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List of Abbreviations

AGEs	advanced glycation endproducts
CEL	N^{ε} -1- carboxyethyl-lysine
CML	N^{ε}-carboxymethyl-lysine
DQ	Dietary questionnaires
DXA	dual-energy X ray absorptiometry
EPIC	European Prospective Investigation into Cancer and nutrition
MG-H1	N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl) ornithines
mrMDS	modified relative Mediterranean Diet Score
PANACEA	Physical Activity, Nutrition, Alcohol, Cessation of smoking, Eating out of home in relation to Anthropometry

Abstract

Purpose Advanced glycation endproducts (AGEs) can be formed in foods by the reaction of reducing sugars with proteins, and have been shown to induce insulin resistance and obesity in experimental studies.

We examined the association between dietary AGEs intake and changes in body weight in adults over an average of 5 years of follow-up.

Methods A total of 255,170 participants aged 25-70 years were recruited in 10 European countries (1992-2000) in the PANACEA study (Physical Activity, Nutrition, Alcohol, Cessation of smoking, Eating out of home in relation to Anthropometry), a sub-cohort of the EPIC (European Prospective Investigation into Cancer and Nutrition). Body weight was measured at recruitment and self-reported between 2-11 years later depending on the study center. A reference database for AGEs was used containing UPLC-MS/MS-measured 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) in 200 common European foods. This reference database was matched to foods and decomposed recipes obtained from country-specific validated dietary questionnaires in EPIC and intake levels of CEL, CML, and MG-H1 were estimated. Associations between dietary AGEs intake and body weight change were estimated separately for each of the three AGEs using multilevel mixed linear regression models with center as random effect and dietary AGEs intake and relevant confounders as fixed effects.

Results A one-SD increment in CEL intake was associated with 0.111 kg (95% CI 0.087 to 0.135) additional weight gain over 5 years. The corresponding additional weight gain for CML and MG-H1 was 0.065 kg (0.041 to 0.089) and 0.034kg (0.012, 0.057), respectively. The top six food groups contributing to AGEs intake, with varying proportions across the AGEs, were cereals/cereal products, meat/processed meat, cakes/biscuits, dairy, sugar and confectionary, and fish/shellfish.

Conclusion In this study of European adults, higher intakes of AGEs were associated with marginally greater weight gain over an average of 5-years of follow-up.

Keywords dietary advanced glycation endproducts, weight change, obesity, adults, Europe

Introduction

In 2016, more than 39% of the world population was affected by overweight or obese (body mass index, BMI ≥ 25 kg/m^2) and it is projected that the prevalence of obesity will increase further in the years to come [1]. This is of concern because the risk of heart disease, stroke, diabetes, and of certain cancers escalates steadily with increasing BMI [2]. Although modest weight loss through changes in diet and physical activity is possible [3], few people manage to maintain these changes in weight over the long term [4]. Prevention of weight gain and obesity are therefore of substantial public health importance.

Overweight and obesity arise as consequence of an imbalance between energy intake and expenditure over a prolonged period [5]. Established risk factors contributing to an energy imbalance include high intakes of energy-dense food and low physical activity [6]. However, an ever increasing proportion of people in virtually all regions of the world have access to low-cost, but highly-processed, energy-dense and nutrient-poor food products [7]. That such foods may facilitate overeating has been confirmed in a randomized controlled trial, where a diet composed of ultra-processed foods lead to significantly greater energy intake and weight gain as compared with an unprocessed diet[8]. However, it is currently uncertain what factors trigger overeating of such foods. A by-product of food processing are advanced glycation endproducts (AGEs) [9]. Dietary AGEs are formed, in particular, during heating, by the non-enzymatic reaction of sugars with proteins [10,11]. Major sources of AGEs are foods that undergo dry heat processing to improve flavour and aroma such as crisps, crackers, or cereal products, but also meat and meat-derived products [12]. Well-characterized AGEs are N$^{\varepsilon}$-carboxymethyl-lysine (CML), N$^{\varepsilon}$-1-carboxyethyl-lysine (CEL), and N$^{\delta}$-(5-hydro-5-methyl-4-imidazolon-2-yl) ornithines (MG-H1), which are formed by the reaction of proteins with sugar, sugar-derived intermediates such as methylglyoxal, glyoxal and 3-deoxyglucosone, and with lipid peroxidation products [9,13].

