Consumption of a Diet Low in Advanced Glycation End Products for 4 Weeks Improves Insulin Sensitivity in Overweight Women

Alicja Budek Mark,[1] Malene Wibe Poulsen,[1] Stine Andersen,[1] Jeanette Marker Andersen,[1,2] Monika Judyta Bak,[1,3] Christian Ritz,[1] Jens Juul Holst,[4] John Nielsen,[2] Barbora de Courten,[3,5,6] Lars Ove Dragsted,[1] and Susanne Gjedsted Bügel[1]

OBJECTIVE

High-heat cooking of food induces the formation of advanced glycation end products (AGEs), which are thought to impair glucose metabolism in type 2 diabetic patients. High intake of fructose might additionally affect endogenous formation of AGEs. This parallel intervention study investigated whether the addition of fructose or cooking methods influencing the AGE content of food affect insulin sensitivity in overweight individuals.

RESEARCH DESIGN AND METHODS

Seventy-four overweight women were randomized to follow either a high- or low-AGE diet for 4 weeks, together with consumption of either fructose or glucose drinks. Glucose and insulin concentrations—after fasting and 2 h after an oral glucose tolerance test—were measured before and after the intervention. Homeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity index were calculated. Dietary and urinary AGE concentrations were measured (liquid chromatography tandem mass spectrometry) to estimate AGE intake and excretion.

RESULTS

When adjusted for changes in anthropometric measures during the intervention, the low-AGE diet decreased urinary AGEs, fasting insulin concentrations, and HOMA-IR, compared with the high-AGE diet. Addition of fructose did not affect any outcomes.

CONCLUSIONS

Diets with high AGE content may increase the development of insulin resistance. AGEs can be reduced by modulation of cooking methods but is unaffected by moderate fructose intake.

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Consumption of highly processed foods has increased over the past 20 years (1), which has probably resulted in an increased exposure to advanced glycation end products (AGEs). AGEs are formed from the Maillard reaction, a process that is important for the formation of aroma, flavor, and the color of foods (2). Cooking temperature is a critical factor; high-heat cooking, such as baking, roasting, frying,

[1]Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark
[2]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[4]Novo Nordisk Foundation Center for Basic Metabolic Research, Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[5]Baker IDI Heart and Diabetes Institute, Melbourne, Australia
[6]Steno Diabetes Center, Copenhagen, Denmark

Corresponding author: Susanne Gjedsted Bügel, shb@life.ku.dk.

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A.B.M. and M.W.P. contributed equally to this work.

and grilling, is a potent promoter of advanced glycation (3). High levels of AGEs are therefore found in many common foods, such as bakery products, cooked meat, and roasted coffee (3,4). Extracts of dietary AGEs have been shown to induce oxidative stress and inflammation in vitro (5), and dietary AGEs are therefore believed to be inflammatory pro-oxidants in vivo (6). There is accumulating evidence from animal studies indicating that a diet high in AGEs, produced by heating foods in the diet, contributes to increased insulin levels, insulin resistance (IR), and type 2 diabetes (7,8). Furthermore, a recent study found that methylglyoxal derivatives induced IR, impaired insulin signaling, and induced proinflammatory phenotypes in successive generations of mice (9), which further implicate dietary AGEs in the etiology of type 2 diabetes. Associations between plasma concentrations of AGEs and markers of IR have been reported in humans (3,7,8,10). Beneficial effects of an AGE-restricted diet on glucose metabolism have been demonstrated in type 2 diabetes patients, but markers of IR did not improve in a control group of healthy individuals randomized to the same diet (11). In another study, homeostasis model assessment (HOMA) of IR was increased after a high-heat–treated diet, compared with a low-heat–treated diet (12), but the change in HOMA-IR was not adjusted for changes in anthropometric measurements or additional differences between the diets.

AGEs are formed endogenously, particularly in diabetes, as the result of increased carbonyl stress (13). A possible effect of fructose on endogenous AGE formation has not been investigated, although fructose is an important reactant for several of the AGE precursors (14). We speculated that a high intake of fructose may exacerbate endogenous AGE formation due to mitochondrial overload, because hepatic fructose uptake is not strictly regulated in the same way as glucose (15) and may thus potentially lead to an increased formation by the glycolytic pathway of reactive trioses, known to be AGE precursors.

