Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The CODAM study

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Original article

Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The CODAM study

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SUMMARY

Background & aims: Advanced glycation endproducts (AGEs) are formed by the reaction between reducing sugars and proteins. AGEs in the body have been associated with several age-related diseases. High-heat treated and most processed foods are rich in AGEs. The aim of our study was to investigate whether dietary AGEs, are associated with plasma and urinary AGE levels.

Methods: In 450 participants of the Cohort on Diabetes and Atherosclerosis Maastricht study (CODAM study) we measured plasma and urine concentrations of the AGEs Nε-(carboxymethyl)lysine (CML), Nε-(1-carboxyethyl)lysine (CEL) and Nδ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) using UPLC-MS/MS. We also estimated dietary intake of CML, CEL and MG-H1 with the use of a dietary AGE database and a food frequency questionnaire (FFQ). We used linear regression to investigate the association between standardized dietary AGE intake and standardized plasma or urinary AGE levels, after adjustment for age, sex, glucose metabolism status, waist circumference, kidney function, energy- and macro-nutrient intake, smoking status, physical activity, alcohol intake, LDL-cholesterol and markers of oxidative stress.

Results: We found that higher intake of dietary CML, CEL and MG-H1 was associated with significantly higher levels of free plasma and urinary CML, CEL and MG-H1 (β CML = 0.253 (95% CI 0.086; 0.415), β CEL = 0.194 (95% CI 0.040; 0.339), β MG-H1 = 0.223 (95% CI 0.069; 0.373) for plasma and β CML = 0.223 (95% CI 0.049; 0.393), β CEL = 0.180 (95% CI 0.019; 0.332), β MG-H1 = 0.196 (95% CI 0.037; 0.349) for urine, respectively). In addition, we observed non-significant associations of dietary AGEs with their corresponding protein bound plasma AGEs.

Conclusion: We demonstrate that higher intake of dietary AGEs is associated with higher levels of AGEs in plasma and urine. Our findings may have important implications for those who ingest a diet rich in AGEs.

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1. Introduction

Advanced glycation endproducts (AGEs) are a large and heterogeneous family of sugar-modified proteins, which have been linked to a plethora of age-related diseases including diabetes, atherosclerosis, chronic obstructive pulmonary disease and Alzheimer's disease [1–3].

In 1912, the French chemist Louis Camille Maillard discovered the reaction between reducing sugars and free amino acids on proteins [4], leading to the formation of AGEs. At body temperature,

the Maillard reaction takes place at a very slow pace. In contrast, in foods which had a prolonged exposure to high heat AGEs were formed rapidly [5]. The consumption of processed foods that were cooked at high temperatures has increased over the past decades [6]. As a consequence, the exposure to dietary AGEs have also increased and may be a risk factor for chronic diseases [7]. Indeed, recent studies have shown associations of dietary AGEs with insulin sensitivity [8], abdominal obesity and hypertriglyceridemia [9] and with poorer memory in Alzheimer's disease [10]. AGEs in the body may contribute to development of age-related diseases through several mechanisms, such as interaction with the receptor for AGEs (RAGE) [11] and crosslinking on long-lived proteins [12]. However, whether dietary AGEs are substantially absorbed in the digestive tract and released into the circulation to contribute to any of these effects remains unclear.

The studies so far addressing whether high intake of dietary AGEs lead to increased plasma and urinary AGEs have not been conclusive [13–22], because most of these studies have been small and have not taken into account confounding factors such as kidney function or dietary energy intake, which may explain the associations [19,20]. Moreover, different analytical techniques to analyze AGE content in food and plasma samples were used in these studies, which may have led to differences in AGE concentrations [21,22].

Taken these considerations into account, the aim of the current study was to establish whether higher intake of dietary AGEs are associated with higher levels of plasma and urinary AGEs. In a previous study, we developed a new dietary AGE database of three major AGEs N ϵ -(carboxymethyl)lysine (CML), N ϵ -(1-carboxyethyl)lysine (CEL) and N δ -(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1), as analyzed in 190 specific food items, based on a state-of-the-art ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) technique [23]. In the present study, we linked information on AGE-content of these food items to a food frequency questionnaire (FFQ) and calculated the consumption of AGEs in a cohort of 465 participants. We subsequently examined the association of dietary AGE intake with three major plasma and urinary AGEs (CML CEL and MG-H1), adjusting for major potential confounders.

