneous dicarbonyls have mainly been studied in experimental and in animal models. Studies in animals show that high amounts of oral MGO increases vascular inflammation[60,75] and induces vascular changes including impaired vasodilation[60,76], thickening of the glomerular basement membrane[77], endothelial dysfunction, and reduced NO bioavailability[60]. One of the potential underlying mechanisms is via inhibition of the IRS1/Akt/eNOS pathway and thereby blunting nitric oxide (NO) production[28]. Moreover, oral MGO administration induced retinopathy-like changes, such as pericyte loss, formation of acellular capillaries, microglial activation, and early neuronal dysfunction[78]. In line, oral MGO induced insulin resistance[19] and administration of exogenous MGO, either subcutaneously or intraperitoneally, induced β-cell dysfunction, insulin resistance and impaired glucose metabolism and insulin sensitivity[18,28]. Less is known about the effects of oral GO and 3-DG, but one study reported that oral GO induced intestinal tumour growth only after prenatal GO exposure in utero[79]. In another study a single dose of 3-DG reduced the secretion of glucagon-like peptide 1 (GLP-1), which is important in glucose metabolism[80].

In contrast, several experimental studies report beneficial effects of exposure to the dicarbonyl MGO (as reviewed in[81]). A recent study in mice showed that long-

term exposure to oral MGO led to a moderate increase in plasma MGO, yet was accompanied by increased survival, less age-related solid tumors, and absence of diabetes and renal insufficiency[59]. Another study in rats showed a similar increase in plasma MGO after long-term administration with oral MGO, but no differences in oxidative stress and higher levels of antioxidants[61]. In line, anti-oxidative effects of low-doses of MGO were reported *in vitro*[32,33,82].

Potentially, consumption of dietary dicarbonyls might lead to small increases in plasma dicarbonyls, which may induce the beneficial, hormetic effects of MGO, as described in section 1.1.2. This process could be similar to for example the hormetic effect of physical activity, which induces production of reactive oxygen species (ROS). High levels of ROS are harmful, but small increases may activate adaptive responses to oxidative stress and subsequently induce antioxidant defense systems[83,84], and may thereby explain part of the beneficial health effects of physical activity. Hormetic effects via induction of regulatory stress response proteins have been described for other dietary compounds, of which alcohol might be the most well-known[85,86]. Thus, dietary dicarbonyls may also have beneficial health effects when consumed in a low dose but detrimental health effects when consumed in a high dose.

2.4 Indirect contribution of the diet to endogenous dicarbonyl formation

The diet may also contribute to endogenous dicarbonyl formation, for example through induction of postprandial glucose peaks (Figure 1.2). We previously showed that plasma dicarbonyl concentrations increase after an oral glucose tolerance test[87] and after a mixed-meal test[88], with glucose as the primary source for this dicarbonyl formation. In addition, we showed that postprandial dicarbonyl stress can be reduced by improving glucose metabolism through a very low caloric diet[88]. This suggests that dietary alterations that reduce postprandial glucose excursions are promising in limiting dicarbonyl stress.

Postprandial blood glucose concentrations are primarily affected by carbohydrate consumption. Carbohydrates differ in their ability to affect blood glucose. This ability is captured by the measures glycemic index (GI) and glycemic load (GL). GI is defined as the potential of carbohydrate-containing foods to increase postprandial blood glucose[89]. GL additionally takes into account the amount of carbohydrates a product contains, and is therefore indicative of not only the quality, but also the quantity of carbohydrates[90]. In animal feeding studies, diets with a high GI have been linked to AGE accumulation in plasma and tissue[91-93]. In humans, a diet with a low GI and/or GL has been shown effective to lower postprandial hyperglycaemia in individuals with type 2 diabetes[94], and may also

have beneficial effects on lowering dicarbonyl stress, a research gap that will be addressed in this thesis.

3. Contribution of impaired detoxification to dicarbonyl stress

Detoxification of dicarbonyls limits dicarbonyl stress. MGO is detoxified to D-lactate by the glyoxalase system, consisting of two enzymes, glyoxalase-1 (Glo1) and glyoxalase-2 (Glo2), of which Glo1 is the rate-limiting enzyme (Figure 1.4)[95]. Reduced glutathione (GSH) serves as a substrate in this detoxification. This system is active in the cytoplasm of all mammalian cells. GO is also detoxified by this system, albeit to a lesser extent. 3-DG is detoxified mainly to 3-deoxyfructose by aldoketoreductases[5].

Regulation of Glo1 expression and activity is a complex process that determines the capacity to detoxify MGO. Expression and activity of Glo1 are, amongst others, responsive to insulin and antioxidants, with downregulation in hyperglycaemic or hypoxic states and upregulation via the antioxidant-system transcription factor Nrf2[35,96]. Inhibition of Glo1 increases MGO accumulation and expression of inflammation and endothelium dysfunction markers, and decreases cellular viability[97-100].

Several experimental studies have linked dysfunction of Glo1 to a higher prevalence of diabetes[101]. In rats, overexpression of Glo1 decreased diabetes-induced accumulation of MGO and the MGO-derived AGE N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), decreased oxidative stress and endothelial dysfunction, and attenuated early renal impairment[99,102]. In humans, low Glo1 activity was associated with painful diabetic neuropathy[103], plaque rupture (as reviewed in[104]), and coronary artery disease[100]. Interestingly, a recent integrative genomics study revealed *GLO1* as a key regulatory gene in coronary artery disease-related processes[105].

Thus, the glyoxalase system, and in particular its rate-limiting component Glo1, may be a key determinant of inter-individual susceptibility to elevated exogeneous and endogenous MGO levels (Figure 1.2).

4. Aims and outline of this thesis

The aim of this thesis was to examine the associations of dicarbonyl intake from the diet with endogenous concentrations of dicarbonyls and AGEs, as well as with low-grade inflammation, microvascular function, insulin sensitivity, β-cell function, and the presence of prediabetes or type 2 diabetes in a human cohort study; The Maastricht study. The first step in elucidating these relationships, is accurate estimation of the intake of dicarbonyls from the diet. This requires a comprehensive database containing dicarbonyl contents of foods, that covers all food items from dietary recalls. Therefore, in **chapter 2**, we compiled an extensive food composition database containing MGO, GO, and 3-DG content of 223 foods and drinks commonly consumed in a Western diet (see Figure 1.5 for schematic overview of chapters). For this, we validated and used an ultra high-performance liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS) method. In **chapter 3**, we examined the associations of dietary intakes of MGO, GO, and 3-DG with corresponding plasma dicarbonyl concentrations and with skin autofluorescence (an estimate of skin AGEs) in individuals from The Maastricht Study. In **chapter 4**, we examined the associations of dietary dicarbonyl intakes with low-grade inflammation and with microvascular function in individuals from The Maastricht Study. In **chapter 5**, we examined the associations of dietary dicarbonyl intakes with insulin sensitivity, β-cell function, and prediabetes or diabetes in individuals from The Maastricht Study.

Besides intake of dicarbonyls via the diet, food intake may also contribute to dicarbonyl stress in other ways. One of these is the endogenous formation of dicarbonyls in plasma during postprandial glucose excursions. In **chapter 6**, we describe the associations of dietary glycaemic index and glycaemic load with dicarbonyls and AGEs in plasma and in urine, in individuals of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM). We hypothesize that individuals with a habitual diet higher in glycaemic index or glycaemic load are exposed to more/higher postprandial blood glucose peaks, resulting in increased formation of dicarbonyls and AGEs.

In addition to a higher intake of dicarbonyls or a higher endogenous formation of dicarbonyls, detoxification of dicarbonyls also plays a role in the exposure to dicarbonyl stress. MGO is detoxified to D-lactate by the glyoxalase system, of which glyoxalase 1 (Glo1) is the rate-limiting enzyme[95]. Genetic variation in *GLO1* may alter the expression and/or the activity of Glo1, and may thus represent life-long exposure to a higher or lower detoxification potency and MGO stress. Using genetic variation as an exposure allows better examination of causality, which is usually hampered by the bidirectional association between Glo1 and MGO, as MGO can also

upregulate or downregulate Glo1[33]. In **chapter 7**, we examined the association of nine single nucleotide polymorphisms (SNPs) that cover the total common variability in *GLO1* with gene expression of GLO1 in white blood cells. In addition, we studied the associations of these SNPs with severity of MGO-stress, assessed as concentrations of MGO, D-lactate, and MGO-derived AGEs in fasting plasma, as the formation of MGO after an oral glucose tolerance test (OGTT), and as concentrations of MGO-derived AGEs and D-lactate in urine. These analyses were performed in individuals of the CODAM study. The results of this thesis are summarized and discussed in **chapter 8**.

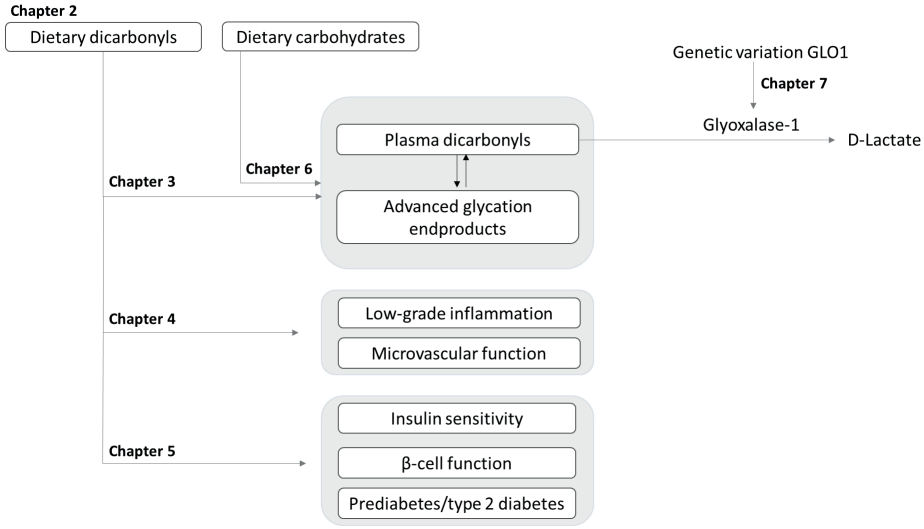


Figure 1.5 **Schematic representation of the associations studies in this thesis, with the chapters indicated in bold**. GLO1; Glyoxalase-1 gene.

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Chapter 2

Quantification of dicarbonyl compounds in commonly consumed foods and drinks; presentation of a food composition database for dicarbonyls

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Abstract

Dicarbonyls are reactive precursors of advanced glycation endproducts. They are formed endogenously and during food processing. Currently, a comprehensive database on dicarbonyls in foods that covers the entire range of food groups is lacking, limiting knowledge about the amount of dicarbonyls that is ingested via food. The aim of this study was to analyze the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) in commonly-consumed products in a Western diet. We validated a UHPLC-MS/MS method to quantify MGO, GO, and 3-DG. We present a dietary dicarbonyl database of 223 foods and drinks. Total dicarbonyl concentrations were highest in dried fruit, Dutch spiced cake, and candy bars (>400 mg/kg). Total dicarbonyl concentrations were lowest in tea, dairy, light soft drinks, and rice (<10 mg/kg). The presented database of MGO, GO and 3-DG opens the possibility to accurately estimate dietary exposure to these dicarbonyls, and explore their physiological impact on human health.

Introduction

Reactive dicarbonyl compounds can modify amino acids in proteins and protein residues, resulting in the formation of advanced glycation endproducts (AGEs). The dicarbonyl compounds methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) are major precursors in the formation of AGEs. The formation and accumulation of these dicarbonyls as well as AGEs in the body plays a role in the development of several age-related chronic inflammatory diseases such as diabetes, cardiovascular disease[1], cancer, and disorders of the central nervous system (as reviewed in[2,3]). Besides endogenous formation, dicarbonyls are also generated during processing and preparation of foods, as part of non-enzymatic browning (Maillard reaction), the caramelization reaction, lipid-peroxidation, or enzymatically by microorganisms in fermented foods (as reviewed in[4]).

The contribution of dietary (exogenous) dicarbonyls to the total amount of dicarbonyls in the body and their impact on human health are unknown. The first step in elucidating this, is to explore the association of dietary dicarbonyl intake with plasma/tissue dicarbonyls and AGEs, and health outcomes, in human cohort studies. This requires a comprehensive database on dicarbonyl concentrations in foods that covers all food items from dietary recalls.

The presence of dicarbonyls in certain foods and drinks, often specific, sugar-rich or fermented products, has been evaluated in several studies. For example, dicarbonyl concentrations have been reported for honey[4-9], coffee[10-12], soft drinks[13,14] and fermented products such as beer[15,16], wine[17,18], and soy sauce[11,19]. These studies include various dicarbonyls, most often MGO, GO, and 3-DG, but also 3-deoxygalactosone (3-DGal), glucosone, diacetyl, and 3,4-dideoxyglucosone-3-ene, and are performed using different analytical techniques, including liquid chromatography–mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC-MS), and high performance liquid chromatography with UV detection (HPLC-UV). The most extensive study to date quantified MGO, 3-DG, and 3-DGal in 173 foods and drinks. They report highest concentrations in balsamic vinegar, honey, and candy[20]. However, this database focuses on sugar-rich foods, and does not include food groups which make up a large part of the habitual diet such as meat, fish, vegetables, fruits, ready-made meals, breakfast cereals, dairy, and tea. Additionally, it does not contain data on the major dicarbonyl GO.

Thus, there is currently no extensive database available that reports on dicarbonyl concentrations in the full scale of commonly-consumed foods and drinks. Such a database will enable us to reliably estimate the dietary intake of dicarbonyls, e.g. in human studies that have food diaries and/or food frequency questionnaires available, and to evaluate their impact on human health. Therefore,

we generated a database of the three major dicarbonyls, MGO, GO, and 3-DG, in a wide range of foods and drinks commonly consumed in the Western diet, measured with ultra high-performance liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS).

Materials and methods

Materials

Methylglyoxal (MGO) solution (~40%), glyoxal (GO) solution (~40%), formic acid (mass-spectrometry grade) and perchloric acid (PCA, 70%, p.a.) were obtained from Sigma-Aldrich (Steinheim, Germany). D_8-o-phenylenediamine (oPD) (98.6%) was obtained from CDN-isotopes (Quebec, Canada). 3-Deoxyglucosone (3-DG) (95%) was obtained from Santa Cruz (Santa Cruz, California). O-phenylenediamine was obtained from Merck (Darmstadt, Germany). Water and acetonitrile (ULC/MS quality) were obtained from BiosolveChimie (Dieuze, France).

Selection of foods and drinks for dicarbonyl analysis

Foods and drinks for dicarbonyl analysis were selected based on the items included in the Food Frequency Questionnaires of the Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC)[21], and the Dutch National Food Consumption Survey[22]. A total of 180 foods and 43 drinks were chosen mainly based upon their habitual and frequent consumption by the Dutch population.

Preparation of foods and drinks for dicarbonyl analysis

Foods and drinks were obtained from local supermarkets and prepared according to the instructions on the manufacturer's label. After preparation, food products were mechanically chopped and homogenized using a conventional hand-held kitchen blender. One complete serving of each product was homogenized, to represent the entire product and not a disproportionate amount of e.g. outer layer. Carbonated drinks were decarbonized by mechanical shaking and releasing gas until no gas remained. Homogenized products were stored at -20°C until analysis.

Optimization of sample preparation and extraction method of dicarbonyls from different food matrices

To investigate the most optimal, fast, and simple sample preparation technique, three different methods were evaluated. To this end, the performance of these three methods was compared for five divergent food matrices - cheese, chocolate, cashew nuts, Dutch spiced cake ("ontbijtkoek"), and raisins. All food products were homogenized, and subsequently weighted, extracted, and quantified in triplicate. Method 1 was a standard liquid extraction procedure and is described in detail in the section "Sample preparation and extraction of dicarbonyls from foods and drinks". For method 2 we used liquid nitrogen frozen food products and crushed these products with a mortar and ~15 mg food product was used for liquid extraction with 120 µL oPD reagent in an Eppendorf cup. For method 3 we prepared a 50% (m/m, %) food homogenate in water and applied sonication for 1 minute at a frequency of 20 kHz. Approximately 30 mg of this food homogenate was mixed with 120 µL oPD reagent in an Eppendorf cup. All three reaction mixtures were allowed to stand overnight (20 h) at room temperature and shielded from light. The standard liquid extraction (method 1) was selected as our standard procedure, and this extraction method was used for all foods and drinks in the database, described in more detail below.

Sample preparation and extraction of dicarbonyls from foods and drinks

For the analysis of foods, a sample of approximately 15 mg was taken from the blended food and weighted exactly using an analytical balance. For the analysis of drinks, 30 µL was taken by pipetting. Of each product, two individual samples were taken from the homogenate and analyzed separately for duplicate analysis.

Weighted food and drink samples were mixed with 120 µL oPD (10 mg oPD in 10 mL 1.6 mol/L perchloric acid) in an Eppendorf cup. oPD was chosen as a derivatizing agent, because D_8-oPD was used to synthesize our internal standards[23], which are not commercially available. Moreover, the combined deproteinization and derivatization step had a simple sample preparation, was less time-consuming, had lower costs, and derivatives gave high ionization efficiency. After an overnight (20 h) reaction at room temperature and shielded from light, 20 µL internal standard solution of MGO, GO, and 3-DG was added. Samples were vortexed and subsequently centrifuged for 20 minutes at 14000 rpm at 4°C. 10 µL was injected for UHPLC-tandem MS analysis. For foods or drinks with dicarbonyl concentrations above calibration concentrations, the homogenized sample was diluted, and the

analysis was repeated. Foods and drinks with dicarbonyl concentrations above the linear range of the MS detector were additionally diluted with 5% (v/v) PCA.

UHPLC tandem MS analysis

Derivatized MGO, GO and 3-DG were separated by ultra high-performance liquid chromatography (Acquity UPLC, Waters, Milford, USA) and detected in ESI positive multiple reaction monitoring (MRM) mode using a Xevo TQ MS (Waters, Milford, USA). Derivatives were separated on a reversed-phase C18 column (Acquity UPLC BEH C18, 50 x 2.1 mm, 1.7 µm) with a binary gradient of 5 mmol/L formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 700 µL/min. A linear gradient was started at 1% eluent B, which was changed within 5 minutes to 15% eluent B. After cleaning the column with 50% eluent B during 2 minutes, the column was equilibrated for 2 minutes at the initial conditions. The injection volume was 10 µL and column temperature was set at 45°C. Retention times of GO, MGO and 3-DG were 2.09, 2.63 and 1.67 min, respectively, and the corresponding stable isotopes were eluted at 2.06, 2.60 and 1.65 min, respectively. Quantification of MGO, GO and 3-DG was performed by calculating the peak area ratio of each unlabeled peak area to the corresponding internal standard peak area. The characteristic combinations of the parent ions and the product ions (Q1>Q3, m/z) for MGO, GO, and 3-DG were 145.1>77.1, 131.1>77.1, and 235.1>171.1, respectively. The characteristic combination of the parent ions and the product ions (Q1>Q3, m/z) for the internal standards [$^2H_4$]-MGO, [$^2H_4$]-GO, and [$^2H_4$]-3DG were 149.1>81.1, 135.1>81.1, and 239.1>175.1, respectively. Electrospray ionization was done at a capillary voltage of 0.5 kV a source temperature of 150°C and a desolvation temperature of 600°C. For qualitative and quantitative analysis Masslynx software (V4.1, SCN 644, Waters, Milford, USA) was used.

Method validation

Linearity of the quantification method in different matrices was determined by a adding standard mixture of MGO, GO, and 3-DG to water (ULC/MS quality) and to four different food and drink samples (whole grain bread, fried potato, coke, and milk). A six-point calibration curve was prepared for MGO (0 – 0.37 mg/L), GO (0 - 0.41 mg/L), and 3-DG (0 – 1.43 mg/L), with dilution factors of 1, 0.75, 0.5, 0.25, 0.125, and 0; diluted in water. The peak area ratio of MGO, GO, and 3-DG multiplied by the concentration of each corresponding internal standard were plotted as a function of the concentration. Inter- and intra-assay variation was tested in coke, tomato soup and milk on 7 different days (inter-assay) and 10 times on the same

day (intra-assay). Recovery was tested by calculating the response factor (slope) of the calibration curves, prepared as described above. Lower limits of quantification (LOQ) were estimated in three different food products at a signal-to-noise (s/N) ratio of 10.

Variation in dicarbonyl concentrations within and between brands

To examine the robustness and accuracy of our measurements, we quantified multiple batches from the same brand and from different brands in a selection of foods and drinks (beer, orange juice, coke, multi grain bread, whole grain bread, Dutch speculaas cookie, Dutch spiced cake, cheese, raisins, carrot, broccoli, potato, cod fish, and chicken). All products were purchased, prepared, and analyzed independently at different time-points.

Effect of preparation methods of foods and drinks on dicarbonyl concentrations

To examine the effect of preparation method, a selection of foods and drinks (white bread, egg, chicken, fish, vegetables, potatoes, and coffee) were prepared by different commonly applied preparation methods. White bread was analyzed before and after toasting. Eggs were boiled, fried or prepared as an omelet. Chicken and cod fish were pan-fried or steamed (for 10 minutes). Bell pepper, tomato, and onion where analyzed both raw and stir-fried (in olive oil for 5-10 minutes). Broccoli was boiled (for 5 minutes) or stir-fried. Carrots were analyzed raw, boiled and stir-fried. Potatoes were peeled, cut into fries, and subsequently boiled, pan-fried, oven baked, or deep-fried. Filtered coffee (both regular and caffeine free), unfiltered coffee, and machine coffee were analyzed. Additionally, fried potatoes were analyzed at seven different time-points (deep-frying for 2-8 minutes), to monitor dicarbonyl concentrations during frying.

Statistical methods

All samples were analyzed at least in duplicate and reported values represent the mean and standard deviation.

To explore whether sugar-rich products contained higher amounts of sugar-derived dicarbonyls, the relationship of total carbohydrate- and simple sugar (mono- and disaccharide) content with dicarbonyl concentration of foods and drinks was examined using Spearman's correlation coefficient. In addition, the relationships of protein- and fat content with dicarbonyl concentration were

examined. The macronutrients and simple sugars content in products were retrieved either from the food label or from the Dutch food composition table (NEVO), in case of unlabeled products.

Modification of protein or fat by dicarbonyls results in the formation of AGEs. We therefore also examined the relationship between dicarbonyl concentration and AGE concentration in foods and drinks, using our recently published food composition AGE database[24]. This database contains concentrations of the three major AGEs; N_{ε}-(carboxymethyl)lysine (CML), N_{ε}-(1-carboxyethyl)lysine (CEL), and N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), measured with UHPLC-MS/MS. The relationship was examined in products that were included in this AGE database as well as in the present dicarbonyl database (n=112).

Lastly, the relative contribution of the various food groups to daily dicarbonyl intake from the Dutch diet was examined. Average daily intake of each food group was retrieved from the Dutch National Food Consumption Survey (Voedsel consumptiepeiling, 2012-2016, age 1-79 years), except for ready-made meals which were not included in the survey[22]. Daily intake of MGO, GO, and 3-DG was then estimated by multiplying the median of dicarbonyl concentrations for each food group, by the average daily intake of that food group, using the following formula (MGO from vegetables as an example):

Estimated MGO intake from vegetables (grams/day) = median MGO concentration in vegetables (mg/kg) / 1000 * daily vegetable intake (grams/day).

All analyses were performed using R version 3.3.0 (cran.r-project.org).

Results and discussion

Comparison of sample preparation and extraction methods of dicarbonyls from different food matrices

Three different extraction methods for MGO, GO, and 3-DG using oPD reagent were developed and compared (see methods section "Optimization of sample preparation and extraction method of dicarbonyls from different food matrices"). The extraction yield of dicarbonyls from food using a simple liquid extraction method was compared with two extended methods of homogenization, i.e. crushing of the frozen foods with a mortar and sonication. Five divergent food matrices were prepared using these three methods for comparison (Supplementary Figure S2.1). In general, the concentrations of all dicarbonyls were in the same order of magnitude for all three extraction methods. We observed higher MGO concentrations in Dutch spiced cake after sonication of the food product as compared to method 1 and 2. Additionally, we observed higher 3-DG

concentrations in raisins after liquid nitrogen before extraction of the food product (method 2) as compared to both other methods. Apart from the mentioned differences, the three methods yielded similar concentrations for MGO, GO, and 3-DG in all five food products. We therefore concluded that, overall, the extraction yield of the two extended methods was equal to the simple liquid extraction method. Thus, the simplest and least time-consuming extraction method – i.e. liquid extraction, method 1 - was selected as the optimal method and used for the analyses.

Method validation

Calibration curves for MGO, GO, and 3-DG were linear over the concentration ranges (r^2>0.99), in water, and in different food and drink matrices. Mean slope (response factor, Rf) for MGO, GO and 3-DG tested in water, food, and drink matrices were 1.00 ± 0.05 (CV, 5.1%), 1.00 ± 0.12 (CV, 12.4%), and 0.99 ± 0.11 (CV, 10.9%), respectively (Supplementary Table S2.1 and Figure 2.1). Inter-assay variation (CV, %), as determined by replicate analysis of a coke, tomato soup, and milk sample on seven different days for MGO, GO and 3-DG was ~8% (using the same homogenized samples) (Supplementary Table S2.2). Intra-assay variation, as determined by replicate analysis (n=10) of a coke, tomato soup, and milk sample on one day for MGO, GO, and 3-DG, was ~12% (Supplementary Table S2.2). The lower limits of quantification at a signal-to-noise ratio of 10 (s/N=10) on column for MGO, GO, and 3-DG were 200, 16, and 108 pg, corresponding to a concentration of 20, 1.6, and 10 µg/L, respectively.

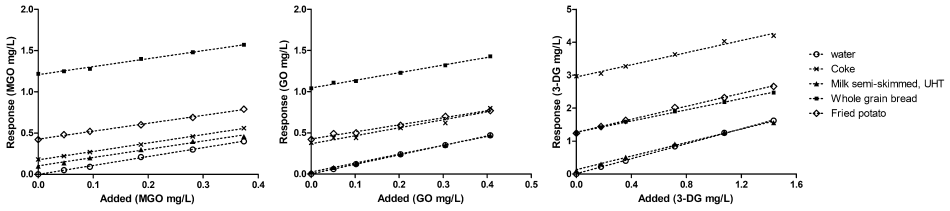

Figure 2.1 Standard addition of MGO, GO, and 3-DG to water, coke, milk, whole grain bread and fried potato. Linearity was determined by adding standard solution of MGO, GO, and 3-DG to water and selected food and drink matrices. The peak area ratio of the analyte/internal standard area of MGO, GO and 3-DG multiplied by the concentration of each corresponding internal standard (Y) were plotted as function of the analyte concentration (X). r^2>0.99. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Dicarbonyl concentration in commonly-consumed foods

A complete list of concentrations of the dicarbonyls in commonly-consumed foods is presented in Supplementary Table S2.3 and summarized in Figures 2.2 and 2.3. Concentrations of dicarbonyls were high in Manuka honey, dried fruits, candy bars, raisin biscuits, Dutch spiced cake, and sweet bread condiments such as bee honey, apple molasses, and sugar syrup (>200 mg/kg). Concentrations of dicarbonyls were low in dairy, white vinegar, olive oil, rice, pasta and potato, fresh fruits, meat (except for stew), fish, and raw or boiled vegetables (<10 mg/kg).

3-DG concentrations ranged from 0.02-2990 mg/kg, and 3-DG was the major dicarbonyl in most foods. MGO concentrations ranged from 0.04-736 mg/kg, and were the highest in Manuka honey, dried apricot, Dutch spiced cake, rusk, apple molasses, and digestive biscuit. GO concentrations ranged from 0.01-37 mg/kg, and were the highest in apple molasses, Dutch spiced cake, sugar syrup, chocolate sprinkles, chocolate, and Manuka honey. In most foods, 3-DG was the major dicarbonyl, but in some foods there was a relatively large contribution of MGO, e.g. in fish, meat (except stew) and most types of cheese, with concentrations exceeding those of GO and 3-DG, while in others presence of GO was high in comparison to the other dicarbonyls, e.g. in most fresh fruits, vegetables, vegetarian products, and nuts.

For bread, dicarbonyl concentration was high in rye bread, croissant, raisin bread, and rusk (ranging from 90-146 mg/kg). In line with these observations, Degen et al. also reported high 3-DG and MGO concentrations in rusk, which they attributed to the combination of high glucose concentrations and intense heat treatment[20]. We observed higher concentrations of dicarbonyls in bread after toasting, and in dark versus light rye bread (prepared by a heat treatment of ~15 versus ~2 hours, respectively). This supports the hypothesis that heat treatment leads to higher concentrations of dicarbonyls. In addition to heat treatment, dicarbonyl formation is affected by the ingredients used. There is no consensus in the literature regarding the influence of different flour types used in bread. Some studies report that the concentrations of 3-DG are independent of flour type[20], whereas other studies reported higher concentrations of 3-DG in products containing oat, teff, or rye in comparison to wheat[25]. We observed high 3-DG concentrations for rye bread and Dutch spiced cake containing rye. In line, 3-DG concentrations were lower in gluten-free Dutch spiced cake, which contains lupine-flour instead of rye (402 versus 473 in regular spiced cake mg/kg). The origin of high concentrations of dicarbonyls in raisin bread are most likely the raisins, as high concentrations of dicarbonyls are present in these samples and other dried fruits (the present study and[20,26,27]).

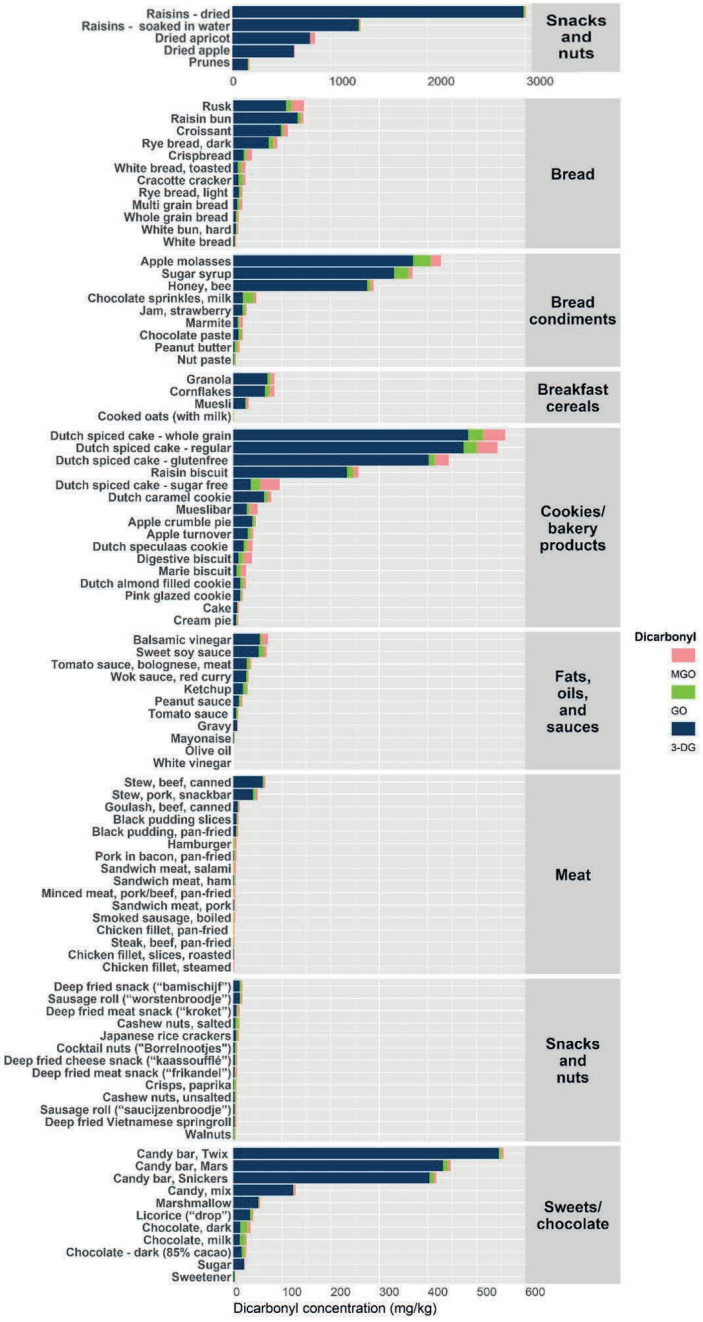

Figure 2.2 MGO, GO, and 3-DG concentration in foods with high concentrations (mg/kg). Concentrations of dicarbonyls in Manuka honey samples are not displayed, and ranged from 25-736 mg/kg for MGO, 9-17 mg/kg for GO, and 490-583 mg/kg for 3-DG. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

For cookies and bakery products, the highest concentrations of dicarbonyls, mainly 3-DG, were detected in Dutch caramel cookie, raisin biscuit, and Dutch spiced cake (total dicarbonyl concentrations 78-558 mg/kg respectively). Sugar-free spiced cake had lower concentrations of dicarbonyls than the regular spiced cake of the same brand (95 versus 543 mg/kg). Dutch spiced cake, digestive biscuits, and muesli bars also contained relatively high concentrations of MGO (17-45 mg/kg). High concentrations of dicarbonyls have previously been reported in cookies containing caramel syrup[20], ammonium bicarbonate, or fructose[28]. This is confirmed in the present study, as glucose-fructose syrup is the main sugar type used in regular Dutch spiced cake and in Dutch caramel cookie, whereas gluten-free spiced cake and digestive biscuits contain inverted sugar syrup and ammonium carbonate.

Breakfast cereals were also high in dicarbonyls, with higher amounts in cornflakes and crunchy granola compared to muesli and cooked oats, likely due to heat treatment and/or use of added sugars.

Sweets and chocolate all contained considerable amounts of total dicarbonyls (26-555 mg/kg), with highest concentrations in chocolate candy bars (Twix, Mars, and Snickers), likely because these bars contain caramel. Notably, chocolate candy bars, chocolate sprinkles and chocolate milk all contained relatively high concentrations of GO, possibly due to the fermentation step in chocolate processing.

In bread condiments, dicarbonyl concentrations were highest in honey, sugar syrup, and apple molasses (288-427 mg/kg). This is in line with literature, and can be attributed to the high concentrations of monosaccharides in honey and sugar syrup and to high thermal impact during industrial production in combination with the presence of molasses as ingredient in apple molasses[7,8].

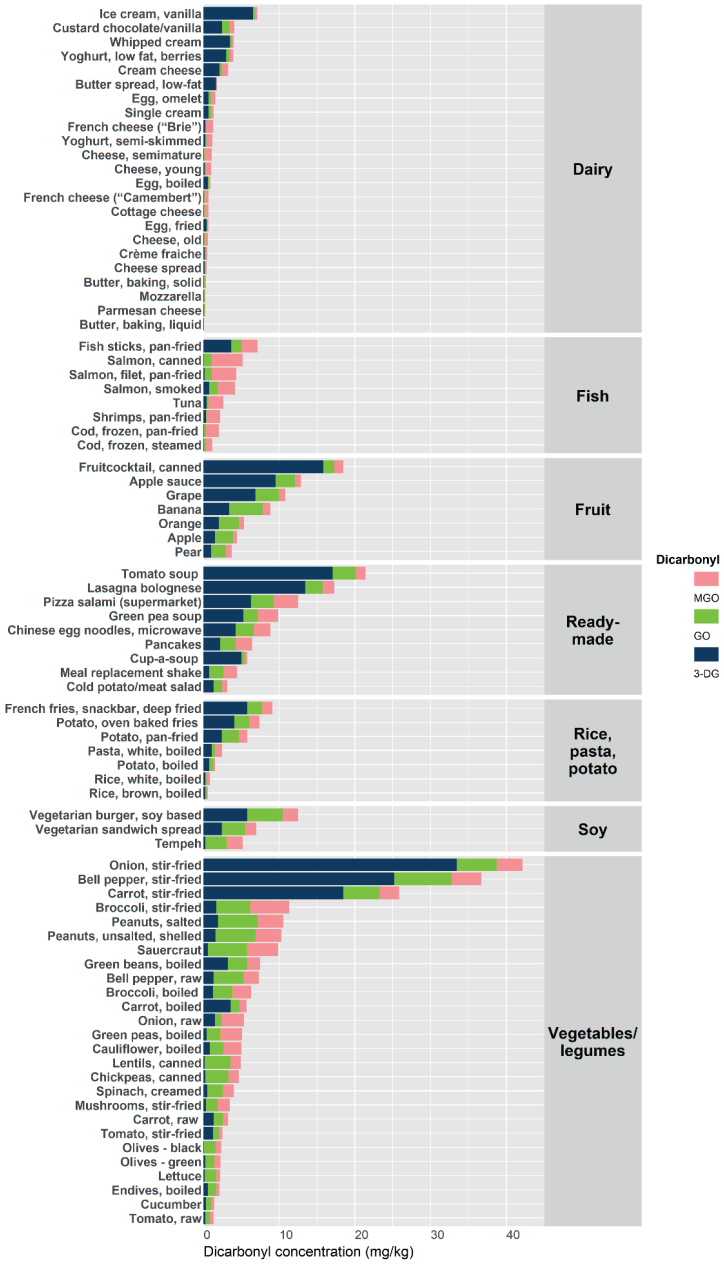

Figure 2.3 MGO, GO, and 3-DG concentration in foods with low concentrations (mg/kg). MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone

Considerable concentrations of 3-DG were detected in balsamic vinegar and soy sauce (~50 mg/kg). Similarly, relatively high concentrations of MGO and GO were detected in balsamic vinegar and soy sauce (12 and 5 mg/kg for MGO and 6 and 11 mg/kg for GO, respectively). These high concentrations of dicarbonyls are in accordance with previous literature, and probably originate from the manufacturing process including fermentation and, in case of soy sauce, the heat-treated caramel or molasses added[10,20]. We observed only a low concentration of MGO in white vinegar, in contrast to a previous report[20]. Total dicarbonyl concentrations were low in other soy products, such as tempeh, although tempeh was relatively high in GO and MGO compared to 3-DG, likely due to the fermentation process.

Meat and fish contained relatively low dicarbonyl concentrations, in line with one previous report on lamb loins[29]. A recent study showed that creatine present in meat acts as a scavenger for dicarbonyls, possibly explaining these low concentrations[30]. MGO was the major dicarbonyl in all meat samples, except for stew, as well as in all fish samples, except for fish fingers, which had a high 3-DG concentration, likely originating from the breadcrumbs. As far as we are aware, concentrations of dicarbonyls in fish for consumption have not been reported previously. Interestingly, there are some studies reporting high concentrations of MGO in zebra-fish and fish oils, possibly via lipid peroxidation of long-chain poly unsaturated fatty acids, such as EPA and DHA[31,32], which might also be the origin of MGO in fish for consumption.

Vegetables had relatively low dicarbonyl concentrations (<50 mg/kg). Interestingly, in most vegetables, MGO and GO concentrations exceeded 3-DG concentrations. The source of this needs to be investigated in more detail, as this is the first study quantifying dicarbonyls in different types of vegetables. Stir-frying of vegetables increased the dicarbonyl content (by 2-8 fold in tomatoes, broccoli, carrots, bell pepper and onion, see also section 'Effect of preparation method on dicarbonyl concentrations').

MGO concentrations were highest in Manuka honey, which is in line with previous literature[7]. MGO concentrations differed between the different Manuka types and did not correspond to the label. In bee honey, MGO concentrations were ~5-100 fold lower and 3-DG concentrations ~2 fold lower than in Manuka honey.

Dicarbonyl concentration in commonly-consumed drinks

A complete list of concentrations of dicarbonyls in commonly-consumed drinks is presented in Supplementary Table S2.4 and visually displayed in Figure 2.4. Dicarbonyl concentrations were highest in beer, a probiotic drink, fruit juices,

sweet liqueur, a sports drink, port, and sweet white wine (8-24 mg/L). Dicarbonyl concentrations were lowest in milk, whiskey, white rum, light soft drinks, and tea (0.14-2 mg/L).

3-DG was the major dicarbonyl in most drinks, except in coffee, white rum, and whiskey, in which MGO concentrations exceeded those of 3-DG. 3-DG concentrations ranged from 0.08-20 mg/L, and were highest in apple juice, a probiotic drink, and beer. MGO concentrations ranged from 0.02-3.2 mg/L, and were highest in coffee, a probiotic drink, and beer. GO concentrations ranged from 0.01-3.2 mg/L, and were the highest in fruit juices, chocolate milk, and a probiotic drink.

Overall, carbohydrate/simple sugar-containing drinks had the highest concentration of dicarbonyls, especially 3-DG. In soft drinks, 3-DG concentration ranged from 0.3-13.8 mg/L, and was highest for sports drink and lowest in light soft drinks. Coke sweetened with stevia showed a similar 3-DG concentration compared to regular coke (2.7 versus 2.5 mg/kg). One high-fructose corn syrup containing drink had higher 3-DG concentrations than regular soft drinks, despite its lower overall simple sugar content. This is in line with literature, reporting the amount and type of sugar or sweetener used as major contributors to dicarbonyl concentration in soft drinks. Different authors reported high dicarbonyl concentrations in regular soft drinks, and even higher in drinks containing high fructose corn syrup[13,14,20]. The higher total concentration of dicarbonyls in orange soft drink compared to other soft drinks, despite its lower simple sugar content in this present study, could potentially be explained by the use of fruit concentrate as an ingredient. Furthermore, the relatively high concentrations of MGO and GO in blackcurrant soft drink might be explained by the presence of fermented berry juice, because in fermented juices, carbohydrates are hydrolysed into their simple sugars, which tend to form dicarbonyls.

The higher dicarbonyl concentrations in fruit juice compared to soft drinks observed in this study, is in line with previous literature, and may be due to the presence of less stable monosaccharides from fruit concentrate compared to sucrose[12,20,33].

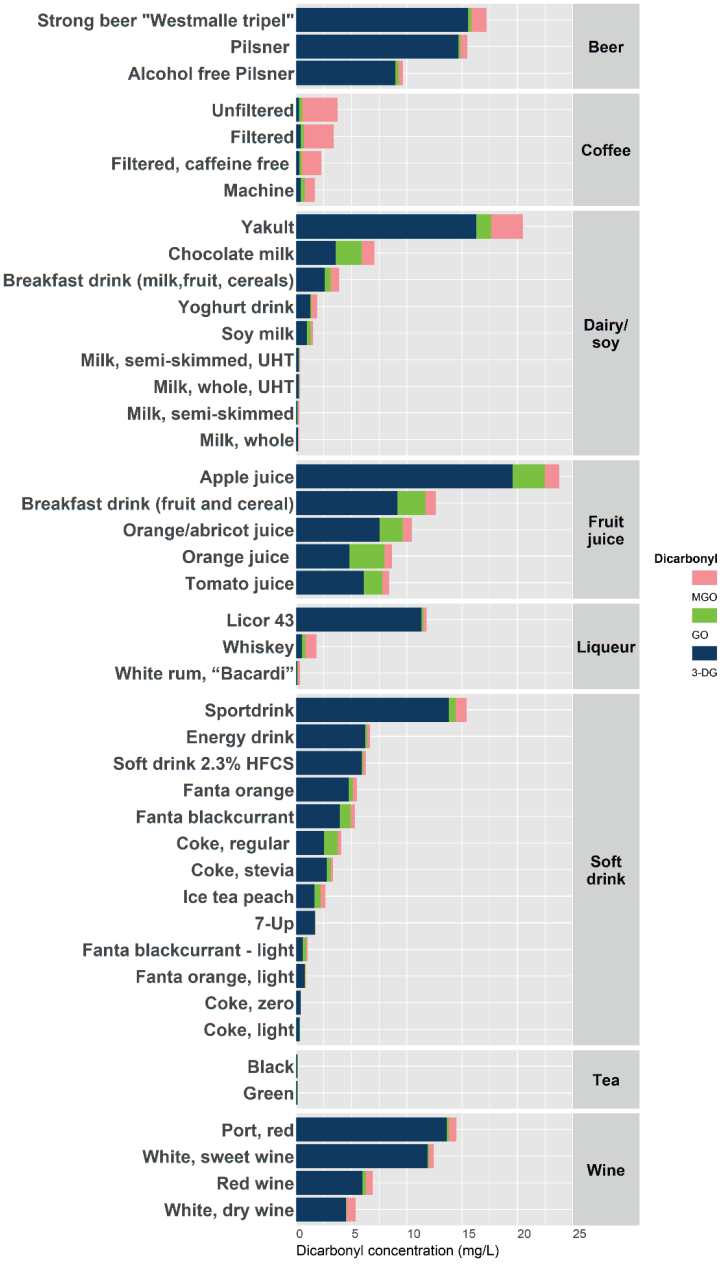

Figure 2.4 **MGO, GO, and 3-DG concentration in drinks (mg/L).** MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Concentrations of dicarbonyls have previously been reported for fermented drinks, such as beer and wine[10,16-18,20,33]. We measured high concentrations of dicarbonyls in beer, with lower concentrations in alcohol-free beer compared to pilsner and strong beer, as was previously reported[16]. We also measured a lower concentration of 3-DG in dry wine compared to sweet and fortified wine, owing to the lower sugar content[18,20,34]. One study in wine stated that particularly the fructose content had a large effect on 3-DG formation compared to glucose[34], but we had no data on sugar type to confirm this. The high MGO concentration in a probiotic drink can likely also be attributed to the fermentation process, and is in line with previous studies[12,33].

MGO was the major dicarbonyl in coffee, with higher concentrations compared to other drinks. MGO concentration was highest in unfiltered coffee, followed by filtered coffee, decaffeinated filtered coffee, and machine coffee. The variation between unfiltered, filtered and machine coffee could be due, at least partly, to differences in amount of ground coffee used to prepare them. The lower concentration seen in decaffeinated filtered coffee is in line with a previous report[35]. The observation of high MGO concentrations in coffee is interesting, since literature on dicarbonyl concentrations in coffee is inconclusive. Most studies report high concentrations of MGO and GO in coffee, which increased during roasting of the coffee beans[10-12,36]. In contrast, Degen et al. detected no MGO, GO or 3-DG in coffee, most likely due to complex sample matrix hindrance, sample preparation differences and detection method used[20]. Contrary to the present study, two studies reported that GO was the most abundant dicarbonyl in coffee[11,12]. In another study on coffee beans, the concentration of MGO was about twice that of GO[36]. As far as we are aware, this is the first study that reported 3-DG concentrations in coffee.

The low concentrations of dicarbonyls observed in tea are in line with literature, and might be due to the presence of scavengers such as polyphenols, which can trap MGO and GO and thereby reduce their formation[12,37,38]. The low concentrations in milk confirm previous literature on dicarbonyls in milk[33]. In line with this study, we observed higher concentrations in complex milk-based foods with added fruit preparations or flavours compared to plain milk products, probably due to the presence of sugar and other ingredients. The low dicarbonyl concentration in soy milk was in contrast to one of these previous studies[12].

Variation in dicarbonyl concentrations within and between brands

To explore variation within and between brands, fifteen foods and drinks of multiple batches from the same brand and different brands were analyzed.

Interestingly, variation between different brands was generally not larger than variation between different batches of the same brand.

In general, foods need more preparation than drinks, and foods that are prepared at home have an additional source of variation compared to foods that are purchased ready-to-eat. Indeed, when comparing variation in dicarbonyl concentrations amongst the different product groups, the variation in MGO and 3-DG concentration was lowest amongst drinks, followed by unprepared foods and home-prepared foods. CVs were 13%, 17%, and 24%, respectively, for MGO and 16%, 19%, and 29%, respectively, for 3-DG, although this was not the case for GO in which CVs were 28%, 15%, and 29%, respectively (based on Supplementary Table S2.5). Additionally, the required sample preparation steps of food products before quantification (i.e. homogenization) and possible interference of the food matrices, may have introduced more variation in foods as compared to quantification of drinks.

For Dutch speculaas cookie, one out of four quantified brands had consistently higher concentrations of all dicarbonyls (Brand C). Interestingly, this brand had a slightly lower carbohydrate and sugar content compared to the other brands, but was the only brand that contained glucose syrup and ammonium bicarbonate, which have both been associated with high dicarbonyl concentrations[20,28].

Although these data show that there is some variation that has to be taken into account when using this database, variation related to different lots of one brand and/or different brands within a drink of food was limited compared to the differences between individual types of drinks and foods.

Effect of preparation method on dicarbonyl concentrations

To explore the effect of preparation method on dicarbonyl concentrations, white bread, egg, potato, French fries, vegetables, chicken, fish, and coffee were prepared by different cooking methods. Toasting of white bread increased the dicarbonyl concentrations by 3-5 fold (Supplementary Table S2.3). In eggs, omelet had highest dicarbonyl concentrations compared to boiled- and fried egg. Pan-fried cod fish and chicken fillet had a slightly higher dicarbonyl concentrations compared to steamed cod fish and chicken fillet. All five vegetables used for comparison had higher MGO, GO and 3-DG concentrations after stir-frying compared to raw/boiled samples, with concentrations being ~2 fold higher for MGO, ~3 fold for GO and ~10 fold for 3-DG. Boiled potato had lowest dicarbonyl concentrations, followed by pan-fried potato and oven baked fries, and lastly French fries. When potato fries were fried for 2-8 minutes in a separate experiment, a gradual increase in MGO, GO, and 3-DG concentration over time was observed (Supplementary Figure S2.2). This is in line

with a previous report, that observed an increase in MGO and GO concentration during frying of dough[39]. Taken together, our data showed an increase in dicarbonyl concentrations during heat treatment.

Composition of commonly consumed foods and drinks: Correlations between dicarbonyls and macronutrient concentrations

To explore the hypothesis that dicarbonyls are mainly present in sugar-rich products, the relationship between total carbohydrate content and dicarbonyl concentration was examined. Total carbohydrate content was positively and significantly associated with all measured dicarbonyls, most strongly with 3-DG (Spearman's rho: 0.6 to 0.8, $p<0.001$ – Supplementary Table S2.6). Correlations were comparable when carbohydrate content was replaced by simple sugar (mono- and disaccharide) content (Spearman's rho 0.3-0.7, $p<0.001$).

In addition to carbohydrates, the presence of protein or fat in foods and drinks might also correlate to the dicarbonyl concentration. Glyoxal can be formed during lipid peroxidation[40]. Additionally, dicarbonyls can modify amino acids in protein or phospholipids in fat, resulting in the formation of AGEs and a concomitant reduction in dicarbonyl concentration[20,34,41]. Correlations of protein and fat content with dicarbonyls MGO and GO were positive, but less strong than for carbohydrates (Spearman's rho: 0.2-0.4, $p<0.05$ – Supplementary Table S2.6). The correlations of protein and fat content with 3-DG were inverse and not statistically significant (Spearman's rho: -0.09 and -0.10, $p=0.14$ and 0.07 – Supplementary Table S2.6).

Thus, the correlation between macronutrient content and dicarbonyl concentration is strongest for carbohydrates, compared to protein and fat. The positive correlation between fat content and GO concentration was in line with one previous study[12]. The inverse correlation of protein and fat with 3-DG might indicate reactivity of 3-DG towards protein or fat residues, a process that would lead to formation of AGEs and a reduction in 3-DG, although this correlation is a very rough estimate and does not take into account other factors such as food processing.

Composition of commonly consumed foods and drinks: Correlations between dicarbonyls concentrations and AGEs

We additionally explored the relationships between concentrations of dicarbonyls and three major AGEs (CEL, CML, and MG-H1) in 112 foods and drinks, using our previously published food composition AGE database[24]. Correlations between dicarbonyls and AGEs were positive and significant (Spearman's rho: 0.3 to 0.6,

p<0.01 – Supplementary Table S2.7). Thus, in general, products high in dicarbonyls were also high in AGEs. This is in line with expectations, as both dicarbonyls and AGEs are mainly present in heat-processed foods and drinks.

Relative contribution of food groups to dicarbonyl intake

To explore the relative contribution of foods and drinks to dietary dicarbonyl consumption, the daily dicarbonyl intake was estimated for each food group, taking into account the average daily consumption of each food group by the Dutch population. The estimated daily intake is ~3 mg for MGO, ~3 mg for GO, and ~9 mg for 3-DG (Supplementary Table S2.8). This was in the same order of magnitude, although lower, as the previously estimated intake in a German population, which was reported to be 5-20 mg/day for MGO and 20-160 mg/day for 3-DG[20]. For MGO, intake comes mainly from coffee consumption, followed by bread, and cookies/bakery products (together >50% of daily intake, Supplementary Figure S2.3). For GO, intake comes mainly from bread, followed by cookies/bakery products, and fruit (together >50% of daily intake, Supplementary Figure S2.3). Interestingly, despite low concentrations of dicarbonyls measured in food groups such as fruits and vegetables, they together contribute to ~20% of daily GO intake. For 3-DG, intake comes mainly from bread, followed by beer, cookies/bakery products, and soft drinks (together >50% of daily intake, Supplementary Figure S2.3).

Conclusion

We present a first comprehensive dietary dicarbonyl database of MGO, GO, and 3-DG in 223 foods and drinks, covering all main food groups, e.g. vegetables, fruit, cheese, fish, meat, milk, and tea. The amount of dicarbonyls was mainly related to the amount of carbohydrates/simple sugars and the type of sugar used. Additionally, heat-treatment increased the dicarbonyl concentrations. Of the three dicarbonyls measured, 3-DG was the most abundant dicarbonyl in most foods and drinks, and estimated daily intake was higher for 3-DG than for MGO or GO (~9, ~3, and ~3 mg/day respectively). Coffee consumption was estimated to be a major contributor to daily MGO intake in the Dutch population, whereas bread is a major contributor to GO and 3-DG intake. This new database is the first that can be used to accurately estimate dicarbonyl intake from the diet by linking it to data from Food Frequency Questionnaires or dietary recalls. This opens the possibility to explore the physiological impact of dietary dicarbonyls on human health in human observational- and intervention studies.

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Supplementary tables

Table S2.1 Mean slopes (response factor) and y-intercepts for MGO, GO, and 3-DG as tested in water, food, and drink matrices.

	Slope			Y-intercept (response, mg/L)		
	MGO	**GO**	**3-DG**	**MGO**	**GO**	**3-DG**
Water	1.078	1.164	1.136	-0.002	0.000	0.008
Coke	1.009	0.972*	0.924	0.176	0.367	2.989
Milk	1.004	1.085	1.029	0.102	0.024	0.132
Whole grain bread	0.966	0.923	0.851	1.210	1.046	1.286
Fried potato	0.944	0.856	0.987	0.429	0.426	1.284
Mean ± SD	1.000 ± 0.051	1.000 ± 0.124	0.985 ± 0.108			
(CV, %)	(5.1)	(12.4)	(10.9)			

Calibration curves showed perfect linearity (r^2>0.99, *r^2=0.97), Figure 2.1. CV, coefficient of variation; 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Table S2.2 Inter- and intra-assay variation of MGO, GO and 3-DG as tested in coke, tomato soup and milk (semi-skimmed).

	Intra-assay (n=10)			Inter-assay (n=7)		
	MGO	**GO**	**3-DG**	**MGO**	**GO**	**3-DG**
Coke	0.190 ± 0.018	0.397 ± 0.064	3.55 ± 0.23	0.205 ± 0.017	0.466 ± 0.090	3.41 ± 0.19
	(9.6)	(16.2)	(6.4)	(8.4)	(19.4)	(5.4)
Tomato-	1.28 ± 0.11	3.08 ± 0.25	17.05 ± 1.77	1.34 ± 0.10	3.04 ± 0.21	16.79 ± 1.53
soup	(8.4)	(8.0)	(10.4)	(7.4)	(7.0)	(9.1)
Milk	0.105 ± 0.007	0.036 ± 0.013	0.127 ± 0.011	0.104 ± 0.003	0.034 ± 0.003	0.128 ± 0.007
	(6.8)	(35.6)	(8.4)	(2.6)	(9.4)	(5.4)

Data are expressed as mean ± SD in mg/L or mg/kg (CV, %). CV, coefficient of variation, MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Table S2.3 MGO, GO, and 3-DG concentration in foods (mg/kg).