Higher AGEs intake may induce weight gain via insulin resistance [14] and hypothalamic inflammation [15]. From animal models and clinical studies in humans, there is evidence that higher exposure to dietary AGEs is associated with impaired insulin sensitivity and weight gain [16-19]. To date there are no prospective data on dietary AGE exposure and weight change available from cohort studies. We, therefore, examined the association of dietary AGEs intake and weight change in a sub-cohort of the EPIC (European Prospective Investigation into Cancer and nutrition) study, the EPIC-Panacea study; PANACEA (Physical Activity, Nutrition, Alcohol, Cessation of smoking, Eating out of home in relation to Anthropometry), where repeated assessments of weight are available.

Methods

Study population

The EPIC study is an ongoing prospective cohort study across 23 centers in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom (UK). From 1992 to 2000 a total of 521,448 men and women were recruited. In France, Norway, Utrecht (Netherlands) and Naples (Italy), only women were recruited. Individuals were selected from the general population with few exceptions. In France, state-school employees were recruited. The Utrecht and Florence (Italy) centers included women invited for a local population-based breast cancer screening program. Some centers in Italy and Spain included members of local blood donor associations. In Oxford (United Kingdom), one-half of the cohort was recruited from lacto-ovo vegetarians and vegans. The rationale and design of the EPIC has been described in detail elsewhere [20,21]. The EPIC study was approved by the Ethical Review Boards of the IARC and the Institutional Review Board of each participating EPIC center.

For the PANACEA study, some of the original 23 centers were combined within countries depending on their follow-up times and/or weight measurement methods, resulting in 16 centers. We excluded pregnant women, participants with missing dietary or lifestyle information, missing data on weight and height or with unreliable anthropometric values at baseline (n=23,713). We further excluded 122,154 individuals with missing weight at follow-up and 2,288 individuals with outlying anthropometry at follow-up: weight change < -5 or > 5 kg/year and BMI at follow up < 16 kg/m². Last, we excluded participants identified as dietary energy over- (n=24,331) or under-reporters (n=93,792) according to Goldberg [22]. This has been shown to reduce BMI-associated bias in energy intake reporting [23], in particular due to underreporting of cakes and cookie consumption [24], which are among the main food groups contributing to AGE intake. More details on follow-up exclusions are given in **Supplemental Figure 1 (Online Resource)** and have been described previously [25,26].

After these exclusions, a sample of 70,570 men and 184,600 women with complete and plausible body weight data was available for analyses.

Anthropometric measures and weight change

Two body weight measures were available for each participant: at baseline and after a median follow-up time of 5 years [min.: 2 years for Heidelberg (Germany), max.:11 years for Varese (Italy)]. All centers used standardized procedures to measure weight and height at baseline, except, in France, Norway, and Oxford center, where participants self-reported their weight. Follow-up weight was self-reported, except for Cambridge (UK) and Doetinchem (The Netherlands) where it was measured [25,26]. The accuracy of self-reported anthropometric measures at baseline and at follow-up was improved with prediction equations derived from participants with both

measured and self-reported weight at baseline [27]. The main outcome of our study was weight change in kg per 5 years, calculated as weight at follow-up minus weight at baseline divided by the follow-up time in years and multiplied by 5 years.

Dietary assessment and estimation of AGEs intake

In the EPIC study, usual food intake was assessed once at baseline using country-specific validated dietary questionnaires (DQ) [21]. In brief, three types of DQ were used to assess habitual food consumption of the past 12 months; a) quantitative DQ were used in northern Italy, Ragusa in Italy, The Netherlands, Germany, Greece, Spain, and France, b) semi-quantitative food-frequency questionnaires were used in Denmark, Norway, Naples in Italy, and Umeå in Sweden, and c) a combination of semi-quantitative food-frequency questionnaires and 7 to 14 day food records were used in the UK and Malmö (Sweden). Harmonization of food grouping and portion sizes for quantification was carried out centrally at IARC. To estimate AGEs intake, we used a reference database containing CEL, CML and MG-H1 content values (in mg/100g of food) in over 200 commonly consumed foods [9]. The content values were obtained from European foods measured by ultra-performance liquid chromatography tandem mass-spectrometry [9]. DQ foods from EPIC were then matched to the AGE database by name and, whenever applicable, by their associated descriptors, particularly those pertaining to preparation and/or processing. Generic or multi-ingredient DQ foods were decomposed into more specific foods or ingredients based on country-specific recipes provided by previous EPIC projects [28]. An end-user EPIC AGEs composition database was then generated containing CEL, CML, and MG-H1 content (in mg/100 g) for all DQ foods and recipes. Finally, dietary AGEs intake of these three compounds was calculated (in mg) for the full EPIC cohort by accounting for the reported frequency and quantity of foods consumed by each EPIC participant.

