Dietary Consumption of Advanced Glycation End Products and Body Composition, Insulin Resistance and Type 2 Diabetes

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ABSTRACT

Background

Limited information is available on the association between consumption of dietary advanced glycation end products (dAGEs) and parameters of body composition, insulin resistance and type 2 diabetes.

Methods

For 4,893 participants of the Rotterdam Study, a population-based cohort, we calculated daily consumption of the dAGEs carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL) and N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MGH1) using data obtained from food-frequency questionnaires. Participants underwent repeated measurement of body composition assessed using dual-energy X-ray absorptiometry (DXA). Up to two measurements were performed for each participant across a mean time period of 5.1 years. As outcome measures, we calculated body mass index (BMI), fat mass index (FMI), fat-free mass index (FFMI), android-to-gynoid fat ratio (AGR), body fat percentage (BF%) and waist-to-hip ratio (WHR). We also analyzed dAGE consumption cross-sectionally in relation to presence of type 2 diabetes as well as the homeostatic models of insulin resistance (HOMA-IR) and pancreatic beta cell function (HOMA-B).

Results

One standard deviation higher energy-adjusted CEL consumption was associated with 0.206 kg/m^2 higher BMI (95% confidence interval 0.105; 0.307), 0.151 kg/m^2 higher FMI (0.075; 0.226), 0.041 kg/m^2 higher FFMI (0.001; 0.082), 0.005 higher AGR (0.001; 0.009), 0.267 higher BF% (0.088; 0.446) and 0.002 higher WHR (0.000; 0.004) after multivariable adjustment. We observed no association between CML or MGH1 consumption and any of the body composition parameters. Consumption of all dAGEs was associated with higher probability of having type 2 diabetes. We observed no associations between dAGE consumption and HOMA-IR or HOMA-B.

Conclusions

Higher dietary CEL consumption is associated with a less favorable body composition profile measured longitudinally, and higher consumption of all studied dAGEs is associated with a higher probability of type 2 diabetes. Further research is needed to better characterize the potential adverse metabolic effects of dAGE consumption.

INTRODUCTION

Advanced glycation end products (AGEs) are a diverse group of molecular compounds which are generally formed through a series of non-enzymatic reactions between sugars and amino acids collectively known as the Maillard reaction.[1–3] They may also be generated through oxidation of lipids or amino acids.[1] Although AGE formation is a normal physiological process during metabolism, if accumulated in high enough quantities AGEs may deposit in tissue (including vascular, cardiac, renal and dermal tissues) and exert pathological effects.[3,4] On a biochemical level, AGEs may induce oxidative stress and inflammation through activation of intracellular signaling cascades.[1]

While AGEs may originate endogenously as byproducts of naturally-occurring metabolic processes, they may also be derived exogenously. For example, tobacco smoking and consumption of certain foods, especially when dry heated, are important exogenous sources of AGEs.[4] The relevance of exogenous AGEs was demonstrated by the finding that dietary consumption of these compounds measurably contributes to the body's total AGE pool.[5] This gave rise to the study of dietary AGEs (dAGEs) in relation to health outcomes. Several analytical methods have been derived to determine AGE content of foods, which led to the development of dAGE databases which list the content of different types of AGEs for different types of food and processing methods.[2] Although the use of different analytical methods causes variability between the measured AGE contents of foods, it appears that fruits, vegetables and high-fat dairy are generally low in AGEs whereas bakery products and processed meat are usually rich in AGEs.[2]

It has been suggested that higher dAGE consumption is associated with more abdominal obesity as assessed by waist circumference.[6] However, dAGEs have not been extensively studied in relation to more refined measurements of body composition and fat distribution or in relation to longitudinal, as opposed to cross-sectional, measures of body composition. Expanding further on the potential role of dAGEs in metabolic disturbances, previous studies have also demonstrated that dAGE restriction might have favorable effects on insulin resistance and inflammation.[7] However, most of these studies were performed within specific patient groups and were therefore limited in terms of generalizability. Therefore, in the present study, we investigated dAGEs in a population-based cohort in relation to longitudinal body composition measured by means of dual-energy X-ray absorptiometry (DXA) which provides accurate estimates of the quantity of fat mass and lean mass and fat distribution.[8] We also investigated the association between dAGEs and measures of insulin resistance as well as type 2 diabetes.

MATERIALS AND METHODS

The Rotterdam Study

The Rotterdam Study is a population-based prospective cohort study that was initiated in 1990 in the Ommoord district in the municipality of Rotterdam, the Netherlands. Its aims and general design have been outlined elsewhere.[9,10] In brief, 7,983 inhabitants of the Ommoord district aged 55 years and older were enrolled in the study at baseline in 1990 (subcohort RS-I). In 2000, a second group of 3,011 inhabitants who had moved into the district or had become 55 years old were enrolled (subcohort RS-II). A third subcohort (RS-III) was added in 2006 when 3,932 inhabitants aged 45-54 years were included in the study. All participants underwent home interviews and comprehensive physical examination at the Rotterdam Study research facility. Generally, these examinations are repeated every 3-6 years. The Rotterdam Study has received approval from the medical ethics committee of Erasmus University Medical Center and the Dutch Ministry of Health, Welfare and Sports. All participants have provided informed consent.[9]