Product group	Specification	MGO	GO	3-DG
Bread	Cracrotte cracker	5.9 ± 0.40	8.4 ± 0.19	11 ± 0.11
	Crispbread ("Knackebrod")	11 ± 1.0	6.5 ± 0.96	21 ± 3.8
	Croissant	8.9 ± 0.69	5.9 ± 0.49	97 ± 1.6
	Multi grain bread*	4.9 ± 0.50	5.6 ± 0.26	8.6 ± 1.1
	Raisin bun	5.1 ± 0.20	6.4 ± 0.39	132 ± 24
	Rusk	26 ± 3.7	11 ± 0.16	109 ± 23
	Rye bread, dark	7.5 ± 1.2	11 ± 1.5	72 ± 13
	Rye bread, light	2.9 ± 0.39	3.9 ± 0.33	12 ± 3.1
	White bread&	2.3 ± 0.74	1.5 ± 0.44	2.4 ± 0.72
	White bread, toasted	8.0 ± 0.53	7.8 ± 0.79	9.7 ± 0.37
	White bun, hard&	2.6 ± 0.30	2.5 ± 0.30	5.5 ± 1.6
	Whole grain bread*	3.2 ± 0.48	3.7 ± 0.91	5.1 ± 1.3
Bread condiments	Apple molasses	21 ± 1.4	37 ± 2.1	369 ± 13
	Chocolate paste	2.6 ± 0.54	7.1 ± 1.6	10 ± 1.0
	Chocolate sprinkles, milk	5.6 ± 0.07	22 ± 0.64	20 ± 0.27
	Honey, bee	6.1 ± 0.11	7.7 ± 0.18	274 ± 9.4
	Jam, strawberry	2.1 ± 0.52	6.3 ± 2.5	19 ± 5.6
	Marmite	6.5 ± 1.1	3.8 ± 2.8	10 ± 6.8
	Nut paste	1.7 ± 0.36	2.4 ± 0.97	1.3 ± 0.41
	Peanut butter	4.5 ± 0.58	6.1 ± 2.0	3.0 ± 1.0
	Sugar syrup	7.6 ± 0.13	31 ± 0.02	330 ± 56
Breakfast cereals	Cooked oats (with milk)	0.73 ± 0.31	0.73 ± 0.32	0.25 ± 0.02
	Cornflakes	9.9 ± 0.66	9.7 ± 0.21	65 ± 6.6
	Granola	7.8 ± 0.24	6.6 ± 0.21	71 ± 9.4
	Muesli	3.6 ± 0.02	3.5 ± 0.55	25 ± 8.1
Cookies/bakery	Apple crumble pie	3.7 ± 0.54	4.3 ± 0.29	39 ± 9.1
	Apple turnover	3.8 ± 0.22	7.5 ± 0.8	30 ± 7.7
	Cake	2.1 ± 0.08	2.1 ± 0.18	7.9 ± 0.39
	Cream pie	1.8 ± 0.02	2.3 ± 0.24	6.1 ± 0.26
	Digestive biscuit	17 ± 1.2	10 ± 0.57	11 ± 1.4
	Dutch almond filled cookie ("gevulde koek")	4.4 ± 1.2	6.4 ± 1.7	15 ± 7.7
	Dutch caramel cookie ("stroopwafel")	6.8 ± 0.56	7.7 ± 0.56	64 ± 1.7
	Dutch speculaas cookie*	11 ± 0.44	7.8 ± 0.78	21 ± 1.4
	Dutch spiced cake - gluten free	29 ± 1.0	12 ± 1.4	402 ± 79
	Dutch spiced cake - sugar free	40 ± 0.82	19 ± 3.9	36 ± 2.0
	Dutch spiced cake - whole grain	45 ± 2.4	31 ± 1.8	482 ± 99
	Dutch spiced cake ("peperkoek")*	43 ± 1.4	27 ± 2.0	473 ± 96
	Marie biscuit	10 ± 0.35	9.4 ± 0.05	6.5 ± 0.45
	Muesli bar	17 ± 1.1	6.4 ± 0.33	27 ± 1.0
	Pink glazed cookie	3.2 ± 0.06	2.8 ± 0.03	14 ± 2.9
	Raisin biscuit	9.7 ± 0.25	14 ± 3.9	233 ± 30
Dairy	Butter spread, low-fat ("halvarine")	0.10 ± 0.03	0.02 ± 0.00	1.6 ± 1.1
	Butter, baking, liquid	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
	Butter, baking, solid	0.11 ± 0.01	0.08 ± 0.00	0.10 ± 0.03
	Cheese ("Jong belegen" 30+)	0.96 ± 0.47	0.09 ± 0.06	0.05 ± 0.02
	Cheese ("Jonge kaas" 48+)*	0.71 ± 0.06	0.20 ± 0.04	0.12 ± 0.06
	Cheese (Old 48+)	0.44 ± 0.03	0.04 ± 0.00	0.08 ± 0.00
	Cheese spread ("Smeerkaas")	0.22 ± 0.06	0.03 ± 0.01	0.16 ± 0.04
	Cottage cheese ("kwark")	0.50 ± 0.04	0.04 ± 0.01	0.09 ± 0.02
	Cream cheese ("Boursin")	0.79 ± 0.03	0.35 ± 0.05	2.1 ± 0.25
	Crème fraiche	0.22 ± 0.01	0.15 ± 0.00	0.14 ± 0.02

Product group	Specification	MGO	GO	3-DG
	Custard chocolate/vanilla ("vla")	0.55 ± 0.01	1.1 ± 0.04	2.4 ± 0.13
	Egg, boiled	0.13 ± 0.01	0.17 ± 0.01	0.57 ± 0.00
	Egg, fried	0.11 ± 0.00	0.15 ± 0.01	0.37 ± 0.02
	Egg, omelet	0.57 ± 0.22	0.32 ± 0.03	0.68 ± 0.09
	French cheese ("Brie")	0.90 ± 0.03	0.13 ± 0.01	0.21 ± 0.01
	French cheese ("Camembert")	0.53 ± 0.42	0.08 ± 0.01	0.08 ± 0.04
	Ice cream, vanilla	0.30 ± 0.02	0.25 ± 0.03	6.5 ± 0.37
	Mozzarella	0.12 ± 0.05	0.04 ± 0.02	0.07 ± 0.04
	Parmesan cheese	0.12 ± 0.00	0.06 ± 0.02	0.05 ± 0.01
	Single cream ("kookroom")	0.30 ± 0.03	0.31 ± 0.05	0.68 ± 0.08
	Whipped cream	0.21 ± 0.01	0.28 ± 0.01	3.5 ± 0.3
	Yoghurt, low fat, berries	0.50 ± 0.03	0.45 ± 0.00	3.0 ± 0.04
	Yoghurt, semi-skimmed	0.76 ± 0.02	0.15 ± 0.03	0.25 ± 0.01
Fats, oils and sauces	Balsamic vinegar	12 ± 0.62	5.7 ± 0.95	54 ± 0.64
	Gravy	0.64 ± 0.06	0.93 ± 0.14	7.6 ± 0.42
	Ketchup	2.5 ± 0.18	6.9 ± 0.48	21 ± 1.7
	Mayonnaise	0.25 ± 0.06	0.60 ± 0.37	1.6 ± 0.12
	Olive oil	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.04
	Peanut sauce	2.9 ± 0.12	4.8 ± 0.33	11 ± 0.64
	Sweet soy sauce	5.0 ± 0.03	11 ± 0.08	52 ± 0.39
	Tomato sauce	1.3 ± 0.17	3.4 ± 0.45	5.8 ± 0.88
	Tomato sauce, bolognese, meat	3.0 ± 0.48	5.8 ± 3.4	28 ± 7.6
	White vinegar	0.04 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
	Wok sauce, red curry	1.7 ± 0.36	3.9 ± 0.75	27 ± 6.0
Fish	Cod, frozen, pan-fried*	1.7 ± 0.03	0.25 ± 0.01	0.06 ± 0.01
	Cod, frozen, steamed	0.9 ± 0.02	0.24 ± 0.02	0.04 ± 0.01
	Fish sticks, pan-fried	2.1 ± 0.65	1.4 ± 0.05	3.7 ± 0.58
	Salmon, canned&	4.1 ± 0.59	0.96 ± 0.61	0.09 ± 0.08
	Salmon, filet, pan-fried	3.2 ± 0.15	0.93 ± 0.05	0.17 ± 0.02
	Salmon, smoked	2.4 ± 0.25	1.1 ± 0.42	0.72 ± 0.15
	Shrimps, pan-fried	1.8 ± 0.52	0.15 ± 0.02	0.29 ± 0.02
	Tuna, canned in sunflower oil	2.0 ± 0.08	0.25 ± 0.01	0.38 ± 0.02
Fruit	Apple	0.47 ± 0.01	2.4 ± 0.02	1.5 ± 0.10
	Apple sauce	0.80 ± 0.12	2.5 ± 0.24	9.5 ± 1.5
	Banana#	1.0 ± 0.07	4.4 ± 0.36	3.4 ± 0.63
	Fruit cocktail, canned&	1.2 ± 0.33	1.5 ± 0.35	16 ± 5.9
	Grape	0.89 ± 0.02	3.1 ± 0.19	6.8 ± 0.48
	Orange	0.75 ± 0.02	2.6 ± 0.04	2.1 ± 0.07
	Pear	0.79 ± 0.07	2.0 ± 0.23	0.99 ± 0.17
Manuka honey	Manuka honey 12+	25 ± 1.1	9.2 ± 0.59	490 ± 62
	Manuka honey 20+	736 ± 16	16 ± 1.3	524 ± 47
	Manuka honey 30+	112 ± 3.5	16 ± 0.94	583 ± 9.2
	Manuka honey 550+	572 ± 7.3	17 ± 0.76	577 ± 15
Meat	Black pudding slices	2.2 ± 0.25	1.3 ± 0.27	7.0 ± 0.51
	Black pudding, pan-fried	2.5 ± 0.03	1.9 ± 0.02	5.8 ± 0.11
	Chicken fillet, pan-fried*	2.3 ± 0.10	0.45 ± 0.07	0.34 ± 0.05
	Chicken fillet, slices, roasted	1.6 ± 0.01	0.30 ± 0.04	0.94 ± 0.24
	Chicken fillet, steamed	1.8 ± 0.20	0.32 ± 0.05	0.12 ± 0.01
	Goulash, beef, canned	2.3 ± 0.12	1.8 ± 0.36	8.9 ± 1.3
	Hamburger, pan-fried	3.9 ± 0.22	2.6 ± 0.06	0.62 ± 0.19
	Minced meat, pork/beef, pan-fried	2.9 ± 0.62	1.0 ± 0.15	0.55 ± 0.02
	Pork in bacon, ("slavink"), pan-fried	3.4 ± 0.01	1.4 ± 0.21	1.7 ± 0.21

Product group	Specification	MGO	GO	3-DG
	Sandwich meat, ham	1.9 ± 0.02	1.2 ± 0.15	1.7 ± 0.39
	Sandwich meat, pork[&]	1.7 ± 0.12	0.91 ± 0.46	1.5 ± 0.40
	Sandwich meat, salami	3.8 ± 0.57	1.1 ± 0.18	0.40 ± 0.06
	Smoked sausage, boiled ("rookworst")	2.6 ± 0.12	0.75 ± 0.08	0.40 ± 0.04
	Steak, beef, pan-fried	1.7 ± 0.16	1.0 ± 0.16	0.25 ± 0.01
	Stew, beef, canned ("zuurvlees")	2.4 ± 0.43	2.2 ± 0.30	61 ± 16
	Stew, pork, snack bar	3.9 ± 0.13	6.2 ± 0.28	41 ± 3.4
Ready-made	Chinese egg noodles, microwave	2.2 ± 0.13	2.4 ± 0.07	4.2 ± 0.29
	Cold potato/meat salad	0.68 ± 0.01	1.1 ± 0.19	1.3 ± 0.06
	Cup-a-soup	0.34 ± 0.01	0.49 ± 0.06	5.0 ± 0.58
	Green pea soup[&]	2.7 ± 0.88	2.0 ± 0.90	5.2 ± 2.3
	Lasagna bolognese	1.5 ± 0.20	2.3 ± 0.49	13 ± 2.8
	Meal replacement shake	1.7 ± 0.24	1.9 ± 0.44	0.75 ± 0.18
	Pancakes	2.2 ± 0.13	2.0 ± 0.16	2.2 ± 0.19
	Pizza salami (supermarket)	3.2 ± 0.19	3.0 ± 0.43	6.3 ± 0.08
	Tomato soup[#]	1.3 ± 0.11	3.1 ± 0.25	17 ± 1.8
Rice, pasta, potato	French fries, snack bar, deep fried[&]	1.3 ± 0.07	2.1 ± 0.76	5.7 ± 0.84
	French fries, home-made, deep-fried 4 min	1.4 ± 0.01	3.3 ± 0.32	2.3 ± 0.29
	Pasta, white, boiled	0.92 ± 0.18	0.45 ± 0.07	1.1 ± 0.24
	Potato, boiled*	0.26 ± 0.03	0.56 ± 0.07	0.72 ± 0.15
	Potato, oven baked*	1.3 ± 0.06	2.0 ± 0.07	4.1 ± 0.37
	Potato, pan-fried*	1.1 ± 0.05	2.3 ± 0.02	2.4 ± 0.10
	Rice, brown, boiled	0.22 ± 0.06	0.14 ± 0.04	0.24 ± 0.02
	Rice, white, boiled	0.37 ± 0.00	0.25 ± 0.03	0.19 ± 0.02
Snacks and nuts	Cashew nuts, salted	2.6 ± 0.00	5.6 ± 0.12	4.4 ± 0.18
	Cashew nuts, unsalted	1.7 ± 0.06	2.6 ± 0.33	2.8 ± 0.05
	Cocktail nuts ("Borrelnootjes")	1.9 ± 0.09	3.1 ± 0.46	3.6 ± 0.38
	Crisps, paprika	1.4 ± 0.04	3.9 ± 0.43	1.8 ± 0.23
	Deep fried cheese snack ("kaassoufflé")	2.6 ± 0.66	2.2 ± 0.71	3.3 ± 0.80
	Deep fried meat snack ("frikandel")	3.8 ± 0.60	1.6 ± 0.14	2.6 ± 0.52
	Deep fried meat snack ("kroket")	4.2 ± 0.29	2.9 ± 0.63	6.7 ± 0.36
	Deep fried snack ("bamischijf")	2.5 ± 0.23	4.2 ± 0.30	13 ± 0.69
	Deep fried Vietnamese spring roll	1.8 ± 0.01	1.8 ± 0.45	2.7 ± 0.19
	Dried apple	6.6 ± 0.26	1.4 ± 0.71	631 ± 49
	Dried apricot	51 ± 6.2	1.5 ± 0.41	797 ± 28
	Japanese rice crackers	2.5 ± 0.11	3.6 ± 0.25	5.8 ± 0.36
	Prunes[&]	5.5 ± 0.44	10 ± 1.0	158 ± 22
	Raisins - soaked in water	7.4 ± 1.1	10 ± 1.3	1296 ± 31
	Raisins - dried*	9.3 ± 1.1	14 ± 0.7	2990 ± 399
	Sausage roll ("saucijzenbroodje")	2.3 ± 0.05	1.8 ± 0.12	2.7 ± 0.03
	Sausage roll ("worstenbroodje")	3.3 ± 0.19	2.5 ± 0.26	13 ± 0.85
	Walnuts	1.1 ± 0.04	3.1 ± 0.17	0.78 ± 0.12
Soy	Tempeh	2.1 ± 0.01	2.9 ± 0.37	0.21 ± 0.00
	Vegetarian burger, soy based	2.0 ± 0.63	4.7 ± 1.3	5.8 ± 1.6
	Vegetarian sandwich sausage spread	1.5 ± 0.17	3.1 ± 0.32	2.4 ± 0.11
Sweets/chocolate	Candy bar, Mars	4.6 ± 0.22	10 ± 0.43	431 ± 121
	Candy bar, Snickers	5.2 ± 1.3	8.7 ± 0.87	403 ± 83
	Candy bar, Twix	5.2 ± 1.4	5.3 ± 1.2	545 ± 148
	Candy, mix	3.7 ± 0.17	1.8 ± 0.16	123 ± 20
	Chocolate - dark (85% cacao)	3.3 ± 1.0	5.9 ± 4.0	17 ± 2.9
	Chocolate, dark[&]	5.3 ± 3.0	15 ± 13	15 ± 8.1
	Chocolate, milk	3.5 ± 0.39	12 ± 2.0	13 ± 1.4

Product group	Specification	MGO	GO	3-DG
	Licorice ("drop")	2.6 ± 0.11	4.1 ± 0.17	35 ± 0.26
	Marshmallow	1.8 ± 0.02	1.6 ± 0.06	52 ± 1.8
	Sugar	0.68 ± 0.01	0.53 ± 0.18	22 ± 1.4
	Sweetener	0.44 ± 0.02	1.0 ± 0.17	2.4 ± 0.91
Vegetables/ legumes	Bell pepper, raw[&]	2.1 ± 0.68	3.9 ± 0.99	1.3 ± 0.59
	Bell pepper, stir-fried[&]	3.9 ± 1.1	7.6 ± 3.5	25 ± 11
	Broccoli, boiled*	2.5 ± 0.32	2.6 ± 0.61	1.2 ± 0.25
	Broccoli, stir-fried	5.1 ± 0.55	4.5 ± 1.2	1.7 ± 0.16
	Carrot, boiled	0.96 ± 0.09	1.1 ± 0.02	3.6 ± 0.39
	Carrot, raw*	0.66 ± 0.06	1.2 ± 0.13	1.4 ± 0.12
	Carrot, stir-fried	2.6 ± 0.44	4.8 ± 1.2	18 ± 4.6
	Cauliflower, boiled	2.4 ± 0.00	1.8 ± 0.19	0.84 ± 0.08
	Chickpeas, canned	1.3 ± 0.03	3.1 ± 0.03	0.23 ± 0.20
	Cucumber	0.40 ± 0.06	0.70 ± 0.10	0.29 ± 0.14
	Endives, boiled	0.46 ± 0.09	1.1 ± 0.30	0.58 ± 0.20
	Green beans, boiled[&]	1.7 ± 0.35	2.5 ± 1.3	3.2 ± 0.97
	Green peas, boiled	2.9 ± 0.01	1.9 ± 0.16	0.36 ± 0.11
	Lentils, canned	1.3 ± 0.14	3.4 ± 0.50	0.12 ± 0.01
	Lettuce	0.55 ± 0.10	1.5 ± 0.34	0.18 ± 0.00
	Mushrooms, stir-fried	1.6 ± 0.03	1.5 ± 0.14	0.31 ± 0.03
	Olives - black	0.72 ± 0.40	1.5 ± 0.04	0.06 ± 0.01
	Olives - green	0.80 ± 0.19	1.2 ± 0.04	0.26 ± 0.01
	Onion, raw	2.9 ± 0.24	0.85 ± 0.13	1.5 ± 0.10
	Onion, stir-fried[&]	3.4 ± 1.6	5.3 ± 2.4	33 ± 12
	Peanuts, salted	3.5 ± 0.05	5.2 ± 0.76	1.9 ± 0.16
	Peanuts, unsalted, shelled	3.3 ± 0.20	5.3 ± 0.10	1.6 ± 0.01
	Sauerkraut[&]	4.1 ± 2.2	5.2 ± 2.7	0.56 ± 0.32
	Spinach, creamed[&]	1.4 ± 0.73	2.1 ± 1.8	0.52 ± 0.39
	Tomato, raw	0.43 ± 0.02	0.62 ± 0.04	0.24 ± 0.04
	Tomato, stir-fried[&]	0.49 ± 0.19	0.76 ± 0.28	1.2 ± 0.54

Concentration in mg/kg food, mean ± SD, n=2; [&]n=4; [#]n=10; *average based on multiple quantifications of this product, when multiple brands were tested, value reflects average of all brands (for all brands see Supplementary Table S2.5). MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Table S2.4 MGO, GO and 3-DG concentration in drinks (mg/L).

Product group	Specification	MGO	GO	3-DG
Beer	Alcohol free pilsner	0.35 ± 0.02	0.32 ± 0.01	9.0 ± 0.06
	Pilsner*	0.61 ± 0.01	0.20 ± 0.04	15 ± 0.40
	Strong beer "Westmalle triple"	1.4 ± 0.03	0.32 ± 0.00	16 ± 0.22
Coffee	Filtered	2.7 ± 0.10	0.27 ± 0.01	0.43 ± 0.02
	Filtered, caffeine free	1.8 ± 0.02	0.21 ± 0.01	0.30 ± 0.00
	Machine	0.89 ± 0.00	0.38 ± 0.00	0.41 ± 0.01
	Unfiltered	3.2 ± 0.09	0.30 ± 0.02	0.26 ± 0.01
Dairy/soy	Breakfast drink (milk, fruit, cereals)	0.75 ± 0.06	0.52 ± 0.01	2.6 ± 0.20
	Chocolate milk	1.2 ± 0.02	2.4 ± 0.01	3.6 ± 0.01
	Milk, semi-skimmed[#]	0.11 ± 0.01	0.04 ± 0.01	0.13 ± 0.01
	Milk, semi-skimmed, UHT	0.06 ± 0.00	0.06 ± 0.00	0.24 ± 0.02
	Milk, whole	0.09 ± 0.01	0.03 ± 0.00	0.14 ± 0.00
	Milk, whole, UHT	0.07 ± 0.00	0.05 ± 0.00	0.23 ± 0.01
	Soy milk	0.21 ± 0.00	0.37 ± 0.01	0.95 ± 0.03
	Yakult - probiotic	2.9 ± 0.10	1.4 ± 0.03	16 ± 0.67
	Yoghurt drink, 0% fat, raspberry[&]	0.53 ± 0.14	0.11 ± 0.03	1.3 ± 0.45
Fruit juice	Apple juice	1.3 ± 0.01	2.9 ± 0.02	20 ± 0.15
	Breakfast drink (fruit and cereal)	0.94 ± 0.18	2.5 ± 0.45	9.2 ± 2.0
	Orange juice*	0.68 ± 0.04	3.2 ± 0.13	4.8 ± 0.14
	Orange/apricot juice	0.84 ± 0.02	2.1 ± 0.17	7.6 ± 0.08
	Tomato juice	0.60 ± 0.04	1.7 ± 0.30	6.1 ± 0.56
Liqueur	Licor 43	0.30 ± 0.03	0.18 ± 0.02	11 ± 0.82
	Whiskey	0.95 ± 0.02	0.35 ± 0.00	0.51 ± 0.02
	White rum, “Bacardi”	0.24 ± 0.01	0.05 ± 0.01	0.08 ± 0.00
Soft drink	7-Up	0.03 ± 0.01	0.02 ± 0.01	1.7 ± 0.02
	Coke, light	0.04 ± 0.00	0.02 ± 0.01	0.31 ± 0.00
	Coke, regular*	0.31 ± 0.01	1.3 ± 0.04	2.5 ± 0.10
	Coke, stevia	0.20 ± 0.01	0.41 ± 0.00	2.7 ± 0.13
	Coke, zero	0.03 ± 0.00	0.01 ± 0.00	0.38 ± 0.01
	Energy drink	0.24 ± 0.02	0.18 ± 0.02	6.3 ± 0.21
	Fanta blackcurrant - light ("Cassis")	0.15 ± 0.00	0.30 ± 0.01	0.59 ± 0.02
	Fanta blackcurrant ("Cassis")	0.41 ± 0.03	0.96 ± 0.04	4.0 ± 0.09
	Fanta orange	0.33 ± 0.00	0.41 ± 0.03	4.7 ± 0.04
	Fanta orange, light	0.05 ± 0.00	0.07 ± 0.00	0.75 ± 0.00
	Ice tea peach	0.43 ± 0.04	0.54 ± 0.07	1.7 ± 0.12
	Soft drink 2.3% HFCS ("Raak")	0.25 ± 0.01	0.14 ± 0.00	5.9 ± 0.18
	Sport drink (“AA”)	0.98 ± 0.03	0.63 ± 0.09	14 ± 0.15
Tea	Black	0.02 ± 0.00	0.03 ± 0.00	0.10 ± 0.01
	Green	0.02 ± 0.00	0.03 ± 0.01	0.10 ± 0.01
Wine	Port, red	0.66 ± 0.01	0.22 ± 0.02	14 ± 0.02
	Red wine	0.66 ± 0.01	0.26 ± 0.04	6.0 ± 0.24
	White, dry wine	0.81 ± 0.02	0.11 ± 0.00	4.5 ± 0.09
	White, sweet wine	0.46 ± 0.01	0.13 ± 0.00	12 ± 0.28

Concentration in mg/L drinks, mean ± SD, n=2; [&]n=4; [#]n=10; *average based on multiple quantifications of this product, when multiple brands were tested, value reflects average of all brands (for all brands see Supplementary Table S2.5). MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Table S2.5 Variation in dicarbonyl concentration within and between brands.

Product	Brand	MGO	GO	3-DG
Drinks				
Beer, pilsner	A	0.69 ± 0.01	0.20 ± 0.01	16 ± 1.0
	A	0.74 ± 0.01	0.37 ± 0.20	20 ± 0.36
	B	0.53 ± 0.00	0.16 ± 0.01	11 ± 0.18
	C	0.59 ± 0.01	0.16 ± 0.00	15 ± 0.30
Fruit juice, orange	A	0.70 ± 0.04	2.4 ± 0.08	4.6 ± 0.10
	A	0.77 ± 0.03	3.9 ± 0.08	3.6 ± 0.11
	B	0.62 ± 0.04	3.2 ± 0.19	5.5 ± 0.17
Soft drink, coke, regular	A	0.19 ± 0.02	0.40 ± 0.06	3.6 ± 0.23
	A	0.25 ± 0.01	0.95 ± 0.07	2.4 ± 0.04
	B	0.40 ± 0.01	1.8 ± 0.01	2.1 ± 0.06
Foods – purchased ready to eat and homogenized without preparation				
Bread, multi grain	A	6.6 ± 0.99	4.6 ± 0.26	8.2 ± 1.5
	B	3.1 ± 0.02	6.5 ± 0.25	9.0 ± 0.7
Bread, whole grain	A	5.0 ± 1.8	4.5 ± 1.5	5.0 ± 0.9
	A	3.1 ± 0.72	4.1 ± 2.3	6.3 ± 2.2
	B	2.9 ± 0.04	3.0 ± 0.43	4.8 ± 0.6
	C	2.7 ± 0.45	3.8 ± 0.43	4.7 ± 1.9
Dutch speculaas cookie	A	11 ± 0.65	6.9 ± 1.9	15 ± 1.1
	B	8.6 ± 0.46	8.3 ± 0.18	15 ± 0.52
	C	16 ± 0.60	8.3 ± 0.82	41 ± 3.3
	D	7.6 ± 0.06	7.5 ± 0.24	14 ± 0.59
Dutch spiced cake	A	37 ± 0.41	27 ± 6.3	479 ± 95
	A	37 ± 2.2	24 ± 1.4	648 ± 135
	A	37 ± 2.7	44 ± 2.3	651 ± 157
	B	46 ± 0.97	23 ± 2.5	436 ± 86
	C	51 ± 1.2	33 ± 0.55	413 ± 82
	D	37 ± 1.7	22 ± 1.8	451 ± 86
Cheese ("Jonge kaas" 48+)	A	0.76 ± 0.02	0.28 ± 0.00	0.20 ± 0.05
	A	1.3 ± 0.21	0.13 ± 0.05	0.22 ± 0.17
	B	0.39 ± 0.01	0.20 ± 0.05	0.03 ± 0.01
Raisins	A	9.7 ± 1.0	15 ± 0.75	2766 ± 212
	B	8.8 ± 1.2	13 ± 0.54	3213 ± 586
Carrot, raw	A	0.60 ± 0.04	1.2 ± 0.02	1.4 ± 0.16
	A	0.73 ± 0.07	1.2 ± 0.24	1.4 ± 0.08
Foods – purchased from supermarket and prepared at home before homogenizing				
Broccoli, boiled	A	2.0 ± 0.02	1.7 ± 0.06	1.2 ± 0.05
	A	3.0 ± 0.62	3.6 ± 1.2	1.2 ± 0.44
Potato, boiled	A	0.29 ± 0.06	0.42 ± 0.03	1.2 ± 0.29
	A	0.22 ± 0.01	0.70 ± 0.10	0.20 ± 0.00
Potato, pan-fried	A	1.3 ± 0.07	2.1 ± 0.01	2.8 ± 0.06
	A	0.83 ± 0.04	2.5 ± 0.02	2.0 ± 0.14
Fish, cod, pan-fried	A	2.6 ± 0.27	0.42 ± 0.06	0.08 ± 0.01
	A	0.84 ± 0.03	0.08 ± 0.01	0.03 ± 0.01
Chicken fillet, pan-fried	A	2.0 ± 0.11	0.51 ± 0.13	0.31 ± 0.07
	A	2.6 ± 0.09	0.40 ± 0.00	0.38 ± 0.02

mg/kg for foods and mg/L for drinks, mean ± SD, n=2. A, B, C, or D are different brands. A versus A reflects difference between batches (multiple products of the same brand purchased and analyzed at different time-points). A versus B additionally reflects variation between brands. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Table S2.6 Spearman correlation of macronutrient concentration in foods and drinks with the dicarbonyls MGO, GO, and 3-DG.

	MGO	GO	3-DG
Carbohydrates	0.55***	0.69***	0.75***
Protein	0.38***	0.20**	-0.09
Fat	0.17*	0.15*	-0.10

Spearman's rho. *p-value<0.05, ** p-value <0.01, *** p-value<0.001. Strength of correlation is color coded with darker colors for stronger correlations, significant positive correlations in blue and significant negative correlations in red. n=219. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone

Table S2.7 Spearman correlation of Advanced Glycation Endproducts concentration in foods and drinks with the dicarbonyls MGO, GO, and 3-DG.

	MGO	GO	3-DG
CML	0.60***	0.53***	0.34***
CEL	0.62***	0.46***	0.25**
MG-H1	0.60***	0.51***	0.30**

Spearman's rho. *p-value<0.05, ** p-value <0.01, *** p-value<0.001. Strength of correlation is color coded with darker colors for stronger correlations, significant positive correlations in blue and significant negative correlations in red. n=112. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

Table S2.8 Estimated daily intake of MGO, GO and 3-DG per food group.

	Food intake (g/day)	**MGO intake (mg/day)**	**GO intake (mg/day)**	**3-DG intake (mg/day)**
Fruit juices and soft drinks	405	0.13	0.24	1.3
Coffee	393	0.87	0.11	0.14
Dairy products	381	0.09	0.05	0.24
Tea	314	0.01	0.01	0.03
Fruit, vegetables, and legumes	255	0.32	0.57	0.54
Bread and breakfast cereals	147	0.80	0.90	2.0
Alcoholic drinks	137	0.08	0.04	1.7
Rice, pasta, potato	119	0.11	0.07	0.13
Meat, fish, and soy	114	0.26	0.12	0.12
Sweets, chocolate, bread condiments, cookies and bakery products	75	0.56	0.52	2.2
Fats, oils, and sauces	41	0.07	0.16	0.46
Snacks and nuts	27	0.07	0.08	0.14
Total	**2407**	**3.4**	**2.9**	**8.9**

Daily intake of MGO, GO and 3-DG estimated by multiplying the median dicarbonyl concentration of each food group by the mean daily intake within the Dutch diet of that food group. Food intake data was obtained from Dutch National Food Consumption Survey. Table is summarized display, combining similar food groups. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Supplementary figures

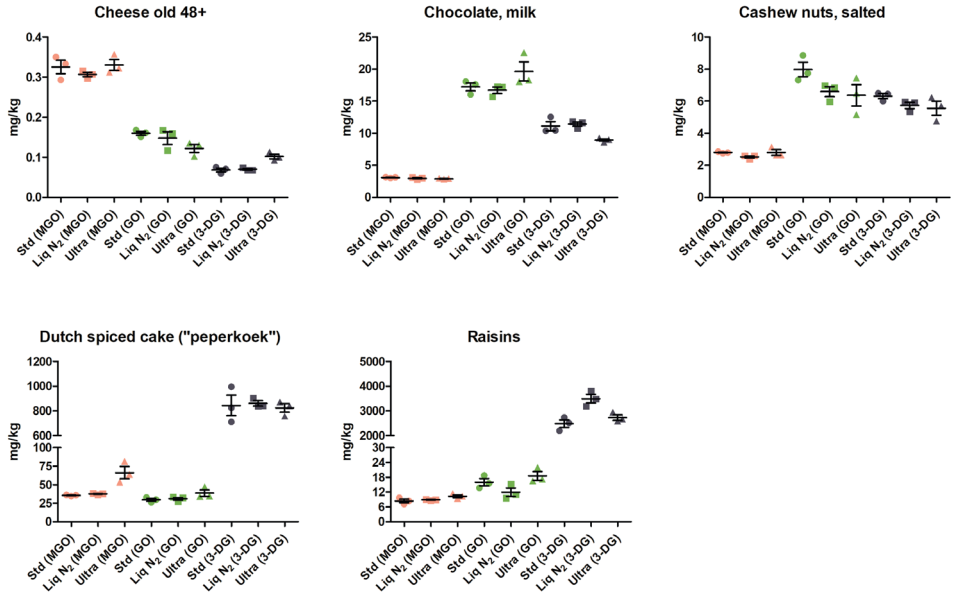

Figure S2.1 Comparison of extraction methods. Dicarbonyl concentrations of five different food matrices, as measured after sample preparation using three different extraction methods, Std: standard extraction method using liquid extraction of ~15mg food product with 120 µL oPD reagent. Liq N_2: comparable to standard extraction method, except using liquid nitrogen and a mortar to crush ~15 mg food product before liquid extraction with oPD reagent. Ultra: comparable to standard extraction method, except preparation of ~30 mg of a 50% (m/m, %) food homogenate in water and subsequent ultra-sonication, before extraction with 120 µL oPD reagent. All extraction methods were applied to five food matrices; cheese, chocolate, cashew nuts, Dutch spiced cake, and raisins. All samples were measured in triplicate (data points in graph) and error bars display standard deviation between triplicates. Pink datapoints are MGO concentrations, green datapoints are GO concentrations and blue datapoints are 3-DG concentrations. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

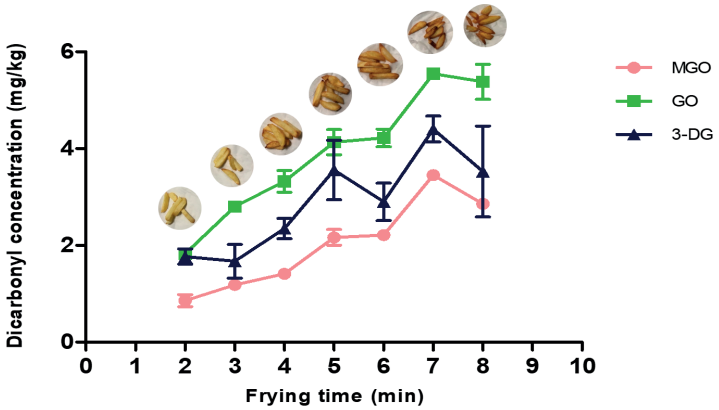

Figure S2.2 MGO, GO, and 3-DG concentration in potato during frying. Potato fries were deep-fried at 180°C and fries were removed from frying-pan after 2, 3, 4, 5, 6, 7, and 8 minutes. Pictures show browning of fries at corresponding time points. Graph shows mean of duplicates and standard deviation. As a reference, dicarbonyl concentrations in boiled potato were 0.26 mg/kg for MGO, 0.56 mg/kg for GO, and 0.72 mg/kg for 3-DG. MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

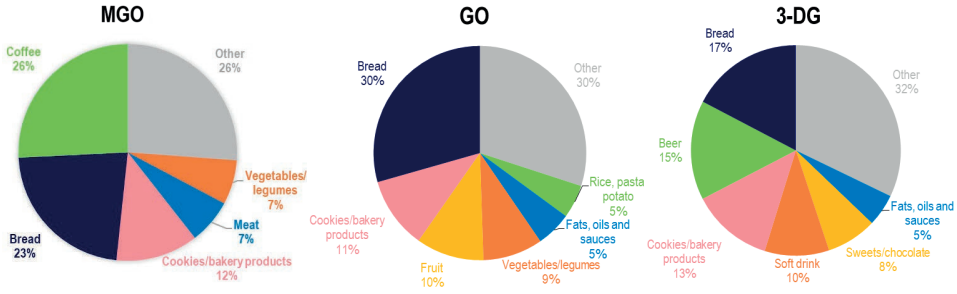

Figure S2.3 Relative contribution to MGO, GO and 3-DG intake per food group. Contribution of food groups to the estimated daily intake of MGO, GO, and 3-DG. Calculated by multiplying the median dicarbonyl concentration of each food group by the average daily intake within the Dutch diet of that food group (mean). Contributions are expressed in percentage of total intake of MGO, GO or 3-DG; food groups with contributions <5% are combined as "other". MGO, methylglyoxal; GO, glyoxal; 3-DG, 3-deoxyglucosone.

Chapter 3

Higher habitual intake of dietary dicarbonyls is associated with higher corresponding plasma dicarbonyl concentrations and skin autofluorescence: The Maastricht Study

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Abstract

Background

Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs). Both dicarbonyls and AGEs are associated with development of age-related diseases. Dicarbonyls are formed endogenously, but also during food processing. To what extent dicarbonyls from the diet contribute to circulating dicarbonyls and AGEs in tissues is unknown.

Objective

To examine cross-sectional associations of dietary dicarbonyl intake with plasma dicarbonyl concentrations and skin AGEs.

Design

In 2566 individuals of the population based Maastricht Study (age: 60±8 yrs, 50% males, 26% type 2 diabetes), we estimated habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG), by combining Food Frequency Questionnaires with our dietary dicarbonyl database of MGO, GO, and 3-DG concentrations in >200 commonly-consumed food products. Fasting plasma concentrations of MGO, GO, and 3-DG were measured by UPLC-MS/MS. Skin AGEs were measured as skin autofluorescence (SAF), using the AGE-Reader. Associations of dietary dicarbonyl intake with their respective plasma concentrations and SAF (all standardized) were examined using linear regression models, adjusted for age, sex, potential confounders related to cardio-metabolic risk factors and lifestyle.

Results

Median intake of MGO, GO, and 3-DG was 3.6, 3.5, and 17 mg/day, respectively. Coffee was the main dietary source of MGO, whereas this was bread for GO and 3-DG. In the fully adjusted models, dietary MGO was associated with plasma MGO (β=0.08, 95%CI (0.02,0.13)) and SAF (β=0.12 (0.07,0.17)). Dietary GO was associated with plasma GO (β=0.10 (0.04,0.16)) but not with SAF. 3-DG was not significantly associated with either plasma 3-DG or SAF.

Conclusions

Higher habitual intake of dietary MGO and GO, but not 3-DG, was associated with higher corresponding plasma concentrations. Higher intake of MGO was also associated with higher SAF. These results suggest dietary absorption of MGO and GO. Biological implications of dietary absorption of MGO and GO need to be determined.

Introduction

Dicarbonyls are highly reactive compounds, and major precursors in the formation of advanced glycation endproducts (AGEs). Both dicarbonyls and AGEs play a role in the pathobiology of age-related diseases, such as type 2 diabetes and cardiovascular diseases[1,2].

Dicarbonyls and AGEs are formed endogenously, mainly during glycolysis but also via lipid peroxidation[3-5]. Moreover, dicarbonyls and AGEs are formed during food processing, mainly during thermal heating in the Maillard reaction or caramelization[6,7]. High dicarbonyl concentrations are present in sugar-rich products, such as honey, cookies, soft drinks, and dried fruits, but also in fermented products, such as soy sauce and balsamic vinegar[6,8]. It is currently unknown whether dietary dicarbonyls contribute to circulating concentrations of dicarbonyls and/or accumulation of AGEs[9]. To evaluate this, we recently compiled a database of the major dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG), in over 200 commonly consumed foods and drinks[8]. Of these dicarbonyls, 3-DG is the most abundant dicarbonyl in most foods and drinks, and MGO and GO are thought to be more potent precursors of AGEs[2]. In the present study, we used this dietary dicarbonyl database for the first time to estimate habitual dietary intake of these dicarbonyls. Subsequently, we investigated in a large observational cohort study whether dietary intake of each of these dicarbonyls was associated with its respective concentration in plasma and with skin AGEs, estimated as skin autofluorescence (SAF), as a reflection of tissue AGEs[10].

Methods

Study population

This study uses data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously[11]. In brief, the study focuses on the etiology, pathophysiology, complications, and comorbid conditions of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged 40 to 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry by mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data from the first 3451 participants, who completed the baseline survey

between November 2010 and September 2013. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (permit 131088-105234-PG) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

For the current analyses, of the 3451 individuals, 160 participants had missing Food Frequency Questionnaire (FFQ) data and 65 reported implausible energy intake, i.e. < 800 or > 4200 kcal/day for males and < 500 or > 3500 kcal/day for females and were excluded from analyses. Another 660 individuals had missing data on one or more of the outcome variables or confounders (BMI (n=2), history of cardiovascular disease (n=58), smoking (n=8), physical activity (n=358), education (n=17), eGFR (n=28), plasma dicarbonyls (n=75), SAF (n=114)). In total, 2566 individuals were included in the analyses (Supplementary Figure S3.1).

Assessment of dietary MGO, GO, and 3-DG intake

Habitual intake of the dicarbonyls MGO, GO, and 3-DG was estimated by combining food intake data from the FFQ used in The Maastricht Study[12] with our previously published dietary dicarbonyl database[8]. This database contains MGO, GO, and 3-DG concentrations of 223 foods and drinks commonly consumed in a Western diet. Foods and drinks were selected based on coverage of the FFQs used in the Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC)[13] and in The Maastricht Study[12].

The validated Maastricht FFQ consists of 253 food items with questions on frequency and consumed amounts, with a 1-year reference period[12]. Frequency questions used an answer model with 11 options, from not used to 7 days/week. Each of the frequency questions was combined with an amount (quantity) question, using an answer model with fourteen standard household servings, from <1/day to >12/day. Average daily consumption of food items were calculated similarly as for the 24-h dietary recalls, thus by multiplying frequency and amount and by transcribing food items into food codes using the Dutch Food Composition Table 2011 (NEVO) to calculate energy and nutrient intakes. This FFQ has been validated, and validation coefficients for each food group were reported elsewhere[12]. Briefly, the median validation coefficient for food groups in our FFQ was 0.64, being >0.50 for 17 of 22 food groups. Overall the validity of the 253-item Maastricht FFQ was deemed satisfactory.

For foods and drinks that were included in both the database and the FFQ (n=150), we used the exact dicarbonyl concentrations. For FFQ items that were not included in the database (n=103), we used the average dicarbonyl concentration of comparable food products from the same food group as an estimate of dicarbonyl concentration. For example, for kiwi the average concentration of each dicarbonyl in all fruits in the database was used. To estimate habitual daily dietary intake of MGO, GO, and 3-DG, we multiplied the dicarbonyl concentration of a food product (mg/g) by the individual's estimated daily intake of that food product based on the FFQ (g/day), and subsequently summed all 253 food items (see formula below, using MGO as example).

$$MGO\ intake\left(\frac{mg}{day}\right) = \sum_{i=1}^{n} \left([MGO]i \left(\frac{mg}{g}\right) * intake\ i \left(\frac{g}{day}\right)\right)$$

Where *i* is food item, [MGO]*i* is MGO concentration for that particular food item from the database and intake *i* is intake of that particular food item derived from the FFQ.

Calculation of the contribution of food groups to dietary dicarbonyl intake

To gain insight into the contribution of specific food groups to dicarbonyl intake, we calculated each individual's habitual daily intake of MGO, GO, and 3-DG from different food groups. Foods and drinks were categorized in 25 food groups based on similarity of dicarbonyl concentrations, i.e. bread, breakfast cereals, cookies/ bakery products, potatoes/rice/pasta, bread condiments, vegetables/legumes, fruits, meat, fish, vegetarian/soy products, milk/dairy products, cheese, egg, ready-made meals, nuts/snacks, fats/oils/savory sauces, sweets/chocolate, dried fruits, tea, coffee, soft drinks, fruit juice, beer, wine, and liqueur. To calculate daily intake of MGO, GO, and 3-DG for each food group, we first multiplied the dicarbonyl concentration of a food product (mg/g) by the individual's daily intake of that food product (g/day) using the formula above and subsequently summed all food products in that particular food group. Herein, we report the population median of daily MGO, GO, and 3-DG intake (in mg/day) from each food group. The relative contribution of each food group is also expressed as percentage: for each individual, [the daily intake of MGO, GO, or 3-DG via each food group] / [total MGO, GO, or 3-DG intake]*100%. Percentages for each food group are reported as population medians, normalized to add up to 100% for all food groups together.

Quantification of dicarbonyls in plasma and SAF

After an overnight fast, EDTA plasma was obtained from venous blood, put on ice, separated after centrifugation (3000×*g* for 15 min at 4°C), and stored at −80°C until the assays were performed. Concentrations of the dicarbonyls MGO, GO, and 3-DG were measured with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS), as described previously[14]. Briefly, plasma was mixed with o-phenylenediamine (in perchloric acid). After an overnight reaction at room temperature, shielded from light, internal standard solution was added. Samples were mixed, centrifuged, and injected for UPLC-MS/MS analysis. Intra- and inter-assay coefficients of variation were 2.9% and 7.3% for MGO, 4.3% and 14.3% for GO, and 2.4% and 12.0% for 3-DG, respectively. The lower limits of quantification (s/N=6) were 9 nmol/L for MGO, 100 nmol/L for GO, and 5 nmol/L for 3-DG.

SAF - an estimate for AGE accumulation in the skin – was measured using the AGE Reader CU (DiagnOptics Technologies BV, Groningen, the Netherlands), as previously described[10]. Participants were asked not to use any sunscreen or self-browning creams on their lower arms within two days before the measurement. The mean of three consecutive measurements was used in the analyses. The intra-class correlation coefficient of three intraindividual consecutive SAF measurements was 0.83 (95% CI 0.65,0.94). Because skin pigment influences the measurement of SAF, in participants with dark-colored skin (i.e. a reflectance of 6%-10%), a validated reflectance-dependent correction was made by the software. Measurements in participants with a mean reflectance below 6% are considered unreliable and are not used for calculation of SAF by the software (n=1). Additionally, a single SAF value above 10 arbitrary units was considered as unreliable; these individual measurements (n=3) were manually excluded, and the mean of the remaining two measurements was used in analyses[15].

Assessment of other covariates

Weight and height were measured by a trained staff member, and body mass index (BMI) was calculated as weight (kg) divided by height^2 (m). Age, sex, smoking behavior, history of cardiovascular disease, educational level, and presence of gastrointestinal tract infection (defined as symptoms of gastrointestinal tract infection in the previous two months) were assessed by means of a self-report questionnaire[11]. Physical activity was assessed using the CHAMPS questionnaire[16]. Medication use was assessed by interview[11]. Glucose metabolism status was defined according to WHO 2006 criteria as normal glucose metabolism, prediabetes (impaired fasting glucose [6.1–7.0 mmol/L] and/or impaired glucose tolerance [2-h

glucose 7.8–11.1 mmol/L]), type 2 diabetes (fasting plasma glucose ≥7.1, 2-h glucose >11.1, or the use of diabetes medication) or other types of diabetes. Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CDK-epi) equation, using both serum creatinine and serum cystatin C[17]. Intake of energy, macronutrients, and the Dutch Healthy Diet index – a measure of diet quality - were assessed using the FFQ described above. The Dutch Healthy Diet index used in The Maastricht Study consists of all fifteen components except coffee, because the coffee component is based on type of coffee (filtered or unfiltered) and our FFQ does not distinguish between types of coffee consumed. Hence, the Dutch Healthy Diet index score ranges from 0 (no adherence) to 140 (complete adherence)[18].

Statistical analyses

The general characteristics of the study population were compared across tertiles of total dietary dicarbonyl intake. For this, a composite score of total dietary dicarbonyl intake was calculated by standardizing each dietary dicarbonyl (MGO, GO, and 3-DG) and averaging these into a composite score, which was used for comparison across tertiles only and not in further analyses. General characteristics are presented for males and females separately, since there was a sex difference in total dietary dicarbonyl intake. Differences in characteristics between tertiles were tested using one-way ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables and χ^2 test for discrete variables.

For all further analyses, each dietary dicarbonyl was considered individually, since they are thought to exert different biological effects. Non-normally distributed outcome variables (plasma MGO, GO, and 3-DG) were ln-transformed to obtain normal distributions. Outcome variables (plasma MGO, GO, and 3-DG, and SAF) and main independent variables (dietary MGO, GO, and 3-DG) were standardized, to allow direct comparison of the strength of the associations. Thus, in the regression analyses, the regression coefficients represent the SD difference in standardized ln-transformed plasma dicarbonyls or standardized SAF per 1 SD higher dietary dicarbonyl intake per day. The regression coefficients are displayed as β with (95% CI).

Because associations between intake of dietary components and (health) outcomes are often non-linear[19-21], we tested for deviation from a linear trend in the associations. For this, we performed a likelihood ratio test for each association, comparing a log-linear model with quintiles of dietary MGO, GO, or 3-DG as a

continuous predictor to a log-linear model with quintiles of dietary MGO, GO, or 3-DG as categorical (dummy) predictor. For all associations, the categorical model did not perform better than the linear model, indicating that the dose-response relationship can be considered linear.

The associations of dietary MGO, GO, and 3-DG intake with their respective plasma concentrations and SAF were examined in multiple linear regression analyses. Associations were first adjusted for age (years) and sex (males/females) [Model 1]. Next, additional adjustments were made for potential confounders related to cardio-metabolic risk factors and lifestyle, i.e. glucose metabolism status (prediabetes, type 2 diabetes, or other types of diabetes as dummy variables with normal glucose metabolism as reference category), eGFR (ml/min/1.73 m^2), history of cardiovascular disease (yes/no), BMI (kg/m^2), smoking (former or current as dummy variables with never as reference category), alcohol intake (g/day), physical activity (total score of all activities, hours/week), use of medication (yes/no for lipid-modifying, and glucose- and blood-pressure-lowering, each), and education (medium or high as dummy variables with low as reference category) [Model 2]. Finally, the analyses were additionally adjusted for total energy intake (kcal/day) [Model 3].

We adjusted for total energy intake by adding total energy intake as a covariate in the model (standard multivariate model)[22]. This model was chosen because using absolute intakes of dietary MGO, GO and 3-DG and adding total energy intake (kcal/day) as a covariate to the model has the advantage that the beta estimates are simplest to interpret, i.e. change in outcome per 1 unit higher intake of MGO (g/day) from the diet. There was no multicollinearity (VIF<3.5).

We tested interaction terms with sex, with glucose metabolism status (prediabetes or type 2 diabetes, with normal glucose metabolism status as reference category), and with eGFR (ml/min/1.73 m^2) to evaluate whether the associations differed according to these factors. Interaction with eGFR was tested because dicarbonyls are excreted from plasma by the kidney. Analyses for which we found an interaction ($p<0.1$) were repeated after stratification on glucose metabolism status (normal glucose metabolism status, prediabetes, or type 2 diabetes), sex, or eGFR (<60 and ≥60 mL/min per 1.73 m^2).

To explore whether observed associations were driven by any of the main food sources of dicarbonyls, we adjusted model 3 sequentially for dicarbonyl intake from each of the main food groups that contributed to >5% of total daily dicarbonyl

intake in this population (for example model 3 with dietary MGO as main independent variable was additionally adjusted for MGO intake from bread (in mg/day)).

Several sensitivity analyses were performed to address the robustness of the outcomes of the main analyses. First, to examine whether intake of other dietary components or better adherence to the Dutch Healthy Diet index explained part of the association, the fully adjusted model was, on top of total energy intake (kcal/day), additionally adjusted for either carbohydrate intake, fat intake, protein intake, fiber intake (all grams/day), or the Dutch Healthy Diet index. In the model where we adjusted for the Dutch Healthy Diet index we did not separately adjust for alcohol intake, which is included in the index. Second, the fully adjusted model was additionally adjusted for pack-years of smoking (with one pack-year of smoking defined as one package (=20 cigarettes) per day, smoked over a course of 1 year), because this was reported to be a determinant of SAF, independent from current smoking status[23]. Third, analyses were adjusted for physical activity as assessed by an accelerometer (ActivPAL) instead of the CHAMPS questionnaire. Accelerometer data are more accurate, but were missing for an additional 338 participants. Fourth, we adjusted for energy intake using the energy density method or the residuals method instead of the standard multivariate model. Total energy intake was also included as a covariate in these models[22]. Fifth, the analyses were repeated after exclusion of 23 individuals with other types of diabetes. Sixth, the analyses were repeated after exclusion of 568 individuals with previously diagnosed type 2 diabetes, as these individuals might have adapted their dietary behavior. Seventh, the analyses with SAF as outcome variable were repeated in a larger subset of the study population, as SAF was available for 5875 individuals. These analyses were not adjusted for eGFR, because this covariate was not available in this larger subset. Last, analyses were repeated after exclusion of 273 individuals with self-reported gastrointestinal tract infection.

All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 24.0). Statistical significance was set at $p<0.05$, except for testing for interaction where statistical significance was set at $p<0.10$.

Results

Characteristics of the study population

Table 3.1 shows that males with a higher dicarbonyl intake were slightly younger, less often had type 2 diabetes, had a lower BMI, were more physically active, were more insulin sensitive and had a higher eGFR. They less often used glucose-

lowering, antihypertensive or lipid-modifying medication. In addition, intake of energy and macronutrients was higher in males with a higher dicarbonyl intake, while there was no difference in adherence to the Dutch Healthy Diet index. Lastly, males with a higher dicarbonyl intake had lower plasma concentrations of 3-DG. Females with a higher dicarbonyl intake had a lower BMI and were more physically active. In addition, intake of energy and macronutrients was higher in females with a higher dicarbonyl intake, and they adhered better to the Dutch Healthy Diet index. The characteristics of individuals not included in the analysis compared to those included were somewhat more often males and smoker, had a lower educational status, and diabetes or a history of cardiovascular disease. In addition, those who were excluded had slightly higher concentrations of MGO and 3-DG in plasma, but lower concentrations of GO, and a slightly higher SAF compared to those included in the study (Supplementary Table S3.1).

Contribution of specific food groups to dicarbonyl intake

Median [IQR] intake of MGO, GO, and 3-DG was 3.6 [3.0-4.3], 3.5 [2.9-4.3], and 17 [12-23] mg/day, respectively. Coffee was the main source of dietary MGO intake (26% of total intake, Figure 3.1 and Supplementary Table S3.2), followed by bread (23%) and vegetables (10%). Bread was the main source of dietary GO (27%), followed by fruits (15%) and vegetables (13%). Bread was also the main source of dietary 3-DG (24%), followed by cookies and bakery products (15%) and sweets and chocolate (11%).

Table 3.1 Population characteristics by tertiles of dietary dicarbonyl intake, separate for males and females.[1]

Characteristic	Tertiles of dietary dicarbonyl intake							
	Males				Females			
	Lowest (n=430)	Middle (n=431)	Highest (n=431)	P-value	Lowest (n=424)	Middle (n=425)	Highest (n=425)	P-value
Demographics								
Age (years)	62 ± 8	61 ± 8	60 ± 8	0.01	58 ± 8	58 ± 8	59 ± 8	0.63
NGM/Prediabetes/T2DM/Other types	39/15/45/1	51/17/31/1	55/16/28/1	<0.001	63/14/21/1	69/14/16/1	70/16/14/1	0.11
Lifestyle								
Smoking (never/ former/current (%))	32/56/12	32/56/11	31/55/14	0.89	39/49/12	40/47/12	40/51/9	0.45
Smoking, packyears	4.5 [0.0-20]	5.7 [0.0-22]	4.8 [0.0-19]	0.96	0.5 [0.0-15]	0.3 [0.0-13]	0.4 [0.0-9.7]	0.50
Waist circumference (cm)	103 ± 12	101 ± 12	100 ± 12	<0.001	91 ± 13	89 ± 13	90 ± 13	0.02
BMI (kg/m^2)	28 ± 4	28 ± 4	27 ± 4	0.002	27 ± 5	26 ± 5	26 ± 5	0.01
Physical activity (h/week)	10 [6-15]	11 [7-16]	12 [8-19]	<0.001	14 [9-19]	15 [10-19]	16 [11-22]	<0.001
Education (low/medium/high (%))	30/30/41	25/30/45	29/30/41	0.54	38/30/32	36/27/38	34/26/41	0.50
Biological								
Fasting glucose (mmol/L)	6.6 ± 1.8	6.4 ± 1.9	6.3 ± 1.9	0.02	5.7 ± 1.5	5.6 ± 1.6	5.5 ± 1.1	0.05
HbA1c (%)	6.2 ± 1.0	6.0 ± 1.0	5.9 ± 1.1	<0.001	5.8 ± 0.83	5.7 ± 0.74	5.7 ± 0.65	0.78
24-h Systolic blood pressure (mmHg)	141 ± 17	139 ± 17	140 ± 17	0.13	131 ± 18	129 ± 17	130 ± 18	0.23
24-h Diastolic blood pressure (mmHg)	79 ± 9.8	78 ± 9.4	79 ± 9.4	0.69	74 ± 9.4	74 ± 9.5	73 ± 9.6	0.36
Insulin sensitivity (Matsuda index)	3.1 ± 2.1	3.5 ± 2.3	3.8 ± 2.3	<0.001	4.1 ± 2.6	4.6 ± 3.2	4.5 ± 2.8	0.13
Cholesterol (mmol/L)	4.8 ± 1.2	5.1 ± 1.1	5.1 ± 1.1	0.003	5.6 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.73
Total-to-HDL cholesterol ratio	3.8 ± 1.2	3.9 ± 1.2	4.0 ± 1.3	0.16	3.4 ± 1.0	3.4 ± 1.1	3.3 ± 1.0	0.45
Triglycerides (mmol/L)	1.6 ± 0.90	1.5 ± 0.84	1.6 ± 1.0	0.21	1.4 ± 0.85	1.3 ± 0.75	1.3 ± 0.83	0.25
eGFR (mL/min/1.73 m^2)	86 ± 15	88 ± 15	88 ± 14	0.03	88 ± 15	88 ± 15	89 ± 14	0.83
History of disease and medication use								
History of CVD (% yes)	21	16	18	0.27	13	15	13	0.98
Gastrointestinal infection[2] (%yes)	10	11	11	0.99	12	10	11	0.18
Glucose-lowering medication (%yes)	35	24	24	<0.001	17	13	11	0.13
Anti-hypertensives (%yes)	54	42	44	0.001	34	30	31	0.60
Lipid-modifying medication (%yes)	52	42	39	<0.001	29	27	26	0.59

Table 3.1 (continued)

Characteristic	Tertiles of dietary dicarbonyl intake							
	Males				Females			
	Lowest (n=430)	Middle (n=431)	Highest (n=431)	P-value	Lowest (n=424)	Middle (n=425)	Highest (n=425)	P-value
Dietary intake								
Energy intake (kcal/day)	1868 ± 383	2369 ± 387	2897 ± 554	<0.001	1595 ± 334	1957 ± 366	2414 ± 459	<0.001
Carbohydrate (g/day)	190 ± 42	246 ± 42	317 ± 63	<0.001	162±35	211±38	274 ± 52	<0.001
Mono- and di-saccharides (g/day)	69 ± 23	96±27	133±42	<0.001	64±20	89±23	125 ± 33	<0.001
Fat (g/day)	74 ± 24	93 ± 26	111 ± 33	<0.001	63±23	75±24	90 ± 28	<0.001
Protein (g/day)	75 ± 17	92 ± 18	108 ± 23	<0.001	68 ± 15	79 ± 17	93 ± 21	<0.001
Fiber (g/day)	22 ± 5	28 ± 5	35 ± 8	<0.001	20 ± 5	26 ± 5	32 ± 7	<0.001
Alcohol (g/day)	9.8 [2.3-20]	13 [5.3-24]	13 [3.9-25]	<0.001	4.2 [0.17-14]	6.0 [0.65-14]	5.5 [0.65-14]	0.24
DHD15-index	80 ± 14	78 ± 14	80 ± 15	0.15	86 ± 13	88 ± 14	89 ± 14	0.03
Dietary MGO (mg/day)	2.9 ± 0.53	3.8 ± 0.46	5.0 ± 0.87	<0.001	2.6 ± 0.43	3.5 ± 0.41	4.5 ± 0.76	<0.001
Dietary GO (mg/day)	2.7 ± 0.51	3.6 ± 0.50	4.9 ± 0.94	<0.001	2.5 ± 0.44	3.4 ± 0.43	4.6 ± 0.76	<0.001
Dietary 3-DG (mg/day)	12 ± 4.1	18 ± 5.1	29 ± 12	<0.001	9.6 ± 3.2	16 ± 4.4	28 ± 13	<0.001
Plasma dicarbonyls and SAF								
Plasma MGO (nmol/L)	331 [290-389]	328 [285-380]	325 [279-378]	0.16	319 [273-372]	312 [273-371]	320 [278-378]	0.55
Plasma GO (nmol/L)	1192 [1012-1480]	1212 [1000-1444]	1184 [1018-1444]	0.92	1249 [1054-1554]	1256 [1041-1505]	1276 [1055-1528]	0.50
Plasma 3-DG (nmol/L)	1405 [1175-1688]	1335 [1151-1611]	1311 [1150-1544]	0.005	1192 [1053-1402]	1169 [1044-1351]	1174 [1055-1350]	0.39
SAF (AU)	2.3 ± 0.5	2.3 ± 0.5	2.2 ± 0.5	0.05	2.1 ± 0.5	2.1 ± 0.4	2.2 ± 0.4	0.13

[1]Data were presented as mean ± standard deviation for normally distributed variables, median [interquartile range] for skewed variables or proportion (%) for categorical variables. [2] Gastrointestinal tract infection defined as self-reported symptoms of gastrointestinal tract infection in the previous two months. 3-DG, 3-Deoxyglucosone; CVD, cardiovascular disease; DHD15-index: Dutch Healthy Diet index 2015; eGFR, estimated glomerular filtration rate; GO, glyoxal; MGO, methylglyoxal; NGM, normal glucose metabolism; SAF, skin autofluorescence; T2DM, type 2 diabetes.