Assessment of other covariates

Data on socio-demographic, lifestyle and other factors, including education level, physical activity and smoking history were collected at baseline through validated questionnaires [21].

Statistical analyses

Habitual dietary AGEs intake of CEL, CML, and MG-H1 were analysed in separate models both on a continuous scale per 1 standard deviation (SD) /day increment (see below) and by categories, where the energy adjusted dietary AGEs intake was divided by quintiles and the lowest intake placed in the first (reference) category.

The association between dietary AGEs intake and body weight change (kg/5 years) was estimated using multilevel mixed linear regression models with center as random effect and dietary AGEs intake and relevant confounders as fixed effects. Models with three different sets of adjustments were fit. The first model (M1) was adjusted for age, sex, and body mass index (BMI) at baseline. Model 2 was further adjusted for total energy intake follow-up time, educational level, levels of physical activity, smoking status at baseline.

Model 3 was additionally adjusted for Mediterranean diet, representing healthy dietary habits, using the modified relative Mediterranean Diet Score (mrMDS) [29].We log (natural)-transformed CEL, CML, and MG-H1 to improve normality and standardized them by dividing each AGE with its standard deviation. Associations with weight change are expressed per 1 SD increment in the log-transformed AGEs intake.

Participants with missing values for physical activity (n=3,663, 1.4%), education (n= 3,490, 1.4%) and smoking status (n= 5,138, 2.0%) at baseline were classified in a separate category and included in the models. Model assumptions and fit were checked visually by plotting the residuals against each of the categorical covariates.

Because of high inter-correlations between CEL, CML and MG-H1, (*Pearson r* 0.8) we calculated standardized principal components for the three dietary AGEs in order to use them together in one model. This allowed us to investigate independent associations among the three dietary AGEs with weight change.

We also conducted center-specific analyses, where respective results were then meta-analyses in random effect models for each AGE [30]. Since the meta-analysed summary estimates were very similar to the pooled analyses, we only present the latter.

We tested *a priori* for effect modification by age (categorised as younger than median age <51 and ≥51 years), sex, and BMI categories at baseline (<25, 25-30, > 30 kg/m²). This was done by including interaction terms between each potential effect modifier and each individual dietary AGE compound (continuous per 1 SD/day) in the models. *P* values for the interaction term were calculated using *F* tests.

We performed a range of sensitivity analyses to assess robustness of our findings and address potential biases **(Supplemental Table 1, Online Resource)**. In order to assess whether observed associations were driven by any of the main food sources of AGEs, we adjusted Model 2 in turn for each of the five main food groups contributing to dietary AGEs intake (**Supplemental Table 2, Online Resource**).

All statistical analyses were performed with STATA 14.1 (College Station, Texas, USA).

Results

Characteristics of the study population

Table 1 shows the main characteristics of the study population at baseline in the lowest and highest quintile of each energy adjusted intake of dietary AGE. Participants in the highest quintile of AGEs intake had slightly higher weight gain, were more likely to be women and more likely to be current smoker at baseline. There were some expected differences in consumption of specific food groups, but not with a Mediterranean dietary pattern (Table 1).

Main food sources of advanced glycation endproducts

Cereals and cereal products, meat and meat products, fish, cakes and biscuits, and dairy were the main food sources of AGEs (**Supplemental Figures 2-4, Online Resource**). Depending on the AGE compound, the proportion of these main food sources varied to some extent. For example, meat and meat products contributed 32% to CEL intake, but only 10% to MG-H1 intake. Cereals and cereal products contributed 38% and 48% to CML intake and MG-H1 intake, respectively.

Intake of AGE´s and 5-year changes in body weight

Between baseline and the 2nd weight assessment on average five years later, the mean weight increase in the study population was 2.1 kg with large variation between participants (SD 5.0 kg). Body weight changes (kg) over an average of 5 years according to baseline dietary AGEs intake are shown in **Table 2**. After adjustment for total energy intake and potential confounders, each of the three AGEs was positively associated with weight gain. Among the three AGEs, CEL intake was associated with the highest (0.111 kg per 1 SD intake increase/5 years, 95% CI 0.087 to 0.135) and MG-H1 intake with the lowest (0.034 kg per 1 SD intake increase/5 years; 95% CI 0.012 to 0.057) increase of weight gain in our main model (Model 3). However, associations from model 2 remained nearly unchanged after further adjustment in model 3 for Mediterranean diet (Table 2).