Measurement of dAGE consumption

In order to estimate total intake of dAGEs we used data from a 389-item semi-quantitative food frequency questionnaire (FFQ) which was admitted to participants in the fifth examination round of subcohort RS-I (RS-I-5; starting 2009), the third examination round of subcohort RS-II (RS-II-3; starting 2011) and the first examination round of subcohort RS-III (RS-III-1; starting 2006). Thus, these measurement points constitute the baseline assessment of our current study. The FFQ was validated among two other Dutch populations and nutrient intake estimates have been demonstrated to correlate well with those obtained from 9-day dietary records and 4-week dietary histories.[11–13] Reported food intake was converted into daily intakes of nutrients and energy for all participants using the most recent version of the Dutch Food Composition Database available at the time of assessment.

The process of assessing dAGE consumption for our population has been described in detail elsewhere.[14] In summary, we used two previously published databases. The first was a Dutch database listing the contents of the AGEs carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL) and N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MGH1) for 190 food items commonly consumed among the Dutch population.[15] The second was a Northern Irish database listing CML contents for 257 frequently consumed foods among Northern Irish young adults.[16] The AGE content of the different types of food in the databases was estimated by first extracting the protein fractions of these foods and then quantifying AGE content using ultra performance

liquid chromatography - tandem mass spectrometry (UPLC-MS/MS). Methods of food processing used by participants were assumed to be similar in the Rotterdam Study compared to the populations used to design the dAGE databases. A four-step approach was then used to ascertain dAGE consumption for our study population. First, FFQ items were matched to the items listed in the Dutch dAGE database. Second, for any item not listed in the Dutch database, we consulted the Northern Irish database. For items with multiple values listed in the dAGE database for different types of a single food item, we used the mean of the listed values. In case the FFQ item was a food group (e.g. "small cookies and biscuits") rather than a single item, and the food group was not listed as such in the dAGE database, we used the mean value of the constituent items of the group listed in the database. Third, for food items in the FFQ that were not listed in either database, we selected a similar food item to be used instead which resembled the missing food item in terms of macronutrient composition (using the Dutch Food Composition Database of 2016). Fourth, for food items consisting of multiple constituent foods, such as certain dishes, AGE contribution of the different constituents was taken into account proportionally to their relative contribution to the combined item, based on packaging information or standard recipes. Then, daily intake (grams/day) of each food item was multiplied by its derived AGE value (mg/100 grams) separately for CML, CEL and MGH1. The resulting values were summed for each participant, thus creating a sum score expressing total daily consumption of each of the three dAGEs (mg/day).

Assessment of body composition, insulin resistance and type 2 diabetes

Body composition was measured by means of DXA (iDXA, GE Healthcare, Chicago, United States) during the same examination round where the FFQ was administered (RS-I-5, RS-II-3, RS-III-1) as well as during the subsequent examination round for each cohort (RS-I-6, starting 2014; RS-II-4, starting 2015; and RS-III-2, starting 2012). Thus, for each participant, up to two body composition and anthropometric measurements were available. Height was recorded with the participant shoeless and in a standing position. Body mass index (BMI) was also calculated, as total weight in kilograms divided by height squared. Waist circumference was measured midway between the lower rib margin and the iliac crest. Hip circumference was measured at the point resulting in the maximum circumference over the buttocks with the tape held horizontal. Waist-to-hip ratio was calculated as the ratio between waist and hip circumference. From the DXA data, we derived the following parameters of body composition and fat distribution: fat mass index (FMI; fat mass in kilograms divided by height in meters squared), fat-free mass index (FFMI; fat-free mass in kilograms divided by height squared), android-to-gynoid fat ratio (AGR; android fat mass in kilograms divided by

gynoid fat mass in kilograms) and body fat percentage (BF%; fat mass in kilograms divided by total body weight in kilograms, expressed as percentage).

At the baseline of our study, fasting serum samples were collected by means of venipuncture. The serum samples were stored in 5 mL aliquots at -80°C. Within one week of sampling, glucose levels (mmol/L) were measured in the samples using the glucose hexokinase method.[17] Insulin levels (μU/L) were determined by means of electrochemiluminescence immunoassay technology (Roche Modular Analytics E170 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Measurements were performed at the Erasmus University Medical Center clinical chemistry laboratory. As a measure of insulin resistance, we used the homeostatic models of insulin resistance (HOMA-IR) and β-cell function (HOMA-B). HOMA-IR was calculated as the product of serum glucose and serum insulin divided by 22.5. We calculated HOMA-B as serum insulin multiplied by 20 divided by serum glucose minus 3.5. Information on type 2 diabetes was collected through interviews at baseline examination as well as through consulting general practitioners' records, hospital discharge letters and glucose measurements performed as part of the Rotterdam Study protocol. We defined type 2 diabetes as fasting serum glucose ≥ 7.0 mmol/L, non-fasting serum glucose ≥ 11.1 mmol/L or use of antidiabetic medication.