We compared the effects on insulin sensitivity of cooking methods targeted at inducing or reducing AGE formation (high- and low-AGE diets, respectively) together with fructose or glucose supplements in a two-factorial design, 4-week parallel dietary intervention in overweight women. The primary end point was HOMA-IR. Secondary end points include the plasma variables glucose, insulin, glucagon-like peptide-1 (GLP-1), and lipid profile, insulin sensitivity index ($S_{i0,120}$), weight, BMI, waist circumference, waist-to-hip ratio, skin autofluorescence (AF), and urinary excretion of AGEs.

RESEARCH DESIGN AND METHODS

Volunteers

A total of 74 overweight women aged 20–50 years were recruited from Copenhagen, Denmark. The inclusion criteria were as follows: BMI between 25 and 40 kg/m^2 and waist circumference $>$88 cm. The exclusion criteria were as follows: weight changes $>$3 kg within the last 2 months, physical activity $>$8 h/week (self-reported), current smoking, use of dietary supplements or medications, known medical condition, vegetarianism, pregnancy or breast feeding, surgery to treat obesity, postmenstrual conditions, blood donation within the last 3 months, and hypersensitivity to para-aminobenzoic acid (PABA). All who responded to the advertisements were prescreened by telephone interview. Of these, 218 subjects were regarded as being eligible and were invited to attend an information meeting. Written informed consent was obtained from 117 volunteers. Of these, two volunteers were excluded because of a nonfasting capillary blood glucose concentration of $>$8.9 mmol/L and BMI $>$40 kg/m^2. Sixteen volunteers withdrew their consent before the beginning of the study for personal reasons. A total of 99 volunteers started the dietary intervention. One volunteer was excluded because of poor compliance, and 24 volunteers dropped out for personal reasons. Seventy-four women completed the study. A sample size of 88 was estimated to be sufficient to detect a 30% change in the difference in HOMA-IR from baseline (SD $<$ 0.6) in a parallel design with a power of 0.80 and a significance level of 0.05. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by The Danish Research Ethics Committee and registered at clinicaltrials.gov (NCT01617304). Not all the originally planned analyses were performed because of economic constraints.

Study Design

This was a two-factorial, parallel, dietary intervention study consisting of two open-labeled arms (comparing high-AGE and low-AGE diets) combined with two double-blinded arms (comparing fructose and glucose). The study was performed at the Department of Nutrition, Exercise and Sports, University of Copenhagen, between March and June 2012. The volunteers were randomized to follow either a high-AGE or a low-AGE diet with supplements of either fructose or glucose. Blood sampling, anthropometric measurements, oral glucose tolerance test (OGTT), and AF measurements were performed at baseline and after 4 weeks of intervention. Halfway through the intervention, telephone calls were made to increase compliance. The volunteers were asked to restrain from rigorous physical activity and alcohol consumption for 48 h before all measurements, and were required to fast (except for 500 mL water) from 8:00 P.M. on the day prior to each blood sampling day.

Dietary Intervention

The volunteers received oral and written instructions on how to comply with either high-AGE or low-AGE diets. Both diets resembled habitual Danish food intake and were similar in nutrient quality, but differed in cooking methods. The instructions included thorough guidance on cooking methods, a food choice list, and predefined recipes for mandatory meals. The high-AGE group was instructed to fry, bake, roast, or grill their foods; to consume toasted bread with a crust; and to choose foods with a high content of AGEs based on the food choice list. The low-AGE group was instructed to boil or steam their food, to consume bread without a crust, and to

choose foods with low content of AGEs based on the food choice list. The food choice list contained examples of foods commonly available in Denmark to be chosen as "preferred," "accepted," or "not allowed." The predefined recipes (Table 1) included 1, 4, and 7 weekly breakfast, lunch, and dinner recipes, respectively. Additionally, the volunteers were instructed to consume 4 weekly portions of muesli or oatmeal in the high-AGE and low-AGE groups, respectively. The volunteers were provided with all the meat and fish for the predefined dinner meals; most of the meat and fish for the predefined lunch meals; and all the necessary eggs, corn oil for cooking, and snacks (Table 1). The remaining foods were purchased and consumed freely, but in compliance with the food choice list. Snacks other than the ones provided were not allowed. The volunteers were instructed to keep their habitual meal portion sizes and not attempt changes in body weight.