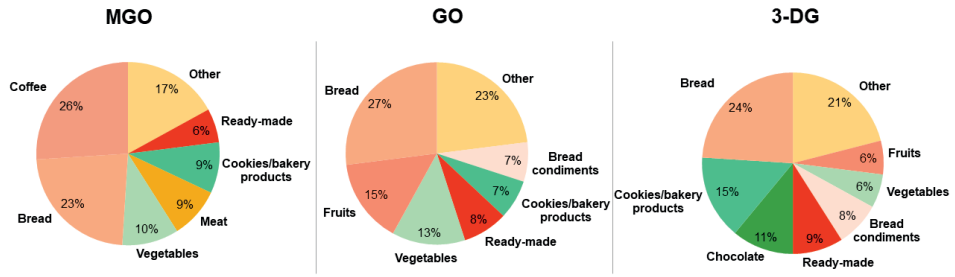

Figure 3.1 Contribution food groups to dietary dicarbonyl intake by participants of The Maastricht Study.[1]

[1] Median [IQR] MGO intake 3.6 [3.0-4.3] mg/day, median GO intake 3.5 [2.9-4.3] mg/day, median 3-DG intake 17 [12-23] mg/day. Bread condiments included spreads such a peanut butter, chocolate spread, jam, nut spread, chocolate sprinkles etcetera. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Associations between intake of MGO, GO, and 3-DG and corresponding plasma concentrations

Higher MGO intake was associated with higher MGO concentrations in plasma in the fully adjusted model (β (95%CI)=0.08 (0.02, 0.13), model 3, Table 3.2). Similarly, higher GO intake was associated with higher plasma GO in the fully adjusted model (β=0.10 (0.04, 0.16), model 3). Higher 3-DG intake was associated with lower plasma 3-DG in the age and sex adjusted model (β=-0.06 (-0.10, -0.02), model 1) but this did not remain after full adjustments (β=0.01 (-0.03, 0.04), model 3).

Glucose metabolism status modified the analyses between dietary MGO and plasma MGO (P interaction for prediabetes 0.001). Stratified analyses revealed that higher MGO intake was associated with higher plasma MGO among individuals with normal glucose metabolism (β=0.13 (0.06, 0.19)) and among individuals with type 2 diabetes (β=0.06 (-0.04, 0.17)), but with lower plasma MGO among individuals with prediabetes (β=-0.16 (-0.32, -0.01)) (Supplementary Table S3.3). We did not observe an interaction with sex or eGFR.

Associations between intake of MGO, GO, and 3-DG and SAF

Higher MGO intake was associated with higher SAF in all models (β=0.12 95% CI (0.07, 0.17), model 3, Table 3.2). Dietary GO and 3-DG intake were not statistically significantly associated with SAF (β=-0.02 (-0.07, 0.04) for GO and β=-0.002 (-0.04, 0.04) for 3-DG, model 3).

Glucose metabolism status modified the associations of GO and 3DG intake with SAF (P interaction type 2 diabetes: 0.07 for GO and 0.01 for 3-DG). After stratification, the association between dietary GO and SAF was not statistically significant in any of the glucose metabolism status groups (Normal glucose metabolism: β=-0.01 (-0.08, 0.06); Prediabetes: β=-0.03 (-0.18, 0.11); Type 2 diabetes: β=-0.04 (-0.16, 0.08), model 3, Supplementary Table S3.3). The association between dietary 3-DG intake and SAF was inverse and statistically significant for individuals with type 2 diabetes only (Normal glucose metabolism: β=0.01 (-0.03, 0.06); Prediabetes: β=0.07 (-0.05, 0.19); Type 2 diabetes: β=-0.10 (-0.19, -0.01), model 3, Supplementary Table S3.3).

Sex modified the association between 3-DG and SAF (P interaction 0.01), but stratified analyses revealed no statistically significant association in males or females (males: β=-0.06 (-0.12, 0.003); females: β=0.04 (-0.01, 0.09), model 3). We did not observe an interaction with eGFR.

Table 3.2 Associations of MGO, GO, and 3-DG intake with corresponding plasma dicarbonyl concentrations and with skin autofluorescence.[1]

	Model	Plasma MGO		SAF	
		β (95% CI)	p	β (95% CI)	p
Dietary MGO					
	Crude	0.03 (-0.01, 0.06)	0.20	0.06 (0.03, 0.10)	0.001
	1	0.02 (-0.02, 0.06)	0.31	0.06 (0.03, 0.10)	0.001
	2	0.03 (-0.005, 0.07)	0.09	0.07 (0.04, 0.11)	<0.001
	3	0.08 (0.02, 0.13)	0.004	0.12 (0.07, 0.17)	<0.001
		Plasma GO		**SAF**	
Dietary GO					
	Crude	0.01 (-0.03, 0.05)	0.60	-0.03 (-0.07, 0.01)	0.11
	1	0.02 (-0.02, 0.06)	0.29	-0.03 (-0.07, 0.002)	0.06
	2	0.02 (-0.02, 0.06)	0.29	-0.001 (-0.03, 0.04)	0.96
	3	0.10 (0.04, 0.16)	0.001	-0.02 (-0.07, 0.04)	0.57
		Plasma 3-DG		**SAF**	
Dietary 3-DG					
	Crude	-0.04 (-0.08, 0.00)	0.05	-0.04 (-0.07, 0.003)	0.07
	1	-0.06 (-0.10, -0.02)	0.001	-0.04 (-0.07, 0.001)	0.05
	2	0.02 (-0.01, 0.05)	0.24	0.003 (-0.03, 0.04)	0.88
	3	0.01 (-0.03, 0.04)	0.70	-0.002 (-0.04, 0.04)	0.91

[1] Data were analyzed using linear regression analysis. Standardized betas (β) were expressed as 1 SD increase in ln-transformed plasma dicarbonyl or 1 SD increase in skin autofluorescence per 1 SD increase in dietary dicarbonyl intake. Model 1: adjusted for age + sex. Model 2: model 1 + glucose metabolism status, eGFR, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, current use of medication (lipid modifying, anti-hypertensive, glucose lowering) and education level. Model 3: model 2 + total energy intake. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; SAF, skin autofluorescence.

Contribution of main food groups to the strength of the associations of dietary dicarbonyl intake with the respective plasma dicarbonyl concentrations or SAF

In additional analyses, fully adjusted analyses were sequentially adjusted for dicarbonyl intake from each of the food groups that contributed >5% to daily MGO, GO, or 3-DG intake. The above-reported associations of dietary MGO intake with plasma MGO (β=0.08, 95% CI (0.02, 0.13), model 3) and dietary MGO intake and SAF (β=0.12 (0.07, 0.17), model 3) were attenuated by ≥60% and no longer statistically significant after additional adjustment for MGO intake from coffee (β=0.02 (-0.05, 0.09) for plasma MGO and β=0.02 (-0.04, 0.09) for SAF, Table 3.3). In contrast, the associations were stronger after adjustment for MGO intake from cookies and bakery products (β=0.14 (0.08, 0.20) and β=0.16 (0.10, 0.22), respectively).

The above-reported association between dietary GO intake and plasma GO (β=0.10 (0.04, 0.16), model 3) was attenuated by 50% and lost statistical significance after additional adjustment for GO intake from fruit (β=0.05 (-0.02, 0.12), Table 3.3). Interestingly, although fruit juice only contributed to ~3% of GO intake, adjustment for GO intake from fruit juice also attenuated the association with plasma GO (β=0.07 (0.01, 0.14) [model 3 + GO intake from fruit juice]). In line with the observations for MGO, the association between dietary GO and plasma GO became stronger after adjustment for GO intake from cookies and bakery products (β=0.14 (0.07, 0.20)). This was also the case after adjustment for GO intake from bread condiments (β=0.14 (0.07, 0.20)).

Sensitivity analyses

The associations of dietary MGO with plasma MGO and SAF did not materially change after additional sequential adjustment for carbohydrate, protein, fat, or fiber intake, or the Dutch Healthy Diet index (Supplementary Table S3.4). The association of dietary GO with plasma GO was stronger after additional adjustment for carbohydrate intake. The association was attenuated and lost statistical significance after additional adjustment for fiber intake. Associations remained similar after additional adjustment for pack-years of smoking and after adjustment for physical activity assessed by the ActivPAL instead of the questionnaire (data not shown). In addition, associations remained similar when dietary MGO, GO, and 3-DG intakes were adjusted for energy using other methods (energy density or residuals method; data not shown).

When the analyses were repeated after excluding individuals with other types of diabetes, individuals with previously diagnosed type 2 diabetes, or individuals with gastrointestinal tract infection, the results did not change. Similarly, when the analyses with SAF as outcome variable were repeated in a larger subset of the study population that had SAF data available, without adjustment for eGFR, the association between dietary MGO intake and SAF remained unchanged.

Table 3.3 Associations of dietary dicarbonyls with plasma dicarbonyls and with skin autofluorescence additionally adjusted for main food groups.[1]

Model	% of dicarbonyl intake [2]	β	95% CI	p
Dietary MGO *with* plasma MGO				
Fully adjusted model (model 3) [3]		**0.08**	**0.02, 0.13**	**0.004**
Model 3 + Coffee	*26*	0.02	-0.05, 0.09	0.59
Model 3 + Bread	*23*	0.07	0.02, 0.13	0.007
Model 3 + Vegetables and legumes	*10*	0.07	0.01, 0.12	0.01
Model 3 + Cookies and bakery products	*9*	0.14	0.08, 0.20	<0.001
Model 3 + Meat	*9*	0.08	0.02, 0.13	0.004
Model 3 + Ready-made	*6*	0.08	0.02, 0.13	0.004
Model 3 + Fruit juice [4]	*1*	0.08	0.02, 0.13	0.005
Dietary MGO *with* SAF				
Fully adjusted model (model 3)		**0.12**	**0.07, 0.17**	**<0.001**
Model 3 + Coffee	*26*	0.02	-0.04, 0.09	0.47
Model 3 + Bread	*23*	0.13	0.08, 0.18	<0.001
Model 3 + Vegetables and legumes	*10*	0.13	0.08, 0.18	<0.001
Model 3 + Cookies and bakery products	*9*	0.16	0.10, 0.22	<0.001
Model 3 + Meat	*9*	0.12	0.08, 0.17	<0.001
Model 3 + Ready-made	*6*	0.12	0.07, 0.17	<0.001
Model 3 + Fruit juice	*3*	0.13	0.08, 0.17	<0.001
Dietary GO *with* plasma GO				
Fully adjusted model (model 3)		**0.10**	**0.04, 0.16**	**0.002**
Model 3 + Bread	*27*	0.10	0.04, 0.16	0.003
Model 3 + Fruits	*15*	0.05	-0.02, 0.12	0.15
Model 3 + Vegetables and legumes	*13*	0.12	0.05, 0.18	0.001
Model 3 + Ready-made	*8*	0.12	0.06, 0.18	<0.001
Model 3 + Bread condiments [5]	*7*	0.14	0.07, 0.20	<0.001
Model 3 + Cookies and bakery products	*7*	0.14	0.07, 0.20	<0.001
Model 3 + Fruit juice	*3*	0.07	0.01, 0.14	0.03

[1] Data were analyzed using linear regression analysis. Standardized betas (β) were expressed as 1 SD increase in ln-transformed plasma dicarbonyl or SAF per 1 SD increase in dietary dicarbonyl intake. [2] % of dicarbonyl intake from each food group as displayed in Figure 3.1 and Supplementary Table S3.2. [3] Model 3: adjusted for age, sex, glucose metabolism status, eGFR, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, current use of medication (lipid modifying, anti-hypertensive, glucose lowering), education level and total energy intake. Additionally, sequentially adjusted for MGO or GO from each of the separate food groups that contribute >5% to total MGO or GO intake. [4] Fruit juice contributed <5% to total MGO or GO intake, but because additional adjustment for fruit juice affected the associations, adjustment for fruit juice is displayed in the table. [5] Bread condiments included spreads such a peanut butter, chocolate spread, jam, nut spread, chocolate sprinkles etcetera. GO, glyoxal; MGO, methylglyoxal; SAF, skin autofluorescence.

Discussion

This study is the first to report on habitual intake of dicarbonyls from the diet in a population-based setting. In this Dutch population, median intake of the dicarbonyl MGO was 3.6 mg/day, with coffee and bread as main dietary sources of MGO. We showed that higher dietary MGO intake was associated with both higher plasma MGO concentrations and with higher SAF. Median intake of GO was comparable with 3.5 mg/day. Bread, fruits, and vegetables were the main dietary sources of GO, and higher dietary GO intake was associated with higher plasma GO concentrations, but not with SAF. Bread, cookies/bakery products, and chocolate were the main dietary sources of 3-DG. Although 3-DG intake was highest with a median intake of 17 mg/day, it was not associated with plasma 3-DG or SAF.

The observed positive associations of dietary MGO and GO intake with their respective plasma concentrations suggest uptake of MGO and GO from the diet. This is in line with previous studies in rats, which showed increased plasma concentrations of MGO after high oral MGO administration via drinking water[24,25]. In a small-scale human study, a single oral dose of honey containing MGO did not lead to changes in urinary MGO excretion[26], but this study had no data on plasma MGO or AGEs. In a similar study with 3-DG, a significant increase in urinary excretion of 3-DG and its metabolite 3-deoxyfructose were reported, with a recovery of approximately 10-15% of the administered 3-DG in urine[27], suggesting dietary absorption of 3-DG. It might be that we did observe positive associations for dietary MGO and GO but not for 3-DG, despite the higher habitual intake of 3-DG, because 3-DG is excreted into the urine before it reacts to proteins, considering it is less reactive than MGO and GO. All in all, these data suggest that these small dicarbonyl compounds are potentially absorbed through the intestinal epithelial wall. These reactive compounds, especially MGO and GO, might additionally contribute to a higher plasma concentration of dicarbonyls via local effects in the gastrointestinal tract, for example by modification of intestinal proteins[28], digestive enzymes[29], or *de novo* formation of AGEs[30], leading to gut inflammation, increased permeability or altered microbiome composition[9,31-33]. Moreover, the concentrations of plasma MGO and GO could potentially be elevated even more indirectly, for example by exhaustion of the glyoxalase pathway - responsible for detoxification of dicarbonyls - in the intestine after long-term exposure to dicarbonyls from the diet[34]. Future studies with stable isotope labeled dicarbonyls are required to determine the exact fate of dietary dicarbonyls.

The implications of a higher plasma MGO concentration and SAF are not completely understood. In our study, a 1 mg/day higher intake of MGO (corresponding to for

example ~400 mL coffee[8]) was associated with a 2-3% higher plasma MGO and SAF. This effect is potentially of relevance, in view of our earlier observation that plasma MGO concentrations differed by only ~5-13% between individuals with diabetes with and without cardiovascular events[35,36]. Elevated MGO has also been associated with the progression of type 2 diabetes[37] and microvascular disease in individuals with and without (pre)diabetes[38]. SAF was found to be positively associated with low-grade inflammation, the degree of atherosclerosis[39], and progression of chronic kidney disease[40]. In contrast, a recent study in mice showed that long-term exposure to dietary MGO led to a moderate increase in plasma MGO, yet was accompanied by increased survival, less age-related solid tumors, no diabetes, and no renal insufficiency[41]. Interestingly, recently the concept of hormesis has been proposed for MGO, meaning favorable biological effects at a low exposure to MGO and toxic effects at a high exposure[42]. Indeed, experimental studies in model organisms show that low-dose MGO promotes life-span whereas high-dose reduces lifespan[37,43]. Thus, dicarbonyl intake may have beneficial health effects when consumed in a low dose but detrimental health effects when consumed in a high dose.

Coffee and bread were both major dietary sources of MGO in this population, yet the associations of dietary MGO with plasma MGO concentration and with SAF were mainly driven by coffee intake. Interestingly, a previous study reported that coffee consumption was an independent predictor of SAF[44]. Our study shows that MGO from coffee might explain, at least partly, this association between coffee consumption and SAF. Bread was the main source of dietary GO, but GO from bread did not drive the association between dietary GO and plasma GO. In contrast, this association seems to be driven by intake of fruit, which is the second largest source of dietary GO, and fruit juice, which contributed only ~3% of total GO intake.

The observation that fruit juice (for GO) and coffee (for MGO) were important drivers of the associations with corresponding plasma dicarbonyls suggests that dicarbonyls are more easily absorbed from drinks and simple food matrices than from food products with complex food matrices that contain fat and proteins. Coffee was a main driver despite the observation of a recent *in-vitro* study that melanoidins in cacao, bread, and especially in coffee are effective scavengers of dicarbonyls[45].

Surprisingly, some of the associations became stronger after adjustment for dicarbonyl intake from certain food groups including cookies and bakery products. The explanation for this is currently unknown, but another study that examined the association between dietary AGEs and weight gain also reported slightly stronger

associations after adjustment for intake of cakes and biscuits, as well as cereals and cereal products[46].

Some of the associations were modified by glucose metabolism status, but after stratification for glucose metabolism group, we observed no clear pattern among these groups, and consider this as a chance finding.

A major strength of this study is the application of the most extensive dietary dicarbonyl database currently available, which was linked to a comprehensive FFQ. This is the first dicarbonyl database that covers all food groups. Some studies have reported on other dicarbonyls in foods, such as 3-DGal[6,47], but little is known about these dicarbonyls, and they often have been measured only in specific food groups, such a milk or sugar-rich foods and drinks. Thus, we did not have enough data on the entire range of food groups to accurately estimate intake of these dicarbonyls. Another strength is the large and deep-phenotyped human cohort with a wide range of potential confounders. Also, our results were robust over a wide range of sensitivity analyses. This study also has limitations. Firstly, the food composition database contains average values of dicarbonyls in foods and is, therefore, unable to take the true effects of industrial and household food preparation on dicarbonyl intake into account. Nevertheless, our database contains dicarbonyl concentrations of foods prepared using various common preparation methods[8]. Secondly, we used an extensive food composition database that was compiled to cover our FFQ, but measurement errors remain a possibility, for example in the measurement of the dicarbonyl concentrations of each food item, in the estimation of food intake with the FFQ, or in the measurement of dicarbonyl concentrations in plasma. Such random error may lead to regression dilution bias, hence the real associations may be stronger than our observed associations. Thirdly, FFQs assess habitual food intake of the previous year and we measured fasting plasma dicarbonyl concentrations, which are less affected by prior meals, but both were measured at one time-point and we had no repeated measures available. Additionally, the cross-sectional design of the study does not allow the assessment of causality. Moreover, although we carefully adjusted for a large set of potential confounders, residual confounding remains a possibility. Our results were, however, robust in several sensitivity analyses. Our population included white individuals aged 40 to 75 years, limiting generalizability to other populations. Finally, we measured SAF, which is thought to be an estimate of tissue AGEs, but had no data on AGEs in specific tissues, AGEs in plasma, or dicarbonyls and AGEs in urine.

In conclusion, dietary MGO and GO were positively associated with their corresponding concentrations in plasma. Dietary MGO, but not GO or 3-DG, was

positively associated with SAF. These cross-sectional associations suggest that dicarbonyls may be absorbed from the diet, with potential preference for absorption from certain food items, and contribute to the pool of dicarbonyls in the circulation and subsequent formation of AGEs. This needs to be confirmed in future human tracer studies and longitudinal studies. Considering the potentially adverse health outcomes that have been associated with plasma dicarbonyls and SAF, but also the proposed hormesis with protective effects at lower concentrations, our current findings warrant future studies on dietary dicarbonyls and health outcomes.

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Supplementary tables

Table S3.1 Population characteristics of final study population and excluded individuals.[1]

Characteristics	Final study population (n=2566)	Missing in study population (n)	Excluded individuals (n=885)	Missing in excluded individuals (n)	$P_{\text{-value}}$
Age (years)	60 ± 8	0	60 ± 9	0	0.83
Sex (% male)	50	0	55	0	0.03
NGM/Prediabetes/T2DM/Other types (%)	58/15/26/1	0	50/13/35/2	0	<0.001
Smoking (never/former/current (%))	36/53/12	0	31/49/20	50	<0.001
Waist circumference (cm)	96 ± 14	1	97 ± 14	3	0.009
BMI (kg/m^2)	27 ± 5	0	27 ± 5	3	0.10
Physical activity (h/week)	13 [8.3-19]	0	13 [8.3-19]	438	0.86
Education (low/medium/high (%))	32/29/40	0	39/27/34	74	<0.001
Fasting glucose (mmol/L)	5.5 [5.1-6.4]	1	5.7 [5.1-7.0]	0	<0.001
HbA1c (%)	5.5 [5.4-6.2]	6	5.7 [5.4-6.5]	7	<0.001
24-h Systolic blood pressure (mmHg)	135 ± 18	2	135 ± 18	0	0.71
24-h Diastolic blood pressure (mmHg)	76 ± 10	2	76 ± 10	0	0.44
Insulin sensitivity (Matsuda index)	3.5 [2.0-5.3]	322	3.5 [2.0-5.4]	202	0.86
Cholesterol (mmol/L)	5.25 ± 1.2	0	5.1 ± 1.2	4	0.03
Total-to-HDL cholesterol ratio	3.6 ± 1.2	0	3.7 ± 1.2	4	0.05
Triglycerides (mmol/L)	1.43 ± 0.88	0	1.4 ± 0.81	4	0.90
eGFR (mL/min/1.73 m^2)	88 ± 15	0	88 ± 16	33	0.48
Medical history of CVD (% yes)	16	0	19	67	0.03
Gastrointestinal infection (%yes)	12	275	14	123	0.16
Glucose-lowering medication (%yes)	21	0	31	0	<0.001
Anti-hypertensives (%yes)	39	0	43	0	0.04
Lipid-modifying medication (%yes)	36	0	38	0	0.17
Energy intake (kcal/day)	2185 ± 599	0	2167 ± 635	226	0.49
Carbohydrate intake (g/day)	234 ± 70	0	231 ± 75	226	0.41
Fat intake (g/day)	84 ± 31	0	84 ± 31	226	0.88
Protein intake (g/day)	86 ± 23	0	85 ± 24	226	0.55
Fiber intake (g/day)	27 ± 8	0	27 ± 9	226	0.25
Alcohol intake (g/day)	8.6 [1.6-19]	0	6.8 [0.9-18.6]	226	0.10
Dietary 3-DG intake (mg/day)	19 ± 11	0	20 ± 15	226	0.17
Dietary GO intake (mg/day)	3.6 ± 1.1	0	3.6 ± 1.2	226	0.21
Dietary MGO intake (mg/day)	3.7 ± 1.0	0	3.7 ± 1.1	226	0.19
DHD15-index	84 ± 15	0	82 ± 15	226	0.13
Plasma MGO (nmol/L)	324 [279-377]	0	334 [287-384]	125	0.003
Plasma GO (nmol/L)	1224 [1030-1497]	0	1188 [992-1453]	125	0.02
Plasma 3-DG (nmol/L)	1249 [1097-1492]	0	1309 [1134-1624]	125	<0.001
SAF (AU)	2.2 ± 0.47	0	2.3 ± 0.55	181	<0.001

[1] Data are presented as mean ± standard deviation for normally distributed variables, median [interquartile range] for skewed variables or proportion (%) for categorical variables. 3-DG, 3-Deoxyglucosone; CVD, cardiovascular disease; DHD15-index: Dutch Healthy Diet-index 2015; eGFR, estimated glomerular filtration rate; GO, glyoxal; MGO, methylglyoxal; NGM, normal glucose metabolism; SAF, skin autofluorescence; T2DM, type 2 diabetes.

Table S3.2 Contribution food groups to dietary dicarbonyl intake by participants of The Maastricht Study.

Food group	Intake of food group (g/day) [1,2]	MGO intake from food group (mg/day)[1]	Contribution to total MGO intake (%) [3]	GO intake from food group (mg/day)	Contribution to total GO intake (%)	3-DG intake from food group (mg/day)	Contribution to total 3-DG intake (%)
Beer	16 [0-114]	0.01	*0.4*	0.004	*0.1*	0.25	*3*
Bread	148 [111-196]	0.69	*23*	0.76	*27*	2.2	*24*
Bread-condiments	12 [2.3-26]	0.08	*3*	0.19	*7*	0.73	*8*
Coffee	488 [250-650]	0.87	*26*	0.14	*5*	0.19	*2*
Cookies/ bakery-products	25 [12-43]	0.25	*9*	0.20	*7*	1.3	*15*
Dairy[4]	182 [98-304]	0.07	*2*	0.05	*2*	0.28	*3*
Fruit juice	33 [4.5-109]	0.03	*1*	0.08	*3*	0.24	*3*
Fruits	165 [92-261]	0.12	*4*	0.44	*15*	0.50	*6*
Meat	107 [73-146]	0.28	*9*	0.12	*4*	0.22	*2*
Nuts, seeds and snacks	17 [8.3-30]	0.04	*1*	0.05	*2*	0.06	*1*
Potato, rice, and pasta	150 [108-199]	0.08	*3*	0.12	*3*	0.21	*2*
Ready-made	31 [13-69]	0.16	*6*	0.22	*8*	0.80	*9*
Sweets/ chocolate	12 [4.6-22]	0.04	*1*	0.06	*2*	1.0	*11*
Vegetables/ legumes	180 [128-244]	0.29	*10*	0.38	*13*	0.53	*6*
Wine	29 [1.0-114]	0.02	*1*	0.01	*0.2*	0.21	*2*
Others[5]	-	0.08	*3*	0.05	*2*	0.22	*2*

[1] Median [IQR] intakes of the study population. [2] Deattenuated correlation coefficients (%) from the validation study of this FFQ were 0.64 for bread, 0.61 for (non)-alcoholic beverages, 0.81 for fruits, 0.75 for vegetables, 0.63 for cakes and cookies, 0.79 for sugar, honey, jams, and candy, 0.90 for milk and milk products, 0.78 for meat, 0.17 for nuts, seeds, and snacks, 0.61 for potatoes, 0.50 for grains, and -0.12 for composite dishes. [3] Note: percentages were normalized to add up to 100%. [4] Dairy is including milk and yoghurt but excluding cheese and eggs. [5] Others is the sum of food groups with ≤1% contribution to all MGO, GO, or 3-DG intake (breakfast cereals, cheese, dried fruits, egg, fats, oils and sauces, fish, liqueur, soft drinks, tea, vegetarian products). 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Table S3.3 Associations for which we observed an interaction stratified for glucose metabolism status.[1]

	Normal glucose metabolism (n=1480)		Prediabetes (n=392)		Type 2 diabetes (n=671)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Dietary MGO with plasma MGO	0.13 (0.06, 0.19)	<0.001	-0.16 (-0.32, -0.01)	0.04	0.06 (-0.04, 0.17)	0.24
Dietary GO with SAF	-0.01 (-0.08, 0.06)	0.78	-0.03 (-0.18, 0.11)	0.65	-0.04 (-0.16, 0.08)	0.52
Dietary 3-DG with SAF	0.01 (-0.03, 0.06)	0.56	0.07 (-0.05, 0.19)	0.23	-0.10 (-0.19, -0.01)	0.04

[1] Data were analyzed using linear regression analysis. Standardized betas (β) represent 1 SD increase in ln-transformed plasma dicarbonyl or 1 SD increase in skin autofluorescence per 1 SD higher dietary dicarbonyl intake. Results are for fully adjusted model (model 3): adjusted for age, sex, glucose metabolism status, eGFR, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, current use of medication (lipid modifying, antihypertensive, glucose lowering), education level and total energy intake. Individuals with other types of diabetes were excluded in these analyses because of the small sample size (n=23). 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; SAF, skin autofluorescence.

Table S3.4 Associations of dietary dicarbonyls with plasma dicarbonyls and with skin autofluorescence - additionally adjusted for other dietary components.[1]

		Plasma MGO β (95% CI)	p	SAF β (95% CI)	p
Dietary MGO					
	Fully adjusted model (model 3) [2]	**0.08 (0.02, 0.13)**	**0.004**	**0.12 (0.07,0.17)**	**<0.001**
	Model 3 + Carbohydrates	0.10 (0.04, 0.15)	0.001	0.13 (0.08, 0.18)	<0.001
	Model 3 + Fat	0.10 (0.04, 0.15)	0.001	0.13 (0.08, 0.18)	<0.001
	Model 3 + Protein	0.07 (0.02, 0.12)	0.009	0.12 (0.07, 0.17)	<0.001
	Model 3 + Fiber	0.08 (0.03, 0.14)	0.004	0.13 (0.08, 0.18)	<0.001
	Model 3 + DHD [3]	0.08 (0.03, 0.13)	0.004	0.12 (0.08, 0.17)	<0.001
		Plasma GO		**SAF**	
Dietary GO					
	Fully adjusted model (model 3)	**0.10 (0.04, 0.16)**	**0.001**	**-0.02 (-0.07, 0.04)**	**0.58**
	Model 3 + Carbohydrates	0.17 (0.10, 0.25)	<0.001	-0.04 (-0.10, 0.03)	0.32
	Model 3 + Fat	0.14 (0.06, 0.21)	0.001	-0.05 (-0.12, 0.02)	0.15
	Model 3 + Protein	0.11 (0.05, 0.17)	<0.001	-0.01 (-0.07, 0.04)	0.63
	Model 3 + Fiber	0.06 (-0.01, 0.14)	0.09	-0.05 (-0.12, 0.02)	0.15
	Model 3 + DHD	0.07 (0.003, 0.13)	0.04	-0.01 (-0.06, 0.04)	0.70
		Plasma 3-DG		**SAF**	
Dietary 3-DG					
	Fully adjusted model (model 3)	**0.01 (-0.03, 0.04)**	0.68	**-0.002 (-0.04, 0.04)**	**0.91**
	Model 3 + Carbohydrates	0.03 (-0.01, 0.06)	0.14	-0.006 (-0.05, 0.04)	0.77
	Model 3 + Fat	0.03 (-0.01, 0.06)	0.15	-0.009 (-0.05, 0.03)	0.66
	Model 3 + Protein	0.006 (-0.03, 0.04)	0.70	0.001 (-0.04, 0.04)	0.96
	Model 3 + Fiber	0.02 (-0.01, 0.05)	0.20	-0.007 (-0.05, 0.03)	0.71
	Model 3 + DHD	0.01 (-0.02, 0.04)	0.49	-0.001 (-0.04, 0.04)	0.94

[1] Data were analyzed using linear regression analysis. Standardized betas (β) represent 1 SD increase in ln-transformed plasma dicarbonyl or 1 SD increase in skin autofluorescence, per 1 SD higher dietary dicarbonyl intake. [2] Model 3: adjusted for age, sex, glucose metabolism status, eGFR, history of cardiovascular disease, BMI, smoking, alcohol intake, physical activity, current use of medication (lipid modifying, antihypertensive, glucose lowering), education level and total energy intake. [3] The model additionally adjusted for Dutch Healthy Diet index was not adjusted for alcohol intake, because this is already included in the index. DHD, Dutch Healthy Diet index; GO, glyoxal; MGO, methylglyoxal; SAF, skin autofluorescence.

Supplementary figure

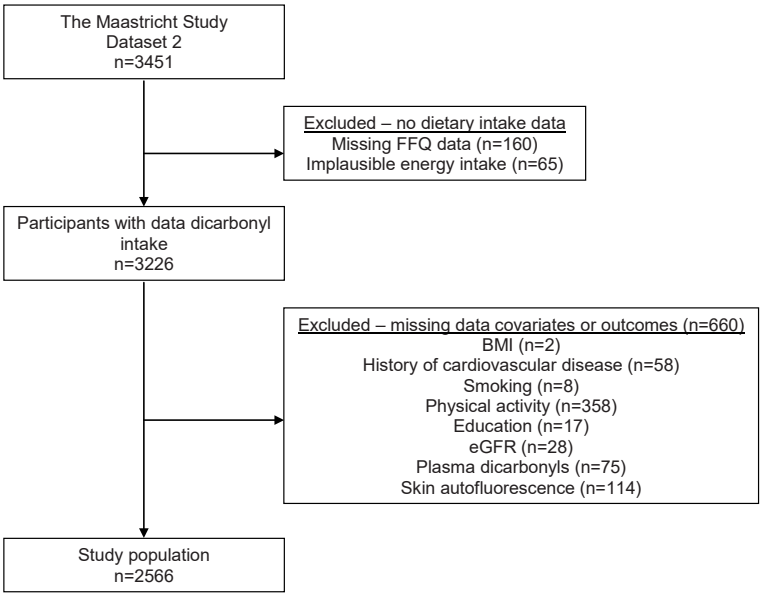

Figure S3.1 Flowchart selection study population.

Chapter 4

Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation: The Maastricht Study

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Abstract

Background

Dicarbonyls are major reactive precursors of advanced glycation endproducts (AGEs). Dicarbonyls are formed endogenously, but also during food processing. Circulating dicarbonyls and AGEs are associated with inflammation and microvascular complications of diabetes, but for dicarbonyls from the diet these associations are currently unknown.

Objective

To examine the associations of dietary intake of dicarbonyls with low-grade inflammation and microvascular function.

Design

In 2792 participants (60±8 years, 50% men, 26% type 2 diabetes) of the population-based cohort The Maastricht Study, we estimated the habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) by linking Food Frequency Questionnaires to our food composition database of the MGO, GO, and 3-DG content of >200 foods. Low-grade inflammation was assessed by six plasma biomarkers, compiled in a z-score. Microvascular function was assessed by a z-score of four plasma biomarkers, as flicker light-induced dilation and diameters in retinal micro vessels, as heat-induced skin hyperemic response, and as urinary albumin excretion. Cross-sectional associations of dietary dicarbonyls with low-grade inflammation and with microvascular function were investigated using linear regression adjusting for age, sex, potential confounders related to cardio-metabolic risk factors, lifestyle and dietary factors.

Results

Fully adjusted analyses revealed that higher intake of MGO was associated with a lower z-score for inflammation (std. β (95%CI)=-0.05 (-0.09, -0.01)) with strongest inverse associations for hsCRP and TNF-α (both -0.05 (-0.10, -0.01)). In contrast, higher dietary MGO intake was associated with impaired retinal venular dilation after full adjustment (std. β (95%CI)=-0.07 (-0.12, -0.01)), but not with the other features of microvascular function. GO and 3-DG intakes were not associated with any of the outcomes.

Conclusion

Higher habitual intake of MGO was associated with less low-grade inflammation. This suggests that food–derived MGO may induce anti-inflammatory effects. This novel, presumable beneficial, association requires further investigation.

Introduction

Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs)[1]. Dicarbonyls are formed endogenously, during glycolysis and lipid peroxidation, but they are also formed in food processing, mainly during heat treatment[2,3]. We recently showed that a higher habitual intake of the dicarbonyls MGO and GO was associated with higher concentrations of these dicarbonyls in plasma. Additionally, higher MGO intake was associated with higher autofluorescence in the skin, an estimate for AGE accumulation in tissue[4]. This suggests that these dietary dicarbonyls contribute to dicarbonyls and AGEs in the body.

The concentrations of dicarbonyls and AGEs in the circulation are associated with inflammation and microvascular dysfunction, which are important risk factors for microvascular complications such as retinopathy, nephropathy, and neuropathy (as reviewed in[1]). Moreover, we previously showed that higher plasma concentrations of the major dicarbonyl MGO were associated with albuminuria, eGFR, and retinopathy[5], as well as with incident cardiovascular disease[6,7].

It is currently unknown if, and to which extent, dicarbonyls derived from the diet also contribute to inflammation and to microvascular function. Long-term exposure to high amounts of oral MGO in animals induced adverse vascular effects, such as endothelial dysfunction[8] and glomerular basement membrane thickness in the kidney[9], and inflammation[8,10]. In contrast, other studies in animals showed no adverse effects after long-term MGO administration, despite elevated MGO concentrations in the circulation, and even showed an increase in antioxidant systems[11] and slightly increased survival[12]. Recent experimental studies also reported favorable effects of MGO including antioxidative effects and prolonged lifespan[13-16]. Thus, dicarbonyls intake might have both beneficial as well as undesirable consequences.

The consequences of dicarbonyls from the diet has, to the best of our knowledge, never been studied in humans. Therefore, in the current study we examined in a large population-based cohort the associations of dietary dicarbonyls with plasma biomarkers of low-grade inflammation and with markers of microvascular function, defined in plasma as biomarkers of endothelial function, in the venules and arterioles of the retina as diameter and flicker light-induced dilation, in the skin as heat-induced hyperemia, and in the kidney as urinary albumin excretion.

Methods

Study population

This study uses data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously[17]. In brief, the study focuses on the etiology, pathophysiology, complications, and comorbid conditions of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged 40 to 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry by mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data from the first 3,451 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (permit 131088-105234-PG) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Assessment of dietary MGO, GO, and 3-DG intake

Habitual intake of the dicarbonyls MGO, GO, and 3-DG was estimated as previously described[4]. In brief, we combined food intake data from the Food Frequency Questionnaire (FFQ) used in The Maastricht Study[18] with our previously published dietary dicarbonyl database, containing MGO, GO, and 3-DG concentrations of 223 foods and drinks[19].

To estimate habitual daily dietary intake of MGO, GO, and 3-DG, we multiplied the dicarbonyl concentration of a food product (mg/g) by the individual's estimated daily intake of that food product based on the FFQ (g/day), and subsequently summed all 253 food items (see formula below, using MGO as example).

$$MGO\ intake\left(\frac{mg}{day}\right) = \sum_{i=1}^{n} \left([MGO]i \left(\frac{mg}{g}\right) * intake\ i \left(\frac{g}{day}\right) \right)$$

Where *i* is food item, [MGO]*i* is MGO concentration for that particular food item from the database and intake *i* is intake of that particular food item derived from the FFQ.

Assessment of low-grade inflammation

Generalized low grade inflammation can be assessed as higher concentrations of proinflammatory cytokines in plasma. Plasma samples were collected after an overnight fast and stored at -80°C until measurements. We measured six plasma biomarkers of low-grade inflammation: high sensitivity C-reactive protein (hsCRP), serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (sICAM-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α). These biomarkers were measured in EDTA plasma samples with commercially available 4-plex sandwich immunoassay kits (Meso Scale Discovery (MSD), Rockville, MD, US), as reported before[20].

Assessment of microvascular function

Microvascular function can be assessed noninvasively in various organs. In the eye, it can be assessed as wider or narrower retinal arteriolar and venular diameters, or impaired flicker light–induced retinal arteriolar and venular dilation response. For retinal venular diameter, microvascular dysfunction is reflected by widening, whereas for retinal arteriolar diameter this is less clear, but it is thought that the development of diabetic microvascular dysfunction is reflected by initial widening and later narrowing[21]. In the skin, it can be assessed as impaired heat-induced skin hyperemia[22]. Microvascular function can also be assessed as higher urinary albumin excretion – a measure of kidney microvascular dysfunction[21] - and as higher plasma biomarkers of endothelial function[23].

For the retinal and skin measurements, all participants were asked to refrain from smoking and drinking caffeine-containing beverages three hours before the measurement. A light meal (breakfast or lunch), low in fat content, was allowed at least 90 minutes before the start of the measurements[22].

Plasma biomarkers of endothelial function

Four plasma biomarkers of endothelial function were measured: sICAM-1, sVCAM-1, soluble E-selectin (sE-selectin), and von Willebrand factor (vWF). sICAM-1, sVCAM-1, and sE-selectin were measured in EDTA plasma samples with commercially available 4-plex sandwich immunoassay kits with different standards and antibodies (Meso Scale Discovery, Rockville, Maryland). vWF was quantified in citrate plasma using ELISA (Dako, Glostrup, Denmark). Concentrations of vWF were expressed as a percentage of vWF detected in pooled citrated plasma of healthy volunteers[20,22].

Retinal microvascular diameters

All fundus photographs were taken with an auto-focus, auto-shot, and auto-tracker fundus camera (Model AFC-230, Nidek, Gamagori, Japan) in an optic disc-centered field of view of 45 degrees in a darkened room, as described previously[22]. Static retinal vessel analysis (one image of the left or right eye, randomly chosen) was performed with the RHINO software (Eindhoven, The Netherlands). Optic disc detection and arteriole/venule classification were corrected manually. Retinal vessel diameters were measured 0.5-1.0 disc diameter away from the optic disc margin and were presented as central retinal arteriolar and venular equivalent (CRAE and CRVE, respectively) in measurement units (MU). The scale factor is based on the optic disc diameter, which is assumed to be 1800 μm. CRAE and CRVE represent the equivalent single-vessel parent diameter for the six largest arterioles and largest venules in region of interest, respectively. The calculations were based on the improved Knudston-Parr-Hubbard formula[24]. Fundus photographs of insufficient quality, e.g. obstructed by lashes or defocused, were evaluated and discussed with a second observer and excluded on mutual agreement.

Flicker light-induced retinal microvascular dilation response

The retinal arteriolar and venular dilation response to flicker light exposure was assessed by the Dynamic Vessel Analyzer (DVA, Imedos, Jena, Germany), as previously described[22]. Briefly, pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine at least 15min prior to the start of the examination. For safety reasons, participants with an intraocular pressure more than 30 mmHg were excluded from the measurements. Per participant, either the left or the right eye was selected depending on the time of day the measurement was performed and without reference to participant characteristics. A straight arteriolar or venular segment of approximately 1.5 mm in length located 0.5–2 disc diameter from the margin of the optic disc in the temporal section was examined. Vessel diameter was automatically and continuously measured for 150 seconds. A baseline recording of 50 seconds was followed by a 40-second flicker light exposure period (flicker frequency 12.5 Hz, bright-to-dark contrast ratio 25 : 1), followed by a 60-second recovery period. Baseline retinal vascular diameters and flicker light-induced retinal vascular dilation were automatically calculated with the integrated DVA software (version 4.51, Imedos, Jena, Germany). Baseline retinal arteriolar/venular diameter was calculated as the average diameter of the 20–50 second recording and was expressed in measurement units, where one measurement unit is equal to 1 mm of the Gullstrand eye.

The flicker light-induced retinal vascular dilation was expressed as the percentage retinal vascular dilation over baseline and based on the average dilation achieved at time-points 10 and 40 seconds during the flicker stimulation period. This dilation response depends on a process called neurovascular coupling, which involves endothelial function[22,25].

Heat-induced skin hyperemic response

Skin blood flow was measured as described previously by means of a laser-Doppler system (Periflux 5000, Perimed, Järfalla, Sweden) equipped with a thermostatic laser-Doppler probe (PF457, Perimed) at the dorsal side of the wrist of the left hand[22]. The laser-Doppler output was recorded for 25 minutes with a sample rate of 32 Hz, which gives semiquantitative assessment of skin blood flow expressed in arbitrary perfusion units. Skin blood flow was first recorded unheated for 2 minutes to serve as a baseline. After the 2 minutes of baseline, the temperature of the probe was rapidly and locally increased to 44°C and was then kept constant until the end of the registration. The heat-induced skin hyperemic response was expressed as the percentage increase in average perfusion units during the 23-minute heating phase over the average baseline perfusion units. Skin perfusion during a period of local heating is thought to be mainly endothelium-dependent[26,27] and this method is commonly used as a test of skin microvascular function[28].

Urinary albumin excretion

Two 24 hour urine collections were used to assess urinary albumin excretion. Urinary albumin concentration was measured with a standard immuno-turbidimetric assay by an automatic analyzer (due to a change of supplier by the Beckman Synchron LX20, Beckman Coulter Inc., Brea, USA, and the Roche Cobas 6000, F. Hoffmann-La Roche, Basel, Switzerland) and multiplied by collection volume to obtain the 24 hour urinary albumin excretion[22]. A urinary albumin concentration below the detection limit of the assay (2 mg/l for the Beckman Synchron LX20 and 3 mg/l for the Roche Cobas 6000) was set at 1.5 mg/l before multiplying by collection volume. Only urine collections with a collection time between 20 and 28 hours were considered valid. If needed, urinary albumin excretion was extrapolated to a 24 hour excretion. Increased urinary albumin excretion (i.e. albuminuria) is as a risk marker for generalized endothelial dysfunction[29].

Assessment of other covariates

Glucose metabolism status was assessed by a 75 gr oral glucose tolerance test (OGTT) and defined according to WHO 2006 criteria as normal glucose metabolism, prediabetes (impaired fasting glucose [6.1–7.0 mmol/L] and/or impaired glucose tolerance [2-h glucose 7.8–11.1 mmol/L]), type 2 diabetes (fasting plasma glucose ≥7.1, 2-h glucose >11.1, or the use of diabetes medication) or other types of diabetes.

Weight and height were measured by a trained staff member, and body mass index (BMI) was calculated as weight (kg) divided by height^2 (m). Age, sex, smoking behavior, history of cardiovascular disease, educational level, and presence of gastrointestinal tract infection (defined as self-reported symptoms of gastrointestinal tract infection in the previous two months) were assessed by means of a self- report questionnaire[17]. Physical activity was assessed using the CHAMPS questionnaire[30]. Medication use was assessed by interview[17]. Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CDK-epi) equation, using both serum creatinine and serum cystatin C[31]. Intake of energy, macronutrients, and the Dutch Healthy Diet index – a measure of diet quality - were assessed using the FFQ described above. The Dutch Healthy Diet index used in The Maastricht Study consists of all fifteen components except coffee, because the coffee component is based on filtered versus unfiltered coffee and our FFQ does not distinguish between these types of coffee. Hence, the Dutch Healthy Diet index score ranges from 0 (no adherence) to 140 (complete adherence)[32]. Office and 24-hour ambulatory blood pressure, total cholesterol, HDL cholesterol, triglycerides, fasting plasma glucose, and glycosylated hemoglobin (HbA1c) were determined as described elsewhere[17].

Statistical analyses

The general characteristics of the study population are shown for the total sample and compared across tertiles of total dietary dicarbonyl intake. For this, a standardized composite score of total dietary dicarbonyl intake was calculated by standardizing each dietary dicarbonyl (MGO, GO and 3-DG), then averaging this into an overall dietary dicarbonyl z-score. Differences in characteristics among individuals in the tertiles were tested using one-way ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables and chi-squared test for discrete variables. We also evaluated to what extent participants were excluded from the analyses due to missing covariates differed from those who were included.

For all further analyses, each dietary dicarbonyl was considered individually, since they are thought to exert different biological effects[33]. Standardized composite scores were created for plasma biomarkers of low-grade inflammation and endothelial function, for reasons of statistical efficiency and to reduce the influence of the biological variability of each biomarker. Before calculating the composite scores, all non-normally distributed markers (i.e. hsCRP, SAA, sICAM-1, IL-6, IL-8, TNF-α, sE-selectin) were ln-transformed. The standardized composite scores were calculated by standardizing each individual biomarker, then averaging this into overall standardized composite scores for either low-grade inflammation or endothelial function, and standardized again. The low-grade inflammation composite score consisted of the biomarkers hsCRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α, and the endothelial function composite score consisted of the biomarkers sVCAM-1, sICAM-1, sE-selectin and vWF.

For the other outcome variables, urinary albumin excretion was ln-transformed to obtain a normal distribution. Absolute change over baseline (delta) retinal venular dilation was used as outcome in the association with MGO intake, because baseline retinal venular dilation was significantly associated with MGO intake (Supplementary Table S4.1) and spurious associations between determinants and outcomes expressed as percentage may occur when determinants are associated with baseline measures[34]. Perfusion during the 23-minute heating phase (in PU) was used as outcome in the association with 3-DG intake, because baseline skin blood flow was also significantly associated with 3-DG intake (Supplementary Table S4.1). Analyses in which absolute perfusion was used as outcome were additionally adjusted for baseline skin blood flow in all regression models (this will not lead to autocorrelation because there was only a weak association between baseline skin blood flow and heat-induced skin hyperemia)[34]. All outcome variables and main independent variables were standardized, to allow direct comparison of the strength of the associations.

We examined the associations of each standardized dietary dicarbonyl (MGO, GO, and 3-DG) with standardized low-grade inflammation and with standardized features of microvascular function, using multiple linear regression analyses. Associations were first adjusted for age (years), sex (men/women), glucose metabolism status (prediabetes, type 2 diabetes, or other types of diabetes as dummy variables with normal glucose metabolism as reference category), and baseline skin blood flow (only for analyses of heat-induced skin hyperemia) [Model 1]. Next, additional adjustments were made for potential confounders related to lifestyle, i.e. BMI (kg/m^2), smoking ('former' or 'current' as dummy variables with 'never' as reference category), alcohol intake (grams/day), physical activity (total

score of all activities, hours/week), total energy intake (kcal/day), and education ('medium' or 'high' as dummy variables with 'low' as reference category) [Model 2]. Finally, the analyses were additionally adjusted for other cardiovascular risk factors: triglycerides (mmol/L), systolic blood pressure (mmHg), total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs (yes/no) [Model 3].

Because the associations between intake of dietary components and (health) outcomes are often non-linear[35], we tested for deviation from a linear trend in our associations. For this, regression models using dietary dicarbonyls as continuous and categorical exposures were compared with a likelihood ratio test. None of the models showed a significantly better fit of the data when using dietary dicarbonyls as categorical exposures, and therefore dietary dicarbonyls were entered as continuous exposures in all models.

For each outcome measure we tested interaction terms with sex, glucose metabolism status (prediabetes and type 2 diabetes as dummy variables with normal glucose metabolism status as reference), and eGFR (ml/min/1.73m^2, thereby also adding eGFR as a covariate in the fully adjusted model), to evaluate whether the associations differed according to these factors. Analyses for which we found an interaction ($p<0.1$) stratified for sex, glucose metabolism status (normal glucose metabolism status, prediabetes, or type 2 diabetes), or eGFR (<60 and ≥60 mL/min per 1.73 m^2).

In additional analyses, we explored whether observed associations were driven by any of the main food sources of dicarbonyls. For this, we adjusted model 3 sequentially for dicarbonyl intake via each of the main food groups that contributed to ≥5% of the total daily dicarbonyl intake in this population (e.g. additional adjustment for MGO intake from coffee)[4].

Several sensitivity analyses were performed to assess the robustness of the outcomes of the main analyses. First, to examine whether the associations could be attributed to intake of other dietary components or better adherence to the Dutch Healthy Diet index, the fully adjusted model was, on top of energy intake (kcal/day), additionally adjusted for either carbohydrate intake, fat intake, protein intake (grams/day), or the Dutch Healthy Diet index. In the model where we adjusted for the Dutch Healthy Diet index we did not separately adjust for alcohol intake, which is included in the index. Second, we additionally adjusted the fully adjusted models for eGFR (ml/min/1.73m^2), urinary albumin excretion (mg/24h, except for analyses in which urinary albumin excretion was the outcome), retinopathy (yes/no), and history of cardiovascular diseases (yes/no). These covariates may introduce overadjustment, as they also reflect microvascular

function. Third, the analyses were repeated after exclusion of individuals with other types of diabetes, after exclusion of individuals with current infection (defined as CRP>10, only for analyses with low-grade inflammation as outcome), and after exclusion of individuals with self-reported gastrointestinal tract infection. Fourth, the analyses were repeated after exclusion of individuals with previously diagnosed type 2 diabetes, as these individuals might have adapted their dietary behavior or might be more prone to underreport their food intakes. Fifth, we explored possible confounding by antihypertensive medication through further specification into RAAS inhibitors and other types of antihypertensive medication. Sixth, several covariates were substituted by alternative measurement methods. Glucose metabolism status was substituted for HbA1c, fasting plasma glucose, or post-load glucose. Office blood pressure was substituted for blood pressure obtained during 24-hour ambulatory measurements. Educational level was substitute for equivalent income or occupational status. Physical activity obtained from the CHAMPS questionnaire was substituted by accelerometer data (ActivPAL). We did not include accelerometer-assessed physical activity in the main analyses because data was missing for a relatively large number of participants. BMI was substituted by waist circumference.

In addition, the association between dietary dicarbonyl intake and low-grade inflammation was verified in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM), since dietary intake data and plasma biomarkers of low-grade inflammation were available in this cohort[36]. CODAM includes 574 individuals with a moderately increased risk for type 2 diabetes and cardiovascular disease, who were extensively characterized at baseline between 1999 and 2002. Baseline data of 515 individuals were included in these analyses (see flowchart Supplementary Figure S4.1). We examined the cross-sectional associations of each dietary dicarbonyl (MGO, GO, and 3-DG) with low-grade inflammation using multiple linear regression, with adjustment for the same covariates as in The Maastricht Study, except for education, which was not available in this cohort.

Beta coefficients are reported with their 95% confidence intervals and represent the SD difference in standardized outcome per 1 SD higher intake of MGO, GO, or 3-DG per day. Statistical significance was set at $p<0.05$, except for testing for interaction where statistical significance was set at $p<0.10$. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 26.0).

Results

Characteristics of the study population

Of the 3451 participants, 225 were excluded because they did not complete the FFQ (n=160) or reported implausible energy intake, i.e. < 800 or > 4200 kcal/day for males and < 500 or > 3500 kcal/day for females (n=65) (Figure 4.1). Another 434 individuals were excluded because of missing data on one or more of the outcome variables or confounders. Of the remaining 2792 participants, plasma biomarkers of low-grade inflammation, plasma biomarkers of endothelial function, retinal microvascular diameters, flicker light-induced retinal microvascular dilation, heat-induced skin hyperemia, and urinary albumin excretion data were available in subpopulations of 2765, 2745, 2438, 2078, 1357, and 2774 participants, respectively. These subpopulations were comparable with regard to age, sex, and cardiovascular risk profile (Supplementary Table S4.2). Individuals with missing data on any of the covariates were more often men, and had somewhat higher cardiovascular risk factors, including more often type 2 diabetes, more current smokers, lower education, higher fasting plasma glucose, a higher total-to-HDL cholesterol ratio, and more medication use (Supplementary Table S4.2).

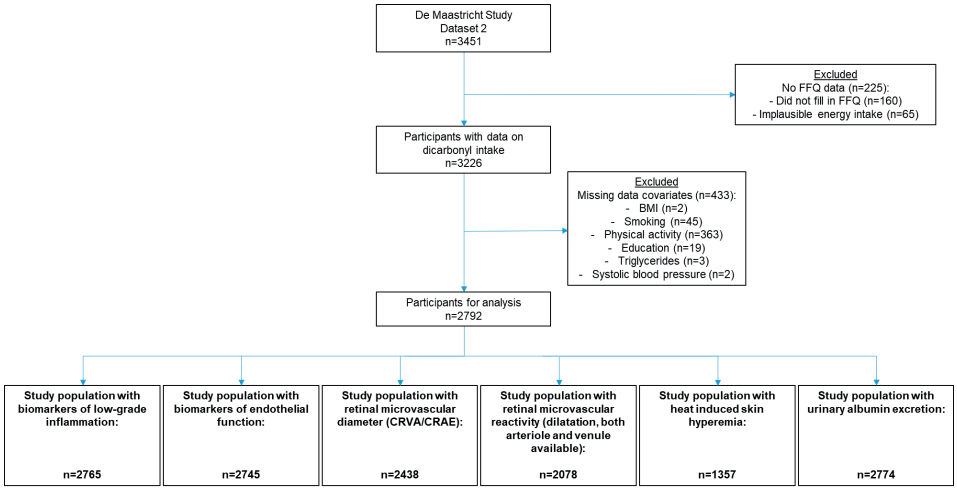

Figure 4.1 **Flowchart of study population**. Arteriolar dilation available for n=2104 and venular dilation available for n=2147. CRAE: central retinal arteriolar equivalent, CRVE: central retina venular equivalent, FFQ: Food Frequency Questionnaire.

Median [IQR] dietary intake was 4.0 [3.2-4.8] mg/day for MGO, 3.5 [2.9-4.3] mg/day for GO, and 17 [12-23] mg/day for 3-DG (Table 4.1). Individuals in the

highest tertile of overall dietary dicarbonyl intake, were more often men, less often had type 2 diabetes, were more physically active, less often used glucose-lowering medication, had higher intake of energy and all other dietary components, but did not differ in adherence to the Dutch dietary guidelines. These participants also had lower plasma hsCRP, SAA, and IL-8 levels.