Population for analysis

Of all Rotterdam Study participants who participated in the baseline examination rounds of the current study (n = 7,972), there were 5,508 participants for whom valid dietary data was available; participants whose self-reported daily energy intake was smaller than 500 kcal or greater than 5000 kcal were excluded. Of these 5,508 participants, 377 did not undergo a DXA measurement or had no information on type 2 diabetes status available. A further 238 participants were excluded from analysis because their BMI exceeded 35 during body composition assessment. Individuals with a BMI larger than 35 exceed the surface area limitation of the DXA device and their body composition can therefore not be estimated accurately. Thus, there were 4,893 individuals available for analysis. Among this total sample, 4,881 individuals also had data available on waist-to-hip ratio. Data on serum levels of glucose and insulin from fasting serum samples (and thus HOMA-IR and HOMA-B) were available for a subset of 4,620 of the total sample of 4,893 individuals, measured once.

Measurement of covariates

We accounted for the following baseline covariates in our analyses, based on general knowledge of their association with both exposure and outcome or based on previous literature: age, sex, Rotterdam Study subcohort, highest attained level of education,

smoking status, degree of physical activity, dyslipidemia, estimated glomerular filtration rate (eGFR), presence of type 2 diabetes and adherence to dietary guidelines (as a measure of overall diet healthiness). Smoking status was assessed during home interviews and participants were classified as never, former or current users of tobacco products. Highest attained level of education was also recorded during home interviews and categorized as primary education, lower general/vocational education, intermediate vocational education or higher vocational education/university. Physical activity was assessed by means of the LASA questionnaire for physical activity and quantified as metabolic equivalent of task (MET) hours per week.[18] Dyslipidemia was defined as total serum cholesterol > 6.5 mmol/L or use of lipid-lowering medication. Serum levels of cholesterol and creatinine were determined in fasting serum samples using a Roche Modular P800 chemistry analyzer. Use of medication was assessed at home interviews and by consulting pharmacy dispensing records. We calculated eGFR based on serum levels of creatinine according to the CKD-EPI formula.[19] Degree of adherence to the Dutch Dietary Guidelines was expressed as a diet quality score on a scale ranging from 0-14 based on FFQ data, as described in detail previously.[11]

Statistical analysis

We used a linear mixed model approach to analyze the association between intake of the dietary AGEs (CML, CEL and MGH1) and body composition outcomes. In these mixed models, we specified random intercepts and random slopes (for time between repeated measurements). Multivariable linear regression analysis was used to analyze AGE intake in relation to measures of insulin resistance (HOMA-IR and HOMA-B). To investigate the association between AGE intake and prevalence of type 2 diabetes, we specified logistic regression models with type 2 diabetes as the outcome. In all models, we investigated potential non-linear effects of the independent variables on the dependent variables by including three-knot natural cubic splines in our models where their use resulted in a significantly ($p < 0.05$) better fit of the model to the data. We investigated whether there was significant interaction between AGE intake and the covariates age, sex, time between repeated measurements (if applicable) and prevalence of type 2 diabetes (if applicable) by introducing the product of AGE intake and the respective covariates to the regression models. Because interaction between AGE intake and prevalence of type 2 diabetes was observed on multiple body composition outcomes in our models, we also repeated our analyses excluding individuals with prevalent type 2 diabetes. In all analyses, we adjusted for covariates in a stepwise manner. First, we specified a model adjusted only for age, sex, Rotterdam Study cohort and, in the case of the linear mixed models, time between repeated measurements. Afterwards, we specified another model additionally adjusted for smoking status, degree of physical activity, dyslipidemia, eGFR, presence of type 2 diabetes

and adherence to dietary guidelines. In addition, for all models using FFMI as the outcome, we also adjusted for body fat percentage to account for correlation between fat free mass and body weight. In the logistic regression models using type 2 diabetes as the outcome, we also adjusted for BMI and did not include type 2 diabetes as a covariate in the model. For all analyses, we transformed intake of the respective AGEs such that they were independent of daily energy intake using the residual method.[20] The variables were standardized afterwards such that one unit increase of the AGE variable expresses one standard deviation (SD) higher consumption of the respective dAGE independent of daily energy intake. We performed ten-fold multiple imputation with chained equations to account for missing values in the covariates. Results are presented as regression coefficients (β) or odds ratios (OR) with corresponding 95% confidence intervals (CI). All analyses were performed using R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of the study population

The characteristics of the baseline assessment of our study population are displayed in Table 4.2.1. Overall, among the 4,893 individuals available for analysis, there were 2,120 men (43.3%) and 2,773 women (56.7%). Mean intake of CML, CEL and MGH1 was 3.4 (SD 1.4), 3.1 (1.3) and 29.1 (11.7) mg/day, respectively. Supplementary Figure 4.2.1 displays the contribution of different food groups derived from the FFQ to the calculated consumption of CML, CEL and MGH1 averaged across all participants. The three greatest contributors to total CML consumption were sweets, whole grains and milk. For CEL, these were unprocessed meat, sweets and whole grains, and for MGH1 these were whole grains, sweets and vegetables. Among the total study population of 4,893, there were 1,641 individuals who underwent one DXA measurement (either at baseline or during the second measurement round) and 3,252 individuals who underwent DXA measurement twice. For the latter group, mean time between the two measurements was 5.1 years (SD 0.7; range 3.0 – 8.2 years).