Monosaccharides

Each volunteer was provided with 84 bottles containing 20 g fructose or 22 g glucose monohydrate powder (Glostrup Apotek, Glostrup, Denmark) in a double-blinded manner. The volunteers were

Table 1—Predefined meals and snacks for the high- and low-AGE group

	High-AGE			Low-AGE		
Meal	Recipe name	Servings*	Provided food product in total	Recipe name	Servings*	Provided food product in total
Breakfast	Fried eggs and bacon	1	15 Eggs (Danæg, Denmark)	Boiled eggs and bacon	1	15 Eggs (Danæg, Denmark)
	Muesli meal	4	4 × 500 g Crüsli (Quark)	Oatmeal	4	2 × 1 kg Oats (7 mornings, Denmark)
Lunch	Fried sausages	1	†	Boiled sausages	1	†
	Fried chicken breast	1	4 × 1 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)	Boiled chicken breast	1	4 × 1 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)
	Fried eggs	1	15 Eggs (Danæg, Denmark)	Egg salad	1	15 Eggs (Danæg, Denmark)
	Fish meatballs	1	9 Fish meatballs of ~60 g (Royal Greenland A/S, Denmark)	Shrimp salad	1	4 × 250 g Shrimp (Royal Greenland A/S, Denmark)
Dinner	Breaded cod fillet with baked potatoes and vegetables	1	4 × 2 Cod fillets of ~150 g (Royal Greenland A/S, Denmark)	Steamed cod fillet with vegetables and boiled potatoes	1	4 × 2 Cod fillets of ~150 g (Royal Greenland A/S, Denmark)
	Fried chicken breast with rice in curry sauce	1	4 × 2 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)	Pasta salad with boiled chicken	1	4 × 2 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)
	Pizza with fried chicken	1	4 × 2 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)	Pita bread with boiled chicken and fresh vegetables	1	4 × 2 Chicken breast without skin ~125 g (Lantmännen Danpo A/S, Denmark)
	Fried salmon with vegetables and rice	1	4 × 2 Salmon fillets ~100 g (Royal Greenland A/S, Denmark	Salmon soup with tomatoes and crust-free bread	1	4 × 2 Salmon fillets ~100 g (Royal Greenland A/S, Denmark
	Fried meatballs with baked potatoes and vegetables	1	4 × 250 g Minced pork meat 8–12% fat (Danish Crown A/S, Denmark)	Greek-style meatballs in tomato sauce with boiled rice	1	4 × 250 g Minced pork meat 8–12% fat (Danish Crown A/S, Denmark)
	Fried noodles with fried minced meat and vegetables	1	4 × 250 g Minced beef meat 8–12% fat (Danish Crown A/S, Denmark)	Pasta with tomato meat sauce	1	4 × 250 g Minced beef meat 8–12% fat (Danish Crown A/S, Denmark)
	Pork chops with bruschetta bread and baked tomatoes	1	4 × 2 Pieces of pork chops ~125 g (Danish Crown A/S, Denmark)	Pork with coconut milk, boiled vegetables and rice	1	4 × 2 Pieces pork chops ~125 g (Danish Crown A/S, Denmark)
Snack	Tortilla chips	125 g	4 × 125 g (Urtekram, Denmark)	Rice cakes	100 g	4 × 100 g (Urtekram, Denmark)
	Cashew nuts	75 g	4 × 75 g (SystemFrugt, Denmark)	Pistachio nuts	80 g	4 × 80 g (Trend, Denmark)
	Oat biscuits	‡	300 g (MyChoice, Denmark)	Muffins	‡	300 g (Dancake, Denmark)
	Oreo biscuits	‡	2 × 154 g (Kraft, Denmark)	Sponge cake	‡	2 × 160 g (Karen Volf, Denmark)

*Number of servings per week. †Had to be purchased by the volunteers. ‡Optional.

instructed to consume three sugar supplements daily in connection with main meals, after dissolution of the monosaccharide powder in water. Additional added sugar and sugar-containing foods were prohibited. However, sugar-containing snacks with either a high or low content of AGEs were provided in case of sugar-craving episodes (Table 1). Beverages with artificial sweeteners were allowed, except for cola beverages in the low-AGE group. Compliance with the sugar supplement intake was >85%, as calculated based on returned bottles.