2. Materials and methods

2.1. Study population

Cross-sectional analyses were performed on data from the Cohort on Diabetes and Atherosclerosis Maastricht study (CODAM), which includes 574 individuals with an elevated risk for T2DM and cardiovascular disease and described in detail elsewhere [24]. Participants were instructed to withhold their lipid-lowering medication for a fortnight prior the first visit, and not to consume any alcoholic drinks, coffee and/or tea, not to smoke, and withhold all other medication the day before. The habitual dietary intake over the last twelve months of all participants was established by a self-administered food frequency questionnaire (FFQ) which queried 194 foods [25].

Individuals were excluded if they did not qualify to report the FFQ successfully ($n = 56$, i.e. more than 10% items missing on the FFQ). Participants who reported an energy intake outside the range of 800–4200 kcal/day for men and 600–3500 kcal/day for women were also excluded ($n = 6$). Due to sample availability, 450 participants were used for statistical analysis (Fig. 1).

Fasting and 2-h postload glucose concentrations were used to classify the study participants' glucose metabolism status (GMS), described in details elsewhere [26]. Questionnaires were used to assess smoking behavior (never, ever, or current smoker) and use of medication (lipid-, glucose-, and blood pressure-lowering

Fig. 1. Flowchart for the exclusion of participants in the current CODAM study.

medication). Plasma creatinine levels were measured with the Jaffe diagnostic test (Roche Diagnostics, Mannheim, Germany), and the estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27]. Plasma malondialdehyde (MDA) levels were measured with a reagent kit for high-performance liquid chromatography analyses (Chromsystems Instruments and Chemicals, Munich, Germany), and total antioxidative status (TAS) was measured in serum with an enzymatic kit (Randox Diagnostics, County Antrim, U.K.). LDL cholesterol was calculated with the Friedewald formula [28], after measurement of fasting total cholesterol, HDL-cholesterol and triglyceride levels.

All subjects gave written informed consent. The study was approved by the local Medical Ethical Committee of the University of Maastricht and University Hospital Maastricht.

2.2. Food, plasma and urinary AGE quantification

Protein-bound AGEs in individual food items and in plasma, and free AGEs in plasma and in urine were analyzed as described in

detail elsewhere [23,29]. In short, foods were obtained from local supermarkets or restaurants and prepared according to the instructions on the label. For UPLC-MS/MS analysis a representative sample of each food item, equivalent to 5 mg of protein, was used. For protein-bound and free plasma AGEs, 25 μ L and 50 μ L plasma was used, respectively. Forty microliters were used for free urinary AGE analysis. All samples were derivatized with butanolic hydrochloric acid and subsequently detected in ESI positive multiple reaction monitoring (MRM) mode using a Xevo TQ MS (Waters, Milford, USA). Quantification of CML, CEL and MG-H1 was performed by calculating the peak area ratio of each unlabeled peak area to the corresponding internal standard peak area. In plasma, the intra- and inter-assay variation 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%. In urine, the intra- and inter-assay variation of free CML, CEL and MG-H1 were between 3.7 and 6.6%. In food matrix, the intra- and inter-assay variation of protein-bound CML, CEL and MG-H1 were between 1.7 and 29.1%.

2.3. Assessment of AGE intake

For UPLC-MS/MS protein-bound AGE analysis, food items were selected based on the items included in the FFQ of CODAM, which queried 194 foods commonly consumed in the Netherlands. Each food item in the FFQ was quantified as grams of food per day and analyzed for AGE content. The AGE intake was estimated for each participant by multiplying the food intake in g/day with the concentration of AGEs in mg/g in each representative individual food item. To calculate the total AGE intake per participant, the estimated AGE levels of the various food items were added together. Some of the food items had unspecific descriptions in the FFQ (e.g. “sweet spreads”). These items were further defined as an average of this food group (e.g. for “sweet spreads” the average AGE content of apple syrup, chocolate spread, chocolate sprinkles, fruit sprinkles and jam was used). Moreover, some of the food items in the FFQ were not analyzed for AGEs. These food items were matched with food items based on ingredient profile. To this end, estimation was made for the AGE content of these missing food items.