Dietary AGE consumption and body composition

Results for the analyses of the association between dAGEs and body composition and fat distribution parameters are displayed in Table 4.2.2. We observed no association between CML or MGH1 consumption and any of the body composition measures across time after adjustment for covariates in model 2. Higher CEL consumption was associated with higher BMI (β 0.206, 95% CI 0.105; 0.307). This was mainly explained by the fact that higher CEL was associated with higher FMI (β 0.151, 0.075; 0.226)

Table 4.2.1. Baseline characteristics of the study population (n = 4,893).

Characteristic	Mean (SD) or N (%)
Age (years)	66.6 (11.0)
Sex	
Male	2120 (43.3%)
Female	2773 (56.7%)
Education	
Primary	375 (6.8%)
Lower vocational	188 (38.6%)
Intermediate vocational	1501 (30.7%)
Higher vocational / university	1129 (23.1%)
Dyslipidemia	
Absent	2725 (55.7%)
Present	2168 (44.3%)
Smoking status	
Never	1554 (31.8%)
Former	2465 (50.4%)
Current	847 (17.9%)
Physical activity (MET hours/week)[1]	40.2 (16.0; 79.9)
Diet Quality Score (scale 0-14)	6.9 (1.9)
Glucose (mmol/L)[2]	5.7 (1.2)
Insulin (uIU/L)[1,2]	10.5 (7.5; 15.1)
Estimated Glomular Filtration Rate (mL/min)	78.2 (15.7)
Type 2 Diabetes status	
Absent	4278 (87.4%)
Present	615 (12.6%)
Body Mass Index (kg/m^2)[3]	26.9 (3.5)
Waist-to-Hip ratio[4]	0.89 (0.1)
Fat Mass Index (kg/m^2)[3]	9.4 (2.9)
Fat-Free Mass Index (kg/m^2)[3]	17.4 (2.0)
Android-to-Gynoid Fat Ratio[3]	0.6 (0.2)
Body Fat Percentage (%)[3]	34.6 (7.5)
HOMA-IR[1,2]	2.5 (1.8; 3.8)
HOMA-β[1,2]	111.1 (79.2; 154.8)
Carboxymethyl-lysine (CML) intake (mg/day)	3.4 (1.4)
Carboxyethyl-lysine (CEL) intake (mg/day)	3.1 (1.3)
N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MGH1) intake (mg/day)	29.1 (11.7)

Values are displayed as mean (SD) or N (%), unless otherwise indicated. [1]Displayed as median (interquartile range). [2]Displayed for a subset of 4,483 individuals who underwent DXA at the baseline examination round. [3]Displayed for a subset of 4,620 individuals from whom fasting serum samples were collected. [4]Displayed for a subset of 4,471 individuals with who underwent DXA data at the baseline examination round and had data on waist-to-hip ratio available.

Table 4.2.2. Associations between dietary advanced glycation end-products (dAGEs) consumption and fat mass index, fat-free mass index, android-to-gynoid fat ratio, body mass index, body fat % and waist-to-hip ratio.

	Body Mass Index	p-value	Fat Mass Index	p-value	Fat-Free Mass Index[1]	p-value	Body Fat Percentage	p-value	Android-to-Gynoid Fat Ratio	p-value	Waist-to-Hip Ratio[2]	p-value
CML												
Model 1	-0.050 (-0.145; 0.046)	0.306	-0.037 (-0.109; 0.034)	0.303	-0.015 (-0.056; 0.027)	0.490	-0.231 (-0.459; -0.002)	0.048	-0.004 (-0.008; 0.000)	0.083	-0.002 (-0.004; 0.000)	0.062
Model 2	-0.035 (-0.129; 0.060)	0.474	-0.017 (-0.087; 0.053)	0.637	-0.018 (-0.060; 0.023)	0.382	-0.171 (0.395; 0.054)	0.136	-0.002 (-0.006; 0.002)	0.396	-0.001 (-0.003; 0.001)	0.515
CEL												
Model 1	0.176 (0.081; 0.271)	< 0.001	0.122 (0.051; 0.193)	0.001	0.058 (0.017; 0.100)	0.005	0.199 (0.029; 0.368)	0.022	0.006 (0.002; 0.010)	0.007	0.003 (0.001; 0.004)	0.012
Model 2	0.206 (0.105; 0.307)	< 0.001	0.151 (0.075; 0.226)	< 0.001	0.046 (0.005; 0.087)	0.026	0.267 (0.088; 0.446)	0.003	0.005 (0.001; 0.009)	0.013	0.002 (0.000; 0.004)	0.017
MGH1												
Model 1	-0.004 (-0.099; 0.092)	0.936	-0.022 (-0.093; 0.050)	0.552	0.012 (-0.029; 0.054)	0.558	-0.058 (-0.215; 0.100)	0.472	-0.001 (-0.005; 0.003)	0.688	-0.003 (-0.005; -0.001)	0.009
Model 2	0.031 (-0.066; 0.128)	0.525	0.014 (-0.058; 0.086)	0.712	0.014 (-0.028; 0.057)	0.506	0.034 (-0.126; 0.194)	0.673	0.002 (-0.002; 0.006)	0.396	-0.001 (-0.003; 0.001)	0.247