Blood Sample Collection and Analyses

Fasting blood samples were drawn by venipuncture at baseline and after a 4-week intervention. Subsequently, a 75-g OGTT was performed after a 12-h fast, with blood samples drawn at 0 and 120 min. The fasting blood samples were collected in dry tubes for the measurement of insulin and lipid profiles, in EDTA tubes on ice for the measurement of total GLP-1, and fluoride citrate tubes for the measurement of glucose. All samples were centrifuged at 2,300*g* for 10 min at 4°C and stored at −80°C until analysis. Plasma glucose and lipids (total cholesterol, HDL, LDL, and triglycerides) levels were measured with an enzymatic colorimetric method (ABX Pentra Glucose HK CP; Horiba, Montpellier, France) and analyzed with an ABX Pentra 400 (Horiba). Serum insulin was measured by a chemiluminescent immunometric assay with an Immulite 1000 Immunoassay System (Siemens Healthcare Diagnostics, West Sacramento, CA). In seven volunteers, the insulin levels were below the detection limit of 14.4 pmol/L, and values of two-thirds of the detection limit were used for statistical calculations. Intra-assay and interassay coefficients of variation (CVs) for glucose, lipids, and insulin were <2.8%. Plasma total GLP-1 level was analyzed by radioimmunoassay using antibody 89390 (16). Intra-assay and interassay CVs were <6% and <15%, respectively.

Insulin Sensitivity

HOMA-IR was used as an index of changes in insulin sensitivity, and was calculated from fasting glucose and insulin levels according to the following equation: HOMA-IR = (glucose [mmol/L] · insulin [μU/mL])/22.5 (17).

$S_{i0,120}$ was calculated using fasting and 120 min glucose and insulin values obtained from the OGTT (18). The following formula was used: $S_{i0,120} = m/MPG/logMSI$, where m is a glucose uptake rate in peripheral tissues and is equal to m = (75,000 mg + [glucose {milligrams per deciliter} at 0 min − glucose {milligrams per deciliter} at 120 min] × 0.19 × body weight)/120 min; MPG is the mean plasma glucose concentration [millimoles per liter] at 0 and 120 min; and MSI is the mean serum insulin concentration [milliunits per liter] at 0 and 120 min.

Urine Collection and Analyses

Twenty-four-hour urine samples were collected prior to and at the end of the study, were kept cold in thermo bags throughout the collection period, and were stored at −80°C immediately after return of the samples until analysis. To measure the completeness of urine collection, each volunteer was administered 3 × 80 mg PABA (University Pharmacy, University of Copenhagen) to be ingested with the main meals during each urine collection. PABA was analyzed with a colorimetric method on a spectrophotometer (19). Intra-assay and interassay CVs were 3.7%. A recovery of ≥85% PABA in urine was considered an acceptable compliance with the urine collection (19).

Concentrations of AGEs were determined by liquid chromatography tandem mass spectrometry. The samples were preconcentrated by solid phase extraction (SPE) using an Oasis HLB LP 96-well plate (60 mg; Waters, Hedehusene, Denmark). The SPE cartridges were preconditioned with 1 mL methanol followed by two washes with the same volume of water. A total of 100 μL urine together with 10 μL internal standards (30 μg/mL) was loaded onto the SPE cartridge and eluted with 300 μL 20% methanol/water. The loading and the eluate were combined, and the solvent was evaporated. The samples were then redissolved in 200 μL 26 mmol/L ammonium formate. The samples were analyzed on a 2.1 mm × 15 cm hypercarb column (3 μm particle size; Thermo Fischer Scientific, Waltham, MA) using an ultra-performance liquid chromatography–triple quadrupole detector system (Waters, Milford, MA) operated in the multiple-reaction mode. The gradient used was 0–20% acetonitrile/26 mmol/L ammonium formate in 0–3 min and 20–60% in 3–10.2 min, and then immediately back to 100% 26 mmol/L ammonium formate to recalibrate the column for 3.8 min before the next injection. The flow rate was 0.1 mL/min, and the transitions used for quantification of N^{ε}-carboxymethyl lysine (CML) and methylglyoxal-derived hydroimidazolone (MG-H1) were 205 > 130 and 229 > 166, respectively. Acetonitrile and methanol OPTIMA Liquid Chromatography Mass Spectrometry Grade were purchased from Fisher Chemical (Leicestershire, U.K.). Water used for all solutions was purified by using a Millipore ultra-pure water system. Intra-assay and interassay CVs for CML were 2.1% and 5.9%, and for MG-H1 they were 3.0% and 5.2%, respectively.