Analyses by quintiles of each dietary AGEs intake confirmed the findings using intake on a continuous scale, where participants in the highest quintile of CEL, CML, and MG-H1 gained more weight as compared to participants of the lowest quintile (Table 2). The main findings were also robust to a range of sensitivity analyses (Supplemental Table 1, Online Resource). Further adjustment for in turn each of the five main food sources, (meat, fish, cakes, cereals and dairy) also resulted in similar associations with weight change, except after adjustment for meat/meat products, which led to an attenuation by approximately half (Supplemental Table 2, Online Resource).

Age and BMI at baseline did not modify associations between CEL, CML, and MG-H1 and weight change. A significant *P* interaction value was obtained between sex and all three AGEs (each *P* interaction < 0.001), where the association with weight gain was more pronounced in women than in men. The stratified results in women and men for all three AGEs in Model 3 are shown in **Supplemental Table 3 (Online Resource)**.

The results of the combined analysis of the three dietary AGEs with regard to weight change are shown in **Table 3**. Principal component (PC) 1, representing an average high intake of all three AGEs together, showed a positive association with weight gain. The same applied for PC3 reflecting higher intake of CEL. PC2, reflecting a high intake of CML with concurrent low intake of CEL and MG-H1, was not associated with weight change.

Discussion

In this prospective analysis, we found that higher dietary intake of AGEs was associated with marginally greater weight gain over an average of 5 years of follow-up in adults from 10 European countries. Observed associations were strongest for CEL, where a high vs. low dietary CEL intake corresponded to a 10% greater body weight increase relative to the population average weight gain. This increase of weight appears trivial at an individual level, but at population level associations were comparable to those observed for high adherence to the Mediterranean diet in the same study population, albeit opposite in direction [31]. Dietary AGEs may be important compounds that lead to energy imbalance and weight gain, and should therefore be studied further in public health research.

To date, no prospective epidemiological study has evaluated the relationship between dietary AGEs intake and weight change over time. In vivo models in mice fed with either a high AGE or a low AGE diet, showed a significantly higher weight gain among the high AGE diet groups [17,18], which is congruent with our findings.

In a randomized controlled trial, a diet composed of ultra-processed foods lead to significantly greater energy intake and weight gain as compared with an unprocessed diet [8]. The putatively high amounts of AGEs in highly processed foods might be one of the potential components triggering energy overconsumption. However, potential mechanism by which higher dietary intake of AGEs may promote weight gain are not well understood. There is suggestive evidence from experimental models and human intervention studies that higher AGE intake can lead to insulin resistance [32-34]. In a randomized crossover diet-controlled intervention trial with 62 volunteers, one month of consuming a high-heat-treated diet, with CML as a marker, induced insulin resistance [32]. Similarly, a double-blind, randomized, crossover trial in 20 participants found that insulin sensitivity increased after a two-week isoenergetic- and macronutrient-matched low-AGE diet, whereas it showed a tendency to decrease after the two-week high-AGE diet [33]. High-normal insulin levels may inhibit lipolysis and promote lipogenesis in adipocytes [14]. A link between AGEs, insulin resistance and weight gain has also been shown in an in vivo study in *Drosophila*, where elevated methylglyoxal, which forms irreversible adducts such as MG-H1 in vivo, lead to progressive development of insulin resistance and weight gain [34]. Hypothalamic inflammation might be another pathway whereby higher AGEs intake could promote weight gain. In a rodent study, the

combination of an overconsumption of fat and sugar triggered hypothalamic inflammatory responses, meditated by excessive CML and MG-H1 production in hypothalamic neurons [15]. In a hypothalamic inflammatory state, the signaling of two key hormones in energy homeostasis, i.e. insulin and leptin, is compromised, which in turn can trigger an adaptive increase of food intake relative to energy expenditure that favors weight gain [35]. It is of note that we adjusted our analysis for total energy intake, which is recommended in order to reduce bias related to self-reported diet [36]. However, this is not unproblematic in case of weight gain as outcome because of over-adjustment for a mediator. Indeed, as shown in our sensitivity analysis, the magnitude of associations between dietary AGEs intake and weight change more than tripled in models without energy adjustment (Supplemental Table 1).