N = 4,893. Values are displayed as regression coefficients (β) with corresponding 95% confidence intervals per 1 standard deviati on increase in consumption of the respective dAGEs, corrected for daily energy intake. CML; Carboxymethyl-lysine. CEL; Carboxyethyl-lysine. MGH1; N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine. Model 1: adjusted for age, sex, Rotterdam Study cohort and time between repeated measurements. Model 2: additionally, adjusted for smoking status, physical activity, highest attained level of education, dyslipidemia, estimated glomerular filtration rate (eGFR), presence of type 2 diabetes and adherence to dietary guidelines. [1]Additionally adjusted for body fat percentage in all models. [2]There were n = 4,881 individuals with data on waist-to-hip ratio available for analysis.

and to a minor extent also with higher FFMI (β 0.046, 0.005; 0.087). These findings also translated into an association between CEL intake and higher BF% (β 0.267, 0.088; 0.446). Higher CEL intake was also associated with fat distribution in terms of higher AGR (β 0.005, 0.001; 0.009). Finally, we also found an association between CEL intake and WHR (β 0.002, 0.000; 0.004). Results of these analyses after exclusion of individuals with prevalent type 2 diabetes are displayed in Supplementary Table 4.2.1. Overall, upon exclusion of these individuals, the differences compared to the main analyses were minor; higher CEL consumption remained associated with increases in all parameters of body composition.

Dietary AGE consumption in relation to insulin resistance and diabetes status

Table 4.2.3 displays the results of the analyses pertaining to dAGE consumption and measures of insulin resistance. Among the subset of 4,620 individuals with data on HOMA-IR and HOMA-B, we found no association between CML, CEL or MGH1 consumption and HOMA-IR or HOMA-B. Exclusion of individuals with prevalent type 2

Table 4.2.3. Associations between dietary advanced glycation end-products (dAGEs) consumption and homeostatic models of insulin resistance (HOMA-IR) and β-cell function (HOMA-B).

	HOMA-IR	p-value	HOMA-B	p-value
CML				
Model 1	-0.059 (-0.140; 0.022)	0.156	-0.741 (-3.089; 1.608)	0.537
Model 2	-0.021 (-0.104; 0.062)	0.620	-0.493 (-2.849; 1.864)	0.682
CEL				
Model 1	0.031 (-0.050; 0.113)	0.450	0.090 (-2.270; 2.449)	0.941
Model 2	0.021 (-0.062; 0.104)	0.624	0.532 (-1.817; 2.882)	0.657
MGH1				
Model 1	-0.065 (-0.146; 0.017)	0.120	0.820 (-1.542; 3.182)	0.496
Model 2	-0.007 (-0.091; 0.078)	0.878	1.430 (-0.998; 3.858)	0.248

N = 4,620. Values are displayed as regression coefficients (β) with corresponding 95% confidence intervals per 1 standard deviation increase in consumption of the respective dAGEs, corrected for daily energy intake. CML; Carboxymethyl-lysine. CEL; Carboxyethyl-lysine. MGH1; Methylglyoxal-derived Hydroimidazolone. Model 1: adjusted for age, sex and Rotterdam Study cohort. Model 2: additionally adjusted for smoking status, physical activity, highest attained level of education, dyslipidemia, estimated glomerular filtration rate (eGFR), presence of type 2 diabetes and adherence to dietary guidelines.

diabetes status did not substantially change these observations (Supplementary Table 4.2.2). However, we did observe that higher intake of each of the dAGEs studied was associated with a higher probability of type 2 diabetes (Table 4.2.4). These associations persisted after adjustment for BMI as a potential intermediate as well as adjustment for other confounders. For example, higher CEL intake was associated with a higher probability of type 2 diabetes (OR 1.21, 95% CI 1.12; 1.31) in model 2.

Table 4.2.4. Associations between dietary advanced glycation end-products (dAGEs) consumption and prevalence of type 2 diabetes.

	Type 2 diabetes (n cases = 615)	p-value
CML		
Model 1	1.09 (1.01; 1.19)	0.035
Model 2	1.13 (1.04; 1.23)	0.004
CEL		
Model 1	1.20 (1.11; 1.30)	< 0.001
Model 2	1.21 (1.12; 1.31)	< 0.001
MGH1		
Model 1	1.13 (1.04; 1.23)	0.005
Model 2	1.18 (1.08; 1.29)	< 0.001

N = 4,893. Values are displayed as odds ratios (OR) with corresponding 95% confidence intervals per 1 standard deviation increase in consumption of the respective dAGEs, corrected for daily energy intake. CML; Carboxymethyl-lysine. CEL; Carboxyethyl-lysine. MGH1; Methylglyoxal-derived Hydroimidazolone. Model 1: adjusted for age, sex and Rotterdam Study cohort. Model 2: additionally adjusted for smoking status, physical activity, highest attained level of education, dyslipidemia, estimated glomerular filtration rate (eGFR), presence of type 2 diabetes, adherence to dietary guidelines and BMI.