2.4. Statistical analyses

All statistical analyses were carried out using the IBM SPSS Statistics Software, version 23.0 (IBM Corporation, Armonk, NY, USA). General characteristics of the CODAM population were compared across tertiles of the dietary AGE score with the use of ANOVA or χ^2 -tests, for continuous or categorical data, respectively. We log_e-normalized all plasma and urinary AGEs to reduce the potential influence of outliers and to obtain a normal distribution, needed for calculation of Z-scores. An AGE score was calculated that represents an individual's overall intake of AGEs via their habitual diet. For this, first the Z-scores were calculated for each dietary AGE (CML, CEL and MG-H1) as follows: for each individual dietary AGE, a Z-score was calculated according to the formula: [(individual dietary AGE – population mean dietary AGE)/population standard deviation dietary AGE]. The resulting Z-scores, of each dietary AGE (CML, CEL and MG-H1), were then averaged into an overall dietary AGE Z-score and this overall dietary AGE Z-score was subsequently standardized.

We used linear regression analyses to investigate the association between dietary and plasma or urinary AGE levels, after adjustments for participant characteristics (age, sex, glucose metabolism status and waist circumference: model 1) and additionally for kidney function (estimated glomerular filtration rate (eGFR): model 2), and further for potential dietary and lifestyle confounding factors (energy intake and protein-, saturated fat-, carbohydrate- and

fiber-intake, all macronutrients expressed as energy percentage and smoking, physical activity and alcohol intake: model 3), and for potential confounding by lipid metabolism and oxidative stress (LDL-cholesterol, malondialdehyde (MDA) and total antioxidant status (TAS): model 4). Participants with missing values ($n = 14$) on co-variables were excluded listwise. We also tested whether associations differed for sex (sex $\times$ dietary AGE), eGFR (eGFR $\times$ dietary AGE) and the presence of impaired glucose metabolism or diabetes (presence of impaired glucose metabolism or diabetes $\times$ dietary AGE), by adding interaction terms to our models (p -values for interaction were <0.1). Overall, we found no consistent interactions (data not shown).

3. Results

Table 1 shows the general characteristics of the total population of the CODAM study as stratified according to tertiles of dietary AGEs. Although we observed important quantitative differences in dietary AGE levels, with MG-H1 being most abundant, we observed strong correlations between the AGE intake (CML vs CEL r_{pearson} : 0.811, CML vs MG-H1 r_{pearson} : 0.795 and CEL vs MG-H1 r_{pearson} : 0.880; for all $p < 0.001$). Plasma free-, plasma protein-bound- and urinary AGEs did not differ between the tertiles of dietary AGEs (Table 1). Male participants had higher intake of dietary AGEs ($p < 0.001$, Table 1). In addition, HDL cholesterol, protein intake and fiber intake were lower in the individuals with higher intake of dietary AGEs ($p < 0.05$, Table 1) and saturated fat-, energy- and alcohol intake were higher in the individuals with higher intake of dietary AGEs ($p < 0.05$, Table 1).

3.1. Associations between dietary AGE intake and protein-bound plasma AGEs

After adjustment for age, sex, glucose metabolism status and waist circumference, we found no significant association between dietary CML and protein-bound plasma CML levels (Table 2, model 1). Further adjustment for eGFR did not change these results (Table 2, model 2). In the fully adjusted model we observed a weak positive association of dietary CML and protein-bound plasma CML, but this was not significant (Table 2, model 4). We observed similar findings for dietary CEL and protein-bound CEL (Table 2).

3.2. Associations between dietary AGE intake and free plasma AGEs

After adjustment for age, sex, glucose metabolism status and waist circumference we observed positive associations between dietary intake of CML, CEL and MG-H1 and their free levels in plasma (Table 3, model 1). Further adjustment for eGFR (Table 3, model 2), energy- and macro-nutrient intake, lifestyle factors, lipid metabolism and markers of oxidative stress (Table 3, model 3 and 4) did not change the results overall. In the fully adjusted model, these associations were approximately twice as strong for CML, CEL and MG-H1 (Table 3, model 4 vs model 1).