Table 4.1 Population characteristics of those with information on all confounders.[1]

Characteristics	Total population (n=2792)	Dietary dicarbonyl intake (tertiles) [2]			
		Lowest (n=930)	Middle (n=931)	Highest (n=931)	P-value
Demographics					
Age (years)	60 ± 8	60 ± 8	60 ± 8	60 ± 8	0.50
Sex (% male)	50	42	51	58	<0.001
Glucose status					0.01
Normal glucose metabolism (%)	58	54	58	62	
Prediabetes (%)	15	15	15	15	
Type 2 diabetes mellitus (%)	26	30	26	22	
Other types of diabetes (%)	1	1	1	1	
Diabetes duration (years)	4 [1-11]	4 [1-11]	5 [2-10]	4 [1-11]	0.78
Lifestyle					
Smoking (%)					0.85
Never	36	36	37	35	
Former	52	51	52	53	
Current	12	13	12	12	
Waist circumference (cm)	95 ± 14	96 ± 14	95 ± 14	96 ± 13	0.29
BMI (kg/m^2)	27 ± 5	27 ± 5	27 ± 5	27 ± 4	0.01
Physical activity (h/week)	13 [8.3-19]	12 [7.5-18]	13 [8.3-18]	14 [8.5-20]	<0.001
Education (%)					0.22
Low	32	34	31	31	
Medium	29	30	29	28	
High	39	36	40	41	
Biological					
Fasting glucose (mmol/L)	5.5 [5.1-6.4]	5.6 [5.1-6.6]	5.5 [5.1-6.4]	5.5 [5.1-6.2]	0.31
HbA1c, %	5.6 [5.4-6.2]	5.7 [5.4-6.3]	5.6 [5.4-6.2]	5.6 [5.3-6.1]	0.27
24-h Systolic blood pressure (mmHg)	135 ± 18	135 ± 19	134 ± 18	135 ± 18	0.44
24-h Diastolic blood pressure (mmHg)	76 ± 10	76 ± 10	76 ± 10	77 ± 10	0.33
HOMA-IR	1.4 [1.0-2.1]	1.4 [1.0-2.3]	1.4 [1.0-2.1]	1.4 [1.0-2.1]	0.35
Cholesterol (mmol/L)	5.3 ± 1.2	5.2 ± 1.2	5.3 ± 1.2	5.3 ± 1.1	0.70
Total-to-HDL cholesterol ratio	3.6 ± 1.2	3.6 ± 1.1	3.6 ± 1.1	3.7 ± 1.2	0.02
Triglycerides (mmol/L)	1.2 [0.90-1.7]	1.2 [0.88-1.7]	1.2 [0.88-1.7]	1.2 [0.86-1.7]	0.40
eGFR (mL/min/1.73 m^2)	88 ± 15	87 ± 15	88 ± 15	89 ± 14	0.15
History of CVD (% yes)	16	16	17	16	0.87
Presence of gastrointestinal infection (%yes)	12	13	11	10	0.83
Retinopathy (%)	2	1	1	2	0.39
(Micro)albuminuria (%)	8	8	8	8	0.92

Table 4.1 (continued)

Characteristics	Total population (n=2792)	Dietary dicarbonyl intake (tertiles) [2]			P-value
		Lowest (n=930)	Middle (n=931)	Highest (n=931)	
Medication use					
Glucose-lowering medication (%yes)	21	23	22	18	0.03
Anti-hypertensives (%yes)	39	43	38	38	0.05
Lipid-modifying medication (%yes)	35	38	35	33	0.11
Dietary intake					
Energy intake (kcal/day)	2184 ± 602	1717 ± 381	2167 ± 416	2668 ± 561	<0.001
Carbohydrate, total (g/day)	233 ± 70	175 ± 40	229 ± 43	296 ± 62	<0.001
Fat, total (g/day)	84 ± 31	68 ± 24	85 ± 26	101 ± 32	<0.001
Protein (g/day)	86 ± 23	72 ± 17	85 ± 18	101 ± 23	<0.001
Fiber (g/day)	27 ± 8.1	21 ± 5.0	27 ± 5.4	34 ± 7.8	<0.001
Alcohol intake (g/day)	8.5 [1.5-19]	6.9 [0.80-17]	8.7 [1.9-19]	9.4 [2.3-20]	<0.001
Dutch Healthy Diet Index	83 ± 15	84 ± 14	83 ± 15	84 ± 15	0.76
Dietary MGO (mg/day)	4.1 ± 1.2	3.0 ± 0.61	4.0 ± 0.60	5.3 ± 1.2	<0.001
Dietary GO (mg/day)	3.7 ± 1.1	2.6 ± 0.51	3.6 ± 0.53	4.8 ± 0.96	<0.001
Dietary 3-DG (mg/day)	17 [12-23]	10 [8.1-13]	17 [14-20]	26 [21-33]	<0.001
Plasma biomarkers of low-grade inflammation					
hsCRP (µg/ml)	1.2 [0.61-2.7]	1.3 [0.68-3.1]	1.2 [0.59-2.7]	1.1 [0.57-2.5]	0.01
SAA (µg/ml)	3.3 [2.1-5.4]	3.4 [2.2-5.6]	3.2 [2.0-5.5]	3.2 [1.9-5.1]	0.02
sICAM1 (ng/ml)	338 [290-398]	341 [292-405]	339 [289-393]	336 [291-389]	0.19
IL-6 (pg/ml)	0.58 [0.39-0.88]	0.60 [0.41-0.89]	0.58 [0.38-0.90]	0.57 [0.38-0.86]	0.07
IL-8 (p g/ml)	4.1 [3.3-5.3]	4.2 [3.3-5.5]	4.0 [3.2-5.2]	4.1 [3.3-5.2]	0.01
TNF-α (pg/ml)	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.5]	2.2 [1.9-2.6]	0.27
Microvascular measurements					
Plasma biomarkers of endothelial function					
sICAM-1 (ng/ml)	338 [291-398]	341 [292-405]	339 [289-393]	336 [291-389]	0.23
sVCAM-1 (ng/ml)	427 ± 101	426 ± 103	429 ± 100	427 ± 100	0.90
sE-selectin (ng/ml)	107 [75-143]	107 [72-145]	106 [74-141]	109 [78-143]	0.48
vWF (%)	132 ± 48	133 ± 47	132 ± 49	132 ± 48	0.56
Retinal microvascular measurements					
CRAE (µm)	142 ± 20	143 ± 20	142 ± 19	142 ± 20	0.41
CRVE (µm)	214 ± 31	215 ± 32	213 ± 30	215 ± 32	0.46
Baseline arteriolar diameter (MU)	115 ± 15	115 ± 15	114 ± 16	116 ± 15	0.19
Baseline venular diameter (MU)	146 ± 21	147 ± 21	146 ± 21	146 ± 20	0.96
Flicker light-induced arteriolar dilation response (%)	3.0 ± 2.8	3.1 ± 2.9	3.2 ± 2.8	2.9 ± 2.7	0.29
Flicker light-induced venular dilation response (%)	3.9 ± 2.2	4.1 ± 2.3	3.8 ± 2.2	3.9 ± 2.2	0.06
Absolute arteriolar dilation response (delta)	4.4 ± 3.6	4.4 ± 3.7	4.5 ± 3.6	4.3 ± 3.4	0.43
Absolute venular dilation response (delta)	7.7 ± 4.1	7.9 ± 4.3	7.5 ± 4.0	7.5 ± 4.1	0.16
Skin microvascular measurements					
Baseline skin blood flow before heating (PU)	11 ± 6.6	12 ± 7.4	11 ± 5.6	11 ± 6.7	0.07
Skin hyperemia during heating (PU)	113 ± 58	114 ± 59	112 ± 55	113 ± 59	0.90
Skin hyperemic response (%)	1129 ± 773	1137 ± 794	1156 ± 768	1094 ± 760	0.47
Kidney microvascular measurement					
Urinary albumin excretion (mg/24h)	6.5 [4.0-11.6]	6.3 [4.0-12]	6.5 [3.9-12]	6.9 [4.2-11]	0.85

[1] Data are presented as mean ± standard deviation, median [interquartile range] or percentage. P-value

of ANOVA, Kruskal-Wallis, or Chi Square tests for differences between tertiles. [2] For the tertiles, a composite score of total dicarbonyl intake (MGO, GO, and 3-DG) was created. Data were available for: waist, n=2791, duration of diabetes, n=606, HOMA-IR, n=2621, eGFR, n=2768, HbA1c, n=2785, fasting plasma glucose, n=2790, history of CVD, n=2777, gastrointestinal infection, n=2500, hsCRP, SAA, sICAM-1, IL-8, TNF-α, sVCAM-1, e-Selectin, n=2766, IL-6, n=2765, vWF, n=2762, retinal microvascular diameters, n=2438, flicker light-induced arteriolar dilation, n=2104, flicker light-induced venular dilation, n=2147, baseline skin blood flow and skin hyperemic response, n=1357, urinary albumin excretion, n=2774. 3-DG: 3-deoxyglucosone, CRAE: central retinal arteriolar equivalent, hsCRP: high sensitivity C-reactive protein, CRVE: central retinal venular equivalent, CVD: cardiovascular diseases, eGFR: estimated glomerular filtration rate, sE-selectin: soluble E-selectin, GO: glyoxal, sICAM-1: soluble intracellular adhesion molecule-1, IL-6: interleukin-6, IL-8: interleukin-8, MGO: methylglyoxal, NGM: normal glucose metabolism, SAA: serum amyloid A, sVCAM-1: soluble vascular adhesion molecule-1, T2DM: type 2 diabetes mellitus, TNF-α: tumor necrosis factor alpha, vWF: von Willebrand factor.

Associations between dietary dicarbonyl intake and plasma biomarkers of low-grade inflammation

Higher intake of MGO was associated with a lower z-score for inflammation in the fully adjusted model (std. β (95%CI)=-0.05 (-0.09, -0.01), Table 4.2). Higher intakes of GO and 3-DG were also associated with a lower z-score for inflammation in the crude model (std. β (95%CI)=-0.06 (-0.09, -0.02) and -0.09 (-0.12, -0.05), respectively), but these associations were attenuated after further adjustments (std. β (95%CI)=-0.01 (-0.06, 0.04) and -0.03 (-0.07, 0.002), respectively, model 3). Intake of MGO was inversely associated with each individual biomarker of inflammation except sICAM, with strongest and statistically significant associations for hsCRP and TNF-α (std. β (95%CI)=-0.05 (-0.10, -0.01) and -0.05 (-0.10, -0.01), respectively, Supplementary Table S4.3).

In addition, we evaluated the association between dietary dicarbonyl intake and plasma biomarkers of low-grade inflammation in an independent cohort, the CODAM study. Dicarbonyl intake in CODAM was similar to The Maastricht Study, with median [IQR] intakes of 3.8 [3.1-4.6] mg/day for MGO, 3.4 [2.8-4.1] mg/day for GO, and 16 [11-22] mg/day for 3-DG. The associations between MGO, GO, and 3-DG intake and low-grade inflammation were inverse, although not statistically significant (std. β MGO=-0.05 (-0.16, 0.05), GO=-0.09 (-0.22, 0.04)), and 3-DG=-0.01 (-0.11, 0.09), model 3, Supplementary Tables S4.4 and S4.5).

Table 4.2 Association between dietary MGO, GO, and 3-DG intakes and a composite score of low-grade inflammation.[1]

	Model	Biomarkers of low-grade inflammation (composite score) [2] Std. β (95% CI)
Dietary MGO		
	Crude	-0.05 (-0.09, -0.01)
	1	-0.03 (-0.06, 0.01)
	2	-0.05 (-0.10, -0.01)
	3	-0.05 (-0.09, -0.01)
Dietary GO		
	Crude	-0.06 (-0.09, -0.02)
	1	-0.02 (-0.06, 0.01)
	2	-0.02 (-0.07, 0.03)
	3	-0.01 (-0.06, 0.04)
Dietary 3-DG		
	Crude	-0.09 (-0.12, -0.05)
	1	-0.05 (-0.08, -0.02)
	2	-0.03 (-0.07, 0.01)
	3	-0.03 (-0.07, 0.002)

[1] Standardized betas (β) were expressed as 1 SD change in composite score of low-grade inflammation per 1 SD higher dietary dicarbonyl intake. All variables were standardized for comparison. [2] Composite score of low-grade inflammation consisted of the biomarkers hsCRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α. Model 1: adjusted for age + sex + glucose metabolism status. Model 2: model 1 + BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level. Model 3: model 2 + triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. The population consisted of 2765 individuals. 3-DG, 3-deoxyglucosone, GO, glyoxal, MGO, methylglyoxal.

Associations between dietary dicarbonyl intake and plasma biomarkers of endothelial function

Intakes of MGO and GO were not associated with the z-score for endothelial function (std. β (95%CI)=-0.01 (-0.06, 0.03) and 0.03 (-0.03, 0.07), respectively, model 3, Table 4.3). Higher intake of 3-DG was associated with a lower z-score for endothelial function in the crude model (std. β=-0.05 (-0.09, -0.01)), but this association was attenuated after further adjustment (std. β=-0.01 (-0.05, 0.02), model 3). The associations of dietary MGO, GO, and 3-DG with individual biomarkers of endothelial function were also not statistically significant (Supplementary Table S4.6).

Association between dietary dicarbonyl intake and retinal microvascular function

Higher intake of MGO was associated with impaired flicker light-induced retinal venular dilation in all models (std. β absolute change over baseline (95%CI)=-0.07 (-0.12, -0.01), model 3, Table 4.3), but not with retinal arteriolar dilation (std. β %-change over baseline=-0.01 (-0.06, 0.05), model 3). Results were similar when %-change of retinal venular dilation was used as an outcome (data not shown). Intake of GO and 3-DG were not associated with flicker light-induced arteriolar and venular dilation (GO std. β %-change over baseline=-0.07 (-0.13, 0.001) and -0.06 (-0.13, 0.01), respectively. 3-DG: -0.004 (-0.05, 0.04) and -0.02 (-0.07, 0.03), respectively, model 3). Intakes of MGO, GO, and 3-DG were not associated with retinal arteriolar diameters (CRAE) or retinal venular diameters (CRVE) (MGO: std. β=0.04 (-0.01, 0.09) and 0.04 (-0.01, 0.09). GO: -0.01 (-0.07, 0.05) and 0.002 (-0.06, 0.06). 3-DG: -0.01 (-0.05, 0.04) and 0.01 (-0.03, 0.06), respectively, model 3).

Associations between dietary dicarbonyl intake and heat induced skin hyperemia

Higher intake of MGO was associated with impaired heat induced skin hyperemia in the crude model (std. β %-change over baseline=-0.05 (-0.11, -0.01), Table 4.3), but this attenuated after further adjustment (-0.02 (-0.08, 0.05), model 3). Intake of GO and 3-DG were not associated with heat induced skin hyperemia (GO: std. β %-change over baseline=0.03 (-0.05, 0.10), model 3). 3-DG: std. β absolute average heating response=0.01 (-0.05, 0.07), model 3).

Associations between dietary dicarbonyl intake and urinary albumin excretion

Higher intake of MGO was associated with higher excretion of urinary albumin in the crude and in the age, sex, and glucose metabolism status adjusted model (std. β=0.05 (0.01, 0.08), model 1, Table 4.3), but did not remain statistically significant after further adjustment (std. β=0.04 (-0.001, 0.09), model 3). Intake of GO and 3-DG were not associated with urinary albumin excretion (GO: std. β=-0.05 (-0.10, 0.01). 3-DG: -0.01 (-0.05, 0.03), model 3).

Table 4.3 Associations between dietary MGO, GO, and 3-DG intakes and measures of microvascular function.[1]

	Biomarkers of endothelial function [2]	CRAE	CRVE	Retinal arteriolar average dilation[3]	Retinal venular average dilation[3]	Heat-induced skin hyperemia [4]	Urinary albumin excretion [5]
Dietary MGO	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**
Crude	0.003 (-0.03, 0.04)	-0.01 (-0.04, 0.05)	0.02 (-0.02, 0.06)	-0.004 (-0.05, 0.04)	-0.05 (-0.09, -0.01)	-0.05 (-0.11, -0.01)	0.05 (0.02, 0.09)
1	0.002 (-0.03, 0.04)	0.03 (-0.01, 0.07)	0.04 (-0.001, 0.08)	-0.02 (-0.06, 0.03)	-0.05 (-0.09, -0.004)	-0.02 (-0.07, 0.03)	0.05 (0.01, 0.08)
2	-0.02 (-0.06, 0.03)	0.04 (-0.01, 0.09)	0.04 (-0.01, 0.09)	-0.01 (-0.07, 0.04)	-0.07 (-0.12, -0.02)	-0.02 (-0.08, 0.05)	0.04 (-0.001, 0.09)
3	-0.01 (-0.06, 0.03)	0.04 (-0.01, 0.09)	0.04 (-0.01, 0.09)	-0.01 (-0.06, 0.05)	-0.07 (-0.12, -0.01)	-0.02 (-0.08, 0.05)	0.04 (-0.001, 0.09)
Dietary GO	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**
Crude	0.007 (-0.03, 0.05)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.03)	-0.03 (-0.07, 0.02)	-0.02 (-0.07, 0.04)	-0.01 (-0.05, 0.03)
1	0.02 (-0.02, 0.06)	-0.001 (-0.04, 0.04)	0.000 (-0.04, 0.04)	-0.03 (-0.08, 0.01)	-0.03 (-0.07, 0.02)	0.01 (-0.05, 0.06)	-0.01 (-0.04, 0.03)
2	0.012 (-0.03, 0.08)	-0.01 (-0.07, 0.05)	0.000 (-0.06, 0.06)	-0.06 (-0.13, 0.01)	-0.07 (-0.14, -0.01)	0.03 (-0.05, 0.10)	-0.04 (-0.10, 0.01)
3	0.03 (-0.03, 0.07)	-0.01 (-0.07, 0.05)	0.002 (-0.06, 0.06)	-0.06 (-0.13, 0.01)	-0.07 (-0.13, 0.001)	0.03 (-0.05, 0.10)	-0.05 (-0.10, 0.01)
Dietary 3-DG	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**	**Std. β (95% CI)**
Crude	-0.05 (-0.09, -0.01)	-0.03 (-0.07, 0.01)	-0.01 (-0.05, 0.03)	-0.003 (-0.05, 0.04)	0.003 (-0.04, 0.05)	0.02 (-0.03, 0.08)	-0.02 (-0.06, 0.02)
1	-0.03 (-0.06, 0.01)	-0.02 (-0.05, 0.03)	0.003 (-0.04, 0.04)	-0.02 (-0.07, 0.02)	0.002 (-0.04, 0.05)	0.03 (-0.03, 0.08)	-0.01 (-0.04, 0.03)
2	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)	0.01 (-0.03, 0.05)	-0.02 (-0.07, 0.03)	-0.003 (-0.05, 0.05)	0.01 (-0.05, 0.07)	-0.004 (-0.04, 0.04)
3	-0.01 (-0.05, 0.02)	-0.01 (-0.05, 0.04)	0.01 (-0.03, 0.06)	-0.02 (-0.07, 0.03)	-0.004 (-0.05, 0.04)	0.01 (-0.05, 0.07)	-0.01 (-0.05, 0.03)

[1] Standardized betas (β) were expressed as 1 SD change in the outcome variables per 1 SD higher dietary dicarbonyl. All variables were standardized for comparison. Model 1: adjusted for age + sex + glucose metabolism status. Model 2: model 1 + BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level. Model 3: model 2 + triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. The endothelial function population consisted of 2745 individuals, the CRAE/CRVE population consisted of 2438 individuals, the retinal dilation population consisted of 2078 individuals, the heat-induced skin hyperemia population consisted of 1357 individuals, the urinary albumin excretion population consisted of 2774 individuals. [2] Composite score of plasma biomarkers of endothelial function, consisted of the biomarkers sVCAM-1, sICAM-1, sE-selectin and vWF. [3] Baseline retinal venular diameter before flicker light was associated with dietary MGO intake (see supplementary Table S4.1), thus absolute change of retinal venular dilation over baseline (delta) was used as outcome in the association between MGO intake and retinal venular dilation, and percentage change over baseline was used as outcome for the other associations. [4] Baseline skin hyperemia was significantly associated with dietary 3-DG (see supplementary Table S4.1), so for the association between dietary 3-DG and skin hyperemia the average total heating response in the arm (in PU, in the interval 2-25 minutes) was used as outcome measure with additional adjustment for baseline skin hyperemia in all models, instead of the percentage change. [5] Urinary albumin excretion was ln-transformed. Higher urinary albumin excretion indicates worse microvascular function. 3-DG, 3-deoxyglucosone, CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, GO, glyoxal, MGO, methylglyoxal.

Interaction analyses

Glucose metabolism status modified the associations of dietary GO with retinal venular diameter, with retinal arteriolar dilation, and with urinary albumin excretion (P-interaction 0.02-0.07). Stratified analyses revealed a significant association between higher GO intake and impaired retinal arteriolar dilation in individuals with type 2 diabetes (normal glucose metabolism: std. β=-0.06 (-0.14, 0.03), prediabetes: 0.02 (-0.16, 0.20), and type 2 diabetes: -0.13 (-0.26, -0.01), Supplementary Table S4.7). Similarly, higher GO intake was associated with lower urinary albumin excretion in individuals with type 2 diabetes only (normal glucose metabolism: std. β=0.04 (-0.03, 0.10), prediabetes: -0.07 (-0.21, 0.08), and type 2 diabetes: -0.13 (-0.26, -0.01)).

Additionally, glucose metabolism status modified the associations of dietary 3-DG with retinal venular diameter, with retinal venular dilation, with skin hyperemia, and with urinary albumin excretion (P-interaction 0.01-0.09). Stratified analyses revealed no significant associations in any of the glucose metabolism groups (Supplementary Table S4.7).

Sex modified the association between dietary GO and retinal arteriolar dilation (P-interaction=0.06), but stratified analyses revealed no significant association for men or women (data not shown). eGFR did not modify any of the associations.

Additional analyses

We explored the contribution of several main food groups (≥5% of daily dicarbonyl intake) to the strength of the observed associations. The strength of the above-reported association between dietary MGO intake and low-grade inflammation remained similar after adjustment for MGO intake from any individual food group (Table 4.4). The above-reported association between dietary MGO intake and retinal venular dilation (std. β=-0.07 (-0.12, -0.01)) was attenuated and lost statistical significance after adjustment for MGO intake from coffee (std. β=-0.04 (-0.12, 0.05), Table 4.4).

In our sensitivity analyses, the observed association between dietary MGO and biomarkers of low-grade inflammation remained of similar strength after the majority of sensitivity analyses. However, statistical significance was lost when we adjusted for physical activity using ActivPAL data instead of the CHAMPS questionnaire, when we excluded individuals with gastrointestinal tract infection, or when we excluded individuals with previous diagnosed type 2 diabetes, most likely due to limited power (Supplementary Table S4.8). The observed association between dietary MGO and retinal venular dilation remained of similar strength, but

was no longer statistically significant after excluding individuals with previously diagnosed type 2 diabetes or when we adjusted for 24-hour ambulatory blood pressure instead of office blood pressure, again probably because of limited power (Supplementary Table S4.9).

Table 4.4 Association of dietary MGO with low-grade inflammation and retinal venular dilation additionally adjusted for main food groups.[1]

			Biomarkers of low-grade inflammation (composite score) [2]	Retinal venular dilation (delta)
Dietary MGO	**Model**	**% of MGO intake [3]**	**Std. β (95% CI)**	**Std. β (95% CI)**
	Fully adjusted model (model 3) [4]		-0.05 (-0.10, -0.01)	-0.07 (-0.12, -0.01)
	Model 3 + Coffee	26	-0.05 (-0.12, 0.01)	-0.04 (-0.12, 0.05)
	Model 3 + Bread	23	-0.05 (-0.09, -0.01)	-0.07 (-0.12, -0.01)
	Model 3 + Vegetables and legumes	10	-0.05 (-0.09, -0.004)	-0.07 (-0.13, -0.02)
	Model 3 + Meat	9	-0.05 (-0.09, -0.01)	-0.07 (-0.12, -0.01)
	Model 3 + Cookies and bakery products	8	-0.04 (-0.09, 0.003)	-0.07 (-0.13, -0.01)
	Model 3 + Ready-made	5	-0.05 (-0.09, -0.01)	-0.07 (-0.12, -0.01)

[1] Standardized betas (β) were expressed as 1 SD change in composite score of low-grade inflammation or in 1 SD change in flicker light-induced retinal venular dilation (delta) per 1 SD higher dietary MGO intake. All variables were standardized for comparison. [2] Composite score of low-grade inflammation consisted of the biomarkers hsCRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α. [3] % of MGO intake via each food group (as reported in [4]). [4] Fully adjusted model (model 3): adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. Additionally, sequentially adjusted for MGO intake via each of the separate food groups that contributed ≥5% to total MGO intake. MGO, methylglyoxal.

Discussion

In this population-based cohort, we observed that higher habitual intake of MGO was associated with lower low-grade inflammation scores, but not consistently with features of microvascular function. GO or 3-DG intakes were not associated with low-grade inflammation or microvascular function.

The association between higher MGO intake and lower low-grade inflammation scores was somewhat unexpected, considering that animal studies report that high amounts of pure oral MGO increases vascular inflammation[8,10]. Nevertheless, our results were corroborated by the observation of a similar inverse, although not significant, association between MGO intake and inflammation in the independent CODAM cohort. A possible explanation for this association between higher MGO intake and a lower degree of inflammation, can be potential anti-oxidative effects of MGO, which were reported both *in vitro*[14,16,37] and in an animal study[11]. These anti-

oxidative effects are thought to occur via an MGO-induced defense mechanism involving the KEAP1-Nrf2 pathway[14,16,37]. The transcription factor Nrf2 is a key regulator in oxidative stress, increasing the production of specific antioxidant enzymes and glyoxalase-1, both involved in cellular protection against glycation. Thus, small increases in plasma MGO after exogenous exposure to food-derived MGO may induce the defense system against glycation and thereby prevent protein damage caused by glycation. Another mechanism behind these anti-oxidative effects could be via formation of the methylglyoxal-derived hydroimidazolone MG-H3[38]. MG-H3 is one of the adducts that is formed after modification of the amino acid arginine by MGO, and was found to possess antioxidant properties *in-vitro*[39].

In the current study, MGO intake was not significantly associated with most microvascular features in the retina, skin, plasma, and kidney. We only found that higher MGO intake was associated with impaired retinal venular (but not arteriolar) dilation, suggesting a detrimental effect of MGO intake on retinal venular function. Venular dilation after flicker-light stimulation is an endothelium-dependent response, and this observation hence points towards dysfunction of retinal venular endothelium[21,25]. MGO might contribute to impaired vasodilation via dysregulation of endothelial NO synthase (eNOS), leading to reduced nitric oxide (NO) production and thus reduced vasoreactivity[1,40-42]. In accordance with this, studies in animals have shown that exogenous administration of MGO induces vascular changes including impaired vasodilation[8,43], reduced NO bioavailability, and endothelial dysfunction[8]. Moreover, oral MGO administration induced retinopathy-like changes, such as pericyte loss, formation of acellular capillaries, microglial activation, and early neuronal dysfunction[44]. A possible explanation why we only observed an impairment of retinal venular dilation and not of other microvascular features is that retinal vessel dilation is a sensitive marker of generalized microvascular function[45,46], and the association with microvascular function might only be detected for this outcome. However, we cannot exclude the possibility that the association between higher MGO intake and impaired retinal venular dilation is a chance finding due to multiple testing. Therefore, the association between retinal venular dilation has to be interpreted with caution and this observation requires confirmation in other studies.

Some associations were modified by glucose metabolism status, but after stratification for glucose metabolism group, there was no clear pattern, so this may be a chance finding.

Strengths of this study include the availability of a large and deep-phenotyped human cohort with validated, state-of-the-art techniques to assess inflammation and microvascular function in various organs, and extensive assessment of potential confounders. Another strength is the application of the most extensive food composition database for dicarbonyls currently available world-wide, in which the highly-sensitive UPLC-MS/MS was used for quantification of MGO, GO and 3DG, and which was specifically developed to match the comprehensive 253-item FFQ used in this cohort. Moreover, we were able to verify the association between dietary dicarbonyl intake and low-grade inflammation in an independent cohort.

This study also has certain limitations. Food items that are high in MGO may also contain other anti-inflammatory or anti-oxidant components, for example caffeine in coffee[47]. Because coffee was the main contributor to MGO intake[4], we cannot exclude that the presence of caffeine or other anti-inflammatory components from coffee, overruled the potential adverse effects of MGO. Nevertheless, additional adjustment for MGO intake from coffee did not change the strength of the association between MGO intake and inflammation. Also, the food composition database contains average values of dicarbonyls in foods and is, therefore, unable to take effects of industrial and household food preparation on dicarbonyl intake into account. Nevertheless, both our dicarbonyl database and the FFQ contain foods prepared using various common preparation methods[19]. Another limitation is the cross-sectional design of the study, which does not allow the assessment of causality. In addition, although we carefully adjusted for a large set of potential confounders, residual confounding remains a possibility.

In conclusion, higher habitual intake of MGO was associated with a lower degree of low-grade inflammation, but was not consistently associated with microvascular function. This suggests that food–derived MGO may induce anti-inflammatory effects. This beneficial association requires further investigation.

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Supplementary tables

Table S4.1 Associations of dietary dicarbonyl intakes with baseline retinal vessel diameters and with baseline skin blood flow.[1]

	Retinal arteriolar baseline diameter (MU) Std. β (95% CI)	Retinal venular baseline diameter (MU) Std. β (95% CI)	Skin baseline blood flow (PU) Std. β (95% CI)
Dietary MGO			
Crude	0.02 (-0.02, 0.06)	0.05 (0.01, 0.09)	0.001 (-0.05, 0.05)
1	0.03 (-0.02, 0.07)	0.05 (0.001, 0.09)	-0.001 (-0.05, 0.05)
2	0.02 (-0.04, 0.07)	0.06 (0.01, 0.12)	-0.02 (-0.08, 0.05)
3	0.01 (-0.04, 0.07)	0.06 (0.01, 0.12)	-0.02 (-0.08, 0.05)
Dietary GO			
Crude	0.02 (-0.02, 0.06)	-0.006 (-0.05, 0.04)	-0.01 (-0.06, 0.05)
1	0.03 (-0.02, 0.07)	-0.01 (-0.05, 0.04)	-0.01 (-0.06, 0.04)
2	0.03 (-0.04, 0.10)	0.01 (-0.06, 0.07)	-0.05 (-0.14, 0.03)
3	0.02 (-0.04, 0.09)	0.01 (-0.06, 0.08)	-0.06 (-0.14, 0.03)
Dietary 3-DG			
Crude	0.002 (-0.04, 0.05)	-0.03 (-0.07, 0.02)	-0.04 (-0.09, 0.01)
1	0.01 (-0.04, 0.05)	-0.03 (-0.07, 0.02)	-0.05 (-0.10, 0.01)
2	0.01 (-0.04, 0.06)	-0.01 (-0.06, 0.04)	-0.07 (-0.13, -0.01)
3	0.02 (-0.03, 0.07)	-0.01 (-0.06, 0.04)	-0.07 (-0.12, -0.01)

[1] Standardized betas (β) were expressed as 1 SD change in outcome variable per 1 SD higher dietary dicarbonyl intake. These analyses were not of primary interest, but were needed to determine what outcome variable to use for retina and skin analyses. Model 1: adjusted for age + sex + glucose metabolism status. Model 2: model 1 + BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level. Model 3: model 2 + triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. 3-DG, 3-deoxyglucosone, GO, glyoxal, MGO, methylglyoxal, MU, measurement units, PU, perfusion units.

Table S4.2 Comparison of population characteristics of the various subpopulations.[1]

Characteristics	Total included population in main analyses (n=2792)	Subpopulations							
		Excluded population (n=659)	Low-grade inflammation population (n=2765)	Endothelial function population (n=2745)	Retinal vessel diameter population (n=2438)	Retinal vessel dilation population (n=2078)	Skin hyperemia population (n=1357)	Urinary albumin excretion population (n=2774)	$P_{excluded}$
Demographics									
Age (years)	60 ± 8	60 ± 9	60 ± 8	60 ± 8	60 ± 8	60 ± 8	60 ± 8	60 ± 8	0.63
Sex (% male)	50	57	50	50	50	51	51	50	0.001
Glucose metabolism status									<0.001
Normal glucose metabolism (%)	58	47	58	58	58	59	56	58	
Prediabetes (%)	15	14	15	15	15	15	16	15	
Type 2 diabetes mellitus (%)	26	37	26	26	27	25	27	26	
Other types of diabetes (%)	1	2	1	1	1	1	1	1	
Diabetes duration (years)	4 [1-11]	6 [2-11]	4 [1-11]	4 [1-11]	4 [1-11]	4 [1-10]	4 [1-11]	4 [1-11]	0.05
Lifestyle									
Smoking (%)									<0.001
Never	36	28	36	36	36	36	34	36	
Former	52	50	52	52	52	52	55	52	
Current	12	22	12	12	12	11	11	12	
Waist circumference (cm)	95 ± 14	98 ± 14	95 ± 14	95 ± 14	95 ± 14	95 ± 14	96 ± 13	95 ± 14	<0.001
BMI (kg/m^2)	27 ± 5	28 ± 5	27 ± 5	27 ± 5	27 ± 5	27 ± 4	27 ± 4	27 ± 5	0.01
Physical activity (h/week)	13 [8.3-19]	13 [7.8-18]	13 [8.3-19]	13 [8.3-19]	13 [8-18]	13 [8-19]	13 [8.3-18]	13 [8.3-19]	0.55
Education (%)									<0.001
Low	32	41	32	32	32	31	32	32	
Medium	29	27	29	29	29	29	28	29	
High	39	23	40	40	39	40	40	39	

Table S4.2 (continued)

Characteristics	Total included population in main analyses (n=2792)	Subpopulations							$P_{excluded}$
		Excluded population (n=659)	Low-grade inflammation population (n=2765)	Endothelial function population (n=2745)	Retinal vessel diameter population (n=2438)	Retinal vessel dilation population (n=2078)	Skin hyperemia population (n=1357)	Urinary albumin excretion population (n=2774)	
Biological									
Fasting glucose (mmol/L)	5.5 [5.1-6.4]	5.8 [5.2-7.2]	5.5 [5.1-6.4]	5.5 [5.1-6.4]	5.5 [5.1-6.4]	5.5 [5.0-6.4]	5.5 [5.0-6.5]	5.5 [5.1-6.4]	<0.001
HbA1c, %	5.6 [5.4-6.2]	5.8 [5.4-6.5]	5.6 [5.3-6.1]	5.6 [5.4-6.1]	5.6 [5.4-6.2]	5.6 [5.4-6.1]	5.7 [5.4-6.3]	5.6 [5.4-6.2]	<0.001
24-h Systolic blood pressure (mmHg)	135 ± 18	136 ± 19	135 ± 18	135 ± 18	135 ± 18	135 ± 18	136 ± 18	135 ± 18	0.15
24-h Diastolic blood pressure (mmHg)	76 ± 10	76 ± 10	76 ± 10	76 ± 10	76 ± 10	76 ± 10	76 ± 9.6	76 ± 10	0.90
HOMA-IR	1.4 [1.0-2.1]	1.5 [1.0-2.2]	1.4 [1.0-2.1]	1.4 [1.0-2.1]	1.4 [0.97-2.11]	1.4 [1.0-2.1]	1.4 [0.94-2.1]	1.4 [1.0-2.1]	0.01
Cholesterol (mmol/L)	5.3 ± 1.2	5.1 ± 1.2	5.2 ± 1.2	5.2 ± 1.2	5.2 ±1.2	5.3 ± 1.2	5.3 ± 1.2	5.3 ± 1.2	0.83
Total-to-HDL cholesterol ratio	3.6 ± 1.2	3.8 ± 1.2	3.6 ± 1.2	3.6 ± 1.2	3.6 ± 1.1	3.6 ± 1.2	3.6 ± 1.1	3.6 ± 1.2	0.02
Triglycerides (mmol/L)	1.2 [0.87-1.7]	1.2 [0.90-1.8]	1.2 [0.88-1.7]	1.2 [0.89-1.7]	1.2 [0.87-1.7]	1.2 [0.87-1.7]	1.2 [0.88-1.7]	1.2 [0.88-1.7]	0.23
eGFR (mL/min/1.73 m^2)	88 ± 15	88 ± 16	88 ± 15	88 ± 15	88 ± 15	88 ± 14	88±15	88 ± 15	0.92
Medical history of CVD (% yes)	16	20	16	16	16	15	17	16	0.03
Medical history of gastrointestinal disease (%yes)	12	14	11	11	12	12	12	12	0.49
Retinopathy (%)	2	3	1	2	2	2	2	2	0.77
(Micro)albuminuria (%)	8	12	8	8	8	8	8	8	0.001

Table S4.2 (continued)

Characteristics	Total included population in main analyses (n=2792)	Subpopulations							
		Excluded population (n=659)	Low-grade inflammation population (n=2765)	Endothelial function population (n=2745)	Retinal vessel diameter population (n=2438)	Retinal vessel dilation population (n=2078)	Skin hyperemia population (n=1357)	Urinary albumin excretion population (n=2774)	$P_{excluded}$
Medication use									
Glucose-lowering medication (%yes)	21	33	21	21	22	20	22	21	<0.001
Anti-hypertensives (%yes)	39	45	39	39	40	37	41	39	0.01
Lipid-modifying medication (%yes)	35	41	36	36	36	34	38	36	0.01
Dietary intake									
Energy intake (kcal/day)	2184 ± 602	2165 ± 637	2184 ± 599	2183 ± 599	2180 ± 605	2177 ± 596	2179 ± 585	2184 ± 601	0.56
Carbohydrate, total (g/day)	233 ± 70	231 ± 76	233 ± 70	233 ± 70	234 ± 70	233 ± 69	232 ± 68	233 ± 70	0.52
Fat, total (g/day)	84 ± 31	85 ± 31	84 ± 31	84 ± 31	84 ± 31	84 ± 31	84 ± 30	84 ± 31	0.84
Protein (g/day)	86 ± 23	84 ± 24	86 ± 23	86 ± 23	86±23	86 ± 23	86 ± 22	86 ± 23	0.14
Fiber (g/day)	27 ± 8.1	26 ± 8.5	27 ± 8.1	27 ± 8.1	27±8.1	27 ± 8.0	27 ± 7.8	27 ± 8.1	0.10
Alcohol intake (g/day)	8.5 [1.5-19]	6.7 [0.79-19]	8.6 [1.6-19]	8.6 [1.5-19]	8.6 [1.6-19]	8.7 [1.6-19]	8.8 [1.5-20]	8.6 [1.6-19]	0.07
Dietary MGO (mg/day)	4.1 ± 1.2	4.0 ± 1.3	4.1 ± 1.2	4.1 ± 1.2	4.1±1.2	4.1 ± 1.2	4.1 ± 1.2	4.1 ± 1.2	0.40
Dietary GO (mg/day)	3.7 ± 1.1	3.6 ± 1.2	3.7 ± 1.1	3.7 ± 1.1	3.7±1.1	3.7 ± 1.1	3.7 ± 1.1	3.7 ± 1.1	0.19
Dietary 3-DG (mg/day)	17 [12-23]	16 [11-23]	17 [12-23]	17 [12-23]	17 [11-23]	17 [12-23]	17 [12-23]	17 [12-23]	0.16
Dutch Healthy Diet Index	83 ± 15	82 ±14	83 ± 15	83 ± 15	84±15	84 ± 15	83±15	83 ± 15	0.04

Table S4.2 (continued)

Characteristics	Total included population in main analyses (n=2792)	Subpopulations							
		Excluded population (n=659)	Low-grade inflammation population (n=2765)	Endothelial function population (n=2745)	Retinal vessel diameter population (n=2438)	Retinal vessel dilation population (n=2078)	Skin hyperemia population (n=1357)	Urinary albumin excretion population (n=2774)	$P_{excluded}$
Plasma biomarkers of inflammation									
hsCRP (µg/ml)	1.2 [0.61-2.7]	1.4 [0.65-3.1]	1.2 [0.61-2.7]	1.2 [0.61-2.7]	1.2 [0.60-2.6]	1.2 [0.61-2.7]	1.2 [0.63-2.7]	1.2 [0.61-2.7]	0.02
SAA (µg/ml)	3.3 [2.1-5.4]	3.3 [2.0-5.6]	3.3 [2.1-5.4]	3.3 [2.1-5.4]	3.3 [2.1-5.4]	3.3 [2.1- 5.4]	3.3 [2.2-5.5]	3.3 [2.1-5.4]	0.85
sICAM1 (ng/ml)	338 [291-398]	345 [296-415]	338 [290-398]	338 [291-398]	339 [293-399]	338 [291- 398]	344 [297-401]	338 [290-398]	0.01
IL-6 (pg/ml)	0.58 [0.39-0.88]	0.66 [0.43-1.0]	0.58 [0.39-0.88]	0.58 [0.39-0.88]	0.58 [0.39-0.88]	0.57 [0.38-0.87]	0.58 [0.39-0.86]	0.58 [0.39-0.88]	<0.001
IL-8 (pg/ml)	4.1 [3.3-5.3]	4.3 [3.5-5.6]	4.1 [3.3-5.3]	4.1 [3.3-5.2]	4.1 [3.2-5.3]	4.0 [3.2-5.2]	4.1 [3.3-5.2]	4.1 [3.3-5.3]	<0.001
TNF-α (pg/ml)	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.6]	2.2 [1.9-2.5]	2.2 [1.9-2.6]	0.02
Microvascular measurements									
Plasma biomarkers of endothelial function									
sICAM-1 (ng/ml)	338 [291-398]	345 [296-415]	338 [290-398]	338 [290-398]	339 [293-399]	338 [291- 398]	344 [297-401]	338 [290-398]	0.01
sVCAM-1 (ng/ml)	427 ± 101	433 ±105	428 ± 101	429 ± 101	428 ± 100	428 ± 103	435 ± 101	427 ± 101	0.21
sE-selectin (ng/ml)	107 [75-143]	112 [78-151]	107 [75-143]	107 [75-143]	108 [75-144]	107 [75-142]	107 [75-143]	107 [75-143]	0.01
vWF (%)	132 ± 48	136 ± 51	132 ± 48	132 ± 48	132 ± 48	124 [99-157]	132 ± 47	132 ± 48	0.07
Retinal microvascular measurements									
CRAE (µm)	142 ± 20	142 ± 23	142 ± 19	142 ± 20	142 ± 20	143 ± 19	143 ± 20	142 ± 20	0.62
CRVE (µm)	214 ± 31	215 ± 33	214 ± 31	214 ± 31	214 ± 31	215 ± 31	215 ± 32	214 ± 31	0.94
Baseline arteriolar diameter (MU)	115 ± 15	117 ±16	115 ± 16	115 ± 15	115 ± 16	115 ± 15	116 ± 16	115 ± 15	0.02
Baseline venular diameter (MU)	146 ± 21	148 ± 21	147 ± 21	147 ± 21	147 ± 20	147 ± 21	147 ± 21	146 ± 21	0.09
Flicker light-induced arteriolar dilation response (%)	3.0 ± 2.8	2.7 ± 2.9	3.1 ± 2.8	3.1 ± 2.8	3.1 ± 2.8	3.0 ± 2.8	2.9 ± 2.8	3.1 ± 2.8	0.01

Table S4.2 (continued)

Characteristics	Total included population in main analyses (n=2792)	Subpopulations							
		Excluded population (n=659)	Low-grade inflammation population (n=2765)	Endothelial function population (n=2745)	Retinal vessel diameter population (n=2438)	Retinal vessel dilation population (n=2078)	Skin hyperemia population (n=1357)	Urinary albumin excretion population (n=2774)	$P_{excluded}$
Flicker light-induced venular dilation response (%)	3.9 ± 2.2	3.7 ± 2.2	3.9 ± 2.2	3.9 ± 2.2	3.9 ± 2.2	3.9 ± 2.2	3.8 ± 2.1	3.9 ± 2.2	0.02
Absolute arteriolar dilation response (delta)	4.4 ± 3.6	4.0 ± 3.7	4.4 ± 3.6	4.4 ± 3.6	4.4 ± 3.5	4.4 ± 3.6	4.2 ± 3.5	4.4 ± 3.6	0.03
Absolute venular dilation response (delta)	7.7 ± 4.1	7.4 ± 4.1	7.7 ± 4.1	7.7 ± 4.1	7.7 ± 4.1	7.6 ± 4.1	7.4 ± 3.9	7.7 ± 4.1	0.30
Skin microvascular measurements									
Baseline skin blood flow before heating (PU)	11 ± 6.6	11 ± 5.7	11 ± 6.5	11 ± 6.6	11 ± 6.6	11 ± 6.2	11 ± 6.6	11 ± 6.5	0.32
Skin hyperemia during heating (PU)	113 ± 58	103 ± 54	113 ± 58	113 ± 58	113 ± 59	114 ± 59	113 ± 58	113 ± 57	0.08
Skin hyperemic response (%)	1129 ± 773	1083 ± 750	1128 ± 770	1127 ± 771	1121 ± 781	1142 ± 774	1129 ± 773	1129 ± 772	0.33
Kidney microvascular measurement									
Urinary albumin excretion (mg/24h)	6.5 [4.0-12]	7.6 [4.3-14]	6.5 [4.0-12]	6.5 [4.0-12]	6.5 [3.9-12]	6.4 [3.9-11]	6.7 [4.1-12]	6.5 [4.0-12]	0.001

[1] Data are presented as mean ± standard deviation, median [interquartile range] or percentage. P-value of ANOVA (for normally distributed continuous variables), Mann-Whitney U Test (for non-normally distributed continuous variables), or Chi Square test (for categorical variables) for differences between included and excluded individuals. 3-DG: 3-deoxyglucosone, CRAE: central retinal arteriolar equivalent, hsCRP: high sensitivity C-reactive protein, CRVE: central retinal venular equivalent, CVD: cardiovascular diseases, eGFR: estimated glomerular filtration rate, sE-selectin: soluble E-selectin, GO: glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; sICAM-1: soluble intracellular adhesion molecule-1, IL-6: interleukin-6, IL-8: interleukin-8, MGO: methylglyoxal, NGM: normal glucose metabolism, SAA: serum amyloid A, T2DM: type 2 diabetes mellitus, TNF-α: tumor necrosis factor alpha, sVCAM-1: soluble vascular adhesion molecule-1, vWF: von Willebrand factor.

Table S4.3 Association between dietary MGO, GO, and 3-DG intakes and individual plasma biomarkers of low-grade inflammation.[1]

	hsCRP	SAA	sICAM	IL6	IL-8	TNF-α
	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)
Dietary MGO	-0.05 (-0.10, -0.01)	-0.03 (-0.08, 0.01)	0.004 (-0.04, 0.05)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.02)	-0.05 (-0.10, -0.01)
Dietary GO	-0.04 (-0.09, 0.02)	0.002 (-0.05, 0.06)	0.02 (-0.04, 0.07)	0.02 (-0.03, 0.07)	-0.05 (-0.11, 0.004)	-0.01 (-0.06, 0.05)
Dietary 3-DG	-0.03 (-0.07, 0.003)	0.01 (-0.03, 0.05)	-0.003 (-0.04, 0.04)	-0.03 (-0.07, 0.004)	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)

[1] All biomarkers were ln-transformed. All biomarkers and dietary dicarbonyls were standardized. Results are for fully adjusted model (model 3), adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. N=2765. 3-DG, 3-deoxyglucosone, hsCRP: high sensitivity C-reactive protein, GO, glyoxal, sICAM-1: soluble intracellular adhesion molecule-1, IL-6: interleukin-6, IL-8: interleukin-8, MGO, methylglyoxal, SAA: serum amyloid A, TNF-α: tumor necrosis factor alpha, sVCAM-1: soluble vascular adhesion molecule-1.

Table S4.4 Association between dietary MGO, GO, and 3-DG intakes and low-grade inflammation in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM).[1]

	Model	Biomarkers of low-grade inflammation (composite score)[2]	
		Std. β	95% CI
Dietary MGO			
	Crude	-0.06	-0.15, 0.03
	1	0.01	-0.08, 0.09
	2	-0.05	-0.16, 0.05
	3	-0.05	-0.16, 0.05
Dietary GO			
	Crude	-0.07	-0.16, 0.02
	1	-0.03	-0.11, 0.06
	2	-0.08	-0.21, 0.05
	3	-0.09	-0.22, 0.04
Dietary 3-DG			
	Crude	-0.03	-0.12, 0.06
	1	0.01	-0.08, 0.10
	2	-0.004	-0.11, 0.10
	3	-0.01	-0.11, 0.09

[1] Standardized betas (β) represent 1 SD change in composite score of low-grade inflammation per 1 SD higher dietary dicarbonyl intake. Model 1: adjusted for age + sex + glucose metabolism status. Model 2: model 1 + BMI, total energy intake, smoking status, alcohol intake, physical activity. Model 3: model 2 + triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. N=515. [2] Composite score of low-grade inflammation consisted of the biomarkers CRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α. 3-DG: 3-deoxyglucosone, GO: glyoxal, MGO: methylglyoxal

Table S4.5 Association between dietary MGO, GO, and 3-DG intakes and individual plasma biomarkers of low-grade inflammation in CODAM.[1]

	hsCRP	SAA	sICAM	IL6	IL-8	TNF-α
	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)
Dietary MGO	-0.08 (-0.19, 0.03)	-0.03 (-0.14, 0.08)	-0.03 (-0.14, 0.07)	-0.01 (-0.12, 0.09)	-0.03 (-0.14, 0.08)	-0.01 (-0.12, 0.11)
Dietary GO	-0.18 (-0.31, -0.05)	-0.04 (-0.18, 0.09)	-0.04 (-0.17, 0.08)	-0.11 (-0.24, 0.03)	0.07 (-0.07, 0.21)	-0.02 (-0.16, 0.13)
Dietary 3-DG	-0.07 (-0.17, 0.04)	-0.03 (-0.14, 0.07)	0.02 (-0.08, 0.13)	-0.02 (-0.13, 0.09)	0.06 (-0.05, 0.17)	0.01 (-0.11, 0.12)

[1] Standardized betas (β) represent 1 SD change in biomarker of low-grade inflammation per 1 SD higher dietary dicarbonyl intake. All biomarkers were ln-transformed. Results are for fully adjusted model (model 3), adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. N=515. 3-DG: 3-deoxyglucosone, CODAM: Cohort on Diabetes and Atherosclerosis Maastricht, hsCRP: high sensitivity C-reactive protein, GO, glyoxal, sICAM-1: soluble intracellular adhesion molecule-1, IL-6: interleukin-6, IL-8: interleukin-8, MGO, methylglyoxal, SAA: serum amyloid A, TNF-α: tumor necrosis factor alpha, sVCAM-1: soluble vascular adhesion molecule-1.

Table S4.6 Association between dietary MGO, GO, and 3-DG intakes and individual plasma biomarkers of endothelial function.[1]

	sICAM	sVCAM	sE-selectin	vWF
	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)	Std. β (95% CI)
Dietary MGO	0.003 (-0.04, 0.05)	-0.02 (-0.07, 0.02)	0.002 (-0.04, 0.05)	-0.02 (-0.06, 0.03)
Dietary GO	0.01 (-0.04, 0.07)	0.02 (-0.04, 0.08)	0.01 (-0.05, 0.06)	0.03 (-0.02, 0.09)
Dietary 3-DG	-0.01 (-0.05, 0.03)	0.001 (-0.04, 0.04)	-0.01 (-0.05, 0.03)	-0.02 (-0.06, 0.02)

[1] Standardized betas (β) represent 1 SD change in biomarkers of endothelial function per 1 SD higher dietary dicarbonyl intake. sICAM and E-selectin were ln-transformed. Results are for fully adjusted model (model 3), adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. N=2745. 3-DG, 3-deoxyglucosone, GO, glyoxal, MGO, methylglyoxal, sE-selectin, soluble e-selectin, sICAM-1: soluble intracellular adhesion molecule-1, sVCAM-1: soluble vascular adhesion molecule-1, vWF: von Willebrand factor.

Table S4.7 Associations for which we observed an interaction stratified for glucose metabolism status.[1]

	Normal glucose metabolism	Prediabetes	Type 2 diabetes
	Std. β (95% CI)	**Std. β (95% CI)**	**Std. β (95% CI)**
Dietary GO with retinal venular diameter	-0.01 (-0.09, 0.07)	-0.03 (-0.20, 0.14)	0.08 (-0.04, 0.21)
Dietary GO with retinal arteriolar dilation	-0.06 (-0.14, 0.03)	0.02 (-0.16, 0.20)	-0.13 (-0.26, -0.01)
Dietary GO with urinary albumin excretion	0.04 (-0.03, 0.10)	-0.07 (-0.21, 0.08)	-0.13 (-0.26, -0.01)
Dietary 3-DG with retinal venular diameter	0.02 (-0.03, 0.08)	-0.03 (-0.18, 0.13)	-0.10 (-0.22, 0.02)
Dietary 3-DG with retinal venular dilation	0.02 (-0.03, 0.08)	-0.03 (-0.18, 0.13)	-0.10 (-0.22, 0.02)
Dietary 3-DG with skin hyperemia [2]	-0.02 (-0.10, 0.05)	0.13 (-0.02, 0.28)	0.04 (-0.06, 0.14)
Dietary 3-DG with urinary albumin excretion [3]	0.01 (-0.03, 0.05)	-0.01 (-0.12, 0.11)	-0.10 (-0.21, 0.01)

[1] Standardized betas (β) represent 1 SD change in outcome per 1 SD higher dietary dicarbonyl intake. Results are for fully adjusted model (model 3): adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. Individuals with other types of diabetes were excluded in these analyses because of the small sample size (n=23). Sample sizes differ per outcome: For retinal venular diameter: normal glucose metabolism n=1401, prediabetes n=366, type 2 diabetes n=648. For retinal arteriolar and venular dilation: normal glucose metabolism n=1220, prediabetes n=319, type 2 diabetes n=520. For skin hyperemia: normal glucose metabolism n=756, prediabetes n=211, type 2 diabetes n=371. For urinary albumin excretion: normal glucose metabolism n=1604, prediabetes n=418, type 2 diabetes n=725. [2] Skin hyperemia was assessed as absolute average skin heating response, adjusting for baseline blood flow in all models. [3] Urinary albumin excretion was ln-transformed. 3-DG, 3-deoxyglucosone, GO, glyoxal, MGO, methylglyoxal.

Table S4.8 Sensitivity analyses for the associations between dietary MGO and plasma biomarkers of low-grade inflammation.[1]

		Outcome: biomarkers of low-grade inflammation (composite score) [2]	
Dietary MGO	**N**	**Std. β**	**95% CI**
Model 3 [3]	**2765**	**-0.05**	**-0.09, -0.01**
Kcal + DHD15	2765	-0.04	-0.08, -0.003
Kcal + carbohydrates	2765	-0.06	-0.10, -0.02
Kcal + protein	2765	-0.04	-0.08, -0.002
Kcal + fat	2765	-0.05	-0.10, -0.01
+ eGFR	2755	-0.04	-0.08, -0.001
+ retinopathy	2574	-0.05	-0.09, -0.004
+ urinary albumin excretion	2748	-0.05	-0.09, -0.01
+ history of CVD	2750	-0.05	-0.09, -0.01
Exclusion other type of diabetes	2738	-0.05	-0.09, -0.01
Exclusion CRP above 10	2646	-0.06	-0.11, -0.02
Exclusion GI disease	2464	-0.04	-0.08, 0.01
Exclusion previously diagnosed diabetes	2153	-0.04	-0.09, 0.01
HT med. > individual HT med.	2765	-0.05	-0.10, -0.01
GMS > HbA1c (%) [4]	2758	-0.05	-0.09, -0.01
GMS > Fasting glucose	2763	-0.06	-0.10, -0.02
GMS > Post-load glucose	2566	-0.06	-0.10, -0.01
OSBP > 24h BP	2395	-0.06	-0.10, -0.01
Education > income	2273	-0.06	-0.11, -0.02
Education > occupational status	2335	-0.05	-0.09, -0.001
CHAMPS > ActivPAL	2415	-0.04	-0.08, 0.004
BMI > waist	2764	-0.04	-0.09, -0.004

[1] Standardized betas (β) represent 1 SD change in composite score of low-grade inflammation per 1 SD higher dietary MGO intake. [2] Composite score of low-grade inflammation consisted of the biomarkers CRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α. [3] Model 3: adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. [4] '>' indicates that first covariate is replaced by the second, so in this case the covariate GMS is replaced by HbA1c. BP: blood pressure, CVD: cardiovascular disease, CRP: C-reactive protein, DHD15: Dutch Healthy Diet Index 2015, eGFR: estimated glomerular filtration rate, GI: gastrointestinal, GMS: glucose metabolism status, HT med.: anti-hypertensive medication, MGO: methylglyoxal, OSBP: office systolic blood pressure.

Table S4.9 Sensitivity analyses for the associations between dietary MGO and flicker light-induced retinal venular dilation.[1]

		Outcome: Retinal venular dilation (delta change)	
Dietary MGO	**N**	**Std. β**	**95% CI**
Model 3 [2]	**2078**	**-0.07**	**-0.13, -0.01**
Kcal + DHD15	2078	-0.07	-0.12, -0.01
Kcal + carbohydrates	2078	-0.07	-0.13, -0.01
Kcal + protein	2078	-0.06	-0.12, -0.01
Kcal + fat	2078	-0.07	-0.13, -0.01
+ eGFR	2062	-0.07	-0.12, -0.01
+ retinopathy	2031	-0.07	-0.12, -0.01
+ urinary albumin excretion	2064	-0.07	-0.13, -0.02
+ history of CVD	2066	-0.07	-0.12, -0.01
Exclusion other type of diabetes	2059	-0.06	-0.12, -0.01
Exclusion GI disease	1850	-0.08	-0.13, -0.02
Exclusion previously diagnosed diabetes	1637	-0.06	-0.12, 0.003
HT med. > individual HT med.	2078	-0.07	-0.12, -0.01
GMS > HbA1c (%) [4]	2075	-0.07	-0.12, -0.01
GMS > Fasting glucose	2077	-0.07	-0.12, -0.01
GMS > Post-load glucose	1947	-0.07	-0.13, -0.01
OSBP > 24h BP	1818	-0.05	-0.11, 0.01
Education > income	1713	-0.07	-0.13, -0.01
Education > occupational status	1753	-0.10	-0.16, -0.04
CHAMPS > ActivPAL	1848	-0.10	-0.16, -0.04
BMI > waist	2077	-0.07	-0.12, -0.01

[1] Standardized betas (β) represent the 1 SD increase in retinal venular dilation per 1 SD higher dietary MGO intake. [2] Model 3: adjusted for age, sex, glucose metabolism status, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of glucose-lowering-, antihypertensive- or lipid-modifying drugs. [4] '>' indicates that first covariate is replaced by the second, so in this case the covariate GMS is replaced by HbA1c. BP: blood pressure, CVD: cardiovascular disease, CRP: C-reactive protein, DHD15: Dutch Healthy Diet Index 2015, eGFR: estimated glomerular filtration rate, GI: gastrointestinal, GMS: glucose metabolism status, HT med.: anti-hypertensive medication, MGO: methylglyoxal, OSBP: office systolic blood pressure.

Supplementary figure

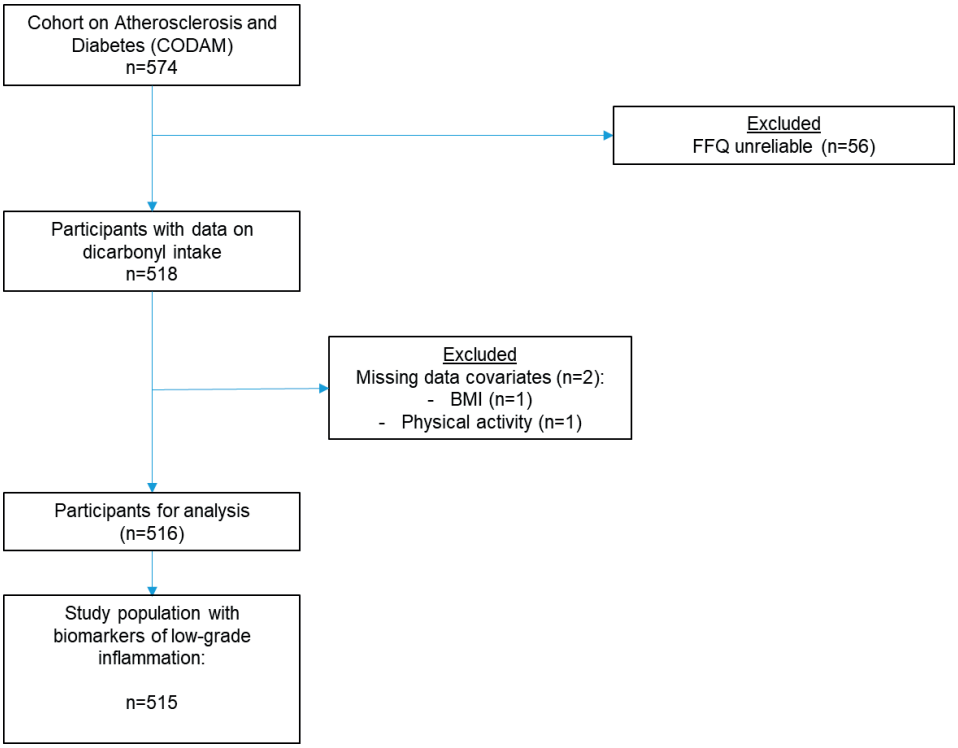

Figure S4.1 Flowchart Cohort on Diabetes and Atherosclerosis Maastricht. FFQ: Food Frequency Questionnaire.