Dietary AGEs

Duplicate dietary portions of the mandatory breakfast, lunch, dinner, and snacks were homogenized, freeze-dried, and stored in sealed plastic bags at −20°C until analyses. The content of CML was determined by liquid chromatography tandem mass spectrometry as described above. The samples were acid hydrolyzed prior to analysis (20).

Other Measurements

Weight, height, and waist and hip circumference were measured. The volunteers were instructed to keep weighed food records during the first and the last 3 days of the dietary intervention, and average daily energy and macronutrient intake was calculated for each subject using the Danish food composition database (DANKOST 2000; Dansk Catering Center, Herlev, Denmark). AF was measured on the forearm in triplicate at baseline and at the end of the dietary intervention using the AGE Reader SU (DiagnOptics Technologies BV, Groningen, the Netherlands), and the mean AF value

was used for the statistical analyses (Supplementary Table 1).

Statistical Analyses

All data were logarithmically transformed prior to analysis in order to achieve approximately normal distributions. However, untransformed data, summarized as the mean ± SEM, are presented in tables.

Primary and secondary outcomes were analyzed using a two-way ANOVA model, initially including the monosaccharide–AGE interaction as well as the main effects of AGE and monosaccharide. Additionally, these models included covariates to adjust for baseline levels, age, and changes in waist circumference and weight from baseline. The interaction and main effects were evaluated by means of *F* tests. Moreover, baseline values of anthropometric and metabolic variables were compared between the groups by two-sample *t* tests. Changes during the dietary intervention in anthropometric and metabolic variables within each group were tested by paired *t* tests. Model validation was based on graphical evaluation of residual plots and normal probability plots. Bivariate associations between urinary AGEs and metabolic variables were assessed by means of Pearson correlation coefficients. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC). Statistically significant differences were obtained if the *P* value was <0.05.

RESULTS

A total of 74 volunteers completed the intervention, and 73 volunteers were included in the final statistical analyses (in 1 volunteer, glucose and insulin values fell outside the 3-SD range, and careful evaluation of further biochemical end points supported the exclusion of this volunteer from the data analysis). This included 37 volunteers in the high-AGE group and 36 volunteers in the low-AGE group, combined with 35 and 38 volunteers receiving fructose and glucose, respectively.

There was no effect of the monosaccharide–AGE interaction ($P = 0.98$) or of the monosaccharide intervention ($P = 0.14$) on HOMA-IR or on any secondary outcomes. Thus, there were only effects of AGEs.

Anthropometric characteristics were not different between the high-AGE and low-AGE groups at baseline (Table 2). Weight, BMI, and waist circumference decreased in both groups during the dietary intervention ($P < 0.05$), but more in the low-AGE group than in the high-AGE group ($P < 0.02$) (Table 2). Mean energy intake and absolute intakes of protein and carbohydrates were similar between the groups throughout the dietary intervention (Table 3). However, fat intake and relative contributions of energy nutrients differed so that volunteers in the low-AGE group consumed ~15% more protein, ~10% more carbohydrates, and ~22% less fat, all expressed as a percentage of energy intake, compared with those in the high-AGE group (all $P < 0.05$).

The estimated mean daily CML intake was 10.7 mg in the low-AGE group and 24.6 mg in the high-AGE group. The mean daily urinary excretion levels of CML and MG-H1 were not different between groups at baseline, and decreased ($P < 0.05$) in the low-AGE group, but not in the high-AGE group, after the intervention (Fig. 1). The effect of the intervention on the urinary AGE excretion (Fig. 1) was stronger after adjustment for macronutrients ($P = 0.02$ for CML and $P = 0.01$ for MG-H1). Urinary excretion levels of CML and MG-H1 were positively correlated with each other at baseline and also after 4 weeks (both $r \sim 0.5$, $P < 0.0001$).