3.3. Associations between dietary AGE intake and free urinary AGEs

In line with the findings for free plasma AGEs, higher dietary intake of CML, CEL and MG-H1 were associated with higher urinary CML, CEL and MG-H1 levels (Table 4, model 1–4). Adjustment for eGFR did not change the results (Table 4, model 2).

4. Discussion

This study is, to our knowledge, the first to demonstrate that higher dietary intake of CML, CEL and MG-H1, as estimated with a

Table 1
Clinical characteristics of total study population.

	Total population	Dietary AGE tertiles			P _{trend}
	(n = 450)	Lowest (n = 151)	Middle (n = 151)	Highest (n = 148)	
Age (years)	59.4 ± 7.0	60.1 ± 6.9	59.0 ± 6.7	58.9 ± 7.4	0.140
Sex (m/f; %)	60.0/40.0	45.7/54.3	59.6/40.4	75.0/25.0	<0.001
NGM/IGM/T2DM (%)	53.6/22.7/23.8	50.3/23.2/26.5	56.3/18.5/25.2	54.1/26.4/19.6	0.376
Body mass index (kg/m ²)	28.3 ± 4.2	28.5 ± 4.5	28.2 ± 4.1	28.3 ± 3.9	0.690
Waist circumference (cm)	98.6 ± 11.6	97.8 ± 12.1	98.4 ± 11.7	99.6 ± 11.0	0.162
Fasting glucose (mmol/L)	6.0 ± 1.4	6.0 ± 1.3	6.0 ± 1.5	6.0 ± 1.4	0.823
HbA1c (%)	5.9 ± 0.8	5.9 ± 0.6	6.0 ± 0.9	6.0 ± 0.8	0.671
Total cholesterol (mmol/L)	5.2 ± 0.9	5.3 ± 0.9	5.1 ± 1.0	5.2 ± 0.8	0.553
HDL cholesterol (mmol/L)	1.20 ± 0.35	1.25 ± 0.36	1.22 ± 0.35	1.15 ± 0.33	0.010
LDL cholesterol (mmol/L)	3.29 ± 0.86	3.32 ± 0.90	3.21 ± 0.89	3.35 ± 0.79	0.757
Triglycerides (mmol/L)	1.4 (1.0–1.9)	1.4 (1.0–1.9)	1.4 (1.0–2.0)	1.4 (1.0–1.9)	0.969
Current smoking (%)	20.2	21.1	22.3	18.5	0.756
Malondialdehyde (μmol/L)	0.18 ± 0.05	0.18 ± 0.06	0.17 ± 0.05	0.18 ± 0.05	0.887
Total antioxidant status (mmol/L)	1.08 ± 0.10	1.08 ± 0.10	1.07 ± 0.10	1.10 ± 0.10	0.090
SBP (mmHg)	139.3 ± 18.7	141.4 ± 17.9	138.4 ± 19.9	138.2 ± 18.0	0.144
DBP (mmHg)	81.3 ± 9.0	81.6 ± 8.2	80.4 ± 9.5	82.0 ± 9.3	0.697
eGFR (mL/min/1.73 m ²)	91.8 ± 17.3	93.1 ± 23.9	90.8 ± 12.6	91.4 ± 12.7	0.379
Glucose-lowering treatment (%)	13.3	13.2	14.6	12.2	0.828
Lipid-lowering treatment (%)	19.1	19.9	21.2	16.2	0.527
Blood pressure-lowering treatment (%)	38.0	41.7	39.1	33.1	0.292
Energy intake (kcal/day)	2191.9 ± 601.0	1677.9 ± 382.0	2148.2 ± 351.6	2750.8 ± 478.5	<0.001
Protein intake (% energy)	15.5 ± 2.2	15.9 ± 2.5	15.6 ± 2.1	15.1 ± 1.9	0.002
Carbohydrate intake (% energy)	44.0 ± 6.2	43.8 ± 6.9	44.4 ± 5.4	43.9 ± 6.2	0.943
Saturated fat intake (% energy)	14.6 ± 2.5	14.2 ± 2.6	14.6 ± 2.5	15.0 ± 2.5	0.006
Fibre intake (% energy)	2.36 ± 0.49	2.47 ± 0.51	2.34 ± 0.49	2.25 ± 0.45	<0.001
Alcoholic drinks (g/day)	107 (11–280)	71 (6–225)	100 (12–284)	135 (29–304)	0.032
Physical activity (min × intensity/week)	1800 (1058–2835)	1703 (1043–2655)	1890 (1140–2865)	1837 (956–2966)	0.404
Plasma free CML (nmol/L)	78 (60–99)	77 (57–101)	77 (63–99)	80 (60–97)	0.626
Plasma free CEL (nmol/L)	45 (37–58)	45 (35–59)	44 (38–55)	47 (38–60)	0.496
Plasma free MG-H1 (nmol/L)	121 (86–173)	113 (82–167)	120 (91–166)	126 (88–191)	0.117
Plasma protein-bound CML (nmol/mmol lys)	35 (30–41)	35 (29–41)	34 (31–41)	35 (29–42)	0.861
Plasma protein-bound CEL (nmol/mmol lys)	24 (19–29)	22 (18–27)	24 (19–29)	24 (20–30)	0.056
Urinary free CML (nmol/mmol creatinine)	954 (749–1220)	944 (735–1257)	941 (739–1244)	966 (756–1175)	0.980
Urinary free CEL (nmol/mmol creatinine)	519 (409–645)	519 (428–635)	510 (384–631)	540 (405–675)	0.296
Urinary free MG-H1 (nmol/mmol creatinine)	2284 (1593–3270)	2251 (1587–3286)	2274 (1535–3190)	2421 (1664–3397)	0.627
Dietary CML (mg/day)	3.10 ± 1.03	2.08 ± 0.47	3.07 ± 0.44	4.17 ± 0.77	<0.001
Dietary CEL (mg/day)	2.32 ± 0.77	1.58 ± 0.31	2.24 ± 0.28	3.15 ± 0.60	<0.001
Dietary MG-H1 (mg/day)	21.7 ± 6.69	15.2 ± 2.85	20.9 ± 2.42	28.9 ± 5.09	<0.001