Chapter 5

Habitual intake of dietary dicarbonyls is associated with greater insulin sensitivity and less type 2 diabetes: The Maastricht Study

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Abstract

Background

Dicarbonyls are reactive precursors of advanced glycation endproducts (AGEs). Dicarbonyls are formed endogenously, but also during food processing. Circulating dicarbonyls are associated with insulin resistance and type 2 diabetes, but consequences of dietary dicarbonyls are unknown.

Objective

To examine the associations of dietary intake of dicarbonyls with insulin sensitivity/resistance, β-cell function, and presence of prediabetes or type 2 diabetes.

Design

In 6282 participants (60±9 yrs, 50% men, 23% type 2 diabetes (oversampled)) of The Maastricht Study, we estimated habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) by linking Food Frequency Questionnaires to our food composition database containing MGO, GO, and 3-DG content of >200 foods. Insulin sensitivity, β-cell function, and glucose metabolism status were measured by a seven-point oral glucose tolerance test (available for n=2390, 2336, and 6282 respectively). Insulin sensitivity was assessed as the Matsuda index. β-cell function was assessed as C-peptidogenic index, overall insulin secretion, glucose sensitivity, potentiation factor, and rate sensitivity. Insulin resistance was measured as HOMA2-IR (n=2611). Cross-sectional associations of dietary dicarbonyls with insulin sensitivity/resistance, β-cell function, and glucose metabolism status were investigated using linear or logistic regression adjusting for age, sex, cardio-metabolic risk-factors, lifestyle and dietary factors.

Results

Higher dietary MGO and 3-DG intakes were associated with greater insulin sensitivity after full adjustment (Std. β (95%CI)=0.08 (0.04, 0.12) and 0.09 (0.05, 0.13), respectively). Accordingly, higher MGO and 3-DG intakes were associated with less insulin resistance (Std. β=-0.05 (-0.09, -0.01) and -0.04 (-0.08, -0.01)). Moreover, higher MGO and 3-DG intakes were associated with less presence of type 2 diabetes, after excluding individuals with previously diagnosed diabetes (OR (95%CI)=0.78 (0.65, 0.93) and 0.81 (0.66, 0.99)). There were no consistent associations of MGO, GO, and 3-DG intakes with β-cell function.

Conclusion

Higher habitual consumption of the dicarbonyls MGO and 3-DG was associated with better insulin sensitivity, less insulin resistance, and with less type 2 diabetes, after excluding individuals with known diabetes. This suggests that food–derived dicarbonyls may play a protective role in type 2 diabetes. These novel observations warrant further exploration in prospective cohorts and intervention studies.

Introduction

Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs)[1]. Dicarbonyls are formed endogenously, during glycolysis and lipid peroxidation, but they are also formed in food processing, mainly during heat treatment[2,3]. We recently showed that a higher habitual intake of the dicarbonyls MGO and GO was associated with higher concentrations of these dicarbonyls in plasma[4]. Additionally, higher MGO intake was associated with higher autofluorescence in the skin, an estimate for AGE accumulation in tissue. This suggests that these dietary dicarbonyls contribute to dicarbonyls and AGEs in the body.

In experimental models, elevated concentrations of dicarbonyls are associated with impaired insulin sensitivity and β-cell function, and induce type 2 diabetes[5]. Moreover, administration of high amounts of MGO in animals induced insulin resistance, impaired glucose tolerance, and reduced insulin secretion by the pancreatic β-cells[6-8]. Potential underlying mechanisms are the functional modification of insulin and the insulin receptor by MGO, direct effects of MGO on β-cells, and interference of MGO in insulin signaling in β-cells (as reviewed in[9]). In contrast, other experimental studies showed favorable effects of MGO, including prolonged lifespan of animals[10-14]. Long-term exposure of mice to exogenous MGO led to a moderate increase in plasma MGO, but these mice did not develop diabetes and, strikingly, even had increased survival[15]. A proposed underlying mechanism of this potential protective effect is upregulation of defense mechanisms by low-dose MGO exposure, via the KEAP1-Nrf2 pathway.

It is currently unknown if and to which extent dicarbonyls derived from the diet affect the progression of type 2 diabetes in humans. Therefore, in the current study we examined the associations of dietary dicarbonyls with insulin sensitivity/resistance, β-cell function, and glucose metabolism status, in an observational population-based cohort, enriched for type 2 diabetes.

Methods

Study population

This study uses data from the Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously[16]. In brief, the study focuses on the etiology, pathophysiology, complications, and comorbid conditions of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals

aged 40 to 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry by mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data from 7689 participants (dataset 3), who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (permit 131088-105234-PG) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Assessment of dietary MGO, GO, and 3-DG intake

Habitual intake of the dicarbonyls MGO, GO, and 3-DG was estimated as previously described[4]. In brief, we combined food intake data from the Food Frequency Questionnaire (FFQ) used in the Maastricht Study[17] with our previously published dietary dicarbonyl database, containing MGO, GO, and 3-DG concentrations of 223 foods and drinks[18].

To estimate habitual daily dietary intake of MGO, GO, and 3-DG, we multiplied the dicarbonyl concentration of a food product (mg/g) by the individual's estimated daily intake of that food product based on the FFQ (g/day), and subsequently summed all 253 food items (see formula below, using MGO as example).

$$MGO\ intake\left(\frac{mg}{day}\right) = \sum_{i=1}^{n} \left([MGO]i \left(\frac{mg}{g}\right) * intake\ i \left(\frac{g}{day}\right)\right)$$

Where *i* is food item, [MGO]*i* is MGO concentration for that particular food item from the database and intake *i* is intake of that particular food item derived from the FFQ.

To gain insight into the contribution of specific food groups to dicarbonyl intake, we calculated each individual's habitual daily intake of MGO, GO, and 3-DG from different food groups, as previously described[4]. For each food group, the contribution to total intake of MGO, GO, or 3-DG was calculated in percent of total intake of that dicarbonyl.

Insulin sensitivity, insulin resistance, β-cell function and glucose metabolism status

Participants underwent a standardized 2 h 75 g oral glucose tolerance test (OGTT) after an overnight fast. For safety reasons, participants using insulin or with a fasting plasma glucose value above 11.0 mmol/L (determined by finger prick) did not undergo the OGTT. Venous blood samples were collected before and 15, 30, 45, 60, 90, and 120 min after oral glucose load. Blood samples for the assessment of insulin and C-peptide levels were collected in ethylenediaminetetraacetic acid (EDTA) tubes, stored on ice, separated after centrifugation (3000× g for 15 min at 4°C), and plasma was stored at −80°C until the assays were performed. Insulin and C-peptide were measured using a custom duplex array (MesoScale Discovery, Gaithersburg, MD, USA), as previously described[19].

Plasma for the assessment of glucose was collected in sodium fluoride/potassium oxalate (NaF/KOx) tubes on ice. Fasting and 120-min-postload plasma glucose were measured in fresh samples and glucose at other time points during the OGTT was measured in samples separated after centrifugation (3000× g for 15 min at 4°C) and stored within 2 h at −80°C until the assays were performed. Fasting and 120-min-postload plasma glucose was measured with the enzymatic hexokinase method using two automatic analysers (i.e., the Beckman Synchron LX20 (Beckman Coulter Inc., Brea, CA, USA) for samples obtained between November 2010 and April 2012, and the Roche Cobas 8000 (Roche Diagnostics, Mannheim, Germany) for samples obtained thereafter). Glucose at other time points during the OGTT was measured with the enzymatic hexokinase method by use of the Roche Cobas 6000 (Roche Diagnostics, Mannheim, Germany), as previously described[19].

Insulin sensitivity and insulin resistance

Insulin sensitivity was assessed by the Matsuda insulin sensitivity index. Matsuda index was calculated as: (10,000/√fasting plasma glucose × fasting plasma insulin × mean glucose concentration during OGTT × mean insulin concentrations during OGTT)[20]. Insulin resistance was assessed as HOMA2-IR and calculated with the HOMA2 calculator version 2.2.3 for Windows[21].

β-cell function

As β-cell function consists of multiple components, it cannot be described by a single measure. Therefore, we used two classical β-cell function indices (C-peptidogenic index and the ratio of the C-peptide to glucose area under the

curve)[22-24] and three mathematical model-based parameters (β-cell glucose sensitivity, β-cell potentiation factor ratio, and β-cell rate sensitivity) according to a previously-described model[25].

C-peptidogenic index ($\Delta CP_{30}/\Delta G_{30}$. The equivalent of the insulinogenic index) reflects early phase insulin secretion and has a good ability to discriminate between normal glucose metabolism and prediabetes or type 2 diabetes (ROC AUCs ≥78%)[22]. The ratio of the C-peptide to glucose area under the curve (CP_{AUC}/G_{AUC}) is a measure of overall insulin secretion.

The mathematical model parameter 'β-cell glucose sensitivity' is the slope of the glucose–insulin secretion dose–response function[23], and represents the dependence of insulin secretion on absolute glucose concentration at any time point during the OGTT. The dose–response relationship is modulated by 'β-cell potentiation', which accounts for higher insulin secretion during the descending phase of hyperglycemia than during the ascending phase of an OGTT, for the same glucose concentration. β-cell potentiation is set as a positive function of time and averages 1 during the OGTT. Therefore, it represents the relative potentiation of the insulin secretion response to glucose. The β-cell potentiation parameter used in the present analysis represents the ratio of the β-cell potentiation factor at the end of the 2-h OGTT relative to the β-cell potentiation factor at the start. 'β-cell rate sensitivity' is a marker of early phase insulin release, and represents the dynamic dependence of insulin secretion on the rate of change in glucose concentration[23].

Glucose metabolism status

Glucose metabolism status was defined according to WHO 2006 criteria as normal glucose metabolism, prediabetes (impaired fasting glucose [6.1–7.0 mmol/L] and/or impaired glucose tolerance [2-h glucose 7.8–11.1 mmol/L]), type 2 diabetes (fasting plasma glucose ≥7.1, 2-h glucose >11.1, or the use of diabetes medication).

Assessment of other covariates

Weight and height were measured by a trained staff member, and body mass index (BMI) was calculated as weight (kg) divided by height^2 (m). Age, sex, smoking behavior, history of cardiovascular disease, educational level, and presence of gastrointestinal tract infection were assessed by means of a self‐report questionnaire[16]. Physical activity was assessed using the CHAMPS questionnaire[26]. Medication use was assessed by interview[16]. Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CDK-epi) equation, using both serum creatinine and serum cystatin C[27]. Intake of

energy, macronutrients, and the Dutch Healthy Diet index – a measure of diet quality - were assessed using the FFQ described above. The Dutch Healthy Diet index used in the Maastricht Study consists of all fifteen components except coffee, because the coffee component is based on type of coffee (filtered or unfiltered) and our FFQ does not distinguish between types of coffee consumed. Hence, the Dutch Healthy Diet index score ranges from 0 (no adherence) to 140 (complete adherence)[28]. Triglycerides, total cholesterol, and HDL and LDL cholesterol, fasting plasma glucose, and glycosylated hemoglobin (HbA1c) were determined as described elsewhere[16].

Statistical analyses

The general characteristics of the total study population are compared across tertiles of total dietary dicarbonyl intake. For this, a standardized composite score of total dietary dicarbonyl intake was calculated by standardizing each dietary dicarbonyl (MGO, GO and 3-DG), then averaging this into an overall dietary dicarbonyl z-score. Differences in characteristics among individuals in the tertiles were tested using one-way ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables and chi-squared test for discrete variables. We also evaluated to what extent participants who were excluded from the analyses because of missing covariates differed from those who were included.

For all further analyses, each dietary dicarbonyl was considered individually, since they are thought to exert different biological effects[29]. Non-normally distributed outcome variables (Matsuda index, HOMA-IR, β-cell glucose sensitivity, β-cell potentiation factor) were ln-transformed to obtain normal distributions. C-peptidogenic index and β-cell rate sensitivity were analyzed in tertiles, because the residuals of linear regression were not normally distributed. All continuous outcome variables and main exposure variables were standardized, to allow direct comparison of the strength of the associations.

We used multiple linear regression to examine the associations of each standardized dietary dicarbonyl (MGO, GO, and 3-DG) with standardized insulin sensitivity (Matsuda index), standardized insulin resistance (HOMA-IR), and standardized β cell function indices (glucose sensitivity, β-cell potentiation factor, and overall insulin secretion). We used multinominal logistic regression to examine the associations of each standardized dietary dicarbonyl with tertiles of β-cell C-peptidogenic index and rate sensitivity (with the highest tertile as reference category), and with presence of prediabetes or type 2 diabetes (with normal

glucose metabolism as reference category). The associations between dietary dicarbonyls and type 2 diabetes were performed in both the full population and after exclusion of individuals with previously diagnosed type 2 diabetes, to address the oversampling of individuals with type 2 diabetes in this cohort, and to explore potential reverse causality because these individuals might have adapted their diet.

Associations were first adjusted for age (years) and sex (men/women) [Model 1]. Next, additional adjustments were made for potential confounders related to lifestyle, i.e. BMI (kg/m^2), smoking (former or current as dummy variables with never as reference category), alcohol intake (grams/day), physical activity (total score of all activities, hours/week), total energy intake (kcal/day), and education level (medium or high as dummy variables with low as reference category) [Model 2]. Lastly, the analyses were additionally adjusted for other cardiovascular risk factors: history of cardiovascular diseases (yes/no), triglycerides (mmol/L), LDL (mmol/L), total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying drugs (yes/no) [Model 3]. In analyses with β-cell function as outcomes, model 3 was also adjusted for insulin sensitivity (Matsuda index).

Because the associations between intake of dietary components and (health) outcomes are often non-linear[30], we tested for deviation from a linear trend in our associations. For this, regression models using dietary dicarbonyls as continuous or categorical exposures (quintiles as one continuous variable or five categorical variables with lowest as the reference) were compared with a likelihood ratio test. The large majority of the models did not show a significantly better fit when using dietary dicarbonyls as categorical exposures, and therefore dietary dicarbonyls were entered as continuous exposures in all models (data not shown).

For each outcome measure we evaluated whether associations differed between males and females, or with varying kidney function, because dicarbonyls are excreted into urine via the kidney and hence dietary dicarbonyls might have more pronounced effects in individuals with impaired kidney function. For this, we added interaction terms with sex (sex*dicarbonyl intake) and with eGFR (eGFR*dicarbonyl intake, $ml/min/1.73\ m^2$; thereby also adding eGFR as a covariate) in the fully adjusted regression models. Analyses for which we found an interaction ($p<0.1$) were repeated after stratification on sex or eGFR (<60 and ≥60 mL/min per $1.73\ m^2$).

In additional analyses, we explored whether observed associations were driven by any of the main food sources of dicarbonyls. For this, we adjusted model 3 sequentially for dicarbonyl intake via each of the main food groups that contributed

to ≥5% of the total daily dicarbonyl intake in this population (e.g. additional adjustment for MGO intake from coffee)[4].

Several sensitivity analyses were performed to assess the robustness of the observations of the main analyses. First, the analyses with glucose metabolism status as outcome were repeated using HbA1c, fasting plasma glucose, or post-load glucose as outcomes. Second, to examine whether associations could be attributed to intake of other dietary components or better adherence to the Dutch Healthy Diet index, the fully adjusted model was, on top of energy intake (kcal/day), additionally adjusted for either carbohydrate intake, fat intake, protein intake (grams/day), or the Dutch Healthy Diet index. In the model where we included the Dutch Healthy Diet index we did not separately adjust for alcohol intake, which is included in the index. Third, the analyses were repeated without adjustment for BMI, because it is unclear whether BMI is a confounder or a mediator, since dietary AGEs intake was associated with weight gain[31], and a similar association might be present for dietary dicarbonyls. Fourth, the analyses were repeated after exclusion of individuals with self-reported gastrointestinal tract infection (defined as self-reported symptoms of gastrointestinal tract infection in the previous two months). Fifth, several covariates were replaced with alternative measurement methods. Educational level was replaced with equivalent income or occupational status. Physical activity obtained from the CHAMPS questionnaire was replaced with accelerometer data (ActivPAL). We did not include accelerometer-assessed physical activity in the main analyses because data was missing for a relatively large number of participants.

Associations are presented as standardized β coefficients (standardized β (95% CI)) for continuous outcome variables and as odds ratios (OR (95% CI)) for categorical outcome variables. Thus, β coefficients represent the SD difference in outcome per 1 SD higher intake of MGO, GO, or 3-DG per day and odds ratios represent the higher odds per 1 SD higher intake of MGO, GO, or 3-DG per day. Statistical significance was set at $p<0.05$ (except for testing for interaction where statistical significance was set at $p<0.10$). All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 26.0).

Results

Characteristics of the study population

Of the 7689 participants, 497 were excluded because they did not complete the FFQ (n=357) or reported implausible energy intake, i.e. < 800 or > 4200 kcal/day for males and < 500 or > 3500 kcal/day for females (n=140) (Figure 5.1). Of the

remaining individuals, 42 individuals were excluded because of they had another type of diabetes than type 2 diabetes, and another 868 individuals because they had missing data on one or more of the covariates. Complete case analyses were performed for the outcomes glucose metabolism status, Matsuda index, HOMA-IR, and β-cell function, and this resulted in study populations of 6282, 2390, 2611, and 2336 individuals for these outcomes, respectively. The smaller sample sizes are because these outcomes were not available for all individuals, and for the OGTT-derived outcomes (Matsuda index and β-cell function) because of the contraindication for an OGTT in individuals on insulin or with a high fasting glucose (see methods). Individuals with missing data on any of the covariates had somewhat higher cardiovascular risk factors, including more often type 2 diabetes, more current smokers, higher BMI, lower education, higher fasting plasma glucose, a higher total-to-HDL cholesterol ratio, more medication use, higher insulin resistance and lower β-cell glucose sensitivity and overall insulin secretion (Supplementary Table S5.1). Overall, the subpopulations were comparable with the total population in regard to age, sex, and cardiovascular risk profile (Supplementary Table S5.2). Individuals in the insulin resistance subpopulation more often had diabetes and more often used medication.

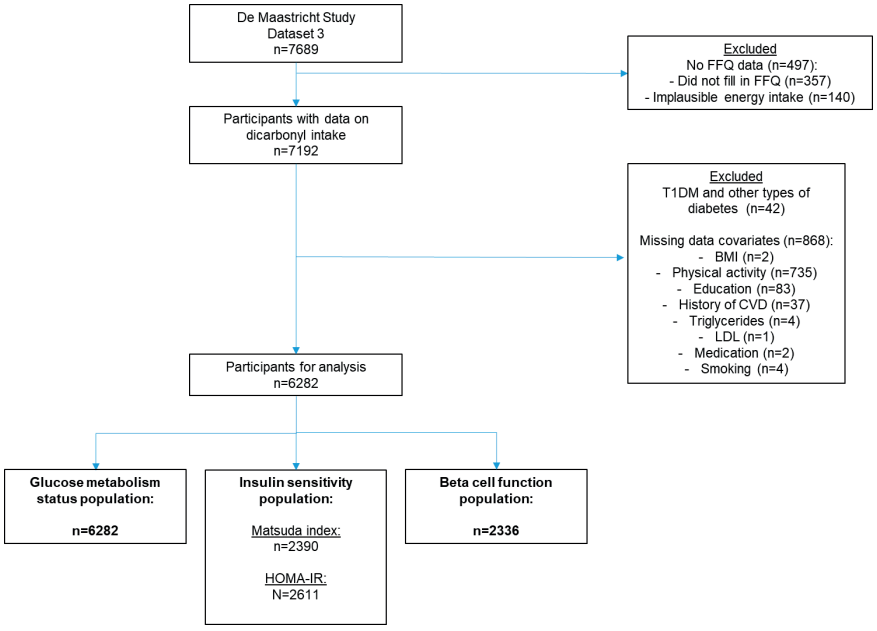

Figure 5.1 Flowchart selection study populations. CVD, cardiovascular diseases; FFQ, food frequency questionnaire; LDL, low-density lipoprotein; T1DM, type 1 diabetes.

Median [IQR] dietary intake was 3.9 [3.2-4.7] mg/day for MGO, 3.4 [2.8-4.2] mg/day for GO, and 16 [11-23] mg/day for 3-DG (Table 5.1). Individuals in the highest tertile of overall dietary dicarbonyl intake were more often men, had less often type 2 diabetes, had a lower BMI, were more physically active, had a higher level of education, and less often used glucose-lowering, anti-hypertensive, or lipid-mediating medication. They also had higher intake of energy and all other dietary components, but did not differ in adherence to the Dutch dietary guidelines. These participants also were more insulin sensitive.

Table 5.1 Population characteristics of those with information on all confounders.[1]

Characteristics	Total population (n=6282)	Dietary dicarbonyl intake[2]			P-value
		Lowest (n=2094)	Middle (n=2094)	Highest (n=2094)	
Demographics					
Age (years)	60±8.6	60±8.8	60±8.6	60±8.4	0.29
Sex (% male)	50	41	52	57	<0.001
Glucose status					
Glucose metabolism status					<0.001
Normal glucose metabolism (%)	62	59	61	66	
Prediabetes (%)	15	15	16	15	
Type 2 diabetes mellitus (%)	23	26	24	20	
Newly diagnosed T2DM (%)	4	5	4	4	<0.001
Diabetes duration (years)	4 [0.0-10]	3.0 [0.0-10]	4.0 [0.0-8.5]	4.0 [1.0-11]	0.54
Lifestyle					
Smoking (%)					0.23
Never	38	39	37	38	
Former	50	48	51	51	
Current	12	13	12	11	
Waist circumference (cm)	95±14	95±14	95±13	94±13	0.02
BMI (kg/m^2)	27±4.4	27±4.6	27±4.4	26±4.3	<0.001
Physical activity (h/week)	13 [8.0-18]	12 [7.3-17]	13 [8.3-18]	14 [8.5-20]	<0.001
Education (%)					0.01
Low	33	35	34	31	
Medium	28	28	27	29	
High	39	37	39	41	
Biological					
Fasting glucose (mmol/L)	5.4 [5.0-6.2]	5.4 [5.0-6.3]	5.4 [5.0-6.2]	5.3 [5.0-6.0]	0.001
HbA1c (%)	5.7±0.83	5.8±0.85	5.7±0.82	5.7±0.82	0.17
24-h Systolic blood pressure (mmHg)	134±18	133±18	134±18	134±18	0.37
24-h Diastolic blood pressure (mmHg)	76±9.8	75±9.8	76±9.6	76±10	0.33
Cholesterol (mmol/L)	5.2±1.1	5.2±1.2	5.2±1.1	5.2±1.1	0.94
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	3.3 [2.7-4.1]	3.4 [2.8-4.3]	3.4 [2.8-4.3]	0.003
LDL (mmol/L)	3.1±1.0	3.0±1.0	3.1±1.0	3.1±0.98	0.25
Triglycerides (mmol/L)	1.2 [0.88-1.69]	1.2 [0.87-1.7]	3.4 [2.8-4.3]	1.2 [0.87-1.7]	0.17
eGFR (mL/min/1.73 m^2)	88±15	87±15	88±15	89±14	0.18
Medical history of CVD (% yes)	16	17	16	15	0.26
Medical history of gastrointestinal disease (%yes)	11	11	10	11	0.68

Table 5.1 (continued)

Characteristics	Total population (n=6282)	Dietary dicarbonyl intake[2]			P-value
		Lowest (n=2094)	Middle (n=2094)	Highest (n=2094)	
Medication use					
Glucose-lowering medication (%yes)	17	18	17	15	0.004
Anti-hypertensives (%yes)	37	40	38	33	<0.001
Lipid-mediating medication (%yes)	31	33	32	29	0.01
Dietary intake					
Energy intake (kcal/day)	2139±594	1680±383	2126±414	2612±554	<0.001
Carbohydrate (g/day)	230±69	172±40	226±42	291±62	<0.001
Fat (g/day)	82±30	66±23	822±26	98±32	<0.001
Protein (g/day)	85±23	70±17	85±18	99±23	<0.001
Alcohol intake (g/day)	8.2 [1.6-18]	6.5 [0.87-16]	8.6 [1.8-19]	9.2 [2.2-20]	<0.001
Dutch Healthy Diet Index	84±15	84±15	83±15	84±15	0.1
Dietary MGO (mg/day)	4.0±1.2	2.9±0.62	3.9±0.60	5.2±1.1	<0.001
Dietary GO (mg/day)	3.6±0.96	2.6±0.53	2.5±0.52	4.7±0.96	<0.001
Dietary 3-DG (mg/day)	16 [11-23]	10 [7.8-13]	16 [13-20]	26 [20-33]	<0.001
Insulin sensitivity/resistance					
Matsuda index	3.5 [2.1-5.3]	3.4 [1.9-5.4]	3.5 [2.1-5.1]	3.7 [2.2-5.5]	0.03
HOMA-IR	1.4 [0.96-2.1]	1.4 [0.97-2.2]	1.4 [0.98-2.1]	1.3 [0.94-2.0]	0.20
β-cell function					
β-cell glucose sensitivity (pmol/min/m^2/mM)	76 [45-109]	73 [42-112]	73 [42-109]	76 [48-109]	0.28
β-cell potentiation factor	1.6±0.69	1.6±0.69	1.6±0.69	1.6±0.70	0.91
β-cell rate sensitivity (pmol/m^2/mM)	532 [196-1012]	486 [178-958]	538 [208-1030]	568 [227-1048]	0.09
C-peptidogenic index (ΔCP_{30}:ΔG_{30} ratio)	465±1165	500±949	443±1312	455±1189	0.60
Overall insulin secretion (CP_{AUC}:G_{AUC} ratio)	197±81	194±78	196±85	199±79	0.50

[1] Data are presented as mean ± standard deviation, median [interquartile range] or percentage. P-value of ANOVA, Kruskal-Wallis, or Chi Square tests for differences between tertiles. [2] For the tertiles, a composite score of total dicarbonyl intake (MGO, GO, and 3-DG) was created. Waist was available for n=6280; HbA1c was available for n=6275; duration of type 2 diabetes was available for n=1150; fasting plasma glucose available for n=6280; blood pressure available for n=6279; eGFR available for n=2728; Matsuda index available for n=2390; HOMA-IR was available for n=3671; β-cell glucose sensitivity, potentiation factor, and rate sensitivity available for n=2443; c-peptidogenic index available for n=2395; C-peptide area under the curve available for n=2391. 3-DG 3-deoxyglucosone, CVD cardiovascular disease, eGFR estimated glomerular filtration rate, GO glyoxal, HOMA-IR Homeostatic Model Assessment for Insulin Resistance, HDL high-density lipoprotein, LDL low-density lipoprotein, MGO methylglyoxal.

Associations of MGO, GO, and 3-DG intake with insulin sensitivity

Individuals who consumed more MGO, GO, or 3-DG had a greater insulin sensitivity, assessed as Matsuda index, after full adjustment (MGO: Std. β (95%CI)=0.08 (0.04, 0.12). GO: 0.08 (0.02, 0.13) and 3-DG: 0.09 (0.05, 0.13), Table 5.2). Accordingly, individuals who consumed more MGO or 3-DG were less insulin resistant, as indicated by a lower HOMA2-IR (MGO: Std. β (95%CI)=-0.05 (-0.09, -0.01) and

3-DG: -0.04 (-0.08, -0.01), Table 5.2). Higher GO intake was associated with less insulin resistance in the age and sex adjusted model, but not after further adjustment (Std. β=-0.04 (-0.09, 0.01), model 3). These associations remained and where somewhat stronger after excluding individuals with previously diagnosed type 2 diabetes (Supplementary Table S5.4). The associations were robust over sensitivity analyses (supplementary tables S5.8 and S5.9).

Table 5.2 Associations of MGO, GO, and 3-DG intake with insulin sensitivity/resistance.[1]

		Insulin sensitivity/resistance	
	Model	**Matsuda index [2] β (95% CI)**	**HOMA-IR [3] β (95% CI)**
Dietary MGO			
	Crude	0.05 (0.01, 0.09)	-0.01 (-0.05, 0.03)
	1	0.08 (0.04, 0.12)	-0.04 (-0.08, 0.00)
	2	0.09 (0.04, 0.13)	-0.06 (-0.10, -0.02)
	3	0.08 (0.04, 0.12)	-0.05 (-0.09, -0.01)
Dietary GO			
	Crude	0.06 (0.02, 0.10)	-0.02 (-0.06, 0.02)
	1	0.07 (0.03, 0.11)	-0.04 (-0.08, -0.003)
	2	0.07 (0.02, 0.13)	-0.04 (-0.09, 0.01)
	3	0.08 (0.02, 0.13)	-0.04 (-0.09, 0.01)
Dietary 3-DG			
	Crude	0.10 (0.06, 0.14)	-0.06 (-0.10, -0.03)
	1	0.11 (0.07, 0.15)	-0.08 (-0.11, -0.04)
	2	0.08 (0.04, 0.12)	-0.03 (-0.07, 0.003)
	3	0.09 (0.05, 0.13)	-0.04 (-0.08, -0.01)

[1] Data were analyzed using linear regression analyses. Standardized βs represent 1 SD higher ln-transformed Matsuda index or ln-transformed HOMA-IR per 1 SD higher dietary dicarbonyl intake. Model 1: adjusted for age + sex. Model 2: model 1 + BMI, smoking, alcohol intake, physical activity, total energy intake and education level. Model 3: model 2 + history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, and use of antihypertensive- or lipid-modifying medication. [2] N=2390 for Matsuda index. [3] N=2611 for HOMA-IR. 3-DG, 3-deoxyglucosone; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal.

Associations of MGO, GO, and 3-DG intake with β-cell function

MGO intake was not associated with any of the indices of β-cell function (Table 5.3). Higher GO intake was associated with lower β-cell potentiation factor after full adjustment (Std. β (95%CI)=-0.07 (-0.13, -0.01), Table 5.3). This association remained after excluding individuals with known type 2 diabetes (Supplementary Table S5.5), but was not robust over sensitivity analyses (Supplementary Table S5.10). In contrast, higher GO intake was associated with higher glucose sensitivity in the age and sex adjusted model, but not after further adjustment (Std. β (95%CI)=0.06 (-0,01, 0,12), model 3). Higher GO intake was also associated with higher C-peptidogenic index in the crude and the age and sex adjusted models, but not after further adjustment (OR (95%CI)=0.89 (0.75, 1,05), model 3).

Table 5.3 Associations of MGO, GO, and 3-DG intake with β-cell function. [1]

		β-cell function						
	Model	**C-petidogenic index**		**Overall insulin secretion**	**Glucose sensitivity**	**Potentiation factor**	**Rate sensitivity**	
		Tertile 1 vs. 3 OR (95% CI)	**Tertile 2 vs. 3 OR (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**Tertile 1 vs. 3 OR (95% CI)**	**Tertile 2 vs. 3 OR (95% CI)**
Dietary MGO								
	Crude	0.96 (0.87, 1.06)	1.04 (0.94, 1.14)	-0.02 (-0.06, 0.03)	0.02 (-0.03, 0.06)	-0.02 (-0.06, 0.03)	0.96 (0.87, 1.06)	1.01 (0.92, 1.12)
	1	0.91 (0.82, 1.01)	1.01 (0.91, 1.11)	-0.002 (-0.04, 0.04)	0.04 (-0.003, 0.08)	-0.001 (-0.04, 0.04)	0.95 (0.85, 1.05)	1.02 (0.92, 1.13)
	2	0.98 (0.86, 1.12)	0.98 (0.87, 1.12)	-0.05 (-0.10, 0.001)	0.02 (-0.03, 0.07)	-0.01 (-0.04, 0.06)	0.98 (0.86, 1.11)	1.01 (0.89, 1.15)
	3	0.98 (0.86, 1.13)	0.98 (0.86, 1.11)	-0.03 (-0.08, 0.02)	0.03 (-0.02, 0.08)	-0.003 (-0.05, 0.05)	0.98 (0.86, 1.11)	1.00 (0.88, 1.14)
Dietary GO								
	Crude	**0.88 (0.80, 0.98)**	0.98 (0.89, 1.08)	0.002 (-0.04, 0.04)	0.03 (-0.01, 0.07)	-0.03 (-0.07, 0.01)	0.93 (0.84, 1.02)	0.95 (0.86, 1.05)
	1	**0.85 (0.76, 0.94)**	0.96 (0.87, 1.06)	-0.03 (-0.03, 0.05)	**0.04 (0.004, 0.09)**	-0.02 (-0.06, 0.02)	0.92 (0.83, 1.02)	0.96 (0.87, 1.06)
	2	0.92 (0.78, 1.08)	0.92 (0.78, 1.07)	-0.03 (-0.09, 0.04)	0.04 (-0.03, 0.10)	**-0.08 (-0.14, -0.01)**	0.92 (0.79, 1.08)	0.89 (0.76, 1.04)
	3	0.89 (0.75, 1.05)	0.92 (0.79, 1.08)	0.002 (-0.06, 0.06)	0.06 (-0.01, 0.12)	**-0.07 (-0.13, -0.01)**	0.88 (0.75, 1.04)	0.88 (0.75, 1.02)
Dietary 3-DG								
	Crude	0.93 (0.84, 1.03)	1.01 (0.91, 1.11)	-0.01 (-0.05, 0.03)	**0.06 (0.02, 0.10)**	0.03 (-0.01, 0.07)	0.92 (0.83, 1.02)	0.99 (0.90, 1.09)
	1	**0.90 (0.81, 0.99)**	0.99 (0.90, 1.09)	0.00 (-0.04, 0.04)	**0.07 (0.03, 0.11)**	-0.03 (-0.01, 0.08)	0.91 (0.82, 1.01)	0.99 (0.90, 1.09)
	2	1.00 (0.89, 1.12)	0.98 (0.88, 1.10)	-0.01 (-0.06, 0.04)	**0.05 (0.01, 0.10)**	0.01 (-0.03, 0.06)	0.97 (0.86, 1.08)	0.99 (0.89, 1.11)
	3	0.99 (0.88, 1.12)	0.98 (0.87, 1.09)	-0.001 (-0.04, 0.04)	**0.06 (0.02, 0.11)**	0.003 (-0.04, 0.05)	0.96 (0.86, 1.08)	0.99 (0.89, 1.10)

[1] Data were analyzed using linear regression analysis for overall insulin secretion, ln-transformed glucose sensitivity and ln-transformed potentiation factor, and using nominal logistic regression analysis for rate sensitivity and C-peptidogenic index. Standardized βs represent 1 SD change in outcome per 1 SD higher dietary dicarbonyl intake. Odds ratio (OR) < 1.00 indicate a greater β-cell rate sensitivity or C-peptidogenic index and OR > 1.00 indicate a worse β-cell rate sensitivity or C-peptidogenic index. Results are for fully adjusted model (model 3), adjusted for adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake, education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use antihypertensive- or lipid-modifying medication and insulin sensitivity. N=2336. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Higher 3-DG intake was associated with higher β-cell glucose sensitivity after full adjustment (Std. β (95%CI)=0.06 (0.02, 0.11), Table 5.3). This association was not statistically significant after exclusion of individuals with known type 2 diabetes (Supplementary Table S5.5), but was robust over sensitivity analyses (Supplementary Table S5.11). Higher 3-DG intake was also associated with higher C-peptidogenic index in the age and sex adjusted model, but not after further adjustment (tertile 1 vs. 3, OR (95% CI)=0.99 (0.88, 1.12), model 3).

Associations of MGO, GO, and 3-DG intake with prediabetes and type 2 diabetes

In the total population, higher MGO intake was associated with less type 2 diabetes in the crude and the age and sex adjusted models (OR (95%CI)=0.84 (0.79, 0.90), model 1, Table 5.4), but this did not remain after further adjustment (OR=0.93 (0.84, 1.02), model 3). Interestingly, after excluding individuals with previously known type 2 diabetes, higher MGO intake was associated with less type 2 diabetes (OR=0.78 (0.65, 0.93), model 3, Table 5.4). In line, higher MGO intake was associated with lower post-load glucose, but not with HbA1c or fasting glucose (Supplementary Table S5.7).

Higher intake of GO was associated with less type 2 diabetes in the total population (OR=0.87 (0.77, 0.99), model 3, Table 5.4). This association did not remain after exclusion of individuals with previously diagnosed diabetes (0.82 (0.66, 1.01), Table 5.4), and was not robust over sensitivity analyses (Supplementary Table S5.12). Higher GO intake was also associated with less prediabetes in the crude and age and sex adjusted models, but this did not remain after full adjustment (OR=0.91 (0.81, 1.03), Table 5.4).

Individuals who consumed more 3-DG had less type 2 diabetes after full adjustment, both in the total population and after excluding individuals with previously diagnosed type 2 diabetes (OR=0.85 (0.76, 0.95) and 0.81 (0.66, 0.99), respectively, Table 5.4). This association was robust over most sensitivity analyses (Supplementary Table S5.12). In line, higher 3-DG intake was associated with less prediabetes in the crude and the age and sex adjusted models, but not in the fully adjusted model (OR=0.96 (0.88, 1.05), Table 5.4). When other measures of glucose metabolism were used as outcomes, higher 3-DG intake was also associated with lower HbA1c, fasting glucose, and post-load glucose (Supplementary Table S5.7).

Interaction analyses

Interaction terms of dietary dicarbonyls with sex and eGFR were added to evaluate whether the associations differed between males and females, or with varying

kidney function. In these analyses, none of the associations were modified by sex. eGFR modified the association between 3-DG intake and type 2 diabetes (P-interaction=0.07). After stratification for eGFR (eGFR<60 or ≥60 mL/min per 1.73 m^2), 3-DG intake was strongly and statistically significantly associated with type 2 diabetes in individuals with impaired kidney function only (eGFR<60, n=114, OR=0.24 (0.09, 0.68). eGFR≥60, n=2614, OR=0.88 (0.76, 1.03), Supplementary Table S5.3). Moreover, eGFR modified the association between 3-DG intake and β-cell glucose sensitivity (P-interaction=0.003). After stratification, the association was again stronger in individuals with impaired kidney function, but only statistically significant for individuals without impaired kidney function (eGFR<60, n=81, Std. β=0.27 (-0.001, 0.54). eGFR≥60, n=2250, Std. β=0.06 (0.01, 0.10), Supplementary Table S5.3). eGFR also modified the associations of 3-DG intake with β-cell rate sensitivity, β-cell potentiation factor, and β-cell insulin secretion and the association between GO intake and insulin resistance (P-interaction=0.01-0.09), but stratification revealed no statistically significant associations (Supplementary Table S5.3).

Table 5.4 Associations of MGO, GO, and 3-DG intake with glucose metabolism status.[1]

	Model	Normal glucose metabolism N=3881	Prediabetes N=946	Known and newly diagnosed type 2 diabetes N=1455	Only newly diagnosed type 2 diabetes N=266
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Dietary MGO					
	Crude	-	0.96 (0.89, 1.03)	0.92 (0.86, 0.97)	0.82 (0.72, 0.93)
	1	-	0.93 (0.86, 1.00)	0.84 (0.79, 0.90)	0.76 (0.66, 0.87)
	2	-	0.94 (0.85, 1.03)	0.92 (0.84, 1.01)	0.77 (0.64, 0.91)
	3	-	0.95 (0.86, 1.05)	0.93 (0.84, 1.02)	0.78 (0.65, 0.93)
Dietary GO					
	Crude	-	0.90 (0.84, 0.97)	0.85 (0.80, 0.90)	0.90 (0.84, 0.97)
	1	-	0.88 (0.82, 0.95)	0.80 (0.75, 0.86)	0.75 (0.66, 0.86)
	2	-	0.92 (0.82, 1.03)	0.92 (0.82, 1.02)	0.84 (0.68, 1.03)
	3	-	0.91 (0.81, 1.03)	0.87 (0.77, 0.99)	0.82 (0.66, 1.01)
Dietary 3-DG					
	Crude	-	0.92 (0.86, 0.99)	0.74 (0.69, 0.80)	0.78 (0.66, 0.92)
	1	-	0.91 (0.84, 0.98)	0.70 (0.64, 0.76)	0.75 (0.63, 0.89)
	2	-	0.98 (0.90, 1.07)	0.87 (0.78, 0.96)	0.84 (0.69, 1.03)
	3	-	0.96 (0.88, 1.05)	0.85 (0.76, 0.95)	0.81 (0.66, 0.99)

[1] Data were analyzed using nominal logistic regression analysis. Odds ratio (OR): Values < 1.00 indicate a lower odds of prediabetes or type 2 diabetes. Model 1: adjusted for age + sex. Model 2: model 1 + BMI, smoking, alcohol intake, physical activity, total energy intake and education level. Model 3: model 2 + history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, and use of antihypertensive- or lipid-modifying medication. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Contribution of main food groups to the strength of the associations

To explore whether the observed associations were driven by any food groups in particular, we sequentially adjusted model 3 for MGO, GO, or 3-DG intake from each of the food groups that contributed >5% to daily MGO, GO, or 3-DG intake (corresponding to the dicarbonyl used as predictor). Overall, the associations remained similar after additional adjustment for dicarbonyl intake from these food groups (Supplementary Table S5.6), and were not driven by dicarbonyl intake from a specific food group.

Discussion

In this population-based cohort, enriched for type 2 diabetes, we observed that higher habitual intakes of the dicarbonyls MGO and 3-DG were associated with greater insulin sensitivity and, accordingly, with less insulin resistance. Moreover, higher intake of MGO and 3-DG was associated with less type 2 diabetes, although for MGO this was restricted to the population in which previously-diagnosed type 2 diabetes was excluded. Higher GO intake was also associated with greater insulin sensitivity and less type 2 diabetes, but this was not robust in the sensitivity analyses. The associations between intake of dicarbonyls and indices of β-cell function on the other hand, were fully not consistent and mostly absent after adjustment for confounders, including prevailing level of insulin sensitivity/ resistance.

The associations of MGO and 3-DG with insulin sensitivity/resistance, and (newly diagnosed) type 2 diabetes may indicate potential protective effects of these dietary dicarbonyls against development of type 2 diabetes. These observations may at first sight appear unexpected, because animal studies report that high amounts of exogenous MGO led to impaired insulin sensitivity, greater insulin resistance[5,7,8], impaired glucose tolerance[5,6], and reduced insulin secretion by the pancreatic β-cells[6]. However, translating these observations to humans is challenging, because the administered doses greatly exceed the estimated daily exposures in humans. For example, in animals administers doses were as high as 1% MGO in drinking water[7], and 50-75 mg/kg body weight (intraperitoneally or subcutaneously)[6,8]. For comparison, in humans, 50-75 mg/kg body weight would translate to an exposure of 3500-5250 mg/kg body weight for an individual of 70 kg, which is approximately 1000-fold higher than the estimated daily intake of 4 mg/day for MGO in this population. Moreover, and in line with the associations observed in the current study, we previously showed in this population that individuals who consumed more MGO had less low-grade inflammation (*unpublished data, Maasen et al.*), also suggesting beneficial effects of dietary MGO

intake. Moreover, in experimental models, low doses of MGO promote lifespan[5,12]. Oral administration of MGO in mice also led to a slightly increase survival, despite higher MGO concentrations in urine and plasma[32].

Interestingly, and in line with these observations, the concept of hormesis was recently proposed for the effects of MGO. Hormesis is described as a beneficial response to low-dose exposures of stressors that are harmful at a higher dose. In line with this concept, recent studies show that small increases of MGO are beneficial and play a role in modulating physiology. For instance, *In vitro,* low concentrations of MGO trigger a response to handle the otherwise harmful effects of MGO by the prevention of its enhanced formation, by detoxification of MGO, and by repair of damage caused when the first two levels of defense are overwhelmed[14]. Early data suggest that a similar defense mechanism exists in human endothelial cells[14]. The mechanism behind these beneficial effects is thought to be an MGO-induced defense mechanism involving the KEAP1-Nrf2 pathway[11,14,33]. The transcription factor Nrf2 is a key regular in oxidative stress, increasing the production glyoxalase-1, involved in the detoxification of MGO. In a recent human trial, stimulation of Nrf2 signaling resulted in an increased glyoxalase-1 protein expression, accompanied by clinically relevant improvements in whole body glucose metabolism[34]. Thus, dietary MGO might have protective effects on insulin sensitivity and glucose metabolism via upregulation of Nrf2 and a subsequent decrease in oxidative stress.

In contrast to MGO, which is considered the most reactive dicarbonyl, little is known about the physiological effects of GO and 3-DG. We previously showed that higher dietary GO intake was associated with correspondingly higher concentrations in plasma, while this was not the case for 3-DG intake[4]. Nevertheless, a small-scale in which participants received a single dose of oral 3-DG reported an increase in urinary excretion of 3-DG and its metabolite[35]. The observation that higher 3-DG consumption associates with greater insulin sensitivity, less insulin resistance, and less type 2 diabetes observed in the current study, suggests that the effects of this dicarbonyl against type 2 diabetes progression are similar to those of MGO, and warrants further investigation.

Possible explanations of the association with higher MGO intake and less type 2 diabetes only after excluding participants with previously-diagnosed type 2 diabetes, are that the latter individuals have received treatment, including dietary advice and hence may have adapted their dietary behavior, causing reverse causality, or may be more likely to underreport their dietary intake. This is in line with the observation that generally all associations were stronger after exclusion of individuals with previously diagnosed diabetes. Furthermore, higher intake of MGO

was also associated with lower post-load glucose concentrations, and higher intakes of GO and 3-DG were associated with post-load glucose, fasting glucose and HbA1c, supporting the associations with (newly diagnosed) type 2 diabetes.

Dicarbonyl consumption was not consistently associated with indices of β-cell function. The association between higher 3-DG intake and higher β-cell glucose sensitivity is suggestive of a positive effect[23], whereas the associations of higher GO intake with lower potentiation factor is suggestive of a negative effect on β-cell function[36]. The latter is in line with an animal study that showed that MGO administration reduced insulin secretion[6]. All in all, we cannot conclude that there is an association between dicarbonyl intake and β-cell function in this study, because GO and 3-DG intakes were each only associated with one of the β-cell function indices and associations were in various directions.

A challenge in nutrition research it that it is not possible to identify the individual association of a single dietary compound on health outcomes. Food items with high dicarbonyl concentrations can contain other components that may protect against type 2 diabetes, for example anti-oxidants in coffee[37]. It is, however, unlikely that the associations observed in this study are completely attributable such anti-oxidants because coffee is a main contributor to MGO intake in this population, but does not greatly contribute to 3-DG intake[4]. Moreover, our explorative evaluations with sequential adjustment for dicarbonyl intake from major food groups, showed that the observed relationships were not driven by particular food groups. Likewise, the associations were independent of intake of other dietary macronutrients and of a general healthier diet. Taken together, both MGO and 3-DG, which are each present in different food items, are consistently associated with greater insulin sensitivity, less insulin resistance and less type 2 diabetes.

Strengths of this study are the availability of a large and deeply-phenotyped human cohort with validated, state-of-the-art techniques and extensive assessment of potential confounders. Impaired β-cell function and insulin sensitivity provide insight into the early processes of type 2 diabetes progression. It is reassuring that in the current study we observed an association with both insulin sensitivity, an early characteristic in the development of type 2 diabetes, and presence of the disease. Another strength is the application of the most extensive food composition database for dicarbonyls currently available, in which the highly-sensitive UPLC-MS/MS was used for quantification of MGO, GO and 3DG, and which was specifically developed to match the comprehensive 253-item FFQ used in this cohort. This study also has certain limitations. The food frequency questionnaire is a self-reported questionnaire, and underreporting or recall-bias may have occurred.

Nevertheless, food frequency questionnaires are the most commonly used method to assess food intake in large cohorts and are able to rank individuals from low to high intake. Additionally, the food composition database contains average values of dicarbonyls in foods and is unable to take effects of industrial and household food preparation on dicarbonyl intake into account. Nevertheless, both our dicarbonyl database and the food frequency questionnaire contain foods prepared using various common preparation methods, such as boiling and stir-frying, thereby covering some degree of variation[18]. Another limitation is the cross-sectional design of the study, which does not allow the assessment of causality. Nevertheless, participants completed the food frequency questionnaire before being informed about their glucose metabolism status, minimizing the risk of reporting bias. Moreover, when we excluded individuals with previously diagnosed type 2 diabetes from our analyses, the associations were similar. Thus, it is unlikely that diet adaptations caused by glucose metabolism status affected our results. Lastly, although we carefully adjusted for a large set of potential confounders, residual confounding remains a possibility.

In conclusion, we observed that higher habitual intakes of MGO, GO, and 3-DG were associated with less type 2 diabetes, and higher MGO and 3-DG were also associated with better insulin sensitivity/less insulin resistance. This suggests that food–derived dicarbonyls may play a protective role in type 2 diabetes. These novel observations require further exploration in prospective cohort and intervention studies.

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Supplementary tables

Table S5.1 Population characteristics of final study population and excluded individuals[1].

Characteristics	Final study population (n=6282)	Excluded individuals (n=1407)	P-included versus excluded
Demographics			
Age (years)	60±8.6	60±9.2	0.93
Sex (% male)	50	53	0.08
Glucose status			
Glucose metabolism status			<0.001
Normal glucose metabolism (%)	62	52	
Prediabetes (%)	15	14	
Type 2 diabetes mellitus (%)	23	31	
Newly diagnosed T2DM (%)	4	4	
Diabetes duration (years)	4 [0.0-10]	5 [1-11]	0.01
Lifestyle			
Smoking (%)			<0.001
Never	38	34	
Former	50	45	
Current	12	21	
Waist circumference (cm)	95±14	97±14	<0.001
BMI (kg/m^2)	27±4.4	28±4.9	<0.001
Physical activity (h/week)	13 [8.0-18]	12 [7.5-18]	0.03
Education (%)			<0.001
Low	33	41	
Medium	28	26	
High	39	33	
Biological			
Fasting glucose (mmol/L)	5.4 [5.0-6.2]	5.6 [5.0-6.9]	<0.001
HbA1c (%)	5.7±0.83	6.0±1.1	<0.001
24-h Systolic blood pressure (mmHg)	134±18	134±19	0.06
24-h Diastolic blood pressure (mmHg)	76±9.8	75±10	0.67
Cholesterol (mmol/L)	5.2±1.1	5.1±1.1	0.01
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	3.5 [2.8-4.3]	0.01
LDL (mmol/L)	3.1±1.0	3.0±1.0	0.03
Triglycerides (mmol/L)	1.2 [0.88-1.69]	1.2 [0.88-1.76]	0.12
eGFR (mL/min/1.73 m^2)	88±15	88±16	0.71
Medical history of CVD (% yes)	16	20	<0.001
Medical history of gastrointestinal disease (%yes)	11	13	0.11
Medication use			
Glucose-lowering medication (%yes)	17	29	<0.001
Anti-hypertensives (%yes)	37	44	<0.001
Lipid-mediating medication (%yes)	31	38	<0.001
Dietary intake			
Energy intake (kcal/day)	2139±594	2161±653	0.34
Carbohydrate, total (g/day)	230±69	231±77	0.54
Fat, total (g/day)	82±30	84±32	0.08
Protein (g/day)	85±23	84±24	0.94
Alcohol intake (g/day)	8.2 [1.6-18]	6.3 [0.81-17]	0.003
Dutch Healthy Diet Index	84±15	82±15	0.002
Dietary MGO (mg/day)	4.0±1.2	4.0±1.3	0.85
Dietary GO (mg/day)	3.6±0.96	3.6±1.24	0.88
Dietary 3-DG (mg/day)	16 [11-23]	15 [10-23]	0.09

Table S5.1 (continued)

Characteristics	Final study population (n=6282)	Excluded individuals (n=1407)	P-included versus excluded
Insulin sensitivity/resistance			
Matsuda index	3.5 [2.1-5.3]	3.3 [2.0-5.2]	0.06
HOMA-IR	1.4 [0.96-2.1]	1.5 [1.0-2.2]	0.01
β-cell function			
β-cell glucose sensitivity (pmol/min/m²/mM)	76 [45-109]	67 [39-100]	<0.001
β-cell potentiation factor	1.6±0.69	1.6±0.64	0.20
β-cell rate sensitivity (pmol/m²/mM)	532 [196-1012]	483 [136-952]	0.13
C-peptidogenic index (ΔCP_{30}:ΔG_{30} ratio)	465±1165	448±684	0.65
Overall insulin secretion (CP_{AUC}:G_{AUC} ratio)	197±81	188±77	0.02

[1] Data are presented as mean ± standard deviation, median [interquartile range] or percentage. P-value of t-test (for normally distributed continuous variables), Mann-Whitney U Test (for non-normally distributed continuous variables), or Chi Square test (for categorical variables) for differences between included and excluded individuals. 3-DG, 3-Deoxyglucosone; CVD, cardiovascular disease; DHD15-index: Dutch Healthy Diet-index 2015; eGFR, estimated glomerular filtration rate; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal; NGM, normal glucose metabolism; T2DM, type 2 diabetes.

Table S5.2 Population characteristics of subpopulations. [1]

Characteristics	Glucose metabolism population (n=6282)	Insulin sensitivity subpopulation (n=2390)	Insulin resistance subpopulation (n=2611)	β-cell function subpopulation (n=2336)
Demographics				
Age (years)	60±8.6	60±8.1	60±8.1	60±8.1
Sex (% male)	50	50	50	50
Glucose status				
Glucose metabolism status				
Normal glucose metabolism (%)	62	62	59	62
Prediabetes (%)	15	16	16	16
Type 2 diabetes mellitus (%)	23	22	26	22
Newly diagnosed T2DM (%)	4	4	4	4
Diabetes duration (years)	4 [0.0-10]	3 [0.0-7.0]	4 [1.0-11]	3 [0.0-7]
Lifestyle				
Smoking (%)				
Never	38	37	36	37
Former	50	52	52	52
Current	12	11	12	11
Waist circumference (cm)	95±14	95±13	96±13	95±13
BMI (kg/m²)	27±4.4	27±4.3	27±4.4	27±4.3
Physical activity (h/week)	13 [8.0-18]	13 [8.3-19]	13 [8.3-19]	13 [8.3-19]
Education (%)				
Low	33	31	32	31
Medium	28	28	29	28
High	39	41	40	41

Table S5.2 (continued)

Characteristics	Glucose metabolism population (n=6282)	Insulin sensitivity subpopulation (n=2390)	Insulin resistance subpopulation (n=2611)	β-cell function subpopulation (n=2336)
Biological				
Fasting glucose (mmol/L)	5.4 [5.0-6.2]	5.5 [5.0-6.1]	5.5 [5.0-6.4]	5.5 [5.0-6.2]
HbA1c (%)	5.7±0.83	5.7±0.63	5.8±0.84	5.7±0.83
24-h Systolic blood pressure (mmHg)	134±18	134±18	134±18	135±18
24-h Diastolic blood pressure (mmHg)	76±9.8	76±10	76±10	76±9.9
Cholesterol (mmol/L)	5.2±1.1	5.3±1.1	5.3±1.2	5.3±1.1
Total-to-HDL cholesterol ratio	3.4 [2.8-4.2]	3.5 [2.8-4.3]	3.5 [2.8-4.3]	3.5 [2.8-4.3]
LDL (mmol/L)	3.1±1.0	3.2±1.0	3.2±1.0	3.1±1.0
Triglycerides (mmol/L)	1.2 [0.88-1.7]	1.2 [0.88-1.7]	1.2 [0.88-1.7]	1.2 [0.89-1.7]
eGFR (mL/min/1.73 m^2)	88±15	88±14	88±14	88±15
Medical history of CVD (% yes)	16	15	16	15
Medical history of gastrointestinal disease (%yes)	11	12	12	12
Medication use				
Glucose-lowering medication (%yes)	17	15	19	15
Anti-hypertensives (%yes)	37	37	39	37
Lipid-mediating medication (%yes)	31	32	35	32
Dietary intake				
Energy intake (kcal/day)	2139±594	2191±603	2179±595	2191±604
Carbohydrate, total (g/day)	230±69	234±70	233±69	234±70
Fat, total (g/day)	82±30	85±31	84±31	85±31
Protein (g/day)	85±23	86±23	86±23	86±23
Alcohol intake (g/day)	8.2 [1.6-18]	8.9 [1.9-19]	8.6 [1.6-19]	9.0 [1.9-19]
Dutch Healthy Diet Index	84±15	84±15	84±15	84±15
Dietary MGO (mg/day)	4.0±1.2	4.1±1.2	4.1±1.2	4.1±1.2
Dietary GO (mg/day)	3.6±0.96	3.7±1.2	3.7±1.2	3.6±1.1
Dietary 3-DG (mg/day)	16 [11-23]	17 [12-23]	17 [12-23]	17 [12-23]
Insulin sensitivity/resistance				
Matsuda index	3.5 [2.1-5.3]	3.5 [2.1-5.3]	3.5 [2.0-5.1]	3.5 [2.1-5.3]
HOMA-IR	1.4 [0.96-2.1]	1.4 [1.0-2.1]	1.4 [1.0-2.1]	1.4 [0.96-2.1]
β-cell function				
β-cell glucose sensitivity (pmol/min/m^2/mM)	76 [45-109]	76 [45-109]	76 [45-112]	76 [45-109]
β-cell potentiation factor	1.6±0.69	1.6±0.70	1.6±0.70	1.6±0.70
β-cell rate sensitivity (pmol/m^2/mM)	539 [196-1012]	529 [196-1006]	529 [196-1006]	535 [202-1015]
C-peptidogenic index (ΔCP_{30}:ΔG_{30} ratio)	465±1165	464±1177	464±1177	469±1183
Overall insulin secretion (CP_{AUC}:G_{AUC} ratio)	197±81	196±81	196±81	197±81

[1] Data are presented as mean ± standard deviation, median [interquartile range] or percentage. 3-DG, 3-Deoxyglucosone; CVD, cardiovascular disease; DHD15-index: Dutch Healthy Diet-index 2015; eGFR, estimated glomerular filtration rate; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal; NGM, normal glucose metabolism; T2DM, type 2 diabetes.