DISCUSSION

In this study, we observed an association between higher CEL consumption and higher BMI across on average 5.1 years of follow-up, mainly driven by higher body fat and changes in fat distribution (i.e. higher AGR). We found no association between CML or MGH1 consumption and any of the body composition or fat distribution parameters. We also found no association between consumption of any of the studied dAGEs and measures of insulin resistance or beta cell function, but we did observe an association

between higher consumption of each of the studied dAGEs and higher probability of type 2 diabetes, which was independent of BMI.

Several mechanisms have been proposed that may explain the association between higher dAGE consumption and the adverse changes (higher BMI, higher body fat percentage and higher AGR) we observed in body composition and fat distribution. The receptor for advanced glycation end products (RAGE) is the most widely studied among the different AGE receptors.[21] Interaction between RAGE and its ligands leads to the activation of pro-inflammatory signaling pathways and induces the generation of reactive oxygen species.[22] Both inflammation and oxidative stress are closely interwoven with obesity.[23,24] It has also been demonstrated that RAGE overexpression accelerates adipocyte hypertrophy in vitro.[25] In a previous animal study, mice which were given a high-AGE diet had increased weight gain and higher visceral adiposity compared to mice given a low-AGE diet.[26] This phenomenon was not observed among genetically modified mice without RAGE expression, indicating that RAGE may indeed represent one of the primary pathways through which AGEs exert their adverse effects.[21,27]

In this study, we observed associations with regards to body composition only for CEL and not for CML or MGH1. It may be possible that the three dAGEs we studied have distinct effects on metabolism and do therefore not affect body composition equally strongly. However, little biological evidence is available to provide mechanistic support for this hypothesis. Another potential explanation for the discrepancies in findings between the different dAGEs may lie in the health effects of their contributing food groups. For instance, consumption of unprocessed meat was a major contributor to total CEL intake, but did not contribute as much to total CML and MGH1 intake (Supplementary Figure 4.2.1), and some previous studies have suggested that higher meat consumption is associated with a higher obesity rate.[28,29] Differences such as these could account for the differential associations between the studied dAGEs and body composition.

In line with our null-finding for CML, a recent cross-sectional study among 265 adults reported that there was no association between CML consumption and body composition parameters (including visceral fat, muscle mass index and fat-free mass) as measured by bioelectric impedance analysis after adjustment for covariates.[30] However, this study did not investigate CEL or any other dAGEs aside from CML. No other studies thus far have investigated dAGEs in relation to measures of body composition other than traditional anthropometrics. In a meta-analysis of randomized controlled trials (RCTs), low-AGE diets were found not to be associated with changes

in the anthropometric indices body mass index, weight or waist circumference when compared to high-AGE diets.[31] However, the included dietary intervention studies generally did not differentiate between different types of dAGEs or investigated only CML, were conducted among specific populations (for example, individuals with type 2 diabetes or renal failure) and the duration of their interventions was generally limited to only several weeks during which a measurable effect on weight might not become apparent.[31] A large multinational observational study later reported that higher consumption of CEL, and to a lesser extent also CML and MGH1, was associated with weight gain as measured on average 5 years later.[32] We extend upon this observation by providing evidence that higher CEL consumption is more strongly associated with increases in fat mass than fat-free mass, and that CEL consumption is also associated with higher AGR.

We observed that higher consumption of each of the studied dAGEs was associated with a higher probability of type 2 diabetes independently of BMI. This association was strongest for CEL. Indeed, it has been demonstrated previously in animal studies that AGEs have the capacity to disrupt insulin signaling and may therefore contribute to the development of type 2 diabetes in vivo.[33,34] Previous meta-analyses of RCTs have indicated that there appears to be a favorable effect of low-AGE diets compared to high-AGE diets on insulin resistance as measured by HOMA-IR, although it should be noted that a substantial amount of the trials included in these studies was limited by poor methodological quality.[7,31] In the present study, dAGE consumption was not associated with insulin resistance or beta cell function as quantified by HOMA. This stands in contrast to previous observational studies that have reported associations between serum CML levels and insulin resistance, and also contradicts our findings with regards to type 2 diabetes.[35,36] Although it has been demonstrated that dAGE consumption contributes to circulating AGE levels, the degree to which AGE accumulation occurs after dietary ingestion is variable and may also depend on, for instance, the phytochemical and fat content of the diet.[21,37] Therefore, a measure of dAGE consumption may not fully accurately reflect the total AGE burden adversely affecting metabolism for a given individual. Ultimately, further studies are needed to elucidate to what extent dAGE consumption affects insulin resistance and risk of type 2 diabetes, and what biological mechanisms may underlie this association.