Data are presented as means ± SD, medians (IQR) or percentages, as appropriate. Linear trend was tested with ANOVA or χ^2 , as appropriate. Skewed continuous variables were analyzed with Kruskal Wallis (triglycerides, and the AGEs) were log_e-transformed prior to analyses.

Table 2
Associations between dietary AGEs and protein-bound plasma AGEs.

	Model	Protein-bound plasma CML		
		β	95%CI	p
Dietary CML	Crude	0.039	−0.054; 0.128	0.42
	1	0.048	−0.041; 0.133	0.30
	2	0.046	−0.042; 0.131	0.32
	3	0.122	−0.042; 0.277	0.15
	4	0.130	−0.032; 0.283	0.12
	Model	Protein-bound plasma CEL		
		β	95%CI	p
Dietary CEL	Crude	0.112	0.017; 0.199	0.02
	1	0.105	0.005; 0.197	0.04
	2	0.101	0.002; 0.194	0.05
	3	0.136	−0.030; 0.293	0.11
	4	0.112	−0.050; 0.268	0.18

Data were analyzed using linear regression analyses. βs are expressed as 1 SD increase of plasma AGE per 1 SD increase of dietary AGE. All plasma AGEs were Ln-normalized in all analyses.

Model 1: adjusted for age, sex, glucose metabolism status, waist circumference.

Model 2: model 1 + eGFR.

Model 3: model 2 + total energy-, protein-, saturated fat-, carbohydrate- and fiber-intake, smoking, physical activity, alcohol intake.

Model 4: model 3 + LDL cholesterol, malondialdehyde, total antioxidant status.

FFQ, is associated significantly with higher free plasma and urinary levels of AGEs, as measured with UPLC-MS/MS. We found a weak non-significant association of dietary AGEs with their corresponding protein bound plasma AGEs. In the current study we estimated dietary CML, CEL and MG-H1. Since these compounds were highly correlated, despite quantitative differences, we assume that these three AGEs were well-representative for dietary AGE contents.