The observed levels of HOMA-IR and $S_{i0,120}$ were similar to those seen previously in normoglycemic volunteers (18,21). Baseline OGTT results revealed five subjects with impaired fasting glycemia. Glucose metabolism variables were not different between the high-AGE and low-AGE groups at baseline (Table 4). Consumption of the high-AGE diet increased fasting insulin levels and HOMA-IR ($P < 0.001$) and decreased $S_{i0,120}$ ($P = 0.04$), compared with the low-AGE diet (Table 4). The effect of the intervention on the changes in the HOMA-IR and fasting insulin levels also remained significant after adjustment for age, change in weight, and change in waist circumference (all $P = 0.001$). Adjusting for dietary intake of macronutrients did not influence the results. Fasting and 2-h glucose levels did not change significantly (Table 4). Plasma lipid, total GLP-1, and AF levels did not differ between groups (Supplementary Table 1).

Baseline urinary excretion of MG-H1 was positively correlated with the 4-week $S_{i0,120}$ and with the dietary intake of carbohydrates ($r = 0.3$, $P < 0.03$), and negatively correlated with dietary intake of protein ($r = -0.3$,

Table 2—Anthropometric characteristics of the study population at baseline and after 4 weeks of dietary intervention

Variables	Time	High-AGE *N* = 37	Low-AGE *N* = 36	*P**	*P*†
Age (years)	Baseline	41.4 ± 1.4	37.9 ± 1.4	—	—
	End	—	—		
Weight (kg)	Baseline	91.8 ± 2.0	92.3 ± 2.2	0.0006	0.0007
	End	90.9 ± 1.7‡	90.2 ± 2.2‡		
BMI (kg/m^2)	Baseline	32.3 ± 0.6	33.2 ± 0.8	0.001	0.001
	End	31.9 ± 0.6‡	32.5 ± 0.7‡		
Waist circumference (cm)	Baseline	106.9 ± 1.5	107.6 ± 1.8	0.02	0.03
	End	105.8 ± 1.3‡	104.8 ± 1.7‡		
Waist-to-hip ratio	Baseline	0.92 ± 0.01	0.93 ± 0.01	NS	NS
	End	0.92 ± 0.01	0.91 ± 0.01		

Data are shown as mean ± SEM (based on untransformed data). **F* test for the effect of diet on outcome change from baseline between groups (log-transformed data). †*F* test for the effect of diet on outcome change from baseline between groups adjusted for age (log-transformed data). ‡Significant paired *t* test for the change from baseline within the group (log-transformed data).

Table 3—Characteristics of the dietary intake of the study population over the 4 weeks of intervention estimated twice from 3-day weighed food records

Variables	High-AGE (*N* = 34)	Low-AGE (*N* = 36)	*P**
Energy (kcal)	1,827 ± 51	1,715 ± 55	NS
Protein (E%)	18.8 ± 0.4	21.6 ± 0.4	<0.0001
Protein (g)	85.4 ± 2.5	91.8 ± 2.9	NS
Carbohydrates (E%)	42.7 ± 0.9	46.9 ± 0.8	0.002
Carbohydrates (g)	195.5 ± 7.1	201.7 ± 7.7	NS
Fat (E%)	37.3 ± 0.8	30.6 ± 0.7	<0.0001
Fat (g)	75.8 ± 2.6	58.5 ± 2.3	<0.0001

Data are shown as mean ± SEM (based on untransformed data). E%, percentage of energy.
**t* test for the difference between the groups (log-transformed data).

P = 0.01). No other correlations were seen between urinary excretion of the analyzed AGEs and other anthropometric and metabolic variables.