Our study includes a large sample of individuals (n = 4,893) who underwent body composition assessment at multiple time points. Thus, our study provides information on the relation between dAGE consumption and trajectories of body composition over time, which provides important additional insights to previously used cross-sectional approaches. In addition, we assessed body composition by means of DXA, an accurate

and well-validated method, rather than only estimations based on weight, waist or hip circumference measurements. We were able to adjust our analyses for a comprehensive selection of demographic, behavioral and metabolic covariates. dAGE consumption was quantified by linking well-validated FFQs to dAGE databases in which AGE content was measured using mass spectrometry, which provides superior accuracy to traditional immunological estimation methods. However, there are also several limitations to our study. We had no information on the food preparation methods used by our participants. Because the dAGE content of food varies according to how the food is prepared, this lack of information may have introduced inaccuracies in our estimations of dAGE consumption.[2] Also, some items in our FFQ had no matching equivalent in the dAGE databases. Although we selected closely matching replacement items for dAGE estimation, some degree of inaccuracy may have remained. Furthermore, only a single repeated measurement of body composition was available per participant. Finally, our study population is a homogeneous sample of middle-aged and elderly Dutch individuals, and the presented results may not directly apply to other populations demographically or ethnically different from ours.

In conclusion, we observed that higher CEL consumption was associated with increased fat mass and a more android-type fat distribution, both measured longitudinally. Higher dAGE consumption was also associated with a higher probability of type 2 diabetes independently of BMI. Our findings warrant future studies directed at characterizing the mechanisms underlying these associations, which may guide potential interventions directed at limiting the adverse influence of dAGE consumption on metabolic health.

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Supplementary Table 4.2.1. Associations between dietary advanced glycation end-products (dAGEs) consumption and fat mass index, fat-free mass index, android-to-gynoid fat ratio, body mass index and body fat %, excluding participants with type 2 diabetes.

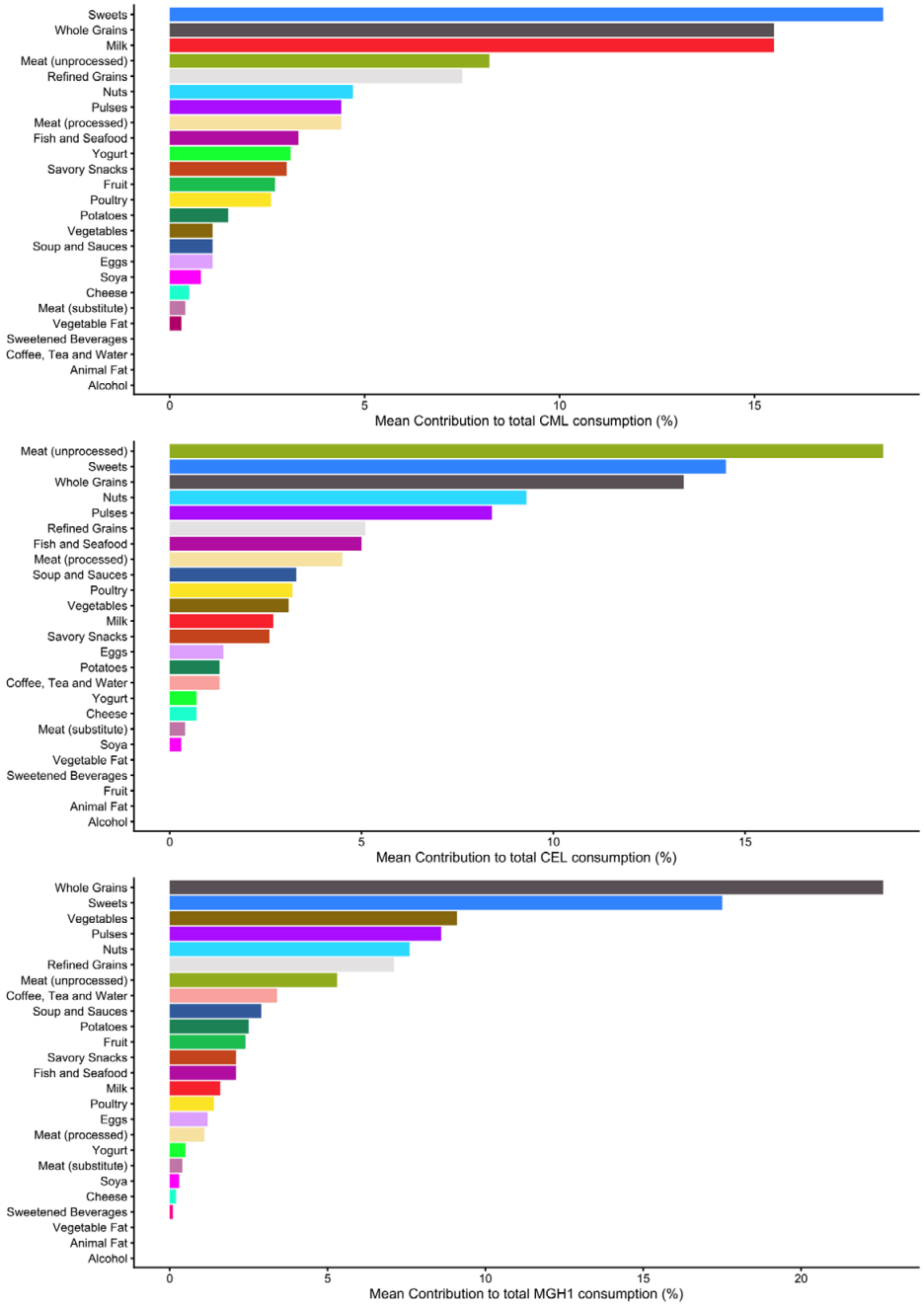
	Body Mass Index (BMI)	p-value	Fat Mass Index	p-value	Fat-Free Mass Index[1]	p-value	Body Fat %	p-value	Android-to-Gynoid Fat Ratio	p-value	Waist-to-Hip Ratio[2]	p-value
CML												
Model 1	-0.043 (-0.144; 0.059)	0.412	-0.025 (-0.102; 0.052)	0.525	-0.019 (-0.062; 0.024)	0.387	-0.175 (-0.427; 0.077)	0.174	-0.005 (-0.010; -0.001)	**0.019**	-0.002 (-0.004; 0.000)	0.051
Model 2	-0.009 (-0.111; 0.094)	0.869	0.007 (-0.070; 0.083)	0.866	-0.014 (-0.058; 0.029)	0.519	-0.083 (-0.331; 0.165)	0.511	-0.002 (-0.007; 0.002)	0.263	0.000 (-0.002; 0.002)	0.698
CEL												
Model 1	0.191 (0.089; 0.293)	**< 0.001**	0.141 (0.064; 0.218)	**< 0.001**	0.056 (0.013; 0.100)	**0.011**	0.244 (0.056; 0.432)	**0.011**	0.005 (0.000; 0.009)	**0.032**	0.002 (0.000; 0.004)	**0.035**
Model 2	0.207 (0.106; 0.308)	**< 0.001**	0.151 (0.075; 0.228)	**< 0.001**	0.059 (0.016; 0.103)	**0.008**	0.266 (0.081; 0.451)	**0.005**	0.006 (0.002; 0.010)	**0.008**	0.003 (0.001; 0.005)	**0.006**
MGH1												
Model 1	0.013 (-0.089; 0.114)	0.804	-0.013 (-0.089; 0.064)	0.748	0.016 (-0.027; 0.059)	0.469	-0.042 (-0.214; 0.129)	0.629	-0.001 (-0.006; 0.003)	0.511	-0.003 (-0.005; -0.001)	**0.007**
Model 2	0.077 (-0.028; 0.181)	0.150	0.040 (-0.039; 0.118)	0.320	0.030 (-0.015; 0.075)	0.188	0.078 (-0.096; 0.252)	0.381	0.002 (-0.002; 0.007)	0.323	-0.001 (-0.003; 0.001)	0.416