Table S5.3 Associations of dietary dicarbonyls with outcomes after stratification for eGFR.[1]

	Associations of	eGFR<60 mL/min per 1.73 m^2 (n=114)	eGFR ≥ 60 mL/min per 1.73 m^2) (n=2614)
Categorical outcomes		**OR (95% CI)**	**OR (95% CI)**
	3-DG intake with type 2 diabetes	0.24 (0.09, 0.68)	0.88 (0.76, 1.03)
	3-DG intake with prediabetes	0.24 (0.06, 0.89)	0.89 (0.78, 1.03)
	3-DG intake with β-cell rate sensitivity [2]	0.45 (0.16, 1.29)	0.96 (0.85, 1.09)
Continuous outcomes		**β (95% CI)**	**β (95% CI)**
	3-DG intake with β-cell glucose sensitivity	0.27 (-0.001, 0.54)	0.06 (0.01, 0.10)
	3-DG intake with β-cell potentiation factor	0.13 (-0.14, 0.40)	-0.002 (-0.05, 0.04)
	3-DG intake with β-cell insulin secretion	0.10 (-0.22, 0.43)	-0.01 (-0.05, 0.04)
	GO intake with HOMA-IR	0.21 (-0.13, 0.55)	-0.03 (-0.08, 0.03)

[1] Associations for which a significant interaction with eGFR was observed were stratified for eGFR <60 and ≥ 60 mL/min per 1.73 m^2. Data were analyzed using linear regression analysis for continuous outcomes, and using nominal logistic regression analysis for categorical outcomes. β-cell glucose sensitivity, β-cell potentiation factor, and HOMA-IR were ln-transformed. Results are for fully adjusted model (model 3). [2] For β-cell rate sensitivity, OR represent odds of tertile 1 vs. tertile 3. 3-DG, 3-Deoxyglucosone; eGFR, estimated glomerular filtration rate; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

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Table S5.4 Associations of MGO, GO, and 3-DG intake with insulin sensitivity/resistance after exclusion of individuals with previously diagnosed diabetes. [1]

	Model	Insulin sensitivity/resistance	
		Matsuda Index [2] β (95% CI)	**HOMA-IR [3] β (95% CI)**
Dietary MGO			
	3	0.09 (0.04, 0.14)	-0.06 (-0.11, -0.02)
Dietary GO			
	3	0.10 (0.04, 0.16)	-0.07 (-0.12, -0.01)
Dietary 3-DG			
	3	0.10 (0.06, 0.14)	-0.06 (-0.10, -0.03)

[1] Data were analyzed using linear regression analyses. Standardized βs represent 1 SD higher ln-transformed Matsuda index or ln-transformed HOMA-IR per 1 SD higher dietary dicarbonyl intake. Results are for fully adjusted model (model 3). [2] N=1974 for Matsuda index. [3] N=2053 for HOMA-IR. 3-DG, 3-deoxyglucosone; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal.

Table S5.5 Associations of MGO, GO, and 3-DG intake with β-cell function after exclusion of individuals with previously diagnosed diabetes.[1]

		β-cell function						
		C-petidogenic index		Overall insulin secretion	Glucose sensitivity	Potentiation factor	Rate sensitivity	
	Model	Tertile 1 vs. 3 OR (95% CI)	Tertile 2 vs. 3 OR (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	Tertile 1 vs. 3 OR (95% CI)	Tertile 2 vs. 3 OR (95% CI)
Dietary MGO								
	3	0.94 (0.82, 1.09)	0.92 (0.80, 1.06)	-0.02 (-0.07, 0.03)	0.08 (-0.01, 0.10)	0.01 (-0.06, 0.06)	0.92 (0.80, 1.07)	0.99 (0.86, 1.14)
Dietary GO								
	3	0.90 (0.77, 1.07)	0.95 (0.80, 1.13)	-0.03 (-0.10, 0.03)	0.02 (-0.05, 0.09)	-0.13 (-0.19, -0.06)	0.95 (0.80, 1.13)	0.89 (0.75, 1.06)
Dietary 3-DG								
	3	1.02 (0.90, 1.15)	0.95 (0.84, 1.08)	-0.02 (-0.07, 0.03)	0.05 (-0.002, 0.10)	-0.01 (-0.06, 0.04)	0.99 (0.87, 1.12)	0.99 (0.89, 1.12)

[1] Data were analyzed using linear regression analysis for overall insulin secretion, ln-transformed glucose sensitivity and ln-transformed potentiation factor, and using nominal logistic regression analysis for rate sensitivity and C-peptidogenic index. Standardized βs represent 1 SD change in outcome per 1 SD higher dietary dicarbonyl intake. Odds ratio (OR) < 1.00 indicate a greater β-cell rate sensitivity or C-peptidogenic index and OR > 1.00 indicate a worse β-cell rate sensitivity or C-peptidogenic index. Results are for fully adjusted model (model 3), adjusted for adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake, education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use antihypertensive- or lipid-modifying medication and insulin sensitivity. N=1922. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Table S5.6 Association of dietary MGO, GO, and 3-DG with insulin sensitivity, insulin resistance and type 2 diabetes additionally adjusted for main food groups.[1]

	Model	% of MGO intake [2]	Insulin sensitivity (Matsuda index) β (95% CI)	Insulin resistance (HOMA-IR) β (95% CI)	Type 2 diabetes OR (95% CI)
Dietary MGO					
	Fully adjusted model (model 3) [3]		**0.08 (0.04, 0.12)**	**-0.05 (-0.09, -0.01)**	**-**
	Model 3 + Coffee	26	0.12 (0.05, 0.19)	-0.07 (-0.13, -0.01)	-
	Model 3 + Bread	25	0.08 (0.04, 0.12)	-0.05 (-0.08, -0.01)	-
	Model 3 + Vegetables and legumes	10	0.08 (0.03, 0.12)	-0.05 (-0.08, -0.01)	-
	Model 3 + Cookies and bakery products	9	0.08 (0.03, 0.13)	-0.05 (-0.09, -0.01)	-
	Model 3 + Meat	9	0.08 (0.04, 0.12)	-0.05 (-0.09, -0.01)	-
	Model 3 + Ready-made	6	0.08 (0.03, 0.12)	-0.05 (-0.09, -0.01)	-
Dietary GO					
	Fully adjusted model (model 3) [3]		**0.08 (0.02, 0.13)**	-	**0.87 (0.77, 0.99)**
	Model 3 + Bread	24	0.08 (0.02, 0.13)	-	0.84 (0.74, 0.95)
	Model 3 + Fruits	15	0.09 (0.03, 0.16)	-	0.84 (0.73, 0.97)
	Model 3 + Vegetables and legumes	13	0.07 (0.01, 0.13)	-	0.87 (0.76, 0.98)
	Model 3 + Ready-made	8	0.07 (0.01, 0.12)	-	0.87 (0.77, 0.98)
	Model 3 + Cookies and bakery products	7	0.07 (0.01, 0.13)	-	0.90 (0.79, 1.02)
	Model 3 + Bread condiments	7	0.08 (0.02, 0.14)	-	0.96 (0.83, 1.01)
Dietary 3-DG					
	Fully adjusted model (model 3) [3]		**0.09 (0.05, 0.13)**	**-0.04 (-0.08, -0.01)**	**0.85 (0.76, 0.95)**
	Model 3 + Bread	24	0.09 (0.05, 0.13)	-0.04 (-0.07, 0.00)	0.82 (0.73, 0.92)
	Model 3 + Cookies and bakery products	15	0.09 (0.04, 0.13)	-0.04 (-0.08, -0.003)	0.84 (0.75, 0.95)
	Model 3 + Sweets and chocolate	11	0.09 (0.05, 0.13)	-0.05 (-0.08, -0.01)	0.86 (0.77, 0.96)
	Model 3 + Ready-made	9	0.09 (0.05, 0.12)	-0.04 (-0.08, -0.01)	0.85 (0.76, 0.94)
	Model 3 + Bread condiments	8	0.08 (0.03, 0.12)	-0.04 (-0.07, -0.01)	0.88 (0.79, 0.99)
	Model 3 + Vegetables and legumes	6	0.09 (0.05, 0.13)	-0.04 (-0.07, -0.01)	0.85 (0.77, 0.95)
	Model 3 + Fruits	6	0.09 (0.05, 0.13)	-0.04 (-0.07, -0.01)	0.85 (0.76, 0.94)

[1] Data were analyzed using nominal regression analysis (for type 2 diabetes as outcome) or linear regression analyses (for continuous outcomes). Standardized βs represent 1 SD higher Matsuda index or HOMA-IR per 1 SD higher dietary dicarbonyl intake. Odds ratio (OR) < 1.00 indicate a lower odds of presence of type 2 diabetes and OR > 1.00 indicate a higher odds of presence of type 2 diabetes. N=2390 for Matsuda index and n=2611 for HOMA-IR and n=6282 for type 2 diabetes. [2] % of MGO intake via each food group (as reported in[4]). [3] Model 3: adjusted for age, sex, BMI, total energy intake, smoking status, alcohol intake, physical activity, educational level, triglycerides, systolic blood pressure, total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying drugs. Additionally, sequentially adjusted for dicarbonyl intake via each of the separate food groups that contribute ≥5% to total dicarbonyl intake. 3-DG: 3-Deoxyglucosone; GO, glyoxal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal.

Table S5.7 Associations of MGO, GO, and 3-DG intake with other measures of glucose metabolism.[1]

	Model	HbA1C β (95% CI)	Fasting glucose β (95% CI)	Post-load glucose β (95% CI)
Dietary MGO				
	3	0.002 (-0.02, 0.03)	0.004 (-0.02, 0.03)	**-0.07 (-0.10, -0.04)**
Dietary GO				
	3	**-0.04 (-0.07, -0.01)**	**-0.05 (-0.08, -0.02)**	**-0.04 (-0.07, -0.01)**
Dietary 3-DG				
	3	**-0.03 (-0.05, -0.003)**	**-0.04 (-0.06, -0.02)**	**-0.03 (-0.06, -0.01)**

[1] Data were analyzed using linear regression analyses. Standardized βs represent 1 SD higher outcome per 1 SD higher dietary dicarbonyl intake. Results are for fully adjusted model (model 3). N=6275 for HbA1c; N=6280 for fasting glucose; N=5967 for post-load glucose. 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

Table S5.8 Sensitivity analyses for the associations between dietary dicarbonyls and insulin sensitivity.[1]

		Outcome: Matsuda index	
Dietary MGO	**N**	**β for dietary MGO**	**95% CI**
Model 3 [2]	2390	**0.08**	**0.04, 0.12**
Kcal + DHD15	2390	0.08	0.03, 0.12
Kcal + carbohydrates	2390	0.08	0.04, 0.13
Kcal + protein	2390	0.08	0.03, 0.12
Kcal + fat	2390	0.08	0.03, 0.13
Without adjustment BMI	2390	0.07	0.03, 0.12
Exclusion GI disease	2136	0.08	0.03, 0.13
Education > income	1969	0.09	0.04, 0.14
Education > occupational status	2017	0.06	0.02, 0.11
CHAMPS > ActivPAL	2069	0.09	0.04, 0.14
Dietary GO	**N**	**β for dietary GO**	**95% CI**
Model 3 [2]	2390	**0.08**	**0.02, 0.13**
Kcal + DHD15	2390	0.06	0.01, 0.12
Kcal + carbohydrates	2390	0.10	0.03, 0.17
Kcal + protein	2390	0.08	0.02, 0.13
Kcal + fat	2390	0.08	0.02, 0.15
Without adjustment BMI	2390	0.11	0.05, 0.16
Exclusion GI disease	2136	0.08	0.02, 0.13
Education > income	1969	0.06	-0.004, 0.12
Education > occupational status	2017	0.07	0.01, 0.13
CHAMPS > ActivPAL	2069	0.10	0.04, 0.15
Dietary 3-DG	**N**	**β for dietary 3-DG**	**95% CI**
Model 3 [2]	2390	**0.09**	**0.05, 0.13**
Kcal + DHD15	2390	0.09	0.05, 0.13
Kcal + carbohydrates	2390	0.09	0.05, 0.14
Kcal + protein	2390	0.09	0.05, 0.13
Kcal + fat	2390	0.09	0.05, 0.13
Without adjustment BMI	2390	0.11	0.07, 0.15
Exclusion GI disease	2136	0.09	0.05, 0.13
Education > income	1969	0.08	0.03, 0.12
Education > occupational status	2017	0.09	0.04, 0.13
CHAMPS > ActivPAL	2069	0.07	0.03, 0.12

[1] Data were analyzed using linear regression analysis. Standardized βs (β) represent the 1 SD increase in ln-transformed insulin sensitivity per 1 SD higher dietary dicarbonyl intake. [2] Model 3: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake and education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying medication. 3-DG: 3-Deoxyglucosone; DHD15: Dutch Healthy Diet Index 2015; GI: gastrointestinal; GO: glyoxal; MGO: methylglyoxal.

Table S5.9 Sensitivity analyses for the associations between dietary dicarbonyls and insulin resistance.[1]

		Outcome: HOMA-IR	
Dietary MGO	**N**	**β for dietary MGO**	**95% CI**
Model 3 [2]	**2611**	**-0.05**	**-0.09, -0.01**
Kcal + DHD15	2611	-0.05	-0.08, -0.01
Kcal + carbohydrates	2611	-0.04	-0.08, -0.01
Kcal + protein	2611	-0.05	-0.09, -0.01
Kcal + fat	2611	-0.04	-0.08, -0.003
Without adjustment BMI	2611	-0.04	-0.08, 0.001
Exclusion GI disease	2334	-0.05	-0.09, -0.01
Education > income	2148	-0.05	-0.10, -0.01
Education > occupational status	2206	-0.04	-0.08, 0.001
CHAMPS > ActivPAL	2267	-0.06	-0.10, -0.02
Dietary 3-DG	**N**	**β for dietary 3-DG**	**95% CI**
Model 3 [2]	**2611**	**-0.04**	**-0.08, -0.01**
Kcal + DHD15	2611	-0.04	-0.07, -0.01
Kcal + carbohydrates	2611	-0.04	-0.07, -0.002
Kcal + protein	2611	-0.04	-0.08, -0.01
Kcal + fat	2611	-0.04	-0.07, -0.001
Without adjustment BMI	2611	-0.06	-0.10, -0.03
Exclusion GI disease	2334	-0.05	-0.09, -0.02
Education > income	2148	-0.03	-0.07, 0.003
Education > occupational status	2206	-0.04	-0.08, -0.01
CHAMPS > ActivPAL	2267	-0.04	-0.08, -0.01

[1] Data were analyzed using linear regression analysis. Standardized βs (β) represent the 1 SD increase in ln-transformed insulin resistance per 1 SD higher dietary dicarbonyl intake. [2] Model 3: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake and education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying medication. 3-DG: 3-Deoxyglucosone; DHD15: Dutch Healthy Diet Index 2015; GI: gastrointestinal; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; MGO, methylglyoxal.

Table S5.10 Sensitivity analyses for the associations between dietary dicarbonyls and β-cell potentiation factor.[1]

		Outcome: β-cell potentiation factor	
Dietary GO	**N**	**β**	**95% CI**
Model 3 [2]	**2336**	**-0.07**	**-0.13, -0.01**
Kcal + DHD15	2336	-0.08	-0.14, -0.02
Kcal + carbohydrates	2336	-0.06	-0.13, 0.02
Kcal + protein	2336	-0.07	-0.13, -0.01
Kcal + fat	2336	-0.07	-0.15, 0.003
Without adjustment BMI	2336	-0.06	-0.12, -0.003
Exclusion GI disease	2088	-0.06	-0.12, 0.01
Education > income	1927	-0.06	-0.13, 0.003
Education > occupational status	1973	-0.07	-0.13, 0.000
CHAMPS > ActivPAL	2012	-0.08	-0.14, -0.01

[1] Data were analyzed using linear regression analysis. Standardized βs (β) represent the 1 SD increase in outcome per 1 SD higher dietary dicarbonyl intake. [2] Model 3: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake and education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying medication and insulin sensitivity. DHD15: Dutch Healthy Diet Index 2015; GI: gastrointestinal; GO: glyoxal.

Table S5.11 Sensitivity analyses for the associations between dietary dicarbonyls and β-cell glucose sensitivity.[1]

		Outcome: β-cell glucose sensitivity	
Dietary 3-DG	**N**	**Std. β for dietary 3-DG**	**95% CI**
Model 3 [2]	**2336**	**0.06**	**0.02, 0.11**
Kcal + DHD15	2336	0.06	0.02, 0.11
Kcal + carbohydrates	2336	0.05	0.01, 0.10
Kcal + protein	2336	0.06	0.01, 0.10
Kcal + fat	2336	0.06	0.01, 0.11
Without adjustment BMI	2336	0.06	0.02, 0.11
Exclusion GI disease	2088	0.06	0.02, 0.11
Education > income	1927	0.06	0.01, 0.11
Education > occupational status	1973	0.05	0.004, 0.10
CHAMPS > ActivPAL	2012	0.04	-0.004, 0.09

[1] Data were analyzed using linear regression analysis. Standardized βs (β) represent the 1 SD increase in outcome per 1 SD higher dietary dicarbonyl intake. [2] Model 3: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake and education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use of antihypertensive- or lipid-modifying medication and insulin sensitivity. 3-DG: 3-Deoxyglucosone; DHD15: Dutch Healthy Diet Index 2015; GI: gastrointestinal.

Table S5.12 **Sensitivity analyses for the associations between dietary dicarbonyls and type 2 diabetes.[1]**

		Outcome: type 2 diabetes	
Dietary GO	**N**	**OR for dietary GO**	**95% CI**
Model 3 [2]	**6282**	**0.87**	**0.77, 0.99**
Kcal + DHD15	6282	0.98	0.86, 1.10
Kcal + carbohydrates	6282	0.94	0.81, 1.10
Kcal + protein	6282	0.77	0.65, 0.93
Kcal + fat	6282	0.88	0.78, 1.00
Without adjustment BMI	6282	0.82	0.73, 0.93
Exclusion GI disease	5611	0.92	0.81, 1.05
Education > income	5102	0.93	0.81, 1.07
Education > occupational status	2320	0.89	0.73, 1.09
CHAMPS > ActivPAL	5917	0.93	0.82, 1.06
Dietary 3-DG	**N**	**OR for dietary 3-DG**	**95% CI**
Model 3 [2]	**6282**	**0.85**	**0.76, 0.95**
Kcal + DHD15	6282	0.85	0.76, 0.95
Kcal + carbohydrates	6282	0.88	0.78, 0.98
Kcal + protein	6282	0.86	0.77, 0.96
Kcal + fat	6282	0.86	0.77, 0.97
Without adjustment BMI	6282	0.80	0.72, 0.89
Exclusion GI disease	5611	0.86	0.77, 0.97
Education > income	5102	0.89	0.79, 1.00
Education > occupational status	2320	0.88	0.75, 1.03
CHAMPS > ActivPAL	5917	0.87	0.78, 0.97

[1] Data were analyzed using logistic regression analysis. Odds ratio (OR): Values < 1.00 indicate a lower odds of prediabetes or type 2 diabetes. [2] Model 3: adjusted for age, sex, BMI, smoking, alcohol intake, physical activity, total energy intake and education level, history of cardiovascular diseases, triglycerides, LDL, total cholesterol/HDL ratio, use antihypertensive- or lipid-modifying medication. 3-DG: 3-Deoxyglucosone, DHD15: Dutch Healthy Diet Index 2015, GI: gastrointestinal, GO: glyoxal.

Chapter 6

High dietary glycemic load is associated with higher concentrations of urinary advanced glycation endproducts: The CODAM Study

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Abstract

Background

Advanced glycation endproducts (AGEs) and their precursors (dicarbonyls) are associated with the progression of diseases such as diabetes and cardiovascular disease. Plasma concentrations of the dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) are increased after an oral glucose load indicating that consumption of diets high in carbohydrates may induce endogenous formation of dicarbonyls and AGEs.

Objective

To examine the associations of dietary glycemic index (GI) and glycemic load (GL) with concentrations of dicarbonyls and AGEs in plasma and urine.

Design

Cross-sectional analyses were performed in a human observational cohort [CODAM, n=494, 59±7 years, 25% type 2 diabetes]. GI and GL were derived from Food Frequency Questionnaires. Dicarbonyls and AGEs were measured in the fasting state by UPLC-MS/MS. MGO, GO and 3-DG and protein-bound Nε-(carboxymethyl)lysine (CML), Nε-(1-carboxyethyl)lysine (CEL) and pentosidine were measured in plasma. Free forms of CML, CEL and Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) were measured in both plasma and urine. Multiple linear regression was performed with dicarbonyls and AGEs as dependent variables, and dietary GI or GL as main independent variables (all standardized). Models were adjusted for health and lifestyle factors, dietary factors and reciprocally for GI and GL. As this was an explorative study, we did not adjust for multiple testing.

Results

GI was not associated with any of the dicarbonyls or AGEs. GL was positively associated with free urinary MG-H1 (β=0.34, 95% CI (0.12, 0.55)). Furthermore, GL was positively associated with free plasma MG-H1 and free urinary CML (β=0.23, 95% CI (0.02,0.43) and β=0.28, 95% CI (0.06, 0.50)), but these associations were not independent of dietary AGE intake.

Conclusions

A habitual diet higher in GL is associated with higher concentrations of free urinary MG-H1. This urinary AGE is most likely a reflection of AGE accumulation and degradation in tissues, where they may be involved in tissue dysfunction.

Introduction

Advanced glycation endproducts (AGEs) are a heterogeneous group of sugar-modified proteins. The formation of AGEs occurs, at least partly, via the reactive AGE precursors methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG). AGEs are found in the body in both tissue and plasma. Plasma AGEs are present as either glycated proteins or as free glycated amino acids. Free glycated amino acids are a reflection of the degradation of protein-bound tissue AGEs and are excreted in urine[1-3]. Accumulation of AGEs and their precursors plays a role in the development of several age-related chronic inflammatory diseases such as diabetes[4-7], cardiovascular disease[8-12], cancer[13,14] and disorders of the central nervous system[15].

Dicarbonyls are glycolytic intermediates in the metabolic conversion of glucose. We previously reported increased plasma concentrations of MGO, GO and 3-DG after an oral glucose tolerance test (OGTT), which were higher in type 2 diabetes (T2DM) than in controls[16]. Glucose was the primary source of these dicarbonyls and the results were replicated after a mixed-meal test[17] and in patients with type 1 diabetes[18]. Postprandial blood glucose concentrations are primarily affected by carbohydrate consumption. Carbohydrates differ in their ability to affect blood glucose and for this reason the concepts of glycemic index (GI) and glycemic load (GL) have been introduced. Glycemic index (GI) is defined as the potential of carbohydrate-containing foods to increase postprandial blood glucose[19]. GL additionally takes into account the amount of carbohydrates a product contains, and is therefore indicative of not only quality, but also quantity of carbohydrates[20]. In animal feeding studies, diets with a high GI have been linked to AGE accumulation in plasma and tissue[21-23].

Based on the above, we hypothesized that a habitual diet high in GI and/or GL results, via induction of higher postprandial glucose concentrations, in higher dicarbonyls and AGE formation. To investigate the association of dietary carbohydrates on dicarbonyl and AGE accumulation in humans, we examined whether individuals who consume a habitual diet with a high GI or GL had higher concentrations of dicarbonyls and their derived AGEs in their plasma and/or urine.

Subjects and methods

Study population

Cross-sectional analyses were performed in the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM), which includes 574 individuals with a moderately increased risk for type 2 diabetes and cardiovascular disease as

described in detail elsewhere[24]. In short, participants are of Caucasian descent and >40 years of age with one or more of the following characteristics: BMI >25 kg/m^2; use of antihypertensive medication; positive family history of T2DM; postprandial blood glucose level >6.0 mmol/L; history of gestational diabetes and/or glycosuria.

For the present evaluation, individuals were excluded when they reported an unrealistic dietary intake (<800 kcal/day or more than 4200 kcal/day for men and <500 or >3500 kcal/day for women, n=6), the Food Frequency Questionnaire contained >10% missing values (n=56), or data on important variables was missing (n=18). Hence, 494 individuals were included in the current evaluation (see flowchart Figure 6.1). The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre. All participants gave written informed consent.

Primary outcomes of this study were plasma and/ or urinary dicarbonyls and AGEs.

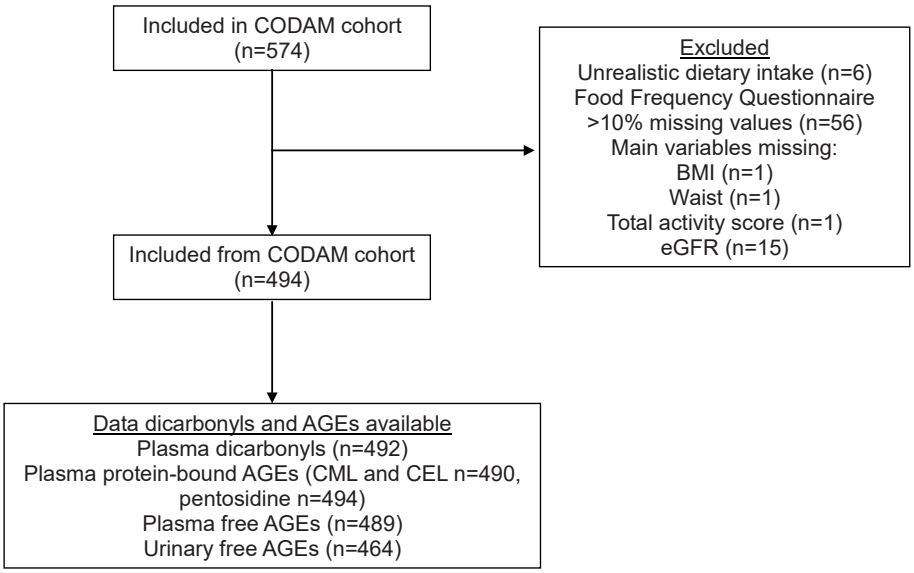

Figure 6.1 Flowchart of study participants.

Dietary assessment

Habitual dietary intake over the past 12 months was estimated by a semi-quantitative 178 food item food frequency questionnaire (FFQ), developed for

Dutch cohorts[25]. The FFQ was previously validated (Spearman's rho: 0.51-0.79) for nutrient intake against 24-hours recalls[25].

Dietary GI and GL were calculated according to the formulas below:

$$GI = \sum_{i=1}^{n} (GIi \times CHOi) / \sum_{i=1}^{n} CHOi \qquad GL = \sum_{i=1}^{n} (GIi \times CHOi) / \sum_{i=1}^{n} / 100$$

where GI*i* is the GI value of food *i* of the GI database in which 50 g glucose was used as reference food[26]. CHO*i* is the amount of available carbohydrates from food *i* calculated as the amount of food consumed (g/d) multiplied by the carbohydrate content from the Dutch food-composition table (NEVO) (g/g), and n is the number of foods eaten per day[27]. GI and GL estimated by this FFQ have been validated against multiple 24 h recalls (r=0.63)[28]. Intakes of energy and nutrients (including alcohol) were calculated according to the extended version of the NEVO 2001. Dietary intake of the AGEs N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), N_{ε}-(carboxymethyl)lysine (CML) and N_{ε}-(1-carboxyethyl)lysine (CEL) was estimated using FFQ data and a dietary AGE database, as previously described[29].

Quantification of dicarbonyls and AGEs in plasma and urine

After an overnight fast, plasma EDTA was obtained from venous blood and stored at -80°C until analysis. Morning urine was also collected and was stored at -20°C until analysis.

Dicarbonyls (MGO, GO and 3-DG), protein-bound AGEs (CML, CEL and pentosidine) and free AGEs (CML, CEL and MG-H1) were measured in plasma. Briefly, for protein-bound and free AGEs, 25 µL and 50 µL plasma was used, respectively. Protein-bound pentosidine was measured using HPLC with a fluorescent detector, all other measurements were done using UPLC-MS/MS. Intra- and inter-assay coefficients of variation (CVs) for pentosidine were 6.5% and 3.1%, respectively. The intra- and inter-assay CVs of protein-bound CML and CEL were between 4.8 and 9.7% and for free CML, CEL and MG-H1 between 2.8 and 7.1%[30].

The free AGEs (CML, CEL and MG-H1) were quantified in 40 µL of urine using UPLC-MS/MS. In urine, the intra- and inter-assay CVs of free CML, CEL and MG-H1 were between 3.7 and 6.6%. To correct for urine concentration, creatinine concentration in urine was analysed based on the Jaffé reaction method[31], and AGE concentrations were expressed as nmol/mmol creatinine.

Assessment of non-dietary covariates

The glucose metabolism status was ascertained on the basis of a standard 75 g OGTT according to the WHO criteria of 1999, as described in detail elsewhere[32]. Individuals were classified as having normal glucose metabolism (NGM), impaired glucose metabolism (IGM, combining impaired fasting glucose [IFG] and impaired glucose tolerance [IGT]) or T2DM. BMI (kg/m^2), waist circumference (cm) and blood pressure (mmHg) were measured as previously described[33,34]. Questionnaires were used to assess smoking behavior (current smoker yes/no), habitual physical activity (total of all activities combined per day) and use of medication (yes/no for the following; lipid-modifying, glucose- and blood pressure-lowering medication). Estimated glomerular filtration rate (eGFR) was calculated with the MDRD formula for isotope dilution mass spectrometry (IDMS) standardized creatinine values[35].

Statistical analysis

The general characteristics of the study population were compared across tertiles of both dietary GI and GL. Differences in characteristics between tertiles were tested using one-way ANOVA for normally distributed continuous variables, Kruskal-Wallis test for non-normally distributed continuous variables and χ^2 test for discrete variables. Reciprocal relationships of individual dicarbonyls and AGEs were examined using Spearman's correlation coefficient. Skewed variables (plasma and urinary dicarbonyls and AGEs, total activity score) were ln-transformed prior to further analyses.

The intake of nutrients was adjusted using the multivariate nutrient density method (expressed as percentage of energy for fat, protein and carbohydrate; expressed as amount per 10 MJ of energy for dietary fiber and GL)[36]. Dietary GI and energy-adjusted dietary GL (main independent variables) as well as dicarbonyls and AGEs in plasma and urine (outcome variables) were standardized to allow direct comparison of the strength of associations.

The associations of GI and GL with dicarbonyls and AGEs were examined in multiple linear regression analyses. Associations were first adjusted for age (years) and sex (men/women) [Model1]. Next additional adjustments were made for potential confounders related to personal health and lifestyle i.e. glucose metabolism status (IGM or T2DM as dummy variables with NGM as reference category), kidney function (eGFR in ml/min/1.73cm^2), BMI (kg/m^2), current smoking (yes/no), alcohol intake (low <20 g/d for women, <30 g/d for men and high >20 g/d for women, and >30 g/d for men), physical activity (total score of all

activities), use of medication (yes/no for lipid-mediating, glucose- and blood pressure lowering) [Model 2]. Last, the analyses were additionally adjusted for potential dietary confounders i.e. total energy intake (kcal/day), fat intake (en%), protein intake (en%), fiber intake (per 10 MJ), and carbohydrate intake (en%; only in the analyses with dietary GI as main independent variable, to evaluate carbohydrate quality independent of total carbohydrate) or GI (only in the analyses with dietary GL as main independent variable, to evaluate carbohydrate load independent of carbohydrate quality) [Model 3]. The associations obtained in the fully adjusted model (model 3) are considered as least likely to be confounded and conclusions have been based on these associations. All models complied with the assumptions of linearity, normal distribution of residuals, homoscedasticity, and no multicollinearity.

Next, differences in the strength of the associations between NGM, IGM and T2DM patients were evaluated by adding interaction terms between glucose metabolism status and GI or GL in the fully adjusted models of the main analyses and analyses were redone after stratification for glucose metabolism status. Also, differences in the strength of the associations between men and women were evaluated by adding interaction terms to the fully adjusted models. We found no such interaction ($P_{interaction}>0.1$), and therefore all results are presented for men and women combined.

Several additional analyses were performed to address the robustness of the outcomes of the main analyses. First, the fully adjusted analyses with GL as main independent variable were repeated without additional adjustment for GI, to evaluate the combined effect of both carbohydrate load and carbohydrate quality. Second, analyses were repeated with carbohydrate intake (en%, standardized) as main independent variable. Third, when significant associations were observed in the main analyses, additional adjustment for intake of dietary AGEs was done. Last, energy adjusted values were replaced by nutrient residuals , computed by regressing absolute nutrient intake on total energy intake[36]. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 24.0) and statistical significance was set at $p<0.05$, except for testing for interaction where statistical significance was set at $p<0.10$.

Results

Characteristics of the study population

The total number of participants in the current evaluation was 494, of whom 263 had NGM, 112 had IGM and 119 were diagnosed with type 2 diabetes. Plasma

dicarbonyls were available for 492 participants, protein-bound CML and CEL for 490 and protein-bound pentosidine for 494. Free plasma AGEs were available for 489 participants and free urinary AGEs for 464 (see Figure 6.1).

General characteristics of the study population according to tertiles of GI and GL are shown in Table 6.1. Participants in the higher tertiles of GI were more often men and had a larger waist circumference. Dietary GL and fat were higher in those with higher GI. Intake of dietary CML and mono- and disaccharides were lower, while intake of polysaccharides was higher in those with higher GI. Participants in the higher tertiles of GL were more often men. Dietary GI and total energy intake, as well as intake of other macro- and micronutrients and dietary AGEs, was higher for those with higher dietary GL.

Dicarbonyls and AGEs according to tertiles of GI and GL are shown in Table 6.2. Supplementary Table S6.1 shows the reciprocal correlations between individual dicarbonyls and AGEs in plasma and urine. In plasma, free AGEs correlated more strongly with each other than did protein-bound AGEs and dicarbonyls. Correlations between plasma free and protein-bound AGEs were weak. Moreover, free urinary AGEs were strongly correlated with free plasma AGEs.

Associations between glycemic index and dicarbonyls and AGEs

Dietary GI was not associated with any of the dicarbonyls or AGEs that we measured in this cohort. This was the case for all models in the total population (Supplementary Table S6.2). Interaction between glucose metabolism status and GI ranged between p=0.02-0.94 and only reached statistical significance ($P_{interaction}$ <0.1) for dicarbonyls 3-DG and MGO and protein-bound CEL in plasma. After stratification for glucose metabolism status, GI was inversely associated with plasma MGO in the IGM group. GI was positively associated with protein-bound plasma CEL in the NGM group. Also, GI was inversely associated with free plasma and urinary CML in the DM group (data not shown).

Table 6.1 General characteristics of the study population across tertiles of glycemic index and glycemic load.[1]

	GI			GL		
	Low (45-57, n=163)	**Mid (57-60, n=165)**	**High (60-68, n=166)**	**Low (35-121, n=162)**	**Mid (121-154, n=166)**	**High (154-304, n=166)**
Age (years)	60±7	58±7	59±7	60±7	60±6	58±7
Sex (%men)	49	60	74	47	61	75
NGM/IGM/T2DM (%)	53/26/21	52/25/23	54/17/29	46/26/28	52/22/26	62/19/19
HOMA-IR	1.51 [1.0-2.25]	1.62 [1.12-2.42]	1.65 [1.18-2.72]	1.5 [1.0-2.7]	1.6 [1.1-2.4]	1.7 [1.1-2.5]
BMI (kg/m^2)	28±4	28±4	29±4	29±5	28±4	28±4
Waist circumference (cm)	97.3±12.1	97.9±11.4	101.4±11.5	98.9±13	97.5±10	100.3±12
SBP (mmHg)	141±18	139±19	140±19	142±19	141±18	137±18
DBP (mmHg)	82±9	81±10	81±8	82±9	81±9	82±9
eGFR (ml/min/1.73cm^2)	91±17	91±17	92±21	92±19	89±17	93±19
Fasting triglycerides (mmol/L)	1.4 [1.0-1.95]	1.4 [1.0-1.9]	1.5 [1.0-2.0]	1.4 [0.9-1.9]	1.4 [1.1-2.0]	1.5 [1.0-2.0]
Use of medication						
Anti-hypertensive (%)	40	39	35	40	42	33
Lipid-modifying (%)	20	21	19	20	21	18
Glucose-lowering (%)	13	13	13	14	15	10
Total activity score (10^3 min*intensity/week)	6.4 [3.9-8.9]	6.4 [3.9-8.7]	5.4 [3.3-8.4]	5.0 [3.3-8.1]	6.9 [4.8-9.3]	6.0 [3.6-8.7]
Current smokers (%)	16	22	24	18	18	27
Total alcohol intake (g/d)	10 [2-24]	10 [2-26]	6 [1-18]	9 [1-25]	8 [2-23]	8 [2-21]
Total energy intake (kcal/d)	2127±577	2247±580	2256±649	1678±369	2142±355	2802±446
Glycemic load	126±38	144±39	153±46	-	-	-
Glycemic index	-	-	-	57±4	58±3	59±3
Nutrient intake (g/d)						
Total carbohydrate intake	231±67	247±67	246±75	170±32	235±18	318±47
Mono- and disaccharides	114±38	110±36	93±40	75±26	102±23	138±37
Polysaccharides	117±39	137±39	153±44	95±22	132±20	180±33
Total fat intake	83±30	90±29	94±32	67±19	86±25	114±27
Saturated fat	34±13	36±12	38±15	27±8	35±11	46±12
Mono-unsaturated fat	29±11	31±11	32±11	23±8	29±9	39±10
Poly-unsaturated fat	17±7	18±7	19±7	13±4	17±6	23±6
Total protein intake	85±23	83±20	85±22	70±17	82±16	101±19
Total fiber intake	25±7	26±7	26±7	20±5	25±4	31±6
Dietary CML (mg/day)	3.3±1.1	3.1±0.9	2.9±1.0	2.3±0.7	3.0±0.8	3.9±0.9
Dietary CEL (mg/day)	2.3±0.8	2.4±0.7	2.3±0.8	1.8±0.6	2.3±0.6	2.9±0.7
Dietary MG-H1 (mg/day)	21.3±7.0	22.2±6.4	21.9±7.0	16.7±4.4	21.2±4.4	27.4±6.4

[1]Values are mean± SD for normally distributed variables, median [interquartile range] for skewed variables or proportion (%) for categorical variables. CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GI, glycemic index, GL, glycemic load; GO, glyoxal; IGM, impaired glucose metabolism; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; MGO, methylglyoxal; NGM, normal glucose metabolism; SBP, systolic blood pressure; T2DM, type 2 diabetes

Table 6.2 Dicarbonyls and AGEs in plasma and urine across tertiles of dietary glycemic index and glycemic load.[1]

	GI			GL		
	Low (45-57, n=163)	**Mid (57-60, n=165)**	**High (60-68, n=166)**	**Low (35-121, n=162)**	**Mid (121-154, n=166)**	**High (154-304, n=166)**
Plasma dicarbonyls						
MGO (nmol/L)	360 [309-403]	352 [322-410]	356 [305-404]	356 [306-405]	371 [323-416]	344 [311-395]
GO (nmol/L)	1105 [922-1350]	1059 [898-1349]	1046 [895-1268]	1075 [912-1329]	1104 [925-1356]	1039 [873-1260]
3-DG (nmol/L)	1158 [1067-1362]	1162 [1050-1322]	1166 [1041-1386]	1182 [1083-1443]	1154 [1030-1326]	1156 [1050-1318]
Plasma protein-bound AGEs						
Plasma CML (nmol/mmol lysine)	33 [29-41]	35 [31-42]	35 [29-40]	34 [29-40]	35 [31-41]	34 [29-41]
Plasma CEL (nmol/mmol lysine)	24 [19-29]	23 [18-28]	24 [20-29]	23 [18-28]	24 [19-30]	24 [19-29]
Plasma pentosidine (nmol/mmol lysine)	0.45 [0.38-0.53]	0.43 [0.36-0.51]	0.42 [0.35-0.55]	0.45 [0.36-0.51]	0.44 [0.38-0.55]	0.41 [0.35-0.50]
Plasma free AGEs						
Free CML (nmol/L)	77 [58-100]	79 [63-98]	77 [59-98]	77 [56-100]	77 [63-99]	79 [61-97]
Free CEL (nmol/L)	44 [36-56]	45 [38-58]	46 [36-61]	43 [36-56]	46 [37-60]	46 [38-56]
Free MG-H1 (nmol/L)	118 [85-170]	120 [86-189]	125 [88-168]	113 [82-165]	121 [85-180]	126 [90-179]
Urinary free AGEs						
Urinary CML (nmol/mmol creatinine)	957 [715-1245]	940 [770-1204]	922 [690-1150]	949 [732-1316]	930 [733-1159]	948 [736-1178]
Urinary CEL (nmol/mmol creatinine)	526 [395-646]	511 [409-646]	504 [402-626]	514 [415-646]	517 [396-620]	503 [391-653]
Urinary MG-H1 (nmol/mmol creatinine)	2260 [1583-3489]	2259 [1617-3420]	2260 [1541-3055]	2294 [1553-3330]	2113 [1467-3183]	2293 [1686-3318]

[1]Values are median [interquartile range]. Plasma dicarbonyls (n=492), plasma protein-bound AGEs (CML and CEL n=490, pentosidine n=494), plasma free AGEs (n=489), urinary free AGEs (n=464). AGE, advanced glycation endproduct; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; GI, glycemic index, GL, glycemic load; GO, glyoxal; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithin.

Associations between glycemic load and dicarbonyls and AGEs

Dietary GL was not associated with plasma dicarbonyls (Table 6.3). There was a positive association between GL and plasma protein-bound CML in the age- and sex-adjusted analyses (β (95%CI)=0.12 (0.04, 0.21), model 1), which was attenuated after adjustment for health- and lifestyle factors (β (95%CI)=0.07 (-0.03,

0.16), model 2). GL was not associated with protein-bound CEL and pentosidine in plasma.

Table 6.3 Associations between dietary glycemic load and plasma dicarbonyls, plasma protein-bound, free and urinary AGEs in the total population.[1]

Model	β	95%CI	P-value
Plasma dicarbonyls			
MGO			
model 1	-0.012	-0.102, 0.079	0.802
model 2	-0.010	-0.107, 0.087	0.834
model 3	-0.036	-0.250, 0.177	0.738
GO			
model 1	-0.048	-0.134, 0.039	0.279
model 2	-0.072	-0.167, 0.023	0.137
model 3	-0.169	-0.378, 0.039	0.111
3-DG			
model 1	-0.077	-0.166, 0.011	0.085
model 2	-0.036	-0.103, 0.032	0.299
model 3	-0.072	-0.219, 0.076	0.341
Plasma protein-bound AGEs			
CML			
model 1	0.124	0.037, 0.211	**0.005**
model 2	0.066	-0.025, 0.157	0.152
model 3	0.067	-0.133, 0.267	0.511
CEL			
model 1	0.065	-0.024, 0.153	0.150
model 2	0.043	-0.058, 0.144	0.401
model 3	0.014	-0.208, 0.235	0.902
Pentosidine			
model 1	-0.016	-0.104, 0.073	0.730
model 2	-0.037	-0.131, 0.058	0.447
model 3	-0.158	-0.364, 0.047	0.131
Plasma free AGEs			
Free CML			
model 1	0.082	-0.004, 0.169	0.061
model 2	0.007	-0.088, 0.102	0.882
model 3	0.097	-0.113, 0.308	0.364
Free CEL			
model 1	0.090	0.004, 0.177	**0.041**
model 2	0.056	-0.037, 0.150	0.239
model 3	0.067	-0.140, 0.275	0.524
Free MG-H1			
model 1	0.183	0.099, 0.267	**<0.001**
model 2	0.105	0.012, 0.198	**0.027**
model 3	0.227	0.023, 0.432	**0.030**
Urinary free AGEs			
Urinary CML			
model 1	0.048	-0.043, 0.139	0.299
model 2	0.042	-0.062, 0.145	0.429
model 3	0.279	0.058, 0.500	**0.013**

Table 6.3 (continued)

Model	β	95%CI	P-value
Urinary CEL			
model 1	0.005	-0.088, 0.098	0.913
model 2	0.013	-0.091, 0.116	0.811
model 3	0.166	-0.055, 0.387	0.142
Urinary MG-H1			
model 1	0.155	0.064, 0.245	**0.001**
model 2	0.123	0.019, 0.226	**0.020**
model 3	0.335	0.117, 0.554	**0.003**

[1]βs are standardized regression coefficients and represent the change in (standardized ln-transformed) dicarbonyls and AGEs per 1 unit increase in standardized GL, as tested by multiple linear regression. Model 1: Standardized value of GL (amount per 10 MJ) adjusted for age + sex. Model 2: model 1 + eGFR + glucose metabolism status + BMI + alcohol intake (grouped) + physical activity + smoking + use of medication. Model 3: model 2 + total energy intake (kcal/day) + fat (en%) + protein (en%) + fiber (amount per 10 MJ) intake + GI. AGE, advanced glycation endproduct; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine. Plasma dicarbonyls (n=492), plasma protein-bound AGEs (CML and CEL n=490, pentosidine n=494), plasma free AGEs (n=489), urinary free AGEs (n=464).

GL was not associated with free plasma CML. There was a positive association between GL and free plasma CEL in the age- and sex-adjusted analyses (β (95%CI)=0.09 (0.004, 0.18), model 1), which was attenuated after adjustment for health- and lifestyle factors (β=0.06 (-0.04, 0.15), model 2). Also, we observed a positive association between GL and plasma free MG-H1, which remained after adjusting for potential confounders (β=0.23 (0.02, 0.43), model 3).

A diet with a higher GL was associated with higher urinary concentrations of CML and MG-H1 in the fully adjusted analyses (β=0.28 (0.06, 0.50) and β=0.28 (0.06, 0.50), model 3, respectively). A similar positive trend was observed for urinary CEL concentrations, but this association was not statistical significant (β=0.17 (-0.06, 0.39), model 3).

Interaction between glucose metabolism status and GL ranged between p=0.03-0.99 and only reached statistical significance ($P_{interaction}$ <0.1) for dicarbonyls MGO and 3-DG, and free CEL in plasma and urine. After stratification for glucose metabolism status, associations were generally strongest in the DM group. GL was significantly associated with all free AGEs in both plasma and urine, in the DM group. GL was also significantly associated with free MG-H1 in plasma and urine in the NGM group (Supplementary Table S6.3).

Additional analyses

Several additional analyses were performed. First, the fully adjusted analyses (models 3) with GL as main independent variable were repeated without adjustment for GI, to evaluate the combined effect of both carbohydrate load and carbohydrate quality. In these analyses GL was not statistically significantly associated with the dicarbonyls or AGEs. Second, the analyses were repeated with carbohydrate intake (en%, standardized) as main independent variable. Similarly to GL, dietary carbohydrate intake was positively associated with free plasma MG-H1 (β=0.22 (0.03, 0.40), model 3). Furthermore, carbohydrate intake was associated with free urinary CML and MG-H1 (β=0.26 (0.06, 0.45), model 3 and β=0.32 (0.13, 0.52), model 3, respectively. Third, the main analyses were additionally adjusted for dietary AGE intake. After additional adjustment for dietary AGE intake, the association between GL and urinary MG-H1 did not materially change (β=0.29 (0.06, 0.52)). The associations between GL and free plasma MG-H1 and free urinary CML were slightly attenuated and not significant (β=0.17 (-0.04, 0.39) and β=0.21 (-0.019, 0.45)). Fourth, energy-adjusted variables (GL, fat, protein, fiber and carbohydrates) were replaced by values adjusted via the residual method. This did not materially change our results, except for the association between GL and free plasma MG-H1, which was not statistically significant (data not shown).

Discussion

In this study, we evaluated the association of carbohydrate quality (assessed as dietary GI) and quantity (assessed as dietary GL) of the habitual diet with dicarbonyls and AGEs. GI was not associated with dicarbonyls or AGEs in fasting plasma or urine. In contrast, a diet higher in GL was associated with higher concentrations of free urinary MG-H1, also after additional adjustment for dietary AGE intake. GL also was associated with higher concentrations of free plasma MG-H1 and free urinary CML, but this was not independent of dietary intake of AGEs. It is noteworthy that GL was not associated with protein-bound plasma AGEs. These associations did not differ significantly between individuals with a different glucose metabolism status.

We previously showed that plasma dicarbonyl concentrations increase after a glucose load and after a mixed meal test[16,17], and that the postprandial dicarbonyl stress can be reduced by improving glucose metabolism through a very low caloric diet[17]. These findings suggest that manipulation of carbohydrate intake may affect the endogenous formation of dicarbonyls and the subsequent conversion of plasma dicarbonyls to AGEs. In the present study, dietary GL was positively associated with the MGO-derived free MG-H1 in urine. Urinary free AGEs are most likely a reflection

of tissue AGE accumulation, since tissue protein-bound AGEs (i.e. glycated proteins) are broken down during proteolysis, are subsequently released into the circulation as free AGEs, and via the kidneys excreted in urine[1-3]. In accordance, GL was also associated with free MG-H1 in plasma. The fact that the associations in both plasma and urine were strongest for MG-H1 adds to the concept that MGO is the main hyperglycemia-induced precursor of AGEs.

Despite the associations with the MGO-derived free AGEs described above, we did not observe a positive association between GL and dicarbonyls in plasma. This might be due to the fact that our samples were collected in a fasted state. Because of the high reactivity of dicarbonyls and their rapid conversion into AGEs, it is likely that the dicarbonyls formed postprandially are not well-reflected in fasting plasma dicarbonyl concentrations. We did not observe positive associations of GL with protein-bound AGEs in plasma. This may indicate that protein-bound AGEs do not accumulate in plasma but are rather rapidly taken up by and incorporated into the various tissues. Thus, plasma protein-bound AGEs might not be a good reflection of total tissue AGEs. The notion that plasma free and protein-bound AGEs represent different (metabolic) entities is also corroborated by their low reciprocal correlations in plasma.

In contrast to previous studies in mice, which reported that a diet with a high GI led to the accumulation of AGEs in tissue and plasma[21-23], we did not observe any associations between dietary GI and dicarbonyls or AGEs in our current observational analyses in humans. There are several possible explanations for the fact that we observed positive associations of dietary GL, but not GI, with AGEs. First, it could be because the range of GI values in our cohort was narrower than the range in GL (the fold increase in median of the highest compared to the lowest tertile of GI is ~1.1, while for GL, this is ~2), which may have resulted in limited contrast to detect associations with dicarbonyls and AGEs. Last, it has been suggested that GL is a better estimate of the total glycemic effect of a diet, since it takes into account not only the GI but also the amount of carbohydrates that is consumed[37]. In our study, GL was more strongly correlated with carbohydrate intake (r=0.91) than with GI (r=0.38). Our findings imply that dietary carbohydrate quantity has a larger impact on dicarbonyls and AGEs in plasma and urine than carbohydrate quality. This was supported by additional analyses, using carbohydrate intake (as en%) as main independent variable, that showed similar associations as observed for GL. To the best of our knowledge, this study is the first to report on the associations of dietary GI and GL with dicarbonyls and AGEs in plasma and urine in humans. A major strength of this study is that we used UPLC-MS/MS to precisely quantify concentrations of dicarbonyls and AGEs in a large and

well-defined cohort study. The availability of data on various dicarbonyls and AGEs, in both plasma and urine samples, gives extensive insight into the associations between dietary GI or GL and AGE formation. Furthermore, the availability of a wide range of covariates enabled adjustment for major potential lifestyle and dietary factors that could confound the associations.

This study also has several limitations. GI and GL were determined based on an FFQ. Although this is the most frequently used method to obtain dietary data in large cohort studies, difficulties in the application of GI values may exist, since GI tables are mainly based on values for Australian and American food products[38]. This may have limited our potential to identify an association between GI and dicarbonyls or AGEs. Furthermore, the cross-sectional study design does not allow the assessment of causality. Although we carefully adjusted for a large set of potential confounders, residual confounding remains a possibility. We do not have direct information on the accumulation of AGEs in tissues. However, in line with our interpretation that free AGEs may reflect tissue AGEs, accumulation of AGEs in several tissues has been reported in mice fed a very high-GI diet compared to a very low-GI diet[21-23]. Also, we acknowledge that this comprehensive evaluation of dietary GI, GL, and AGE accumulation entails multiple statistical tests. This may have increased the chance of false positive findings (type 1 error) while, on the other hand, rigorous adjustment for multiple testing increases the chance that a real biological association would remain undetected (type 2 error). In this study, we explored hypotheses that were generated from previous literature, in order to provide a first line of human evidence as a basis for further research. In the light of this aim, we argue that it would be undesirable to miss an association that does exist in the population. Therefore, and in agreement with current recommendations for observational studies, we did not formally adjust for multiple testing[39]. Last, the sample size was too small to conclude whether the associations between GI and dicarbonyls and AGEs after stratification for glucose metabolism status were biologically relevant or spurious findings.

Future studies, such as a randomized controlled dietary intervention, controlling for both energy intake and carbohydrate quality, could confirm the long-term effects of the quantity of carbohydrates. A meal test comparing a low- versus high glycemic index could further elucidate the immediate effects of dietary glycemic index, as we cannot exclude the possibility that this affects dicarbonyl or AGE formation in the short-term.

In conclusion, these observations suggest that low carbohydrate dietary interventions have the potential to lower formation and accumulation of harmful AGEs, possibly via reduced postprandial glycemia. The association between GL and

carbohydrate intake of the habitual diet, but not GI, and dicarbonyls or AGEs, implies that carbohydrate quantity plays a larger role in the formation of AGEs than quality.

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Supplementary tables

Table S6.1 Correlations between plasma dicarbonyls, plasma protein-bound, free and urinary free AGEs.[1]

	Plasma dicarbonyls			Plasma protein-bound AGEs			Plasma free AGEs			Urinary free AGEs		
	MGO	GO	3-DG	CML	CEL	Pento-sidine	CML	CEL	MG-H1	CML	CEL	MG-H1
Plasma dicarbonyls												
MGO	-	**0.41**	**0.43**	**0.09**	0.07	**0.14**	0.05	**0.15**	0.09	-0.04	0.09	0.01
GO		-	**0.15**	**0.23**	-0.04	**0.09**	**-0.10**	-0.02	-0.04	-0.06	0.00	-0.03
3-DG			-	**-0.12**	-0.01	0.05	0.04	**0.16**	0.03	0.03	**0.18**	0.03
Plasma protein-bound AGEs												
CML				-	**0.38**	**0.35**	0.06	0.03	**0.11**	0.00	-0.02	0.05
CEL					-	0.06	0.06	**0.13**	0.08	-0.02	0.03	-0.01
Pentosidine						-	**0.13**	0.06	**0.11**	0.02	-0.05	0.00
Plasma free AGEs												
CML							-	**0.61**	**0.57**	**0.64**	**0.36**	**0.39**
CEL								-	**0.69**	**0.34**	**0.67**	**0.45**
MG-H1									-	**0.34**	**0.46**	**0.75**
Urinary free AGEs												
CML										-	**0.61**	**0.57**
CEL											-	**0.67**
MG-H1												-

[1] Spearman's rho. Bold: p-value <0.05. Strength of correlation is color coded with darker colors for stronger correlations, negative correlations in red and positive correlations in blue. Plasma dicarbonyls (n=492), plasma protein-bound AGEs (CML and CEL n=490, pentosidine n=494), plasma free AGEs (n=489), urinary free AGEs (n=464). AGE, advanced glycation endproduct; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

Table S6.2 Associations between dietary glycemic index and plasma dicarbonyls, plasma protein-bound, free and urinary AGEs in the total population.[1]

Model	β	95%CI	P-value
Plasma dicarbonyls			
MGO			
model 1	-0.014	-0.107, 0.078	0.758
model 2	-0.033	-0.123, 0.057	0.475
model 3	-0.039	-0.135, 0.057	0.422
GO			
model 1	-0.072	-0.160, 0.015	0.106
model 2	-0.070	-0.158, 0.018	0.120
model 3	-0.062	-0.155, 0.032	0.194
3-DG			
model 1	-0.027	-0.117, 0.064	0.563
model 2	-0.046	-0.108, 0.017	0.151
model 3	-0.057	-0.124, 0.009	0.089

Table S6.2 (continued)

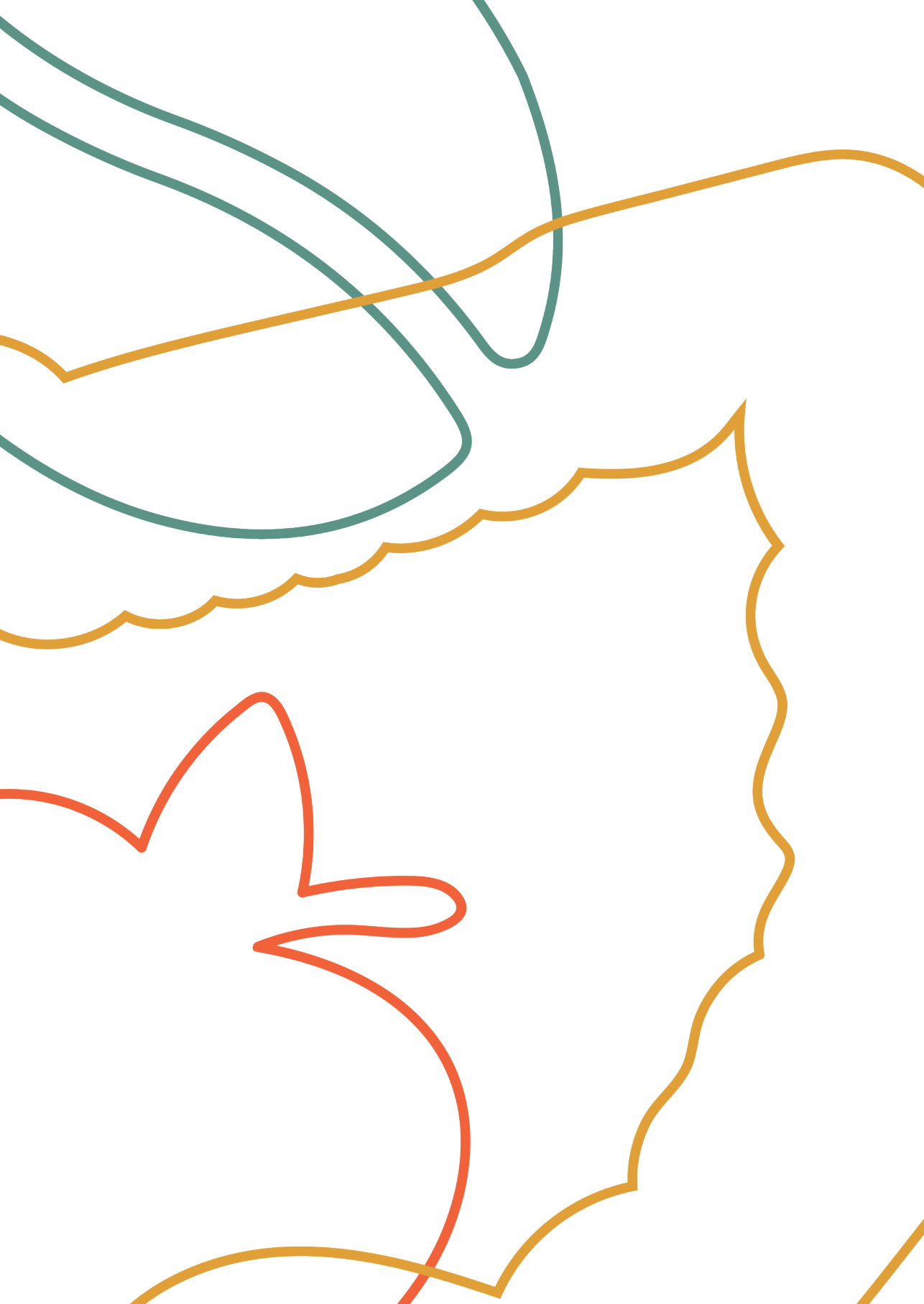
Model	β	95%CI	P-value
Plasma protein-bound AGEs			
CML			
model 1	-0.003	-0.093, 0.087	0.953
model 2	0.011	-0.074, 0.096	0.800
model 3	-0.002	-0.092, 0.088	0.972
CEL			
model 1	0.066	-0.024, 0.157	0.151
model 2	0.051	-0.043, 0.145	0.289
model 3	0.052	-0.047, 0.152	0.304
Pentosidine			
model 1	-0.056	-0.146, 0.034	0.224
model 2	-0.026	-0.114, 0.062	0.564
model 3	-0.043	-0.135, 0.050	0.364
Plasma free AGEs			
Free CML			
model 1	0.013	-0.076, 0.102	0.782
model 2	-0.026	-0.115, 0.063	0.566
model 3	-0.031	-0.126, 0.063	0.513
Free CEL			
model 1	0.029	-0.061, 0.118	0.529
model 2	-0.011	-0.098, 0.077	0.811
model 3	-0.016	-0.109, 0.077	0.734
Free MG-H1			
model 1	0.002	-0.086, 0.091	0.958
model 2	-0.040	-0.127, 0.048	0.375
model 3	-0.059	-0.151, 0.032	0.203
Urinary free AGEs			
Urinary CML			
model 1	-0.039	-0.129, 0.052	0.402
model 2	-0.044	-0.138, 0.050	0.355
model 3	-0.039	-0.138, 0.060	0.442
Urinary CEL			
model 1	-0.032	-0.125, 0.060	0.494
model 2	-0.056	-0.150, 0.038	0.240
model 3	-0.057	-0.156, 0.043	0.261
Urinary MG-H1			
model 1	-0.033	-0.124, 0.058	0.478
model 2	-0.065	-0.158, 0.029	0.177
model 3	-0.072	-0.171, 0.026	0.148

[1] βs are standardized regression coefficients and represent the change in (standardized and ln-transformed) dicarbonyls and AGEs per 1 unit increase in standardized GI, as evaluated in multiple linear regression. Model 1: Standardized value of GI adjusted for age + sex. Model 2: model 1 + eGFR + glucose metabolism status + BMI + alcohol intake (grouped) + physical activity + smoking + use of medication. Model 3: model 2 + total energy intake (kcal/day) + fat (en%) + protein (en%) + carbohydrate intake (en%) + fiber (amount per 10 MJ) intake. Plasma dicarbonyls (n=492), plasma protein-bound AGEs (CML and CEL n=490, pentosidine n=494), plasma free AGEs (n=489), urinary free AGEs (n=464). AGE, advanced glycation endproduct; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

Table S6.3 Associations between dietary glycemic load and plasma dicarbonyls, plasma protein-bound, free and urinary free AGEs after stratification on glucose metabolism status.[1]

	NGM			IGM			T2DM		
Model	**β**	**95%CI**	**P-value**	**β**	**95%CI**	**P-value**	**β**	**95%CI**	**P-value**
Plasma dicarbonyls									
N	262			111			119		
MGO	0.002	-0.335,0.338	0.991	-0.096	-0.493,0.300	0.631	-0.100	-0.509,0.308	0.627
GO	-0.201	-0.490,0.088	0.171	-0.147	-0.515,0.221	0.431	-0.139	-0.666,0.387	0.601
3-DG	-0.016	-0.221,0.188	0.875	0.031	-0.194,0.255	0.787	-0.263	-0.653,0.128	0.185
Plasma protein-bound AGEs									
N	261			110			119		
Protein-bound CML	0.055	-0.124,0.234	0.549	0.014	-0.222,0.251	0.905	0.056	-0.200,0.313	0.665
Protein-bound CEL	-0.015	-0.312,0.282	0.919	-0.008	-0.400,0.383	0.966	0.295	-0.131,0.721	0.173
Protein-bound pentosidine	-0.010	-0.303,0.283	0.944	-0.160	-0.598,0.278	0.470	-0.169	-0.638,0.300	0.477
Plasma free AGEs									
N	259			111			119		
Free CML	0.092	-0.232,0.415	0.577	-0.214	-0.720,0.292	0.404	0.376	0.005,0.746	0.047
Free CEL	0.070	-0.247,0.387	0.663	-0.321	-0.774,0.133	0.163	0.441	0.045,0.837	0.029
Free MG-H1	0.357	0.065,0.649	0.017	-0.178	-0.680,0.324	0.483	0.507	0.104,0.909	0.014
Urinary free AGEs									
N	246			105			113		
Urinary CML	0.245	-0.105,0.596	0.169	-0.016	-0.556,0.523	0.952	0.637	0.307,0.967	0.000
Urinary CEL	0.166	-0.180,0.512	0.346	-0.234	-0.718,0.250	0.340	0.541	0.161,0.921	0.006
Urinary MG-H1	0.410	0.084,0.737	0.014	-0.101	-0.630,0.429	0.707	0.686	0.279,1.09	0.001

[1] βs are standardized regression coefficients and represent the change in (standardized ln-transformed) dicarbonyls and AGEs per 1 unit increase in standardized GL, as evaluated in multiple linear regression. Results are shown for the fully adjusted model (model 3: Standardized value of GL (amount per 10 MJ) adjusted for age + sex + eGFR + glucose metabolism status + BMI + alcohol intake (grouped) + physical activity + smoking + use of medication + total energy intake (kcal/day) + fat (en%) + protein (en%) + fiber (amount per 10 MJ) intake + GI). AGE, advanced glycation endproduct; CML, Nε-(carboxymethyl)lysine; CEL, Nε-(1-carboxyethyl)lysine; 3-DG, 3-Deoxyglucosone; GO, glyoxal; IGM=impaired glucose metabolism; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; NGM=normal glucose metabolism; T2DM=type 2 diabetes mellitus.