CONCLUSIONS

The current study found that consumption of a low-AGE diet over 4 weeks improved IR in overweight women. The addition of fructose did not affect any outcomes. Previous studies have found that high-AGE diets increase IR in mouse models of type 2 diabetes (7) as well as in wild-type mice (8,9). A 4-month–long AGE-restricted diet improved markers of oxidative stress, inflammation, and HOMA-IR in patients with type 2 diabetes, but not in healthy control subjects (11). The study included only 18 healthy control subjects in a parallel design, and this part of the study was most likely underpowered (11). The present parallel study with 73 volunteers had a comparatively higher power to detect a possible difference in markers of IR. Our finding of improved IR is consistent with a previously published crossover study in healthy individuals (12). However, energy intake in that study was higher in the high-heat–treated diet than in the low-heat–treated diet, as were carbohydrate and fat intake. The effects of this on weight were not reported and do not seem to be accounted for in the statistical analyses. In contrast to that study (12), we obtained isocaloric diets in the high-AGE and low-AGE groups, although we could not avoid similar changes in macronutrient composition. In our study, the fat intake was lower (in both percentage of energy and grams), and the protein and carbohydrate intake was higher (only in percentage of energy), in the low-AGE diet. Both groups lost weight and reduced their waist circumferences during the intervention, but significantly more so in the low-AGE group. Nevertheless, the changes in fasting insulin levels and HOMA-IR remained different between the groups when the changes in weight and waist circumference were included in the analyses. We cannot, however, completely exclude the possibility that these changes were due to differences in the macronutrient composition rather than to the differences in AGE intake.

Previous studies have shown that fat-restricted isocaloric diets have beneficial effects on non-HDL cholesterol and inflammation markers, whereas insulin sensitivity, blood pressure, and body weight remain unaffected (22). In contrast, we observed weight loss in both the high-AGE and low-AGE groups, although the most was observed in the low-AGE group. This suggests some degree of undereating in both groups, despite encouragement to maintain habitual energy intake. The estimated low energy intake also indicates under-reporting of dietary intake. Under-reporting during dietary assessment is a well-known problem, particularly in obese individuals (23,24).

Adverse effects of AGEs on insulin sensitivity could be mediated by increased oxidative stress and inflammation (25). Indeed, decreases in markers of oxidative stress and inflammation have been found in several human intervention studies with an AGE-restricted diet (11,12,26–29). The observed effects on insulin sensitivity could also be due to the interaction of AGEs with the insulin-signaling cascade (30,31).

CML and MG-H1 are formed from lysine and arginine, respectively, through different pathways, which make the combined use of these two markers useful for estimates of overall AGE exposure. Unlike CML, MG-H1 is not acid-stable, and enzymatic hydrolysis prior to analysis is often used. Unfortunately, enzymatic hydrolysis of food samples is often inadequate, particularly that of high-heat–treated food with many complex structures (32,33). We attempted to measure MG-H1 levels in the food samples but

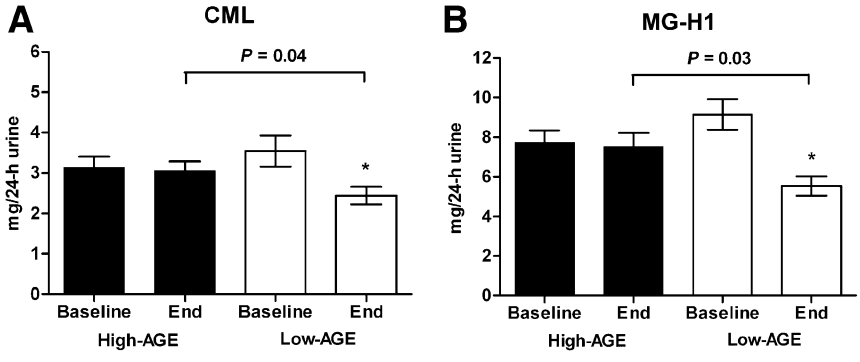

Figure 1—Urinary excretion (mean ± SEM) of CML (*A*) and MG-H1 (*B*) in milligrams per 24 h in high-AGE (black bars, *n* = 37) and low-AGE (white bars, *n* = 36) groups at baseline and after 4 weeks of dietary intervention. *P* values indicate differences between the groups (based on ANOVA models with additional adjustment for baseline). *Significant paired *t* test for the change from baseline within the group (log-transformed data).