N = 4,278 free of type 2 diabetes. Values are displayed as regression coefficients (β) with corresponding 95% confidence intervals per 1 standard deviation increase in consumption of the respective dAGEs, corrected for daily energy intake. CML; Carboxymethyl-lysine. CEL; Carboxyethyl-lysine. MGH1; N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine. Model 1: adjusted for age, sex, Rotterdam Study cohort and time between repeated measurements. Model 2: additionally, adjusted for smoking status, physical activity, highest attained level of education, dyslipidemia, estimated glomerular filtration rate (eGFR) and adherence to dietary guidelines. [1]Additionally adjusted for body fat percentage in all models. [2]There were n = 4,267 individuals free of type 2 diabetes with data on waist-to-hip ratio available for analysis.

Supplementary Table 4.2.2. Associations between dietary advanced glycation end-products (dAGEs) consumption and homeostatic models of insulin resistance (HOMA-IR) and β-cell function (HOMA-B).

	HOMA-IR	p-value	HOMA-B	p-value
CML				
Model 1	-0.042 (-0.102; 0.019)	0.175	-0.133 (-2.631; 2.366)	0.917
Model 2	-0.022 (-0.083; 0.039)	0.476	-0.082 (-2.603; 2.439)	0.949
CEL				
Model 1	0.026 (-0.035; 0.087)	0.401	1.314 (-1.206; 3.834)	0.307
Model 2	0.030 (-0.031; 0.091)	0.333	1.244 (-1.271; 3.759)	0.332
MGH1				
Model 1	-0.032 (-0.092; 0.029)	0.305	2.015 (-0.483; 4.512)	0.114
Model 2	-0.002 (-0.064; 0.060)	0.942	2.374 (-0.200; 4.948)	0.071

Total n = 4,620. Values are displayed as regression coefficients (β) with corresponding 95% confidence intervals per 1 standard deviation increase in consumption of the respective dAGEs, corrected for daily energy intake. CML; Carboxymethyl-lysine. CEL; Carboxyethyl-lysine. MGH1; Methylglyoxal-derived Hydroimidazolone. Model 1: adjusted for age, sex and Rotterdam Study cohort. Model 2: additionally adjusted for smoking status, physical activity, highest attained level of education, dyslipidemia, estimated glomerular filtration rate (eGFR) and adherence to dietary guidelines.



Suppplementary Figure 4.2.1. Relative contribution of different food groups to total dietary advanced glycation end-product consumption. CML; carboxymethyl-lysine. CEL; carboxyethyl-lysine. MGH1; N(δ)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.