Chapter 7

Polymorphisms in glyoxalase I gene are not associated with glyoxalase I expression in whole blood or markers of methylglyoxal stress: The CODAM study

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Abstract

Glyoxalase 1 (Glo1) is the rate-limiting enzyme in the detoxification of methylglyoxal (MGO) into D-lactate. MGO is a major precursor of advanced glycation endproducts (AGEs), and both are associated with development of age-related diseases. Since genetic variation in *GLO1* may alter the expression and/or the activity of Glo1, we examined the association of nine SNPs in *GLO1* with Glo1 expression and markers of MGO stress (MGO in fasting plasma and after an oral glucose tolerance test, D-lactate in fasting plasma and urine, and MGO-derived AGEs CEL and MG-H1 in fasting plasma and urine). We used data of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM, *n*=546, 60 ± 7 y, 25% type 2 diabetes). Outcomes were compared across genotypes using linear regression, adjusted for age, sex, and glucose metabolism status. We found that SNP4 (rs13199033) was associated with Glo1 expression (AA as reference, standardized beta AT=−0.29, p=0.02 and TT=−0.39, p=0.3). Similarly, SNP13 (rs3799703) was associated with Glo1 expression (GG as reference, standardized beta AG=0.17, p=0.14 and AA=0.36, p=0.005). After correction for multiple testing these associations were not significant. For the other SNPs, we observed no consistent associations over the different genotypes. Thus, polymorphisms of *GLO1* were not associated with Glo1 expression or markers of MGO stress, suggesting that these SNPs are not functional, although activity/expression might be altered in other tissues.

Introduction

Glyoxalase 1 (Glo1) is the rate-limiting enzyme in the detoxification of methylglyoxal (MGO) into D-lactate[1]. MGO is a highly reactive compound that is mainly formed during glycolysis and lipid peroxidation. It is a major precursor in the formation of advanced glycation endproducts (AGEs), and both MGO and MGO-derived AGEs are associated with the development of age-related diseases, such as diabetes and its associated complications, cardiovascular disease in particular[2-5].

Detoxification of MGO via the glyoxalase system limits MGO stress; i.e., results in lower MGO concentrations and less formation of MGO-derived AGEs[6,7]. Inhibition of Glo1 increases MGO accumulation, expression of inflammation and endothelium dysfunction markers, and decreases cellular viability[8-11]. The glyoxalase system, and in particular its rate-limiting component Glo1, may be a key determinant of interindividual susceptibility to elevated MGO levels in the setting of hyperglycemia and subsequent development of diabetic complications, as several experimental studies have linked dysfunction of Glo1 to a higher prevalence of diabetes[12].

In rats, overexpression of Glo1 decreased diabetes-induced accumulation of MGO and MG-H1, oxidative stress and endothelial dysfunction, and attenuated early renal impairment[10,13]. In humans, low Glo 1 activity was associated with painful diabetic neuropathy[14], plaque rupture (as reviewed in[15]), and coronary artery disease[11]. Interestingly, a recent integrative genomics study revealed *GLO1* as a key regulatory gene in coronary artery disease-related processes[16].

Genetic variation in *GLO1* may alter the expression and/or the activity of Glo1, and may thus represent life-long exposure to a higher or lower detoxification potency and MGO stress[3]. Nine single nucleotide polymorphisms (SNPs) have been identified, that cover the total common variability in *GLO1*[17]. In this explorative study, we examined the association of these nine SNPs with gene expression of *GLO1* in white blood cells. In addition, we studied the associations of the SNPs with severity of MGO-stress as estimated by concentrations of MGO, D-lactate, and MGO-derived AGEs in fasting plasma, with the formation of MGO after an oral glucose tolerance test (OGTT), and with concentrations of MGO-derived AGEs and D-lactate in urine. These analyses were done using data from individuals of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM).

Materials and methods

Study population

Analyses were performed in the CODAM study, which includes 574 individuals with a moderately increased risk for type 2 diabetes and cardiovascular disease as described in detail elsewhere[18]. In short, participants are of Caucasian descent and >40 years of age with one or more of the following characteristics: BMI >25 kg/m^2; use of antihypertensive medication; positive family history of type 2 diabetes; postprandial blood glucose level >6.0 mmol/L; and history of gestational diabetes and/or glycosuria. All 574 participants were extensively characterized at baseline between 1999 and 2002. At the follow-up examination between 2006 and 2009, with a median follow-up period of 7.0 years (IQR 6.9–7.1), the measurements were repeated in 491 individuals.

For the present evaluation, we used data at baseline, except for the *GLO1* mRNA data, which was only available at follow-up. Individuals who had missing DNA samples and therefore missing data for all genotyped SNPs (n=6) were excluded from the current analyses. Additionally, individuals with missing data on fasting plasma concentrations of MGO, D-lactate, or MGO-derived AGEs were excluded (n=22). Participants with missing data on any of the other outcomes (*GLO1* mRNA, iAUC of MGO, urinary AGEs, and urinary D-lactate) and main independent variables (the nine SNPs) were not excluded to maximize the sample size for each analysis. Hence, the maximal study population consisted of 546 participants (see flowchart Supplementary Figure S7.1). The study was approved by the Medical Ethics Committee of the Maastricht University Medical Centre. All participants gave written informed consent.

Single nucleotide polymorphism selection and genotyping

SNP selection was based on *GLO1 (*Gene accession number NC_000006.11 and NC_000006.12), including 3000 base pairs downstream and upstream as previously described[17]. Common SNPs (minor allele frequency (MAF) >5%, n=28) were selected using HapMap and Haploview. By genotyping nine tag SNPs, these 28 common SNPs were captured at r^2 more than 0.8 in linkage disequilibrium, and therefore, total common genetic variability of *GLO1* was covered (Figure 7. 1)[17]. For genotyping, DNA was extracted from peripheral blood samples according to standard procedures. *GLO1* SNPs were genotyped using the ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Hardy–Weinberg equilibrium was previously assessed using a χ^2 test[17].

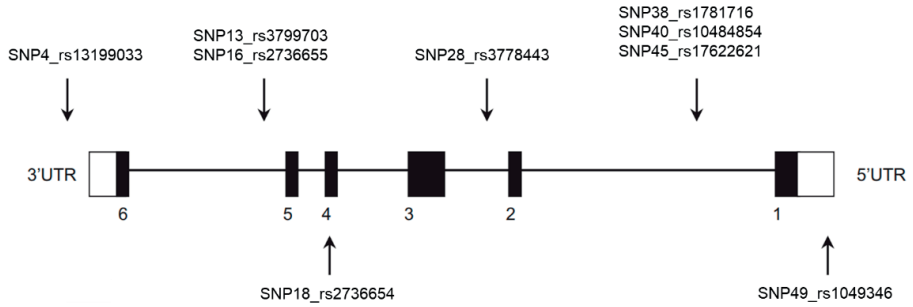


Figure 7.1 Representation of the human glyoxalase 1 gene. Arrows indicate the approximate locations of the nine single nucleotide polymorphisms (SNPs) genotyped in this study. Black boxes represent exons and untranslated regions (UTRs) of the first and last exon are indicated as white boxes. SNP4 is located on the 3′ untranslated region; SNP18 in exon 4 (nonsynonymous); SNP49 in exon 1 (untranslated region); and all other SNPs are located in introns.

GLO1 mRNA expression in whole blood

To ensure adequate stabilization of mRNA, whole blood was collected in PAX gene tubes (Qiagen) and stored at −80°C until further use. RNA was isolated in an automated fashion with the QIAcube, according to the manufacturer's instructions. RNA quantity and quality was assessed in a subset of samples (n=216) to ensure the quality of RNA was adequate for qPCR (RIN was >5 in 99.5%). Next, 500 ng RNA per sample was used to synthesize cDNA using miScript, according to the manufacturer's instructions. Next, qPCR of GLO1 was performed. HRPT1 and WHKY1 were used as reference genes. Primer sequences were for GLO1-forward, 5′-GGTTTGAAGAACTGGGAGTCAAA-3′ and for GLO1-reverse, 5′-ATCCAGTAGCCATCAGGATCTTG-3′; for YWHA-forward, 5′-CGTTACTTGGCTGAGGTTGC-3′ and for YWHA-reverse, 5′-TGCTTGTTGTGACTGATCGAC-3′; and for HRPT1-forward, 5′-AAG-AAT-GTC-TTG-ATT-GTG-GAA-GA-3′ and HRPT1-reverse, 5′-ACC-TTG-ACC-ATC-TTT-GGA-TTA-3′.

Markers of MGO stress

Concentrations of MGO, D-lactate, and free N_{ε}-(1-carboxyethyl)lysine (CEL), free N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), and protein-bound CEL, were measured in fasting plasma samples, using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) as previously described[19-21]. Concentrations of free CEL, free MG-H1, and D-lactate were measured in fasting urine samples, as previously described[21,22]. To correct for urine

volume, creatinine concentration in urine was analyzed based on the Jaffé reaction method[23], and CEL, MG-H1, and D-lactate concentrations were expressed as nmol/mmol creatinine. The intra- and inter-assay variation was between 3.6 and 4.3% for MGO, between 2.9 and 5.2% for D-lactate, between 2.8 and 7.1% for free CEL and MG-H1, and between 4.8 and 9.7% for protein-bound CEL in plasma. The intra- and inter-assay variations of D-lactate, free CEL, and free MG-H1 in urine were between 3.7 and 7.0%.

iAUC of MGO after an OGTT

Individuals underwent a standard 75-g OGTT and venous blood samples were obtained prior to and at 30, 60, and 120 min after the glucose load. Individuals with known type 2 diabetes or with fasting glucose levels >8.5 mmol/L were excluded from undergoing an OGTT. Concentrations of MGO were quantified in plasma samples collected during the OGTT using UPLC–MS/MS. The area under the curve for the OGTT levels of MGO was calculated according to the trapezoidal method[24], where baseline (fasting) levels were subtracted from each individual data point to specify the post-glucose load increases. These data are referred to as iAUC.

Definition of glucose metabolism status

Individuals' glucose metabolism status was ascertained using fasting and 2-h postload glucose concentrations during the OGTT, according to the World Health Organization criteria, as described in detail elsewhere[18]. Briefly, individuals were classified as having normal glucose metabolism when they had normal fasting (<6.1 mmol/L) and 2-h postload (<7.8 mmol/L) glucose concentrations. Individuals with impaired fasting glucose (6.1–7.0 mmol/L), impaired 2-h postload glucose levels (7.8–11.1 mmol/L), or both were classified as having impaired glucose metabolism. When individuals had high fasting plasma glucose levels (≥7.0 mmol/L) and/or high 2-h postload glucose levels (≥11.1 mmol/L) or when they used glucose-lowering medication or insulin, they were classified as having type 2 diabetes.

White blood cell composition

Proportions of white blood cells were estimated based on DNA methylation data, using the EpiDISH package through the algorithm CIBESORT (Teschendorff, Breeze, Zheng, and Beck, 2017).

Statistical analysis

First, reciprocal relationships among all outcome variables (*GLO1* mRNA, plasma fasting concentrations of MGO, D-lactate, free CEL, free MG-H1, protein-bound CEL, iAUC MGO, and urinary concentrations of free CEL, free MG-H1, and D-lactate) were examined using Spearman's correlation coefficient. Skewed outcome variables (i.e., all except fasting plasma MGO) were ln-transformed prior to further analyses.

In the main analyses, outcome variables were compared across the genotypes of the nine *GLO1* SNPs using multiple linear regression analyses. This was done using additive models with the major homozygous genotype as a reference and the other two genotypes as dummy variables. Outcome variables were standardized ((participants' value-population mean)/SD) to allow direct comparison of the strength of associations. The results of the linear models are displayed after adjustment for age (years), sex (men/women), and glucose metabolism status (impaired glucose metabolism and type 2 diabetes as dummy variables with normal glucose metabolism as the reference category).

As a sensitivity analysis, the associations with *GLO1* mRNA expression as an outcome were additionally adjusted for white blood cell type composition, because Glo1 expression can vary between cell types. For this, proportions were added as covariates to the model for all but one of the cell types, to improve model stability because cell proportions sum to 1 (B-cells, NK-cells, CD4T-cells CD8T-cells, monocytes, neutrophils and eosinophils, and dropping granulocytes). Cell composition data was available for 162 individuals.

To correct for multiple testing, false discovery rate- (FDR-) based values were calculated for each outcome, using the *p*-values of the fully adjusted model (18 p-values: 9 SNPs × 2 tests per SNP)[25]. The FDR analysis was performed separately for each outcome, because FDR assumes that the *p*-values corresponding to the null hypothesis tests are independent, and in this study the outcome variables are markers of the same pathway and thus not independent. Therefore, calculation of FDR based values for all outcomes simultaneously would likely lead to overcorrection. Significance of the associations was assessed by a 0.05 threshold of value ($q<0.05$).

All models were checked for the assumptions of linearity, normal distribution of residuals, homoscedasticity, and multicollinearity. In the model with iAUC MGO as the outcome variable, the assumption of normality was violated, and therefore as a sensitivity analysis the association between low/high iAUC MGO concentrations (dichotomized by performing a median split) and the nine *GLO1* SNPs was examined using logistic regression. In the analysis between SNP16 and *GLO1* mRNA, one influential outlier was excluded because Cook's distance was 1.1.

Reported betas are after exclusion of the influential outlier. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 24.0) and statistical significance was set at p<0.05.

Results

General characteristics for the study population are shown in Supplementary Table S7.1. Excluded individuals were slightly older and more often males. They more often had type 2 diabetes, were less physically active and smoked less (data not shown). All genotyped SNPs were in the Hardy–Weinberg equilibrium.

Correlations between GLO1 mRNA expression and markers of MGO stress

Reciprocal correlations among all outcomes are shown in Table 7.1. Glo1 mRNA expression was positively correlated with plasma and urinary concentrations of D-lactate, the product of MGO detoxification (rho=0.2). *GLO1* mRNA expression was also positively correlated with concentrations of its substrate, MGO, in fasting plasma (rho=0.2), and iAUC MGO and MGO-derived AGEs in urine (rho=0.1). Notably, *GLO1* mRNA was measured in samples collected at follow-up, seven years after measurements of the other outcomes. Most other associations were relatively weak (rho ranging from −0.2 to 0.3). We observed a strong reciprocal correlation between the free forms of the MGO-derived AGEs, CEL and MG-H1, measured in plasma (rho=0.7), as well as between CEL and MG-H1 measured in urine (rho=0.6). Additionally, we observed a strong correlation between free CEL measured in plasma and in urine, and the same holds true for free MG-H1 in plasma and urine (both rho=0.7).

Association between GLO1 polymorphisms and GLO1 mRNA expression in whole blood

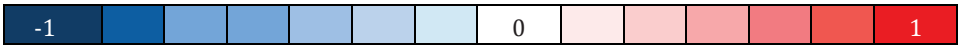
The mean *GLO1* mRNA expression for each genotype of the nine SNPs is shown in Supplementary Table S7.2. After adjustment for age, sex, and glucose metabolism status, carriers of the AT or the TT genotype of SNP4 (rs13199033) had lower *GLO1* mRNA expression than those with the AA genotype (AA as reference, beta AT=−0.29, p=0.02 and beta TT=−0.39, p=0.30; Table 7.2). The effect was additive, but not statistically significant for the TT genotype, likely due to the small sample size (*n*=7).

Carriers of the AG or the AA genotype of SNP13 (rs3799703) had higher *GLO1* mRNA expression than those with the GG genotype, in an additive manner (beta AG=0.17, p=0.14 and beta AA=0.36, p=0.005; Table 7.2).

After additional adjustment for white blood cell composition in a subset of the population with cell composition data available (*n*=162), the strength of the associations remained similar, although none of the associations was statistically significant, likely due to low power.

When we corrected these analyses for multiple testing using FDR, none of these associations remained statistically significant.

Table 7.1 Correlations between *GLO1* mRNA, MGO, D-lactate and AGEs in plasma, and AGEs and D-lactate in urine. [1.]



		Glo1 Expr.	Plasma MGO and D-Lactate			Plasma AGEs			Urinary AGEs and D-Lactate		
		mRNA	MGO	D-lactate	iAUC MGO	Free CEL	Free MG-H1	PB CEL	Free CEL	Free MG-H1	D-lactate
Glo1 expr.	mRNA	mRNA	0.15 **	0.17 **	0.10 *	0.08	0.08	−0.003	0.12 *	0.11 *	0.21 **
Plasma MGO and D-lactate	MGO		MGO	0.07	−0.23 **	0.16**	0.10 *	0.06	0.09 *	−0.01	0.08
	D-lactate			D-lactate	0.15 **	0.17**	0.09 *	0.33 **	0.21 **	0.12 **	0.33 **
	iAUC MGO				iAUC MGO	0.05	−0.02	−0.04	0.12*	0.02	0.10*
Plasma AGEs	Free CEL					Free CEL	0.69 **	0.10 *	0.67 **	0.45 **	0.05
	Free MG-H1						Free MG-H1	0.07	0.45**	0.75 **	0.01
	PB CEL							PB CEL	0.04	0.02	0.05
Urinary AGEs and D-lactate	Free CEL								Free CEL	0.58 **	0.32 **
	Free MG-H1									Free MG-H1	0.20 **
	D-lactate										D-lactate

[1] rho = Spearman's correlation coefficient. * $p < 0.05$, ** $p < 0.01$. Note: all outcomes were measured at baseline (1999–2002), except *GLO1* mRNA expression, which was measured at follow-up (2006–2009). AGE, advanced glycation endproduct; CEL, Nε-(1-carboxyethyl)lysine; Glo1, glyoxalase-1; iAUC MGO, incremental area under the curve of MGO after an OGTT; MGO, methylglyoxal; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; OGTT, oral glucose tolerance test; PB, protein-bound.

Table 7.2 Association of *GLO1* SNPs with *GLO1* mRNA, plasma MGO and D-lactate, and urinary D-lactate[1].

		Glo1 expression		Plasma						Urine	
		GLO1 mRNA[2]		MGO		D-lactate		MGO iAUC		D-lactate	
	N	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
SNP4											
AA	423	-	-	-	-	-	-	-	-	-	-
AT	102	−0.29	*0.02*	0.07	*0.54*	0.05	*0.62*	0.08	*0.52*	0.11	*0.27*
TT	7	−0.39	*0.30*	0.64	*0.08*	−0.08	*0.83*	−0.37	*0.38*	−0.04	*0.91*
SNP13											
GG	148	-	-	-	-	-	-	-	-	-	-
AG	249	0.17	*0.14*	0.02	*0.85*	0.29	*0.004*	−0.01	*0.91*	0.02	*0.85*
AA	126	0.36	*0.005*	0.05	*0.65*	0.08	*0.50*	0.07	*0.59*	−0.03	*0.76*
SNP16											
GG	397	-	-	-	-	-	-	-	-	-	-
AG	127	0.15	*0.17*	−0.01	*0.89*	0.12	*0.23*	0.09	*0.41*	0.04	*0.64*
AA	11	0.21	*0.46*	−0.11	*0.71*	−0.17	*0.57*	0.04	*0.91*	0.06	*0.85*
SNP18											
TT	161	-	-	-	-	-	-	-	-	-	-
GT	277	−0.07	*0.49*	−0.20	*0.04*	−0.03	*0.78*	−0.03	*0.77*	0.03	*0.76*
GG	98	−0.11	*0.44*	−0.16	*0.20*	−0.10	*0.40*	−0.02	*0.88*	−0.005	*0.96*
SNP28											
GG	458	-	-	-	-	-	-	-	-	-	-
AG	76	0.07	*0.61*	0.13	*0.26*	0.16	*0.17*	−0.03	*0.81*	−0.06	*0.62*
AA	4	0.16	*0.75*	0.26	*0.59*	0.34	*0.49*	−0.10	*0.84*	−0.69	*0.12*
SNP38											
GG	428	-	-	-	-	-	-	-	-	-	-
CG	96	0.05	*0.71*	0.11	*0.31*	0.16	*0.67*	0.01	*0.94*	−0.02	*0.86*
CC	4	0.16	*0.75*	0.28	*0.55*	0.17	*0.13*	−0.09	*0.86*	−0.68	*0.13*
SNP40											
CC	273	-	-	-	-	-	-	-	-	-	-
CT	226	−0.09	*0.38*	−0.07	*0.40*	−0.004	*0.97*	−0.04	*0.70*	−0.14	*0.09*
TT	35	−0.03	*0.88*	0.12	*0.48*	−0.08	*0.65*	−0.17	*0.36*	−0.28	*0.09*
SNP45											
GG	199	-	-	-	-	-	-	-	-	-	-
AG	252	−0.16	*0.11*	0.01	*0.91*	0.001	*0.10*	0.000	*0.99*	−0.09	*0.32*
AA	77	−0.27	*0.06*	0.08	*0.53*	0.000	*0.99*	−0.12	*0.39*	−0.08	*0.50*
SNP49											
AA	135	-	-	-	-	-	-	-	-	-	-
AG	284	0.11	*0.31*	0.04	*0.67*	0.04	*0.73*	0.08	*0.46*	0.07	*0.46*
GG	117	0.26	*0.06*	−0.07	*0.57*	−0.10	*0.41*	0.08	*0.58*	0.20	*0.08*

[1] Data are analyzed using linear regression analysis, adjusted for age, sex, and glucose metabolism status (impaired glucose metabolism and type 2 diabetes as dummy variables with normal glucose metabolism as reference). The genotype with the highest frequency was used as reference, with the other two genotypes added as dummy variables. All outcome variables were standardized and, apart from fasting plasma MGO, all outcome variables were ln-transformed prior to standardization. [2] *GLO1* mRNA expression as measured in white blood cells, expressed as ratio of Glo1 versus reference genes. GLO1, glyoxalase-1; iAUC MGO, incremental area under the curve of MGO after an OGTT; MGO, methylglyoxal; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

Association between GLO1 polymorphisms and markers of MGO stress

The mean of each marker of MGO stress for each genotype of the nine SNPs is shown in Supplementary Table S7.2. In line with their higher *GLO1* mRNA expression, carriers of the AG or the AA genotype of SNP13 (rs3799703) had higher concentrations of plasma fasting D-lactate than those with the GG genotype, albeit not additive and only statistically significant for the AG genotype (beta AG=0.29, p=0.004 and beta AA=0.08, p=0.50; Table 7.2). Carriers of the GT or the GG genotype of SNP18 (rs2736654) had lower concentrations of fasting plasma MGO than those of the TT genotype, but this was only statistically significant for the GT genotype (beta GT=−0.20, p=0.04 and beta GG beta=−0.16, p=0.20; Table 7.2). Additionally, carriers of the AG or the GG genotype of SNP49 (rs1049346) had lower free CEL concentrations in plasma than those of the AA genotype, but this was only statistically significant for the AG genotype (beta AG=−0.24, p=0.02 and beta GG=−0.15, p=0.23; Table 7.3). In the sensitivity analyses where the dichotomized variable iAUC MGO was used as the outcome variable, results were similar to the linear regression, with no statistically significant associations (Supplementary Table S7.3).

After correction for multiple testing using FDR none of these associations remained statistically significant.

Table 7.3 Association of GLO1 SNPs with MGO-derived AGEs in plasma and urine.[1]

		Plasma						Urine			
		Free CEL		Free MG-H1		PB CEL		Free CEL		Free MG-H1	
	N	β	p	β	p	β	p	β	p	β	p
SNP4											
AA	423	-	-					-	-	-	-
AT	102	0.16	*0.14*	−0.07	*0.50*	0.17	*0.13*	0.15	*0.19*	−0.06	*0.60*
TT	7	0.16	*0.67*	−0.10	*0.80*	−0.35	*0.37*	0.13	*0.73*	−0.06	*0.87*
SNP13											
GG	148	-	-	-	-	-	-	-	-	-	-
AG	249	0.05	*0.62*	0.003	*0.97*	−0.008	*0.94*	−0.004	*0.97*	−0.006	*0.96*
AA	126	0.10	*0.39*	0.07	*0.53*	0.003	*0.98*	−0.09	*0.49*	−0.10	*0.40*
SNP16											
GG	397	-	-	-	-	-	-	-	-	-	-
AG	127	0.11	*0.27*	0.12	*0.24*	0.01	*0.90*	0.06	*0.57*	0.03	*0.77*
AA	11	0.10	*0.73*	0.20	*0.50*	0.09	*0.78*	−0.47	*0.16*	−0.12	*0.71*
SNP18											
TT	161	-	-	-	-	-	-	-	-	-	-
GT	277	−0.12	*0.22*	−0.02	*0.81*	−0.11	*0.27*	0.02	*0.82*	0.03	*0.74*
GG	98	−0.12	*0.33*	−0.02	*0.87*	0.008	*0.95*	0.08	*0.57*	0.10	*0.44*
SNP28											
GG	458	-	-	-	-	-	-	-	-	-	-
AG	76	0.12	*0.32*	0.14	*0.26*	0.04	*0.76*	−0.01	*0.97*	0.03	*0.81*
AA	4	0.32	*0.51*	0.22	*0.66*	0.11	*0.82*	−0.40	*0.41*	−0.20	*0.68*
SNP38											
GG	428	-	-	-	-	-	-	-	-	-	-
CG	96	0.17	*0.13*	0.12	*0.26*	0.03	*0.81*	0.05	*0.67*	0.05	*0.69*
CC	4	0.32	*0.49*	0.22	*0.65*	0.12	*0.81*	−0.38	*0.43*	−0.20	*0.69*
SNP40											
CC	273	-	-	-	-	-	-	-	-	-	-
CT	226	−0.06	*0.50*	−0.03	*0.77*	−0.06	*0.48*	0.02	*0.83*	−0.03	*0.76*
TT	35	0.05	*0.79*	0.14	*0.43*	−0.03	*0.88*	0.007	*0.97*	0.10	*0.57*
SNP45											
GG	199	-	-	-	-	-	-	-	-	-	-
AG	252	−0.09	*0.36*	−0.10	*0.29*	0.01	*0.88*	−0.01	*0.88*	−0.10	*0.30*
AA	77	0.17	*0.21*	0.003	*0.98*	0.11	*0.42*	0.13	*0.33*	−0.05	*0.71*
SNP49											
AA	135	-	-	-	-	-	-	-	-	-	-
AG	284	−0.24	*0.02*	−0.11	*0.26*	−0.03	*0.78*	−0.12	*0.27*	−0.03	*0.80*
GG	117	−0.15	*0.23*	−0.05	*0.71*	−0.05	*0.69*	−0.08	*0.55*	0.04	*0.75*

[1] Data are analyzed using linear regression analysis, adjusted for age, sex, and glucose metabolism status (impaired glucose metabolism and type 2 diabetes as dummy variables with normal glucose metabolism as reference). The genotype with the highest frequency was used as reference, with the other two genotypes added as dummy variables. All outcome variables were standardized and, all outcome variables were ln-transformed prior to standardization. AGEs, advanced glycation endproducts; CEL, Nε-(1-carboxyethyl)lysine; GLO1, glyoxalase-1; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; SNP, single nucleotide polymorphism.

Discussion

In the present study we examined if polymorphisms in *GLO1* were associated with *GLO1* mRNA expression in whole blood and/or with MGO and MGO-derived AGEs. We hypothesized that if these polymorphisms are functional, they can serve as a proxy of a life-long exposure to enhanced or decreased MGO stress, due to altered detoxification potency for MGO.

We observed associations of SNP4, SNP13, SNP18, and SNP49 with various outcomes, but after correction for multiple testing with FDR none of these associations remained statistically significant. Thus, these findings are possibly chance findings, but notwithstanding the observed associations could be of interest.

There are several possible explanations for the apparent null findings after correction for multiple testing. First, the SNPs of *GLO1* could be non-functional, and have no effect on the activity of the Glo1 enzyme. Second, the effects of these SNPs could be compensated by other mechanisms, for example upregulation of enzyme transcription/activity of Glo1 or other relevant enzymes[26-29]. Third, the -possibly mild- effects of these SNPs could be overshadowed by other conditions that affect Glo1 transcription/activity, such as hyperglycemia, inflammation, hypoxia, or oxidative stress[3]. Fourth, it could be that markers of MGO stress and Glo1 expression in white blood cells do not reflect altered enzyme activity/function in other cells and/or tissues, and results might be different if cellular Glo1 activity and/or AGE accumulation in different tissues were evaluated as outcomes. Last, correction for multiple testing by FDR could be too strict for these analyses. FDR assumes that p-values are independent. Since our outcomes are all markers of the glyoxalase pathway, we calculated the FDR-based *p*-values separately for each outcome. However, due to the dependent nature of genetic data, SNPs in linkage disequilibrium are also correlated to some degree, so the FDR analyses, using p-values of all nine SNPs with two genotypes each, might have been too stringent. Thus, the current, explorative evaluation might be underpowered due to the relative small sample size, and replication in a larger, independent cohort is warranted.

In our current analyses, carriers of the GT or the GG genotype of SNP18 (rs2736654) had lower concentrations of plasma MGO than those of the TT genotype, although only significant (nominal *p*-value) for the GT genotype. Additionally, carriers of the AG or the GG genotype of SNP49 (rs1049346) had lower plasma CEL concentrations than those of the AA genotype, which was significant for the AG genotype (nominal *p* value). In line, we observed a trend of higher *GLO1* mRNA expression and urinary D-lactate for carriers of these genotypes. SNP18 (rs2736654) is a nonsynonymous SNP located in exon 4. SNP49

(rs1049346) is located in the 5′ untranslated region (UTR) and may be involved in disrupting the regulation of expression, resulting in a lower enzyme concentration[30]. Studies on associations of these SNPs with markers of the glyoxalase pathway are inconsistent. Some studies in human on SNP18 reported that carriers of the AA (i.e., TT) genotype had the highest Glo1 activity in blood[31,32], whereas others reported only a trend towards higher Glo1 activity[30], or no association[33]. One of these studies additionally reported higher Glo1 activity and lower AGE concentrations in post-mortem brains of carriers of the AA genotype, in healthy individuals but not in autistic individuals[31]. The same study reported no differences in *GLO1* mRNA expression or protein levels, whereas others reported higher *GLO1* mRNA levels for carriers of the AA genotype[32], which is in line with the trend that we observed. This would suggest that carriers of the AA (i.e., TT) genotype might have been exposed to a smaller lifelong MGO stress. However, contrary to these data in human, in immortalized lymphoblastoid cells the AA genotype showed lower Glo1 activity and higher MGO concentrations[34]. Interestingly, in the present evaluation we also observed higher MGO concentrations for carriers of the AA genotype, despite the higher *GLO1* mRNA for this genotype. Considering that SNP18 is located on an exon, it is plausible that SNP18 alters the functionality of Glo1, resulting in altered MGO concentrations.

A previous human study on SNP49 reported that Caucasian carriers of CT (i.e., AG) and TT (i.e., AA) genotypes had a lower Glo1 enzyme activity compared to those of the CC genotype (i.e., GG), measured in whole blood samples[30]. This is in line with the trend of lower *GLO1* mRNA expression for the AG and the AA genotype in the current evaluation. Considering that SNP49 is located in the 5′UTR, it is conceivable that this *GLO1* promoter polymorphism has a functional influence on transcriptional regulation. However, in contrast, in a human cell line the -7T promoter of SNP49 was found to have a higher activity than the -7C promoter[35].

We observed an additive effect on *GLO1* mRNA expression in carriers of the T allele at SNP4 (rs13199033) (AA > AT > TT). In line, there was a trend of higher fasting plasma MGO concentrations in these individuals (AA < AT < TT). SNP4 (rs13199033) is located in the 3′UTR, whereas the other SNPs are located in introns[17]. Although SNP4 has not been reported as functional SNP, our association with *GLO1* mRNA is in line with its location in the 3′UTR.

To date, little is known about the association between these nine SNPs and diabetic complications. One study reported an increased prevalence of peripheral neuropathy, but not nephropathy or retinopathy, for carriers of the CC genotype of SNP18 in type 2 diabetic patients[36]. A study from our group reported no associations of the nine investigated SNPs with vascular complications in this

population[17]. Moreover, in this previous study we did not observe an association between these nine SNPs and concentrations of the plasma AGEs, CEL and N_{ε}-(carboxymethyl)lysine (CML)[17]. Similarly, we reported only minor associations of the two reported functional SNPs (i.e., SNP18 and SNP49) with plasma concentrations of MGO, free CEL, CML, and MG-H1 and protein-bound CML, CEL, and pentosidine in the same population[37]. Other authors also reported no association between six *GLO1* SNPs and serum CML concentrations[38] and SNP18 and serum MG-H1[33]. We herein extend these findings to a full panel of markers of MGO stress, including MGO, D-lactate, and MGO-derived AGEs in plasma and urine. This comprehensive analysis shows that indeed these two functional SNPs, and the other studied SNPs, after correction for multiple testing, are not associated with lower or higher concentrations of markers of MGO stress, and thus likely do not play a large role in the detoxification capacity of MGO.

When we explored the reciprocal correlations of all outcome variables, we observed that although Glo1 catalyzes the detoxification of MGO into D-lactate, *GLO1* mRNA expression was not only positively correlated with D-lactate, but also with MGO concentrations. Given the seven year difference between sample collection for measurement of Glo1 expression and measurement of the other outcomes, these correlations have to be interpreted with care, but a possible underlying mechanism is the upregulation of Glo1 as a response to elevated MGO concentrations, via KEAP1 modification and subsequent Nrf1 activation, which regulates Glo1 transcription[39].

We previously showed formation of MGO in plasma after an OGTT, with higher peaks for individuals with prediabetes and type 2 diabetes. These higher peaks of MGO were attributed to an increased formation of MGO from higher blood glucose peaks, but decreased detoxification of MGO by Glo1 in these individuals could also play a role. Our observation of equal genotype frequencies between individuals with normal glucose metabolism, impaired glucose metabolism and type 2 diabetes[17], and the lack of association between *GLO1* SNPs and iAUC of MGO after an OGTT do not support the possibility that impaired detoxification capacity of MGO caused by *GLO1* SNPs are an important contributor to the higher MGO peaks observed after an OGTT.

This study had several strengths. First, we quantified an extensive panel of markers of the glyoxalase pathway and MGO stress, using state-of-the-art techniques. Although these are all markers of the same pathway, they might reflect different (metabolic) entities, as shown by the low reciprocal correlations between the markers. Second, using polymorphisms of *GLO1* as a predictor of the Glo1 enzyme mitigates the limitation of reversed-causality often encountered in cross-

sectional studies. The main limitation of this study is its limited power due to the small sample size. Hence we may have missed true positive associations that would be significant in larger study populations (Type II error). Additionally, we measured *GLO1* mRNA expression in white blood cells, but we do not know to what extent this reflects *GLO1* mRNA expression and/or activity in other cells and tissues[28]. In addition, our study only included Caucasian individuals and cannot be extrapolated to different ethnicities.

Conclusions

In conclusion, after correction for multiple testing, polymorphisms in *GLO1* are not associated with *GLO1* mRNA expression and markers of MGO stress, in a Dutch cohort of middle-aged to elderly Caucasian individuals with a moderate risk of cardiometabolic diseases. These null findings have to be interpreted with caution because of the chance of Type II error due to the small sample size, and replication in an independent cohort is warranted.

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Supplementary tables

Table S7.1 General characteristics of the study population.[1]

	Total population (n=546)
Age (years)	60 ± 7
Sex (%men)	61
NGM/IGM/T2DM (%)	52/23/25
BMI (kg/m2)	29 ± 4
Waist circumference (cm)	99 ± 12
Systolic blood pressure (mmHg)	140 ± 19
Diastolic blood pressure (mmHg)	82 ± 9
Fasting plasma glucose (mmol/L)	5.6 [5.2-6.4]
HbA1c (%)	6.0 ± 0.8
eGFR (ml/min/1.73cm^2) [2]	91.1 ± 18.4
Triglycerides (mmol/L)	1.4 [1.0-2.0]
Total cholesterol (mmol/L)	5.2 ± 1.0
HDL cholesterol (mmol/L)	1.2 ± 0.3
Anti-hypertensive medication (%)	38
Lipid-mediating medication (%)	19
Glucose-lowering medication (%)	13
Total activity score (10^3 METs/week)	5.9 [3.7-8.7]
Current smokers (%)	23
Total alcohol intake (g/d)	8.5 [1.3-22.5]
Total energy intake (kcal/d)	2213 ± 666
Plasma D-lactate and MGO	
Fasting plasma D-lactate (µmol/L)	8.7 [6.3-12.9]
Fasting plasma MGO (nmol/L)	366.3 ± 77.8
iAUC MGO during OGTT	9413 [4042-14525]
Plasma AGEs	
Fasting plasma free CEL (nmol/L)	45.4 [36.6-58.4]
Fasting plasma free MG-H1 (nmol/L)	123.6 [86.6-172.6]
Fasting plasma protein-bound CEL (nmol/mmol lysine)	23.1 [18.7-28.9]
Urinary AGEs and D-lactate	
Urinary CEL (nmol/mmol creatinine)	517 [408-642]
Urinary MG-H1 (nmol/mmol creatinine)	2266 [1593-3300]
Urinary D-lactate (nmol/mmol creatinine)	0.8 [0.4-1.7]

[1]Data are presented as mean ± standard deviation for normally distributed variables, median (interquartile range) for skewed variables or proportion (%) for categorical variables. [2] Calculated using the Modification of Diet in Renal Disease (MDRD) formula. AGE, advanced glycation endproduct; CEL, Nε-(1-carboxyethyl)lysine; eGFR, estimated glomerular filtration rate; iAUC MGO, incremental area under the curve of MGO after an oral glucose tolerance test; IGM, impaired glucose metabolism; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; MGO, methylglyoxal; NGM, normal glucose metabolism; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes.

Table S7.2 Means of Glo1 mRNA and markers of MGO stress for each of the three genotypes of the nine SNPs[1].

SNP	Genotype	Glo1 expression		Plasma MGO and D-lactate					Plasma AGEs				Urinary AGEs and D-lactate			
		n	Glo1 mRNA	n	Plasma MGO	Plasma D-lactate	n	iAUC MGO post- OGTT (units)	n	Plasma free CEL	Plasma protein-bound CEL	Plasma free MG-H1	n	Urinary CEL	Urinary MG-H1	n	Urinary D-lactate
SNP4	AA	355	0.95	423	366 ± 78	8.4	376	9.8	423	45	23	123	396	512	2292	398	0.78
rs3199033			[0.74-1.2]			[6.4-12]		[4.1-15]		[37-57]	[19-29]	[85-173]		[405-629]	[1596-3202]		[0.38-1.6]
	AT	82	0.82	102	365 ± 72	9.5	93	8.1	102	46	24	118	97	532	2141	97	0.78
			[0.58-1.0]			[5.6-14]		[3.5-13]		[38-60]	[19-31]	[87-157]		[443-707]	[1603-3331]		[0.44-2.1]
	TT	7	0.82	7	426 ± 72	9.1	6	6.4	7	60	21	158	7	547	2046	7	0.35
			[0.59-1.0]			[4.7-15]		[1.4-9.9]		[35-71]	[15-25]	[63-211]		[323-832]	[1239-3557]		[0.27-1.8]
SNP13	GG	121	0.86	148	363 ± 73	7.9	140	8.4	148	46	23	122	141	484	2274	141	0.75
rs3799703			[0.60-1.1]			[5.4-11]		[2.7-14]		[36-59]	[19-29]	[87-168]		[424-662]	[1551-3330]		[0.38-1.7]
	AG	206	0.93	249	368 ± 82	9.0	216	9.4	249	45	23	120	237	520	2217	239	0.81
			[0.71-1.2]			[7.0-14]		[4.2-15]		[36-56]	[19-29]	[85-170]		[397-642]	[1586-3459]		[0.39-1.8]
	AA	110	0.97	126	368 ± 74	7.9	111	11	126	45	27	123	115	522	2165	115	0.80
			[0.76-1.2]			[5.9-7.9]		[4.6-15]		[39-57]	[19-29]	[89-169]		[401-623]	[1593-3032]		[0.38-1.5]
SNP16	GG	326	0.92	397	368 ± 80	8.7	354	8.9	397	45	23	121	374	511	2274	376	0.80
rs2736655			[0.68-1.1]			[6.4-13]		[4.0-14]		[36-58]	[19-29]	[85-170]		[405-639]	[1569-3368]		[0.38-1.7]
	AG	107	1.0	127	365 ± 72	8.9	113	10	127	46	24	129	120	532	2208	120	0.85
			[0.73-1.2]			[6.4-14]		[4.0-16]		[38-59]	[19-29]	[91-193]		[418-652]	[1649-3264]		[0.40-1.7]
	AA	11	0.96	11	349 ± 51	7.3	10	8.5	11	41	27	135	9	429	2358	9	0.58
			[0.78-1.3]			[5.4-12]		[4.6-13]		[35-63]	[20-29]	[82-173]		[429-522]	[1888-2605]		[0.41-2.5]
SNP18	TT	135	0.92	161	376 ± 73	9.3	143	9.9	161	46	24	121	149	525	2165	149	0.77
rs2736654			[0.74-1.2]			[6.2-12]		[4.8-14]		[39-59]	[20-30]	[87-178]		[397-657]	[1596-3246]		[0.43-1.4]
	GT	230	0.94	277	363 ± 80	8.5	242	8.8	277	45	22	126	262	516	2263	265	0.81
			[0.68-1.2]			[6.4-14]		[4.1-15]		[36-57]	[18-27]	[88-170]		[405-636]	[1666-3315]		[0.36-2.1]
	GG	80	0.90	98	363 ± 73	8.3	93	9.5	98	47	24	115	92	492	2311	91	0.77
			[0.70-1.2]			[6.5-11]		[2.4-15]		[36-59]	[19-30]	[85-176]		[419-665]	[1497-3477]		[0.40-1.6]
SNP28	GG	381	0.92	458	366 ± 70	8.5	412	9.5	458	46	23	124	428	518	2292	430	0.79
rs3778443			[0.71-1.2]			[6.2-13]		[4.2-15]		[37-58]	[19-29]	[88-171]		[409-642]	[1588-3281]		[0.38-1.7]
	AG	63	1.0	76	375 ± 82	9.7	63	8.6	76	48	23	126	73	519	2126	73	0.75
			[0.68-1.3]			[6.8-13]		[3.7-15]		[38-60]	[19-29]	[84-194]		[399-654]	[16449-3489]		[0.36-1.4]
	AA	4	1.0	4	382 ± 30	9.5	4	6.7	4	48	24	110	4	482	2479	4	0.54
			[0.71-1.4]			[5.9-20]		[-1.6-16]		[35-69]	[20-28]	[87-218]		[350-540]	[1244-3212]		[0.35-0.93]

Table S7.2 (continued)

SNP		Genotype		Glo1 expression					Plasma MGO and D-lactate					Plasma AGEs			
		n	Glo1 mRNA	n	Plasma MGO	Plasma D-lactate	n	iAUC MGO post- OGTT (units)	n	Plasma free CEL	Plasma protein-bound CEL	Plasma free MG-H1	n	Urinary CEL	Urinary MG-H1	n	Urinary D-lactate
SNP38	GG	354	0.93	428	365 ± 77	8.4	386	9.1	428	45	23	121	402	517	2292	402	0.78
rs1781716			[0.71-1.2]			[6.2-13]		[4.0-14]		[36-58]	[19-29]	[89-173]		[405-638]	[1584-3259]		[0.38-1.7]
	CG	79	0.94	96	372 ± 81	9.6	81	10	96	49	23	132	90	528	2126	92	0.81
			[0.68-1.3]			[6.8-13]		[4.2-14]		[37-60]	[19-29]	[85-180]		[416-665]	[1662-3637]		[0.47-1.5]
	CC	4	1.0	4	382 ± 30	9.5	4	6.7	4	48	24	110	4	482	2474	4	0.54
			[0.71-1.4]			[5.9-20]		[1.6-16]		[35-69]	[20-28]	[87-219]		[350-540]	[1244-3123]		[0.35-0.93]
SNP40	CC	223	0.94	273	369 ± 75	9.0	242	9.5	273	46	24	120	252	525	2252	254	0.81
rs10484854			[0.73-1.2]			[6.2-13]		[4.2-15]		[38-58]	[19-30]	[88-170]		[401-646]	[1664-3263]		[0.44-1.7]
	CT	194	0.93	226	364 ± 79	8.5	201	9.3	226	44	23	126	216	511	2228	216	0.77
			[0.66-1.2]			[6.5-13]		[3.9-16]		[36-58]	[19-29]	[84-171]		[415-635]	[1532-3142]		[0.35-1.7]
	TT	28	0.84	35	374 ± 83	8.3	33	7.9	35	50	24	143	33	441	2291	33	0.47
			[0.73-1.1]			[5.9-12]		[16-12]		[35-59]	[19-30]	[85-205]		[384-567]	[1370-3505]		[0.28-1.2]
SNP45	GG	163	1.0	199	367 ± 74	8.8	174	10	199	45	24	120	181	525	2310	183	0.84
rs17622621			[0.78-1.2]			[6.2-12]		[4.2-15]		[38-59]	[18-29]	[89-170]		[406-650]	[1746-3213]		[0.44-1.6]
	AG	212	0.88	252	367 ± 82	8.5	226	9.5	252	44	23	118	241	511	2219	241	0.79
			[0.67-1.2]			[6.5-13]		[4.3-15]		[36-55]	[19-29]	[80-170]		[410-632]	[1482-3265]		[0.38-1.7]
	AA	66	0.86	77	369 ± 71	8.7	72	7.3	77	51	24	130	73	511	2187	73	0.50
			[0.70-1.1]			[5.8-14]		[3.4-12]		[39-63]	[19-30]	[88-175]		[410-706]	[1559-3377]		[0.33-1.7]
SNP49	AA	112	0.90	135	365 ± 74	8.8	122	8.3	135	50	23	126	127	511	2125	128	0.62
rs1049346			[0.68-1.1]			[6.4-13]		[3.8-12]		[36-63]	[19-29]	[86-184]		[419-6845]	[1667-3489]		[0.35-1.6]
	AG	240	0.94	284	370 ± 80	8.8	254	9.4	284	44	23	126	272	520	2327	272	0.79
			[0.66-1.2]			[6.5-13]		[4.0-15]		[37-55]	[19-29]	[84-170]		[408-637]	[1500-3416]		[0.38-1.7]
	GG	94	0.97	117	364 ± 74	8.2	101	10	117	45	23	119	104	526	2319	105	0.91
			[0.78-1.2]			[6.0-13]		[4.1-16]		[37-59]	[18-28]	[89-170]		[406-663]	[1909-3174]		[0.48-1.8]

[1]Data are presented as mean ± standard deviation for normally distributed variables and median [interquartile range] for skewed variables AGE, advanced glycation endproduct; CEL, Nε-(1-carboxyethyl)lysine; iAUC MGO, incremental area under the curve of MGO after an oral glucose tolerance test; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; MGO, methylglyoxal; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

Table S7.3 Associations between iAUC MGO after an OGTT and SNPs of GLO1 using logistic regression[1].

	N	Genotype	iAUC MGO (low/high)	
			OR	p
SNP4 (rs3199033)	475	AA	-	-
		AT	0.82	0.40
		TT	0.39	0.32
SNP13 (rs3799703)	467	GG	-	-
		AG	1.00	0.99
		AA	1.38	0.24
SNP16 (rs2736655)	477	GG	-	
		AG	1.24	0.35
		AA	1.45	0.57
SNP18 (rs2736654)	478	TT	-	
		GT	0.82	0.36
		GG	0.89	0.68
SNP28 (rs3778443)	479	GG	-	-
		AG	1.01	0.99
		AA	0.30	0.33
SNP38 (rs1781716)	471	GG	-	-
		CG	1.23	0.40
		CC	0.32	0.36
SNP40 (rs10484854)	476	CC	-	-
		CT	0.90	0.60
		TT	0.98	0.96
SNP45 (rs17622621)	472	GG	-	-
		AG	0.91	0.66
		AA	0.72	0.26
SNP49 (rs1049346)	477	AA	-	-
		AG	1.30	0.26
		GG	1.48	0.17

Odds ratios of logistic regression, using a categorical variable of iAUC MGO (below/above median) as outcome variable and the three genotypes (dummy variables with major homozygous as reference) as main independent variable. Odds ratio indicates the odds of having a MGO concentration above the median for the stated genotype compared to the reference genotype. Results are displayed for fully adjusted model (adjusted for age, sex and glucose metabolism status). iAUC: incremental area under the curve. MGO: methylglyoxal. OR: Odds ratio.

Supplementary figure

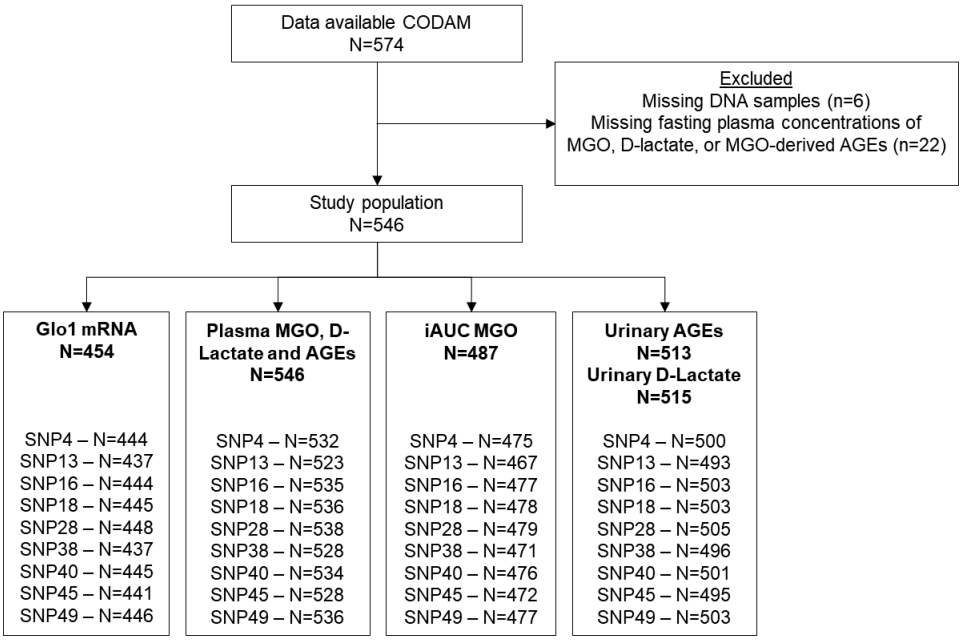

Figure S7.1 Flowchart of CODAM study population.

Chapter 8

Summary and general discussion

Summary and general discussion

The main aim of the research described in this thesis was to investigate the associations of dicarbonyl intake via the diet with presence of dicarbonyls and their subsequent AGEs in the human body, and with low-grade inflammation, microvascular function, insulin sensitivity, β-cell function, and presence of prediabetes and type 2 diabetes.

Presence of dicarbonyls in foods and drinks

Dicarbonyl content in foods and drinks has been evaluated in previous studies, but these studies were often small and limited to specific food groups and did not always use extensively validated methods of extraction and quantification[1-20]. In **chapter 2**, we therefore describe the optimization and validation of an ultra high-performance liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS) method to quantify the major dicarbonyls methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) in foods and drinks. Moreover, we present a first comprehensive food composition database with dicarbonyls content of 223 commonly-consumed products, covering a broad range of food groups, including vegetables, fruit, cheese, meat, fish, and ready-made products.

We found that dicarbonyl concentrations were, generally, the highest in sugar-rich foods and drinks, and in products that were exposed to heat-treatment during food processing. Total dicarbonyl concentrations were highest in dried fruit, chocolate candy bars, Dutch spiced cake and sweet bread condiments such as bee honey, apple molasses, and sugar syrup (>200 mg/kg), and lowest in tea, dairy, light soft drinks, and rice (<10 mg/kg). We found that 3-DG is the most abundant dicarbonyl in most foods and drinks, and this is in agreement with literature[3,6,21]. MGO was the most abundant dicarbonyl in fish, most meats and coffee. GO was the most abundant dicarbonyl in most vegetables, fruits, and nuts. Dicarbonyl content differed with degree of food processing, carbohydrate/sugar content, and type of sweetener used. For example, dicarbonyl content was high in items exposed to intense heat-treatment, particularly those containing molasses, caramel, glucose-fructose syrup, or monosaccharides. We corroborated results from other studies that heat-treatment increases the concentrations of these dicarbonyls[22,23], as we observed an increase in concentrations over time when potatoes were fried. In line, stir-fried food items had higher concentrations than when that same item was raw/boiled (e.g. raw, boiled, and stir-fried carrot). MGO and GO content was relatively high in foods and drinks that included a fermentation step in food

processing, such as coffee and chocolate. Total dicarbonyl content, especially 3-DG, was higher in sugar-rich items than sugar-free or artificially sweetened items.

This database is the most extensive database to date on MGO, GO, and 3-DG content in foods and drinks. Strengths of this database were that we used the validated gold-standard UHPLC-MS/MS technique to quantify dicarbonyl contents, that we included a wide range of foods and drinks covering all food groups, and that food items were selected based on coverage of the food frequency questionnaires (FFQs) used in The Maastricht Study, making it a well-suited tool to accurately estimate dietary dicarbonyl intakes in individuals from this cohort. Hereby, it serves as a first step in elucidating the physiological impact of dicarbonyl intakes on human health.