Studies so far have suggested that dietary AGEs are associated with circulating AGEs [13,15–18,30]. Our current study expands on these findings in several important ways. First, we have used a state-of-the-art UPLC-MS/MS technique to analyze three different dietary AGEs and circulating AGEs. Moreover, we performed UPLC-MS/MS plasma and urinary analyses in a large and well-defined cohort study to investigate the associations between three different dietary AGEs and plasma and urinary AGEs. Another major strength of our study is that we were able to use a database of AGEs, as measured with UPLC-MS/MS [23], in 190 food items to calculate the habitual intake of dietary AGEs based on a FFQ. However, one important limitation of our study was that our FFQ did not include detailed information about food preparation techniques on all food items. We and others have shown that differences in these cooking techniques and duration of heating are fundamental in the formation of AGEs in food [5,23,31]. However, approximately 90% of the

Table 3
Associations between dietary AGEs and free plasma AGEs.

		Free plasma CML		
Model		β	95%CI	p
Dietary CML	Crude	0.055	−0.039; 0.147	0.26
	1	0.099	0.006; 0.190	0.04
	2	0.094	0.004; 0.183	0.04
	3	0.224	0.057; 0.386	0.01
	4	0.253	0.086; 0.415	<0.01
		Free plasma CEL		
Model		β	95%CI	p
Dietary CEL	Crude	0.086	−0.008; 0.176	0.07
	1	0.098	0.003; 0.188	0.04
	2	0.086	−0.007; 0.174	0.07
	3	0.208	0.053; 0.353	0.01
	4	0.194	0.040; 0.339	0.01
		Free plasma MG-H1		
Model		β	95%CI	p
Dietary MG-H1	Crude	0.127	0.033; 0.219	0.01
	1	0.150	0.056; 0.241	<0.01
	2	0.135	0.043; 0.224	<0.01
	3	0.239	0.085; 0.390	<0.01
	4	0.223	0.069; 0.373	<0.01

Data were analyzed using linear regression analyses. β s are expressed as 1 SD increase of plasma AGE per 1 SD increase of dietary AGE. All plasma AGEs were Ln-normalized in all analyses.

Model 1: adjusted for age, sex, glucose metabolism status, waist circumference.

Model 2: model 1 + eGFR.

Model 3: model 2 + total energy-, protein-, saturated fat-, carbohydrate- and fiber-intake, smoking, physical activity, alcohol intake.

Model 4: model 3 + LDL cholesterol, malondialdehyde, total antioxidant status.

Table 4
Associations between dietary AGEs and urinary free AGEs.

		Urinary free CML		
Model		β	95%CI	p
Dietary CML	Crude	0.045	−0.049; 0.138	0.35
	1	0.127	0.033; 0.218	0.01
	2	0.127	0.033; 0.218	0.01
	3	0.211	0.038; 0.380	0.02
	4	0.223	0.049; 0.393	0.01
		Urinary free CEL		
Model		β	95%CI	p
Dietary CEL	Crude	0.056	−0.037; 0.146	0.25
	1	0.121	0.024; 0.211	0.01
	2	0.124	0.027; 0.214	0.01
	3	0.189	0.027; 0.340	0.02
	4	0.180	0.019; 0.332	0.03
		Urinary free MG-H1		
Model		β	95%CI	p
Dietary MG-H1	Crude	0.066	−0.028; 0.157	0.17
	1	0.134	0.039; 0.224	<0.01
	2	0.134	0.043; 0.228	<0.01
	3	0.205	0.045; 0.357	0.01
	4	0.196	0.037; 0.349	0.02

Data were analyzed using linear regression analyses. β s are expressed as 1 SD increase of urinary AGE per 1 SD increase of dietary AGE. All urinary AGEs were Ln-normalized in all analyses.

Model 1: adjusted for age, sex, glucose metabolism status, waist circumference.

Model 2: model 1 + eGFR.

Model 3: model 2 + total energy-, protein-, saturated fat-, carbohydrate- and fiber-intake, smoking, physical activity, alcohol intake.

Model 4: model 3 + LDL cholesterol, malondialdehyde, total antioxidant status.