The composition of nutrients, temperature, moisture, and duration of heat exposure are the main parameters that determine the rate of dietary AGEs formation in foods [12]. The formation takes place spontaneously under certain conditions and food preparation methods, like dry-heat cooking at high temperature [37]. In addition, several factors such as composition of the food, presence of pro- or anti-oxidants, availability of water, as well as pH impact on the rate and diversity of AGE formation [38]. It is assumed that the absorption of dietary AGEs into the circulation in humans is about 10% of ingested AGEs, and the renal excretion of the absorbed amount in healthy participants is about 30% [39]. Scheijen et al. showed 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, demonstrating dietary AGEs are indeed absorbed in the human body [40].

The main food sources of AGEs with different proportions across the three AGEs were cereals or cereal products, meat and processed meat, cakes and biscuits, dairy, sugar and confectionary, as well as fish and shellfish. Previous studies in the same study population investigated associations between some of these food groups and weight change [41,26,31]. In Vergnaud et al., it was shown that a higher meat consumption was positively associated with weight gain in men and women (per 1 SD higher meat consumption associated with approximately 0.25 kg greater weight gain over 5 years) [26]. In our study, meat and meat products contributed to higher CEL intake. Therefore, our findings may in part explain the positive association found in Vergnaud et al. 2010 [26]. However, we cannot exclude that higher meat consumption was at least partially driving observed associations between CEL intake and weight gain, because adjustment for meat/processed meat consumption attenuated associations by half (Supplemental Table 2, Online Resource).

Several limitations of our study should be pointed out. First only self-reported weight at follow-up was available in most centers. To alleviate this potential source of bias, we applied a prediction equation to improve self-reported weight estimates [27]. Furthermore, in the EPIC-Norfolk study (UK Cambridge center of EPIC) a

high correlation between self-reported and measured weight data has been shown (r=0.97 in men and r=0.98 women) [42], likewise the Norway center of EPIC has been presented that self-reported weight and height provide a valid classification of BMI in their cohort of middle-aged Norwegian women, which means that ranking of participants according to self-reported weight was adequate [43]. Moreover, associations with weight gain were strongest in the two centers (Cambridge, UK and Doetinchem, NL) with measured weight at follow-up (data not shown). Second, we were not able to accurately measure changes in body composition (e.g. using dual-energy X ray absorptiometry, DXA); therefore, we had to make the reasonable assumption that encountered weight changes are largely due to changes in body fat mass and not in lean body mass or height [5]. Third, we were not able to elucidate for potential changes in diet during follow-up; yet dimensions of change in weight appear to be more pronounced and more robust if changes in diet can be accounted for [44]. In view of the inherent limitation of all epidemiology studies using self-reported dietary data, measurement error is another drawback. To minimise this bias, adjustments were made for total energy intake, sex, age, dietary patterns and other lifestyle factors notably physical activity and smoking, and for plausibility of dietary energy reporting; the latter has been recently shown in the EPIC-Potsdam sub-study to improve expected associations between intakes of energy-dense foods and BMI [24]. Another limitation might be that given the inevitable correlation between foods and AGEs, residual confounding from other components in the same foods cannot be ruled out. In order to address this potential issue, we in turn adjusted for the five main food groups rich in dietary AGEs and found that the observed associations between dietary AGEs and weight change were to a great extent independent of any single food source.

Strengths of our study include its prospective design with a reasonably long follow-up and the large sample size. To the best of our knowledge, it is the first prospective human study confirming evidence from in vivo animal models that higher dietary AGEs intake is associated with weight gain. We used a food-composition table based on state-of-the-art measured AGEs in commonly consumed foods [9]. Nevertheless, to further increase the knowledge about dietary AGEs and their impact on weight change and other health outcomes, it is important to continue the search for, and validation of biomarkers of dietary AGEs intake in the future.

We conclude that in this prospective study of adults from 10 European countries representing populations with heterogeneous diets, higher intakes of AGEs were associated with marginally greater weight gain over an average of 5-years of follow-up. Further studies are needed to confirm these findings.

Author contributions:

Heinz Freisling and Mazda Jenab developed the overall research plan; Viktoria Knaze and Reynalda Cordova performed the data matching; Casper G. Schalkwijk provided the AGEs database; Reynalda Cordova conducted the statistical analyses. Vivian Viallon contributed to the statistical analyses; Reynalda Cordova and Heinz Freisling wrote the manuscript; Heinz Freisling supervised the data analysis, reviewed/edited the manuscript, and had primary responsibility for final content; and all authors: contributed substantially to data collection, the interpretation of data and the drafting or critical revision of the manuscript for important intellectual content.

Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

Ethics approval

The present study was approved by the ethics committees of the IARC and the individual study centers.

Conflict of Interest Statement

None of the authors declared a conflict of interest.

Data access

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval. For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at: http://epic.iarc.fr/access/index.php.

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Table 1 Main characteristics of the study population according to the lowest (Q1) and highest (Q5) quintile of dietary advanced glycation endproducts (AGEs) intake

	CEL Q1	CEL Q5	CML Q1	CML Q5	MG-H1 Q1	MG-H1 Q5
Dietary AGEs intake (mg/day)	1.2 ± 0.8	3.5 ± 0.7	2.2 ± 0.8	4.4 ± 07	7.3 ± 0.6	9.8 ± 0.7
Follow uptime (years)	5.7 ± 2.6	4.9 ± 2.2	5.4 ± 2.3	5.1 ± 2.3	5.4 ± 2.5	5.3 ± 2.1
Weight change (kg/5 years) [1]	1.8 ± 4.5	2.2 ± 5.0	2.0 ± 4.8	2.1 ± 4.9	1.9 ± 4.7	2.2 ± 4.7
Women (%)	70	73	68	75	70	75
Age (years)	53.2 ± 8.6	51.0 ± 9.9	53.1 ± 9.2	51.9 ± 9.8	53.4 ± 8.3	51.2 ± 10.3
BMI at inclusion (kg/m²)	24.7 ± 3.9	25.2 ± 4.1	25.2 ± 4.1	24.9 ± 3.9	25.0 ± 3.9	24.5 ± 3.8
BMI categories (%)						
< 25 kg/m2	58	54	54	56	55	61
25 < 30 kg/m2	33	33	34	33	34	30
> 30 kg/m2	9	12	12	11	11	9
University degree (%)	26	25	25	24	25	28
Missing	1.1	1.9	1.4	1.7	1.2	1.6
Physically inactive (%)	19	23	22	22	20	18
Missing	0.7	1.0	1.6	0.8	0.5	1.6
Smoking status at baseline (%)						
Never	47	56	44	57	46	55
Former	27	25	26	25	26	28
Current	25	17	28	15	26	15
Missing	1.6	2.2	2.5	1.8	1.6	2.2
Previous illness (%)[2]	7	8	7	8	7	8
Missing	7.2	8.8	6.8	10.4	6.6	10.7
Dietary intake						
Total energy intake (kcal/day)	2202 ± 506	2201 ± 413	2193 ± 510	2192 ± 401	2177 ± 487	2183 ± 408
Vegetables (g/day)	225 ± 160	273 ± 142	285 ± 196	206 ± 126	224 ± 162	225 ± 139

Fruits (g/day)	273 ± 212	246 ± 164	293 ± 222	231 ± 157	257 ± 209	242 ± 162
Legumes (g/day)	12 ± 21	24 ± 29	15 ± 23	19 ± 27	9 ± 17	19 ± 29
Meat/products (g/day)	91 ± 55	121 ± 64	98 ± 57	108 ± 61	113 ± 59	93 ± 59
Dairy (g/day)	347 ± 268	338 ± 210	288 ± 223	415 ± 245	342 ± 261	373 ± 225
Fish (g/day)	34 ± 29	44 ± 37	36 ± 34	41 ± 35	38 ± 32	42 ± 39
Egg/egg products (g/day)	19 ± 17	20 ± 18	20 ± 19	19 ± 17	21 ± 18	17 ± 16
Potatoes (g/day)	94 ± 82	96 ± 69	98 ± 80	92 ± 66	95 ± 78	103 ± 75
Cereals/cereal products (g/day)	226 ± 109	235 ± 109	195 ± 82	250 ± 120	184 ± 83	268 ± 109
Sugar/confectionary (g/day)	55 ± 74	36 ± 29	48 ± 71	42 ± 33	55 ± 73	39 ± 30
Cakes/biscuits (g/day)	29 ± 30	52 ± 46	21 ± 20	65 ± 55	28 ± 30	52 ± 45
Added fat (g/day)	30 ± 18	28 ± 18	34 ± 21	25 ± 16	30 ± 18	27 ± 18
Non-alcoholic beverages (g/day)	1187 ± 848	996 ± 693	1185 ± 822	1027 ± 726	1186 ± 813	1141 ± 711
Alcoholic beverages (g/day)	286 ± 412	129 ± 191	305 ± 428	103 ± 152	301 ± 419	115 ± 168
mrMED score units/day	9 ± 3	9 ± 3	9 ± 3	9 ± 3	8 ± 3	9 ± 3