Contribution of the diet to dicarbonyl stress and AGE accumulation

In **chapter 3**, we used the food composition database described in chapter 2 to estimate habitual intake of dicarbonyls from the diet in individuals from the The Maastricht Study. For this, we matched all 253 food items from the validated FFQ used in this cohort[24], with the corresponding food item in our database. In this population, median [IQR] intakes of MGO, GO, and 3-DG were 3.6 [3.0-4.3], 3.5 [2.9-4.3], and 17 [12-23] mg/day, respectively. Coffee and bread were the main dietary sources of MGO, whereas bread was the main source of GO and 3-DG, all responsible for approximately 25% of intake.

Furthermore, we showed that a higher habitual intake of dietary MGO and GO was associated with higher corresponding plasma concentrations. Additionally, higher intake of MGO was associated with higher skin autofluorescence, an estimate of skin AGEs that is thought to reflect AGEs in tissue[25]. These results suggest dietary absorption of these small dicarbonyl compounds. The associations of dietary MGO with plasma MGO and with skin autofluorescence were mainly driven by coffee intake, whereas the association of dietary GO with plasma GO was mainly driven by fruit and fruit juice intake, rather than from complex solid foods with high concentrations of these compounds. This implies that the food matrix might play a role in the absorption of dicarbonyls, and dicarbonyls may be more easily absorbed from drinks with simple food matrices. Considering the reactivity of dicarbonyls, it is conceivable that these dicarbonyls react with proteins and other food constituents during digestion. Indeed, several food constituents were reported to scavenge dicarbonyls[26 27]. However, little is known about the consequences of these bound dicarbonyls. Thus, it is important to consider the food matrix when predicting effects of dietary dicarbonyls.

Besides absorption of these dicarbonyls through the intestinal epithelial wall, there are also other possible, more indirect, mechanisms by which dicarbonyl intake via the diet may contribute to higher plasma dicarbonyls. For example, the concentrations of plasma MGO and GO could be elevated as a result of diminished detoxification due to exhaustion of the glyoxalase pathway in the intestine after long-term exposure to dicarbonyls from the diet[28]. Future studies with stable isotope labeled dicarbonyls are required to determine the exact gastrointestinal and metabolic fate of dietary dicarbonyls.

Such studies with stable isotope labeled dicarbonyls will also provide more insight into which extent of the circulating pool of dicarbonyls and AGEs in plasma originates from exogenous exposure to dicarbonyls. In **chapter 3** we calculated that a 1 mg/day higher intake of MGO (corresponding to for example ~400 mL coffee (**chapter 2**)) was associated with a 2-3% higher plasma MGO and skin autofluorescence. Notably, concentrations of free dicarbonyls in (fasting) plasma might provide only a snapshot and be an underestimation of actual contributions of dietary dicarbonyls.

Our results are in line with studies in animals that showed that oral MGO resulted in increased plasma MGO concentrations[29-31]. We recently found in mice that long-term administration of a high dose (50 mM) of oral MGO led to a two-fold higher in plasma MGO (*unpublished data, van Grieken and Schalkwijk*). Our results are in contrast with two small-scale human studies in which a single oral dose of honey containing MGO did not lead to changes in urinary MGO excretion[32], but a single oral dose of 3-DG led to an increase of urinary 3-DG excretion[33]. However, these studies had no data on dicarbonyls in plasma or on AGEs. A possible explanation why 3-DG intake was not associated with plasma 3-DG or skin autofluorescence in our study, despite the higher habitual intake of 3-DG, is that 3-DG is rapidly excreted into the urine before it reacts to proteins, considering it is less reactive than MGO and GO. Interestingly, our association between higher MGO intake from coffee and skin autofluorescence is in line with a study that showed that coffee consumption was an independent predictor of skin autofluorescence in the Lifelines cohort[34]. Our study shows that MGO from coffee might explain, at least partly, this association between coffee consumption and skin autofluorescence.

Although in this thesis we mainly focus on the role of consumption of exogenous dicarbonyls via the diet, our diet can also contribute to dicarbonyl stress through modulating endogenous dicarbonyl formation, e.g. via ingestion of food components that are precursors of dicarbonyls (Figure 8.1)[35,36]. Therefore, in **chapter 6**, we examined whether long-term consumption of a diet that induces

high postprandial glucose peaks was associated with higher concentrations of these dicarbonyls and their derivative AGEs in the human body. In the CODAM study we found that a habitual diet with a higher glycemic load was associated with higher concentrations of the free AGE MG-H1 in urine, also after adjustment for dietary intake of AGEs. Higher glycemic load was also associated with higher concentrations of free plasma MG-H1 and free urinary CML, but this was not independent of dietary AGE intake. Urinary AGEs are most likely a reflection of AGE accumulation and degradation in tissues, where they may be involved in tissue dysfunction. These observations suggest that low carbohydrate dietary interventions have the potential to lower formation and accumulation of AGEs, possibly via reduced postprandial glycemia. Carbohydrate quantity seemed to play a larger role in the formation of AGEs than carbohydrate quality.

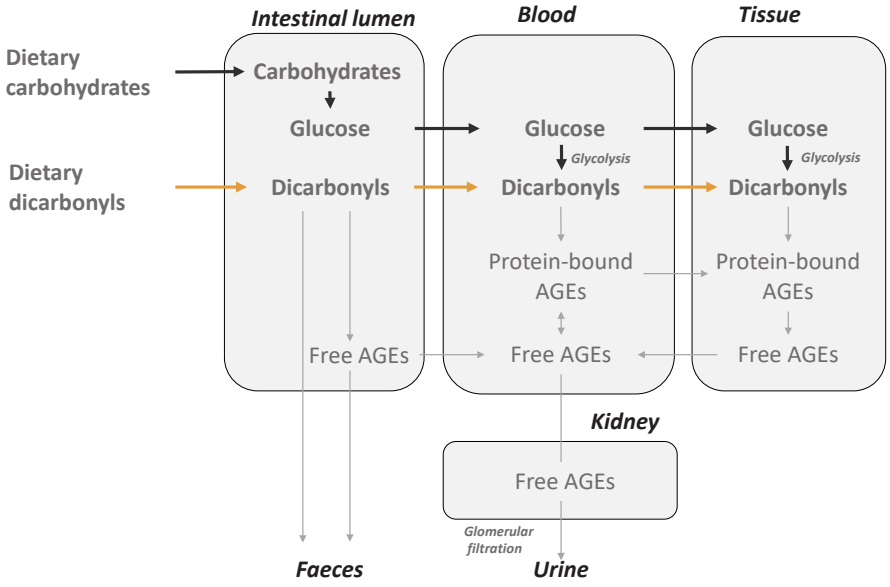

Figure 8.1 **Schematic overview of potential contribution of carbohydrates and dicarbonyls from the diet to endogenous dicarbonyls and advanced glycation endproducts (AGEs).** Dietary carbohydrates cause postprandial glucose peaks, inducing endogenous formation of dicarbonyls. Dietary dicarbonyls might be absorbed in the intestinal lumen, or react with proteins to form free AGEs that can be absorbed. Dietary dicarbonyls and AGEs that are not absorbed can end up in faeces. In the circulation, these reactive dicarbonyls modify amino acids, leading to formation of AGEs in either the free or protein-bound form. These AGEs can be incorporated into various tissues where they can accumulate. Protein-bound AGEs in tissues are broken down during proteolysis, and released into the circulation as free AGEs. Circulating dicarbonyls and AGEs can be excreted via the kidneys into the urine. This overview is not exhaustive and focuses on dietary carbohydrates and dicarbonyls, as these are examined in this thesis.

Contribution of impaired detoxification to dicarbonyl stress and AGE accumulation

Impaired detoxification of dicarbonyls is a driver of dicarbonyl stress. Glyoxalase 1 (Glo1) is the rate-limiting enzyme in the detoxification of methylglyoxal (MGO) into D-lactate[37]. Genetic variation in *GLO1* may alter the expression and/or the activity of Glo1, and may hence represent life-long exposure to a higher or lower detoxification potency and MGO stress[38]. In **chapter 7**, we examined the associations of nine single nucleotide polymorphisms (SNPs) with gene expression of *GLO1* in white blood cells and with markers of MGO-stress, i.e. MGO, D-lactate, and MGO-derived AGEs in fasting plasma, MGO formation during an oral glucose tolerance test (OGTT), and MGO-derived AGEs and D-lactate in urine. In the CODAM study, we observed that two out of these nine SNPs were associated with Glo1 expression, but these associations were not statistically significant after adjustment for multiple testing. We therefore concluded that polymorphisms of *GLO1* were not associated with *GLO1* mRNA expression or markers of MGO stress, suggesting that these SNPs are not functional. Notwithstanding, the observed associations could be of interest and the apparent null findings have several possible explanations. First, we only had data on *GLO1* expression in whole blood, and Glo1 activity/expression might be altered in other tissues. Second, this study is likely underpowered, because changes that occur as a result of SNPs are expected to be modest, and large sample sizes are often required to pick up any effect of SNPs. Third, the correction for multiple testing might be too strict for these analyses (see discussion of multiple testing in the methodological considerations section below). Therefore, replication in a larger, independent cohort is warranted.

Taken together, we can conclude from these chapters that both a habitual diet with a higher MGO or GO intake, and a habitual diet with a higher glycemic load were associated with higher endogenous levels of dicarbonyls or AGEs. Impaired detoxification did not seem to play a major role in dicarbonyl stress and AGEs, although this has to be interpretated with caution considering mentioned limitations. Several research gaps remain to be addressed to draw conclusions on which of these two sources is the largest contributor to the endogenous dicarbonyls and AGEs pool. Both exposures were studied in different cohorts and different measures of dicarbonyls and AGEs were available, complicating comparison of the associations. In addition, dicarbonyl content in foods and drinks was highly correlated to carbohydrate content, and it is impossible to distinguish these contributions in an observational study. Moreover, it is uncertain whether

exogenous dicarbonyls and AGEs have similar biological consequences as endogenously formed dicarbonyls and AGEs.

Potential health effects of dietary dicarbonyls

The associations between dietary dicarbonyls and health outcomes were explored in chapters 4 and 5, and results are schematically displayed in Figure 8.2. In **chapter 4**, we reported that a higher habitual intake of MGO was associated with lower low-grade inflammation scores. This association found in The Maastricht Study was confirmed in the CODAM study. Higher MGO intake was also associated with impaired retinal venular dilation, but not with other features of microvascular function, and this might hence be a chance finding. In **chapter 5**, we reported that individuals who consumed more MGO and 3-DG had a greater insulin sensitivity, in The Maastricht Study. Moreover, higher 3-DG intake was associated with less type 2 diabetes, both in the total cohort and after excluding individuals with known type 2 diabetes. Higher MGO intake was associated with less type 2 diabetes after excluding individuals with known type 2 diabetes. There were no consistent associations between dicarbonyl intakes and β-cell function. Although the results from these chapters suggest that food–derived dicarbonyls might induce both favorable as well as harmful effects, dicarbonyl intakes were more often associated with beneficial effects on markers of type 2 diabetes than with adverse effects.

The associations of higher MGO intake with less low-grade inflammation and with better insulin sensitivity and less type 2 diabetes were at first sight somewhat unexpected, considering the associations of higher MGO intake with higher plasma MGO and skin autofluorescence observed in chapter 3, and the adverse health outcomes that have been associated with elevated plasma dicarbonyls and skin autofluorescence[39-41]. Moreover, most animal studies reported adverse consequences of oral MGO[29,42-47]. Possible explanations for the discrepancies between these studies in animals and our findings in humans, are that experimental studies were often conducted with very high concentrations of MGO, out of the physiological range, whereas the exposure to dicarbonyls in humans is much lower. In addition, most studies in animals used a commercially available batch of MGO, which is highly contaminated with other reactive compounds. Therefore, we cannot exclude the possibility that the adverse effects of MGO administration observed in animals are attributable to the very high concentrations of MGO or the contaminations in the batch of MGO.

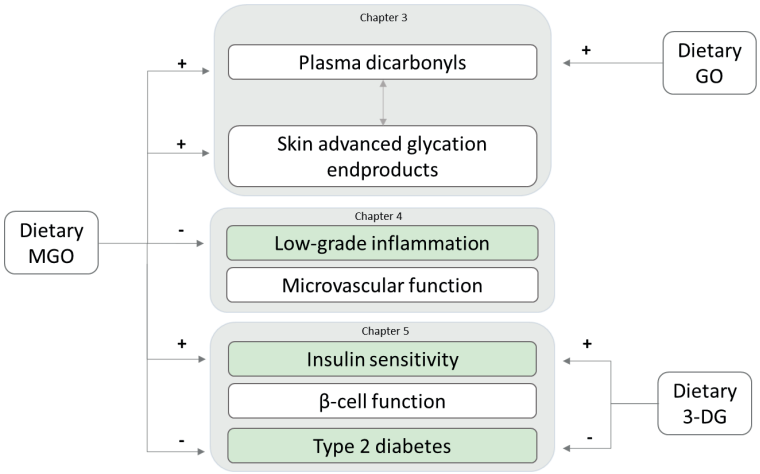

Figure 8.2 **Associations between dietary MGO, GO, and 3-DG intake and outcomes as reported in this thesis.** Arrows with '+' indicate positive associations and arrows with '-' indicate inverse associations. Outcomes in green indicate that we found a presumable beneficial association between dietary dicarbonyls and that outcome. Higher dietary MGO was associated with higher plasma MGO concentrations and with more skin advanced glycation endproducts (chapter 3), with less low-grade inflammation (chapter 4), with better insulin sensitivity and with less type 2 diabetes (chapter 5). Higher GO intake was associated with higher plasma GO concentrations (chapter 3), but not with any of the other outcomes (chapter 4 and 5). Higher 3-DG intake was associated with better insulin sensitivity and with less type 2 diabetes (chapter 5). MGO: methylglyoxal, GO: glyoxal, 3-DG: 3-deoxyglucosone.

Interestingly, we recently corroborated our association between higher MGO intake and greater insulin sensitivity in an animal experiment. In this experiment where we used pure MGO, mice that received 1 mM of oral MGO via drinking water for 13 weeks were more insulin sensitive (*unpublished data, van Grieken and Schalkwijk*). Mice that received a higher dose of 50 mM of MGO via drinking water showed no change in insulin sensitivity.

Although the calculated intake of MGO from low dose of 1 mM in drinking water is still approximately 15 times higher than the dietary exposure to MGO of mice fed with chow diet, this suggests that MGO may have beneficial effects at a lower dose in animals. In line with these observations and our observations in humans, several recent studies in animals also reported that long-term exposure to oral MGO had beneficial consequences, including an increase in antioxidant systems[48] and slightly increased survival[49], despite elevated MGO concentrations in the circulation, as described in the introduction of this thesis. These findings challenge the conviction that dicarbonyls only have adverse consequences. Our results support the idea that

modest increases in plasma MGO, as induced by dietary MGO intake, might have beneficial effects. This may also be the case for the other dietary dicarbonyls, at least GO and 3-DG, because we also found associations of GO and 3-DG intakes with several favorable health outcomes, although these results were less consistent. These differences between MGO, GO, and 3-DG suggest that not all dicarbonyl compounds are equal. Differences between dicarbonyls include their prevalence in foods and drinks, their reactivity, their size, and the AGEs that are formed upon reaction with amino acids. Our observation of most consistent associations for MGO intake might be explained by the high reactivity of MGO, or because MGO-derived AGEs exert more biological effects.

Methodological considerations

Nutritional epidemiology research has several methodological concerns that have to be considered when interpreting the results. In the following section, the most relevant considerations for the research presented in this thesis are discussed.

Study design and population

In chapters 3, 4, and 5, we used data of The Maastricht Study. The Maastricht Study is a population-based observational study with an oversampling of individuals with type 2 diabetes, making it suitable to study (progression of) type 2 diabetes. This study is characterized by deep-phenotyping, allowing extensive adjustment for possible confounders. Outcomes are assessed using validated, state-of-the-art techniques, including multiple biomarkers of low-grade inflammation, multiple features of microvascular function in various organs, multiple indices of β-cell function, and insulin sensitivity as Matsuda index.

Individuals of The Maastricht Study are recruited from the general population, with an oversampling of individuals with type 2 diabetes by study design[50]. We accounted for this oversampling of type 2 diabetes by adjusting for glucose metabolism status in our analyses and by repeating our analyses after exclusion of individuals with previously diagnosed type 2 diabetes. In The Maastricht Study, representation of the source population in the study region is monitored, but selection bias remains a possibility during recruitment in all observational cohorts, for example in case of a higher response rate of healthier individuals. As for the studies in this thesis in particular, we performed complete case analyses, excluding participants with missings in any of the covariates for each outcome. Selection bias might have occurred when missings for exposures, potential confounders, or outcomes were missing not at random. To examine this, we compared general characteristics between excluded and included individuals. Although excluded

individuals were generally somewhat less healthy, missings on most key variables were considered to be at random. A potential source of selection bias by design of The Maastricht Study is that an oral glucose tolerance test (OGTT) was not performed in individuals with elevated fasting plasma glucose (above 198 mg/dL) and/or who used insulin. In chapter 5, we used the Matsuda index as outcome, which is derived from the OGTT, thereby excluding individuals who are considered to have the most severe complications of type 2 diabetes. We therefore also performed robustness analyses using insulin resistance (assessed as HOMA2-IR), HbA1c, fasting glucose, and post-load glucose as outcomes, because these were available in more individuals, and we observed similar results for these outcomes.

Currently, baseline data is available in The Maastricht Study, thus all analyses are cross-sectional. This does not allow the assessment of causality and we cannot exclude that reversed causality occurred. To examine this reversed causality, we repeated our analyses after excluding individuals with known type 2 diabetes, who may have adapted their diet after diagnosis. Generally, this did not change our results. In addition, we observed associations for all dietary dicarbonyls (MGO, GO, and 3-DG), which are all present in different food items. 3-DG intake is highly correlated with sugar intake, but this is the case to a lesser extent for MGO and GO. Moreover, dicarbonyl intake also largely comes from food items that are considered healthy, such as fruit, vegetables and coffee. This was supported by the similar Dutch Healthy Diet Index (DHD15) score for individuals over all tertiles of dicarbonyl intakes, and by the robustness of our associations after additional adjustment for the DHD15 score.

Dietary assessment

Dietary intake in The Maastricht Study is assessed using a validated 253-item semi-quantitative FFQ, making it one of the most comprehensive FFQs in the Netherlands. The Maastricht-FFQ includes questions on frequency and consumed amounts with a 1-year reference period, and was specifically developed for this population. FFQs are the most common method to assess dietary intake in large epidemiological studies, mainly because of the lower costs and participant burden than dietary recalls and food records. In spite of methodological limitations, FFQs can sufficiently rank individuals by their intake of foods, energy, and nutrients, and this ranking is pivotal to explore associations between diet and health outcomes[24]. The Maastricht-FFQ is validated against a mean of 2.8 (range 1-5) 24-hour recalls in 135 participants[24]. Overall, the relative validity of the Maastricht-FFQ was considered to be good, and this validation showed that this FFQ can be used for studying associations both at nutrient and foods levels.

In our studies, the habitual dietary dicarbonyl intakes were estimated by matching food intakes from the FFQs with our food composition database for dicarbonyls. This database was specifically developed to cover food items of the Maastricht-FFQ and is the most extensive database to date, containing MGO, GO, and 3-DG content of 223 foods and drinks and covering all major food groups, making it an accurate tool to estimate dicarbonyl intakes. Of the 253 food items in the Maastricht-FFQ, 150 items were also present in the database and could be directly matched. 103 items were not present in the database, and these items were matched to a comparable food item in the database, or to the average of comparable food items (for example the average of all vegetables in the database as estimation of red cabbage).

Notwithstanding the mentioned strengths of our database and FFQ, it is important to consider potential sources of measurement error when interpreting the estimates of dietary dicarbonyl intake in this population. Dicarbonyls are mainly formed during heat-treatment, and it is therefore important to take preparation methods into account. Our dietary dicarbonyl database and FFQ covered this to a certain extent, because both included various common preparation methods such as boiling or stir-frying. For example, our dietary dicarbonyl database contained raw, boiled, and stir-fried vegetables and raw, steamed, and baked meat and fish products, and several preparation methods were also specified in the FFQ (e.g. raw and boiled vegetables). In addition, we explored variation of dicarbonyl concentrations between different batches and different brands for the same food item, and found that this variation was smaller than the variation between different products. The variation was smallest for drinks and unprepared foods (**chapter 2**), which is reassuring because coffee and bread are the main contributors to dicarbonyl intakes in this population (**chapter 3**) and both belong to these categories. Because coffee was the main contributor to MGO intake, we further explored dicarbonyl content in filtered or unfiltered and caffeinated or decaffeinated coffee, but MGO was abundant in all products. However, there presumably still is some variation in industrial and household preparation of foods and drinks, as both our database and FFQ are unable to capture the duration and intensity of heat treatment applied to the food items consumed by individuals. Although The Maastricht Study-FFQ was validated for intake of the major food groups, it has not been validated for estimation of total dicarbonyls intake, and it would be interesting to evaluate the accuracy of this FFQ in that regard.

Moreover, measurement error might have occurred in the measurement of dicarbonyl content in food items. For example, dicarbonyls might be formed during the quantification procedure or they might react with proteins depending on the

food matrix, leading to over- or underestimation of actual concentrations. To minimize these possibilities, we optimized and validated our quantification method and used the gold-standard method (UHPLC-MS/MS). Although absolute concentrations varied, the order of magnitude of dicarbonyls reported in our database was comparable to literature, with higher concentrations of 3-DG than MGO and GO in most foods and drinks[6]. Furthermore, slight under- or overestimations of actual concentrations are presumably the same for all food items, and thus would not have affected our ranking of individuals from low to high dicarbonyl intakes.

An additional consideration regarding measurement error is that dicarbonyl content of the food items in the food composition database were quantified between 2018 and 2021. The FFQs of The Maastricht Study were conducted between 2010 and 2020, and those of CODAM between 1999 and 2002. It might be that the food composition or industrial processing of foods has changed since then, and that the dicarbonyl content measured in 2018-2020 are an under- or overestimation of those present in the period when the FFQs were conducted. However, the findings in this thesis mainly rely on observations in The Maastricht Study, which was conducted recently, and it is therefore unlikely that dicarbonyl content of foods has changed greatly in those years.

Another potential source of measurement error in our self-reported dietary intake is reporting bias. Underreporting of dietary intake is a common phenomenon in dietary recalls[51]. If the extent of underreporting is similar for all individuals, this does not affect the ranking of individuals according to intakes. However, it is conceivable that underreporting differs between individuals, and for example individuals with overweight or individuals diagnosed with type 2 diabetes who have received dietary advice may be more inclined to underreport their intakes. We aimed to minimize this risk by only informing participants about their glucose metabolism status after completion of the FFQ, by adjusting our associations for total energy intake and BMI, and by repeating the analyses after exclusion of individuals with previously diagnosed type 2 diabetes. These alternative statistical approaches did not change our results and, if anything, associations of dietary dicarbonyl intakes with insulin sensitivity and presence of type 2 diabetes were even stronger after excluding individuals with previously diagnosed diabetes.

In conclusion, the combination of the most extensive dicarbonyl database to date and the validated FFQ used in this thesis likely resulted in reasonable estimates of habitual dicarbonyl intake in this population, but certain measurement error in estimating can never be excluded. The consequences of measurement error depend on whether the error occurs random or systematic, and whether it is in the

exposure or the outcome. Systematic error in an exposure or outcome variable can cause both overestimation and underestimation of the effect size. Random measurement error in an exposure variable biases results towards the null (regression dilution bias)[52]. Because most errors in our exposure (dietary dicarbonyl intakes) as described above are likely to occur at random, results would have been biased to the null. Therefore, if anything, the actual associations may be stronger than reported in this thesis.

Confounding

Confounding occurs when a true association between an exposure and an outcome is altered by one or more extraneous factors (i.e. confounders)[53]. An extraneous factor is a confounder when it is associated with both the main exposure and the outcome, and is not on the causal path between both. We aimed to minimize the risk of confounding by adjusting for factors that were considered potential confounders in our multiple linear regression models. Notwithstanding our efforts to adjust for confounding, in observational nutrition studies it remains challenging to fully eliminate residual confounding by other dietary factors. It has been reported that 'single nutrient' studies may potentially be confounded by the effect of dietary patterns[54]. We aimed to address this issue by adjusting for total energy intake in the fully adjusted models. There are several approaches to adjust for total energy intake. In chapters 3, 4, and 5, we adjusted for total energy intake using the standard multivariate method, because this is the simplest method and facilitates interpretation of the beta estimates. In chapter 3, we verified that using alternative methods, i.e. the energy density method or the residuals methods, yielded similar results. Moreover, we additionally adjusted our full models for the Dutch Healthy Diet Index or for either carbohydrate, fat, or protein intake (in turn to prevent multicollinearity, and on top of total energy intake). The conclusions were drawn from associations that remained robust in these analyses, indicating that these observed associations were independent of compliance to a healthier diet or differences in macronutrient intakes.

Nevertheless, it is inherent to observational nutrition studies that it is impossible to disentangle the effects of one compound from those of other dietary components that are present in the food matrix, because people do not eat isolated nutrients but meals consisting of a variety of foods with multiple nutrients that are likely to interact, as described in Hu et al.[54]. For instance, food items that have a high dicarbonyl content, may also contain other compounds that are associated with anti-inflammatory or other beneficial effects. For example, because dicarbonyls are (amongst others) formed during fermentation and fermented

products are thought to have beneficial health effects[55], we cannot exclude that our observations are attributable to other components formed in the fermentation process. More specifically, coffee was the main contributor to MGO intake in our study. In literature, coffee consumption is associated with skin autofluorescence[56], anti-inflammatory effects[57], and less type 2 diabetes[58]. In this thesis we observed associations between MGO intake and these outcomes. This indicates that the beneficially effects may be -partially- due to the MGO in coffee, but coffee also contains other bio-active compounds such as caffeine and polyphenols[53], and thus we cannot exclude the possibility that our observed associations between MGO intake and outcomes might be attributed to other compounds in coffee. Nevertheless, we observed an association between higher MGO intake and higher plasma MGO concentrations, which is unlikely to be due to other dietary compounds and suggests that MGO is absorbed from the diet. Additionally, our observed associations between higher MGO intake and less inflammation, greater insulin sensitivity and less insulin resistance remained after additional adjustment for coffee intake, suggesting that coffee intake alone did not explain the entire association. Nevertheless, additional adjustment for individual food groups might not be the most apt statistical approach to investigate the contribution of food groups, because of multicollinearity between total dicarbonyl intake and dicarbonyl intake from a single food group. Hence, it would be interesting to further explore statistical approaches that counter a degree of the model instability caused by multicollinearity, such as ridge regression[59].

Besides confounding by dietary factors, the associations might also be confounded by other factors. Individuals with an unhealthy dietary pattern often also adhere to a less healthy lifestyle in general, characterized by more smoking, alcohol consumption and less physical activity, which are also risk factors in the development of low-grade inflammation and type 2 diabetes. Because The Maastricht Study is such a deeply-phenotyped cohort using state-of the-art-measurements, we were able to carefully adjust our analyses for a wide range of potential confounders, and to perform several sensitivity analyses where we substituted confounders by alternative measures. For instance, we replaced physical activity assessed via the CHAMPS questionnaire with ActivPAL data and replaced educational level with occupational level or income. Often, selecting which measurement to use is a trade-off between using the gold-standard and having a larger power, for example using the ActivPAL which is more accurate but only available in a smaller sample. These sensitivity analyses allow to explore both options. Nevertheless, residual confounding remains a possibility.

Multiple testing

Multiple testing is defined as having multiple looks at the data, using various exposures and various outcomes. In all our studies, we performed multiple tests, as we included several dietary exposures (either MGO, GO, and 3-DG in chapters 3-5 or glycemic index and glycemic load in chapter 6) and several outcomes (ranging from four to eight in chapters 3-6). Performing multiple tests increases the likelihood that some of them will be statistical significant due to chance (false positives). One can adjust for multiple testing, which reduces the chance of false positive results, but does so at the cost of increasing the chance of false negative results[60]. In other words, it increases the chance that a true association that does exist in the population will not be detected in the analyses. Whether to adjust for multiple testing or not is a matter of ongoing debate[61-65]. False positive results must be rigorously avoided in the context of clinical, confirmatory studies such as in phase III drug trials. In contrast, our studies explored hypotheses generated from basic science and aimed to provide a first line of human evidence as basis for further research. Given this purpose, increasing the risk to miss true biological associations would be undesirable. Moreover, the conclusions in our studies were drawn from the totality of the evidence rather than from a single statistical test.

All in all, in our studies where we using dietary intakes as exposures (chapter 3-5), we decided not to adjust for multiple testing in our analyses, because we believe that missing true associations, and thereby overlooking possibly important findings, in this stage of research would be worse than doing observations that later cannot be confirmed in further studies. This is in line with the recommendations in the guidelines for observational studies (STROBE)[66]. We recognize the importance of further prospective studies or interventions studies, to confirm or refute our observations, and have mentioned this in our discussions. In addition, we discussed the issue of multiple testing in chapter 6.

We made an exception in chapter 7, where we examined the associations between nine SNPs and ten outcomes, thus performing 180 tests. In this study we adjusted for multiple testing, using false discovery rate (FDR) estimation[67]. This was done separately for each outcome, because the outcomes were all markers of the same pathway and therefore highly correlated, meaning that calculation of FDR based p-values for all outcomes simultaneously would likely lead to overcorrection.

External validity

For comparison with intake in The Maastricht Study, we calculated dietary dicarbonyls intake in an independent cohort, the CODAM study. In CODAM, The Dutch EPIC FFQ is used to estimate food intakes[68], and this FFQ was linked to the same dietary dicarbonyl database. Habitual intake of dietary dicarbonyls in CODAM was similar to The Maastricht Study, with median [IQR] intakes of 3.8 [3.1-4.6] mg/day for MGO, 3.4 [2.8-4.1] mg/day for GO, and 16 [11-22] mg/day for 3-DG. To our knowledge, only one other study estimated dicarbonyl intake, based on a hypothetical German diet, and these intakes were in the same order of magnitude, with intakes of 1.9 mg/day for MGO and of 61.2 mg/day for 3-DG[21]. Currently, our dietary dicarbonyl database is being used to estimate daily MGO, GO, and 3-DG intake in individuals from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, and it will be highly interesting to see whether intakes in this population are comparable, whether similar food groups contribute to these intake, and to explore differences between European countries. However, all these three cohorts are conducted in European countries. Our dietary dicarbonyl database was mainly composed to cover items of the Western diet, and it might be less suited to estimate dicarbonyl intakes in populations with different dietary patterns. Nevertheless, because our database covers all food groups and hence also includes many products such as fruits and vegetables, and because dietary patterns worldwide are shifting towards a Western diet, this database, potentially with the addition of certain products, may still be accurate to estimate dicarbonyl intakes in other countries as well.

Furthermore, we were able to verify the association between higher MGO intake and less inflammation, as observed in The Maastricht Study, in the CODAM study. A limitation regarding external validity is that both The Maastricht Study and CODAM consist of individuals over the age of 40 years, and largely consist of Caucasian individuals, limiting externalization of these associations to other populations. Nevertheless, there is no reason to expect that the associations are different in other age groups or ethnicities.

Conclusion and future directions

In conclusion, in this thesis we showed that individuals who consumed a habitual diet higher in MGO had higher MGO concentrations in plasma and more AGEs in the skin. Moreover, food-derived MGO seemed to induce anti-inflammatory effects, and contribute to a better insulin sensitivity and less type 2 diabetes. Higher GO intake was not associated with any of the health outcomes, in spite of the association with

higher GO in plasma. Individuals who consumed more 3-DG did not have elevated 3-DG, but had a better insulin sensitivity and less type 2 diabetes. These results, for the first time, show an association of dietary dicarbonyl intakes with circulating dicarbonyls and tissue AGEs, as well as with health outcomes. The novel finding of mostly favorable associations between dietary dicarbonyls and markers of type 2 diabetes, indicate that these dietary dicarbonyls might be less harmful to human health than hypothesized, and might even protect against the development of type 2 diabetes and its complications.

Further studies are warranted to confirm and expand on these observations. Examples of required future studies are prospective observational cohorts and intervention studies. Longitudinal data from The Maastricht Study will be available in the future once follow-up health outcomes are measured. Since habitual dietary dicarbonyl intake is already accurately estimated in this study, it would be interesting to also study the longitudinal associations between dietary dicarbonyl intakes and health outcomes. Another interesting cohort to examine these association is the EPIC cohort, for several reasons. The EPIC cohort was conducted in 10 European countries, with varying patterns of the Western diet. EPIC is characterized by a highly detailed FFQ, and with a total study population of 340,234 individuals and 12,403 verified incident cases of type 2 diabetes[69], it has a large power to detect associations. At the time of writing, dicarbonyl intakes are calculated for this study, using our food composition database for dicarbonyls, with an extension of 60 additional food items to accurately cover the FFQs used in EPIC, and we are planning to examine the associations between dietary dicarbonyl intakes and incident type 2 diabetes in this cohort. An additional interesting angle would be comparing dietary patterns low and high in dicarbonyls, complimentary to the focus on single nutrients, such as individual dicarbonyls. This can be done by principal components analyses[54], something that will be part of the analyses in EPIC. Exploring dietary patterns has as an additional advantage that they are more easily translatable to dietary guidelines and recommendations.

A next obvious step would be conducting a randomized controlled trial, in which participants follow either a diet low in dicarbonyls or high in dicarbonyls. Both diets should be matched for total energy intake and macronutrients intake, to limit confounding by intake of energy of other dietary factors. Because coffee is a major contributor to MGO intake, and hardly contributes to intake of energy or other macronutrients, an option would be to provide similar diets, one with coffee (high MGO group) and one with another drink (low MGO group). However, a remaining limitation of such a study design is that it will be impossible to identify the effects of MGO independent from other compounds in coffee. Therefore, it might be interesting to also include another food matrix with high or low MGO

concentrations, such as Manuka honeys with different concentrations of MGO, and a control without MGO. This could also be done for GO and 3-DG, as these dicarbonyls are also present in Manuka honey at high concentrations.

Finally, combining our data in humans with experimental studies in animals and *in vitro* can provide mechanistic insights into the biological pathways underlying the health effects of dicarbonyls intake from the diet.

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Chapter 9

Scientific and societal impact

Scientific and societal impact

Non-communicable inflammatory diseases – primarily cardiovascular diseases, respiratory diseases, cancers and diabetes – pose a major risk to healthy ageing. With an estimated 41 million deaths annually, they account for 71% of all deaths globally[1]. Besides a threat to human health, non-communicable diseases impose a major economic burden on health-care systems, the wider global economy, and on affected individuals, due to medical costs and loss of income[2]. Therefore, prevention of these diseases is imperative to avert these – often premature – deaths and improve quality of life. Diet is an important modifiable risk factor for these diseases, and the availability of nutritious foods and adherence to a healthy diet are key in tackling these diseases. Research aimed at identifying diets and dietary components that play a role in the progression of non-communicable inflammatory diseases, can provide fundamental knowledge which can serve as a basis for guidelines and regulations made by policy makers and governmental bodies, and for industrial techniques applied in food industry.

It is now well-established that elevated concentrations of endogenous dicarbonyls play a role in the development and progression of type 2 diabetes and its complications[3,4]. However, these dicarbonyls are not only formed endogenously, but are also formed during food processing and hence we are exposed to them via the diet[5,6]. This raises the question whether the consumption of these dietary dicarbonyls exerts similar harmful effects on human health as their endogenous counterpart and is therefore a concern to food safety. In this thesis, we have examined this question in more detail. To the best of our knowledge, the studies described in this thesis are the first to investigate the impact of dicarbonyl intake from the diet on health outcomes in humans. Based on our findings, we can conclude that there is no strong evidence for detrimental health effects posed by a higher habitual consumption of dietary dicarbonyls. In contrast, our results showed protective effects regarding low-grade inflammation, insulin sensitivity, and presence of type 2 diabetes. Based on our data we can conclude that there is no need for concern about dietary dicarbonyls for food safety. However, further prospective and intervention studies are definitely needed to confirm this and to study the associations with other outcomes, such as cancer and mental diseases. These outcomes are of particular interest because of the potential role of dicarbonyls in DNA damage by modification and in neurodegenerative mental diseases (as reviewed in[7]).

Although our results are of interest, at this stage, it is too early to make any strong recommendations on the policy maker or consumer levels. When we confirm these results in prospective cohorts and find that dietary dicarbonyls are

associated with higher insulin sensitivity and lower incidence of type 2 diabetes, our research can provide the fundament for prevention strategies for type 2 diabetes and related complications. In that case, the research output can be used to inform multiple stakeholders involved, including inspection bodies, food industry, governments, health-care providers, and consumers. As the World Health Organization mentioned in its global report on diabetes, "a combination of fiscal policies, legislation, changes to the environment and raising awareness of health risks works best for promoting healthier diets and physical activity at the necessary scale"[2]. The current and future research output in this field can be used by inspection bodies (such as Food and Consumer Safety Authorities), to monitor potential food safety concerns for human. Inspection bodies and governmental bodies can draft and implement regulation where necessary. If confirmed in future research, these findings can also provide evidence for dietary recommendations.

The studies describes in this thesis were performed in The Maastricht Study; a population-based observational cohort with individuals from 40-75 years[8]. Therefore these findings are applicable to the general population within this age range, and potentially can be translated beyond this. However, communicating new dietary advice has to be carefully considered in order to provide a clear message for the public, bearing in mind the risk of overwhelming the public and the variety of dietary guidelines already available. In addition, dicarbonyls are present in a broad range of food items, complicating specific dietary recommendations based on dicarbonyls. Given the crucial role of food processing in the formation of dicarbonyls, dicarbonyl content can be altered by food processing, without the need for - often challenging to achieve - dietary changes. The dietary dicarbonyl database presented in this thesis, provides extensive information on the presence of dicarbonyls in a broad variety of food items. It also adds to and confirms existing knowledge about the formation of dicarbonyls during heat treatment, and about processing techniques and ingredients that correlate with high/low dicarbonyl contents in food items. Thus, this database can be informative for food industry when considering processing adaptations in the future.

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Addendum

Nederlandse samenvatting

Dankwoord

Scientific output

Curriculum vitae

De inname van dicarbonylen uit voeding: vriend of vijand van onze gezondheid?

Introductie

Dicarbonylen zijn een groep reactieve (=snel bindende) moleculen. Ze binden aan aminozuren in eiwitten en DNA, waardoor deze veranderen van structuur en/of functie. Wanneer dicarbonylen binden aan eiwitten, worden deze ook wel versuikerde eiwitten genoemd, in het Engels 'advanced glycation endproducts' - afgekort AGE's. Verhoogde concentraties dicarbonylen en AGE's in het lichaam spelen een rol in de ontwikkeling van ouderdomsziektes zoals diabetes en hart- en vaatziekten.

Een verhoogde concentratie dicarbonylen in het lichaam - 'dicarbonylstress' - treedt op wanneer er meer dicarbonylen worden gevormd in het lichaam (endogeen) dan wel opgenomen uit voeding (exogeen), dan er afgebroken worden. De endogene vorming van dicarbonylen vindt vooral plaats tijdens de afbraak van glucose in cellen, tijdens de glycolyse. Voeding is de voornaamste bron van exogene blootstelling. Dicarbonylen worden gevormd tijdens de bereiding van eten, met name bij het verhitten van suikerrijke voedingsproducten. Op dit moment is er nog weinig bekend over de bijdrage van dicarbonylen uit voeding aan dicarbonylstress in het lichaam en de mogelijke effecten hiervan op de gezondheid.

In dit proefschrift hebben we onderzocht of mensen die langdurig veel dicarbonylen binnenkrijgen via voeding ook meer dicarbonylen opnemen en meer AGE's in hun lichaam hebben. Hiernaast hebben we onderzocht wat de gevolgen zijn van de inname van dicarbonylen voor de gezondheid. Dit hebben we gedaan door de verbanden tussen inname van dicarbonylen via voeding en verschillende gezondheidsuitkomsten te bestuderen. We hebben gekeken naar laaggradige inflammatie, de functie van kleine bloedvaten, de productie van het hormoon insuline door de bètacellen van de alvleesklier, de gevoeligheid van het lichaam voor dit hormoon, en de aanwezigheid van prediabetes en type 2-diabetes.

Belangrijkste bevindingen

De bijdrage van voeding aan dicarbonylstress

Een inschatting van de inname van dicarbonylen uit voeding kan gemaakt worden aan de hand van de hoeveelheid dicarbonylen in verschillende voedingsproducten. Tot nu toe zijn de hoeveelheden dicarbonylen vooral gemeten in enkele specifieke voedingsgroepen. Daarom hebben we eerst een uitgebreide database samengesteld

van 223 voedingsproducten waarin we de hoeveelheid van drie belangrijke dicarbonylen gemeten hebben: methylglyoxaal (MGO), glyoxaal (GO) en 3-deoxyglucosoon (3-DG). Deze metingen zijn uitgevoerd met een gevalideerde analytische techniek, namelijk vloeistofchromatografie gecombineerd met massaspectrometrie ('UPLC-MS/MS'). Deze dicarbonylendatabase is beschreven in **hoofdstuk 2.** De gemeten totale dicarbonylconcentraties waren het hoogst in suikerrijke producten en/of producten die sterk verhit worden tijdens bereiding, zoals gedroogd fruit, chocoladerepen met karamel, peperkoek, honing en stroop (>200 mg/kg). Concentraties waren het laagst in thee, zuivel, rijst en frisdranken waarin suiker is vervangen door zoetstoffen (<10 mg/kg). Van de drie gemeten dicarbonylen was 3-DG de meest voorkomende en hing deze concentratie het sterkst samen met de hoeveelheid suiker in het product. Koffie bevatte relatief veel MGO en chocolade relatief veel GO, waarschijnlijk door het roosteren van de koffie- en cacaobonen. Daarnaast worden MGO en GO mogelijk geproduceerd door micro-organismen tijdens de fermentatie van de bonen.

Deze database is wereldwijd de meest uitgebreide database van dicarbonylen in voeding die er momenteel beschikbaar is. Omdat er grote overlap is tussen voedingsproducten uit de database en voedingsproducten opgenomen in bestaande Nederlandse voedingsvragenlijsten, is deze database zeer geschikt om de inname van dicarbonylen vanuit de voeding in de Nederlandse populatie te schatten.

In **hoofdstuk 3** hebben we onze dicarbonyl database gebruikt om de inname van MGO, GO en 3-DG vanuit de voeding te schatten in een grote observationele studie: De Maastricht Studie. Hiervoor hebben we onze database gekoppeld aan de voedingsvragenlijsten van De Maastricht Studie. De geschatte innames van MGO, GO, en 3-DG (mediaan [interkwartielafstand]) in deze populatie waren respectievelijk 3.6 [3.0-4.3], 3.5 [2.9-4.3] en 17 [12-23] mg/dag. Koffie en brood leverden de grootste bijdrage aan MGO-inname en brood leverde de grootste bijdrage aan GO en 3-DG inname.

In **hoofdstuk 3** hebben we aangetoond dat mensen met een hogere gebruikelijke inname van MGO of GO uit voeding ook een hogere concentratie MGO of GO in het plasma hebben. Daarnaast hebben mensen met een hogere MGO inname ook meer AGE's in het weefsel (gemeten als autofluorescentie van de huid). Deze resultaten doen vermoeden dat de dicarbonylen MGO en GO opgenomen worden vanuit de voeding en op die manier een bijdrage leveren aan dicarbonylstress in het lichaam. Het verband tussen MGO-inname en MGO in plasma en AGE's in de huid werd vooral verklaard door koffieconsumptie, ook goed voor meer dan een kwart van de MGO inname. Het verband tussen GO-inname en plasma GO werd vooral verklaard door het eten van fruit en het drinken van

fruitsap. Hoewel GO-inname vooral uit brood afkomstig is, lijkt het verband niet verklaard te worden door broodconsumptie. Deze bevindingen suggereren dat opname van dicarbonylen vanuit voeding afhankelijk is van de voedingsmatrix waarin deze zich bevinden en dat dicarbonylen mogelijk gemakkelijker opgenomen worden uit simpelere voedingsmatrices.

Hoewel we in dit proefschrift met name focussen op de inname van dicarbonylen vanuit voeding, kan voeding ook de mate van de endogene vorming van dicarbonylen in het lichaam beïnvloeden. We hebben daarom onderzocht of een voedingspatroon dat gekenmerkt wordt door hogere postprandiale glucosepieken, samenhangt met hogere concentraties dicarbonylen en AGE's in het lichaam. In **hoofdstuk 6** vonden we dat een voedingspatroon met een hogere glycemische last samenhangt met hogere concentraties van een uit MGO gevormde AGE in urine. AGE's in urine zijn waarschijnlijk een weerspiegeling van de opstapeling van AGE's in het weefsel, waar ze bijdragen aan een verminderde functie van het weefsel. Deze resultaten doen vermoeden dat een koolhydraatarm dieet kan helpen bij het verlagen van dicarbonylstress in het lichaam.

De rol van afbraak van dicarbonylen in dicarbonylstress

Een verminderde afbraak van dicarbonylen kan ook bijdragen aan verhoogde dicarbonylstress. Een belangrijk enzym in de afbraak van MGO naar D-lactaat is glyoxalase-1 (Glo1). Genetische variatie in het *GLO1* gen heeft mogelijk invloed op de expressie en activiteit van het Glo1 enzym, en is daarmee een weerspiegeling van de afbraakcapaciteit van MGO in het lichaam. In **hoofdstuk 7** hebben we het verband bestudeerd tussen negen 'single nucleotide polymorphisms' (SNPs) in het *GLO1* gen en verschillende uitkomstmaten: de expressie van Glo1 in witte bloedcellen en markers van MGO stress. Voor twee van de negen SNPs vonden we een verband met Glo1 expressie, maar dit verband was niet meer statistisch significant nadat we corrigeerden voor multiple testing. Deze resultaten doen vermoeden dat deze polymorfismen van *GLO1* niet samenhangen met Glo1-expressie en MGO-stress, en dus geen functionele SNPs zijn.

Mogelijke gezondheidseffecten van dicarbonylen uit voeding

In de **hoofdstukken 4 en 5** hebben we de cross-sectionele (op één tijdstip gemeten) verbanden tussen de inname van dicarbonylen uit voeding en verschillende gezondheidsuitkomsten onderzocht in De Maastricht Studie. In hoofdstuk 4 vonden we dat een langdurige hogere inname van MGO samenhangt met minder laaggradige inflammatie. Deze bevinding is verrassend, aangezien de meeste studies met kleine proefdieren schadelijke effecten van MGO inname

rapporteren. Onze bevinding wordt gesterkt door een vergelijkbaar verband in een onafhankelijke observationele studie; de CODAM-studie. In hoofdstuk 4 vonden we ook een verband tussen een hogere inname van MGO en een verslechterde vaatfunctie in de venule van de retina, maar we vonden geen verband tussen de functie van kleine bloedvaten in andere vaatbedden. In hoofdstuk 5 zagen we dat mensen met een langdurig hogere inname van MGO of 3-DG gevoeliger zijn voor het hormoon insuline. Daarnaast hadden deze mensen minder vaak type 2-diabetes. We vonden geen verband tussen de inname van dicarbonylen uit voeding en de afgifte van het hormoon insuline door de bètacellen van de alvleesklier.

Conclusies

Uit de resultaten van de studies in dit proefschrift blijkt dat zowel een voedingspatroon met meer dicarbonylen MGO en GO, als een voedingspatroon met meer koolhydraten bijdragen aan verhoogde dicarbonylstress in het lichaam. Hoewel verhoogde dicarbonylstress in de literatuur in verband is gebracht met ongunstige gezondheidsuitkomsten, hing hogere inname van dicarbonylen vanuit voeding in onze studies vooral samen met gunstige gezondheidsuitkomsten, zoals minder inflammatie, betere insulinegevoeligheid en minder type 2-diabetes. Onze resultaten doen vermoeden dat de inname van dicarbonylen uit voeding gunstige effecten heeft op het ontstaan van type 2 diabetes, in tegenstelling tot de verwachting. Om te bepalen of de gevonden verbanden oorzakelijk zijn, is het nodig om data uit grotere bevolkingsonderzoeken te analyseren, waarin mensen door de tijd gevolgd worden. Daarnaast kunnen voedingsinterventies waarbij mensen een dieet krijgen met weinig of veel dicarbonylen meer inzicht geven in de gevolgen van inname van dicarbonylen vanuit voeding.

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Scientific output

List of publications

K. Maasen, J.L.J.M. Scheijen, A. Opperhuizen, C.D.A. Stehouwer, M.M.J. van Greevenbroek, C.G. Schalkwijk. Quantification of dicarbonyl compounds in commonly consumed foods and drinks; presentation of a food composition database for dicarbonyls. Food Chemistry. 2021. 339:128063.

K. Maasen, S.J.P.M. Eussen, J.L.J.M. Scheijen, C.J.H. van der Kallen, P.C. Dagnelie, A. Opperhuizen, C.D.A. Stehouwer, M.M.J. van Greevenbroek, C.G. Schalkwijk. Higher habitual intake of dietary dicarbonyls is associated with higher corresponding plasma dicarbonyl concentrations and skin autofluorescence: The Maastricht Study. American Journal of Clinical Nutrition. 2022;115(1):34-44.

K. Maasen, M.M.J. van Greevenbroek, J.L.J.M. Scheijen, C.J.H. van der Kallen, C.D.A. Stehouwer, C.G. Schalkwijk. High dietary glycemic load is associated with higher concentrations of urinary advanced glycation endproducts: The CODAM Study. American Journal of Clinical Nutrition. 2019;110(2):358-366.

K. Maasen, N.M.J. Hanssen, C.J.H. van der Kallen, C.D.A. Stehouwer, M.M.J. van Greevenbroek, C.G. Schalkwijk. Polymorphisms in Glyoxalase I Gene Are not Associated with Glyoxalase I Expression in Whole Blood or Markers of Methylglyoxal Stress: The CODAM Study. Antioxidants. 2021;10(2):219.

Submitted

K. Maasen, S.J.P.M. Eussen, P.C. Dagnelie, A.J.H.M. Houben, C.A.B. Webers, M.T. Schram, T.T.J.M. Berendschot, C.D.A. Stehouwer, A. Opperhuizen, M.M.J. van Greevenbroek, C.G. Schalkwijk. Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation: The Maastricht Study. American Journal of Clinical Nutrition.

Oral presentations

Annual Meeting of the European Association for the Study of Diabetes (EASD) 2021 (virtual): Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation, but also with impaired retinal microvascular function: The Maastricht Study.

International Maillard Reaction Society conference (iMARS) 2021 (virtual): Higher habitual intake of dietary dicarbonyls is associated with higher concentrations of corresponding plasma dicarbonyls and with skin autofluorescence: The Maastricht Study.

Annual Scientific Meeting of The Maastricht Study 2020 (virtual): Dietary assessment with a focus on Dicarbonyls and Advanced Glycation End Products in The Maastricht Study (co-presentation with Dr. Simone Eussen).

38th International Symposium on Diabetes and Nutrition (DNSG) 2021 (virtual): Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation, but also with impaired retinal microvascular function: The Maastricht Study.

Annual Dutch Diabetes Research Meeting (ADDRM) 2021 (virtual): Habitual intake of dietary dicarbonyls is associated with higher insulin sensitivity and lower odds of type 2 diabetes: The Maastricht Study.

Young AGE researchers meeting (YoungAGErs) 2021 (virtual): Use of the dicarbonyl database in the Maastricht Study: The association of dicarbonyl intake with low-grade inflammation and microvascular function.

Annual Dutch Diabetes Research Meeting (ADDRM) 2020 (virtual): Polymorphisms in glyoxalase 1 gene are not associated with glyoxalase 1 expression or with markers of methylglyoxal stress: The CODAM study.

37th International Symposium on Diabetes and Nutrition (DNSG) 2019 (Kerkrade, the Netherlands): High dietary glycemic load is associated with higher concentrations of plasma and urinary advanced glycation endproducts: The CODAM Study.

Annual Dutch Diabetes Research Meeting (ADDRM) 2019 (Wageningen, the Netherlands): Dietary dicarbonyls are associated with higher levels of plasma dicarbonyls and with Skin Autofluorescence: The Maastricht Study.

Young AGE researchers meeting (YoungAGErs) 2019 (Maastricht, the Netherlands): Presentation of a dietary dicarbonyl database: a tool to study health effects of dicarbonyl intake.

Annual Meeting of the European Association for the Study of Diabetes (EASD) 2018 (Berlin, Germany): Dietary Glycaemic Load is associated with elevated levels of Advanced Glycation Endproducts: The CODAM Study.

International Maillard Reaction Society conference (iMARS) 2018 (Montreal, Canada): Dietary Glycaemic Load is associated with elevated levels of Advanced Glycation Endproducts: The CODAM Study.

Annual Dutch Diabetes Research Meeting (ADDRM) 2018 (Oosterbeek, the Netherlands): High dietary Glycaemic Load is associated with increased levels of urinary Advanced Glycation Endproducts: The CODAM Study.

Young AGE researchers meeting (YoungAGErs) 2018 (Wageningen, the Netherlands): High dietary glycaemic load is associated with increased levels of plasma and urinary methylglyoxal-hydroimidazolone (MG-H1) in Type 2 Diabetes: The CODAM Study.

Poster presentations

International Maillard Reaction Society conference (iMARS) 2021 (virtual): Higher habitual intake of dietary methylglyoxal is associated with less low-grade inflammation, but also with impaired retinal microvascular function: the Maastricht Study.

Dutch Epidemiological Conference (WEON) 2021 (virtual): Higher habitual intake of dietary dicarbonyls is associated with higher corresponding plasma dicarbonyl concentrations and skin autofluorescence: The Maastricht Study.

Annual Meeting of the European Association for the Study of Diabetes (EASD) 2020 (virtual): Higher habitual intake of dietary dicarbonyls is associated with higher concentrations of plasma dicarbonyls and with Skin Autofluorescence: The Maastricht Study.

European Nutrition Conference, Malnutrition in an Obese World: European Perspectives 2019 (Dublin, Ireland): Presentation of a food composition database for dicarbonyls: a tool to study the impact of dicarbonyl intake on health outcomes.

Papendal course Vascular biology 2018 (Papendal, the Netherlands): High dietary Glycaemic Load is associated with increased levels of urinary Advanced Glycation Endproducts: The CODAM Study.

Papendal course Vascular Biology & Pathology 2019 (Papendal, the Netherlands): High intake of dietary dicarbonyls is associated with higher levels of plasma dicarbonyls: The Maastricht Study.

Awards

HS-BAFTA 'Talented PhD candidates' fellowship to visit the International Agency for Research of Cancer (IARC) at Lyon, France for our project: 'The association between dietary dicarbonyls and incident type 2 diabetes: EPIC cohort'. Granted by the School for Cardiovascular Research (CARIM) Maastricht University.

38th International Symposium on Diabetes and Nutrition 2021 (virtual): Young Investigator Award for best oral communication.

37th International Symposium on Diabetes and Nutrition 2019 (Kerkrade, the Netherlands): new investigator award for oral presentation.

Wetenschapsdag Interne Geneeskunde 2019 (Maastricht , the Netherlands): best poster award.

Curriculum Vitae

Kim Maasen werd geboren op 7 augustus 1992 in Nijmegen en groeide hier op met haar ouders Jan en José en jongere broer Jeroen. De eerste steen voor haar interesse in onderzoek werd gelegd op de Nijmeegse Scholengemeenschap (NSG), met een profielwerkstuk waarvoor ze een lespakket ontwikkelde voor basisschoolkinderen over voeding, gezondheid en het milieu. Na haar eindexamen in 2010 begon Kim met haar Bachelor Voeding & Gezondheid aan de Wageningen Universiteit (WUR). Als onderdeel hiervan schreef ze haar thesis over de voedselzekerheidsituatie in Rwanda en volgde ze een extra minor aan Corvinus Universiteit in Budapest, Hongarije. In 2014 behaalde ze haar Bachelor diploma, waarna ze haar studie vervolgde met de Master Voeding & Gezondheid aan de WUR, met als specialisatie moleculaire voeding en toxicologie. Als onderdeel hiervan deed ze een Master thesis in Rwanda en stage in Gambia, beide gericht op de epigenetische effecten van voeding tijdens de eerste 1000 dagen. In 2016 behaalde ze haar Master diploma, waarna ze nog een jaar bleef werken als onderzoeker op haar stageplek bij London School of Hygiene and Tropical Medicine/Medical Research Council (MRC) The Gambia Unit. Van 2018-2022 deed Kim promotieonderzoek onder begeleiding van Prof. Casper Schalkwijk, Dr. Marleen van Greevenbroek en Prof. Coen Stehouwer, bij de afdeling Interne Geneeskunde, binnen CARIM School for Cardiovascular Diseases aan de Universiteit Maastricht (UM). Hier heeft ze zich verder verdiept in voedingsepidemiologie en type 2 diabetes, met behulp van data van de Maastricht Studie. Als onderdeel van haar promotieonderzoek heeft Kim samengewerkt met de International Agency for the Research of Cancer (IARC) in Lyon, Frankrijk, om te kijken naar incidentie van type 2 diabetes in de European Prospective Investigation into Cancer and Nutrition (EPIC) studie.

Kim Maasen was born on the 7th of August 1992 in Nijmegen, the Netherlands, where she grew up with her parents, Jan and José, and younger brother Jeroen. After completing secondary school at the Nijmeegse Scholengemeenschap (NSG) in Nijmegen, she enrolled in the Bachelor of Science (BSc) Nutrition and Health at Wageningen University and Research in 2010. As part of the curriculum, she completed an exchange semester at Corvinus University, Budapest, Hungary, and wrote her thesis on the food security status in Rwanda. In 2014, she obtained her BSc degree, after which she continued with the Master of Science (MSc) program

Nutrition and Health at Wageningen University, with a specialization in Molecular Nutrition and Toxicology. During her MSc, she wrote her thesis in Rwanda and conducted her internship in The Gambia, both focused on the epigenetic effects of nutrition during the first 1,000 days of life. In 2016 she graduated from her MSc, after which she continued working as a researcher for another year at her placement, the London School of Hygiene and Tropical Medicine/Medical Research Council (MRC) The Gambia Unit. From 2018-2022 she conducted her PhD at the department of Internal Medicine and School for Cardiovascular Diseases (CARIM) at Maastricht University and Medical Centre, under supervision of Prof. Casper Schalkwijk, Dr. Marleen van Greevenbroek and Prof. Coen Stehouwer. During her PhD she worked in the field of nutritional epidemiology, exploring the health effects of dietary dicarbonyls. As part of her PhD she visited the International Agency for the Research of Cancer (IARC) in Lyon, France, to examine the association between dietary dicarbonyls and incident type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.