Table 4—Characteristics of glucose metabolism variables of the study population at baseline and after 4 weeks of dietary intervention

Variables	Time	High-AGE (N = 37)	Low-AGE (N = 36)	P*	P†
Fasting glucose (mmol/L)	Baseline	5.5 ± 0.1	5.4 ± 0.1	NS	NS
	End	5.5 ± 0.1	5.5 ± 0.1		
2-h Glucose (mmol/L)	Baseline	6.3 ± 0.2	6.3 ± 0.3	0.09	NS
	End	6.2 ± 0.2	5.8 ± 0.2‡		
Fasting insulin (pmol/L)	Baseline	52.3 ± 6.0	65.7 ± 7.2	0.001	0.001
	End	58.0 ± 5.5‡	60.1 ± 6.9‡		
2-h Insulin (pmol/L)	Baseline	393.8 ± 60.3	435.8 ± 72.6	NS	NS
	End	382.4 ± 53.9	368.1 ± 47.6		
$S_{i0,120}$ (mg · L^2/mmol · mU · min)	Baseline	77.4 ± 3.6	75.7 ± 4.4	0.04	NS
	End	75.0 ± 3.0	81.1 ± 4.1‡		
HOMA-IR (μU · L/mmol · L)	Baseline	2.14 ± 0.3	2.65 ± 0.3	0.001	0.001
	End	2.40 ± 0.2‡	2.43 ± 0.3‡		

Data are shown as mean ± SEM (based on untransformed data). *F test for the effect of diet on outcome change from baseline between groups (log-transformed data). †F test for the effect of diet on outcome change from baseline between groups adjusted for age, difference in weight, and difference in waist circumference (log-transformed data). ‡Significant paired t test for the change from baseline within the group (log-transformed data).

were not able to obtain reliable results. However, the urinary excretion of MG-H1 clearly indicates a significant difference in MG-H1 content between the two diets. The CML content of the diets was two to four times higher than reported in other intervention studies, but with a comparable ratio between the low-AGE and high-AGE diet (12,34). Similarly, urinary excretion of CML was twice as high as previously reported (34). We cannot exclude that CML has formed in the food samples during storage before analysis, which could, in part, explain the high levels in both diets; however, because urine levels were similarly elevated, this was probably not the case. The urinary excretion of CML and MG-H1 indicates that the high-AGE diet corresponded well to the volunteers' habitual diet regarding the content of AGEs. This is consistent with the understanding of common Western diets as being high in AGEs (6). We did not measure AGEs in plasma, because that might reflect only the most recent exposure, but we examined urinary content for AGEs instead, because this reflects at least 24 h of accumulated AGE consumption as well as endogenously formed AGEs.

Despite the differences in urinary excretion of CML and MG-H1, skin AF values were not affected by the intervention (Supplementary Table 1). Skin AF values reflect accumulated fluorescent AGEs, as in diabetes (35), and the lack of measurable changes suggests that the 4-week duration of the study was insufficient to change AGE levels in skin. Furthermore, CML and MG-H1 are not fluorescent, and a change in their urinary excretion levels might not be associated with skin AF.

The main weakness of our study is the change in body weight and fat intake. However, the change in insulin sensitivity was significant even after adjustment for changes in weight and fat intake; but possible confounding from these factors cannot be ruled out, and the results should be interpreted with caution. In addition, we had no control over compliance with the actual meal plan as well as no control over energy and nutrient intake. The difference in fat intake may be due to differences in cooking methods, which should be taken into account in future studies. Furthermore, the open-label design increases the risk of biased results, but blinding was not practically possible with the high-AGE and low-AGE diets.

Both the dietary macronutrient composition and heat-induced changes other than the formation of AGEs in the high-AGE diet may have confounded the results, but these factors are difficult to avoid when comparing real-life cooking methods. Such changes might include exposures to heterocyclic aromatic amines, acrylamide, protein pyrolysates, and possibly other, as-yet unknown compound groups. Furthermore, a lower content of heat-sensitive nutrients (e.g., vitamin C and thiamine) in the high-AGE diet cannot be excluded.

In summary, a 4-week modulation of cooking methods with the purpose of reducing AGE content was sufficient to decrease markers of IR in overweight women. The addition of fructose did not affect the outcome. We conclude that low-temperature cooking methods with limited formation of AGEs may decrease the risk of developing IR, either by decreasing dietary fat intake or by restricting dietary AGE content.

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