P values for continuous variables (ANOVA) and chi-square tests for categoric variables were all <0.001.
Data are expressed as arithmetic mean ± standard deviation (SD) if not stated otherwise
First quintile corresponds to the lowest and quintile five to the highest intake of energy-adjusted AGEs
BMI body mass index (calculated as weight in kilograms divided by height in meters squared), mrMED modified relative Mediterranean diet score (range 0-18; higher score characterizing a Mediterranean diet)
[1] Calculated as weight at follow-up minus weight at baseline divided by the follow-up time in years and multiplied by 5 years
[2] Type 2 diabetes, cardiovascular disease, cancer
CEL: N^{ε}-(1-carboxyethyl)lysine, CML: N^{ε}-(carboxymethyl)lysine, MG-H1: N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine

Table 2 Difference in body weight gain (kg) over 5 years according to baseline dietary advanced glycation endproducts (AGEs) intake in 255,170 men and women

	CEL		CML		MG-H1	
Model 1						
Beta (95%CI) per 1 SD /day	0.089	(0.069, 0.110)	0.052	(0.032, 0.072)	0.021	(0.002, 0.041)
Quintiles of dietary AGEs intake						
Lowest	Reference		Reference		Reference	
Q2	0.091	(0.034, 0.149)	0.047	(-0.010, 0.105)	0.052	(-0.006, 0.110)
Q3	0.154	(0.095, 0.212)	0.078	(0.019, 0.136)	0.055	(-0.003, 0.114)
Q4	0.188	(0.128, 0.248)	0.101	(0.041, 0.161)	0.037	(-0.0230.096)
Q5	0.221	(0.158, 0.285)	0.116	(0.053, 0.178)	0.066	(0.005, 0.128)
P trend (linear)		<0.001		<0.001		0.098
Model 2						
Beta (95%CI) per (1 SD/day)	0.112	(0.088, 0.137)	0.068	(0.044, 0.092)	0.028	(0.006, 0.051)
Quintiles of dietary AGEs intake						
Lowest	Reference		Reference		Reference	
Q2	0.106	(0.047, 0.164)	0.060	(0.002, 0.119)	0.054	(0.004, 0.113)
Q3	0.176	(0.115, 0.236)	0.098	(0.037, 0.158)	0.063	(0.003, 0.124)
Q4	0.222	(0.157,0.286)	0.129	(0.064, 0.193)	0.046	(-0.017, 0.109)
Q5	0.267	(0.195, 0.340)	0.146	(0.075, 0.218)	0.082	(0.014, 0.150)
P trend (linear)		<0.001		<0.001		0.049
Model 3						
Beta (95%CI) per 1 SD/day	0.111	(0.087, 0.135)	0.065	(0.041, 0.089)	0.034	(0.012, 0.057)
Quintiles of dietary AGEs intake						
Lowest	Reference		Reference		Reference	
Q2	0.104	(0.046, 0.163)	0.059	(0.000, 0.117)	0.060	(0.002, 0.119)
Q3	0.173	(0.112, 0.234)	0.094	(0.034, 0.155)	0.072	(0.011, 0.132)
Q4	0.219	(0.154, 0.283)	0.123	(0.059, 0.187)	0.057	(-0.007, 0.120)
Q5	0.264	(0.192, 0.337)	0.138	(0.066, 0.210)	0.098	(0.029, 0.167)
P trend (linear)		<0.001		<0.001		0.016