FFQ food items were either uncooked, such as fruit, raw vegetables and drinks or were pre-processed foods which were produced by different manufacturers, such as cookies, bread or peanut butter. Our FFQ consisted of only 10% of food items which have to be prepared by the participant itself, such as meat, fish, potatoes or pasta. We have included detailed information on the preparation of potatoes in the FFQ, such as boiling, frying or baking. Unfortunately, no detailed information of the preparation, in particular duration of heating, of meat or fish was available. However, our dAGE database [23], which was used to estimate AGE-intake as calculated with FFQ data, consisted of meat- and fish products which were prepared according to the instructions of the manufacturer's label or using the most common preparation technique. Perhaps this will have led to a source of variance in estimating the dietary AGE content, but this was only true for a minority of the food items. Nonetheless, we still found clear and consistent associations between estimated dietary AGE intake and plasma AGE levels.

Our crude analysis only revealed an association between higher dietary AGEs and higher plasma and urinary AGE levels. Particularly after adjustment for sex, age, glucose metabolism and waist (model 1), and further adjustment for the major macronutrients and lifestyle factors (model 3) even stronger associations were found between dietary AGEs and free plasma and urinary AGEs. The strong effect of confounding factors on the reported associations may at least partly explain conflicting findings between previous studies which have not taken into account these confounding factors [19,20].

Dietary AGE-modified proteins are mainly digested into free glycated and non-glycated amino acids and small peptides [32] and thus protein-bound dietary AGEs are expected to enter the circulation predominately in the free form. Subsequently, these AGEs are excreted in the urine [16]. We found no association of dietary AGEs with protein-bound AGEs in plasma, suggesting that the vast majority of the protein-bound AGEs in the human body are formed by the non-enzymatic reaction of glucose, α -oxoaldehydes and other saccharide derivatives with proteins, nucleotides and lipids. On the contrary, we found an association of dietary AGEs with free AGEs in plasma. This is in accordance with a recent study which has showed that free plasma AGEs were mainly released from the protein-bound dietary AGEs and hereby enter the circulation predominantly [32]. In line, we found that associations between dietary AGEs and free plasma AGEs are stronger than with protein-bound plasma AGEs. Two intervention studies were in line with these results [13,16]. Birlouez-Aragon et al., reported a significant increase of 7% protein-bound plasma CML after administering a high AGE diet versus a significant increase of 40% free urinary CML [16]. Furthermore, the associations between the estimated dietary AGE intake with free plasma AGEs is in line with animal intervention studies [33,34]. In these studies, low- and high-AGE diets were tested in animals and significantly increased levels of free plasma CML, but not protein-bound plasma CML, were found [33,34]. In the same study, increased plasma levels of sRAGE and a pro-inflammatory response was reported after administering a high-AGE diet [33] which implicates important biological consequences.

In humans, the beneficial effects of a low AGE-diet have been described [8,13,35]. If a high-AGE diet in humans, and contemporaneously increased levels of free plasma AGEs, can lead to an increased risk for development of chronic diseases is still under investigation. In our observational study we investigated the association of a habitual diet with similar results.

In summary, we have shown that higher levels of dietary AGEs were significantly associated with higher levels of free plasma

and urinary AGEs. These findings are an important step in understanding the metabolic transit of these dietary AGEs. Several studies indicate that dietary AGEs are associated with poor health [8–10,16,20]. Future prospective studies should address whether dietary AGEs are associated with adverse outcomes such as accelerated development of cardiovascular disease, diabetes and other age-related diseases. This line of investigation may eventually lead to new dietary recommendations for both the general public and perhaps specific patients groups in particular.

Statement of authorship

Scheijen: wrote paper.
 Hanssen: contributed to discussion and reviewed and edited the manuscript.
 Greevenbroek: reviewed and edited the manuscript.
 Van der Kallen: reviewed and edited the manuscript.
 Feskens: reviewed and edited the manuscript.
 Stehouwer: reviewed and edited the manuscript.
 Schalkwijk: designed the study, reviewed and edited the manuscript and had primary responsibility for final content.

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Conflict of interest statement

Authors have no potential conflicts of interest.

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