Multilevel linear mixed models with random effect on the intercept and slope according to center.
Overall mean 5- year weight gain corresponded to 2.1 kg (SD 5.0) and positive beta values indicate more weight gain (kg) over the same period.
Model 1 was adjusted for age, sex and BMI at baseline; Model 2 was further adjusted for follow-up-time in years, total energy intake (kcal/day), educational level, levels of physical activity, smoking status at baseline, and plausibility of dietary energy reporting; Model 3 was further adjusted for modified relative Mediterranean diet score.
CEL (mg/day) mean intake ± standard deviation (SD) within the quintiles of log-transformed CEL: Q1= 1.4 (± 0.2), Q2=1.8(± 0.08), Q3=2.2(± 0.1), Q4=2.6 (± 0.2), Q5=3.6 (± 0.8)
CML (mg/day) mean intake (SD) within the quintiles of log-transformed CML: Q1=2.0 (± 0.3), Q2=2.5 (± 0.1), Q3=3.0(± 0.2), Q4=3.6(± 0.2), Q5=4.9 (± 1.0)
MG-H1 (mg/day) mean intake (SD) within the quintiles of log-transformed MG-H1: Q1=13.2(± 1.2), Q2=17.6 (± 1.0), Q3=21.0(± 1.1), Q4=25.4 (± 1.5), Q5=36.1 (± 8.7)
CEL:N^{ε}-(1-carboxyethyl)-lysine, CML:N^{ε}-(carboxymethyl)-lysine,MG-H1: N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine.

Table 3: Difference in body weight gain (kg) over 5 years according to baseline dietary advanced glycation endproducts (AGEs) intake in 255,170 men and women using principal component analysis of the three AGEs

	Model 1		Model 2		Model 3	
	beta per 1 SD/day (95% CI)		*beta per 1 SD/day (95% CI)*		*beta per 1 SD/day (95% CI))*	
PC1	**0.057**	(0.038, 0.077)	**0.064**	(0.044, 0.083)	**0.064**	(0.044, 0.083)
Quintiles of PC1						
Lowest	Reference		Reference		Reference	
Q2	0.075	(0.017, 0.132)	0.079	(0.022, 0.137)	0.080	(0.022, 0.138)
Q3	0.097	(0.038, 0.155)	0.104	(0.046, 0.163)	0.150	(0.046, 0.163)
Q4	0.076	(0.017, 0.135)	0.087	(0.028, 0.146)	0.087	(0.028, 0.147)
Q5	0.172	(0.110, 0.233)	0.187	(0.125, 0.248)	0.187	(0.126, 0.249)
P trend (linear)		< 0.001		< 0.001		< 0.001
PC2	**-0.011**	(-0.032, 0.011)	**-0.008**	(-0.030, 0.013)	**-0.009**	(-0.031, 0.013)
Quintiles of PC2						
Lowest	Reference		Reference		Reference	
Q2	-0.004	(-0.065, 0.065)	0.011	(-0.049, 0.072)	0.010	(-0.050, 0.071)
Q3	0.003	(-0.060, 0.066)	0.027	(-0.036, 0.090)	0.025	(-0.038, 0.088)
Q4	-0.029	(-0.093, 0.036)	-0.006	(-0.071, 0.058)	-0.009	(-0.074, 0.056)
Q5	-0.043	(-0.110, 0.024)	-0.029	(-0.096, 0.038)	-0.033	(-0.101, 0.035)
P trend (linear)		0.155		0.332		0.279
PC3	**0.112**	(0.092, 0132)	**0.103**	(0.082, 0.123)	**0.101**	(0.080, 0.123)
Quintiles of PC3						
Lowest	Reference		Reference		Reference	
Q2	0.125	(0.067, 0.183)	0.105	(0.047, 0.163)	0.103	(0.045, 0.161)
Q3	0.231	(0172, 0.290)	0.202	(0.143, 0.261)	0.199	(0.139, 0.258)
Q4	0.293	(0.233, 0.353)	0.261	(0.201, 0.321)	0.256	(0.194, 0.318)
Q5	0.285	(0.222, 0.347)	0.260	(0.197, 0.323)	0.253	(0.187, 0.318)
P trend (linear)		< 0.001		< 0.001		< 0.001

Principal component analyses with calculated residuals adjusted for energy intake for each dietary AGE. Non-linear transformation was applied, e.g. log transformation for CEL: N^{ε}-(1-carboxyethyl)-lysine, CML: N^{ε}-(carboxymethyl)-lysine, MG-H1: N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine in regression model. Model 1 was adjusted for age, sex and body mass index (BMI) at baseline; Model 2 was further adjusted for follow-up-time in years, total energy intake (kcal/day), educational level, levels of physical activity, smoking status at baseline, and plausibility of dietary energy reporting; Model 3 was further adjusted for modified relative Mediterranean diet score. Eigenvalues of the covariance matrix for the three principal components (PCs) are as follows: PC1: 2.38051, PC2: 0.385845 PC3: 0.233649.