See corresponding editorial on page 1381.

Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial[1–3]

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ABSTRACT

Background: The consumption of advanced glycation end products (AGEs) has increased because of modern food processing and has been linked to the development of type 2 diabetes in rodents.

Objective: We determined whether changing dietary AGE intake could modulate insulin sensitivity and secretion in healthy, overweight individuals.

Design: We performed a double-blind, randomized, crossover trial of diets in 20 participants [6 women and 14 men; mean ± SD body mass index (in kg/m^2): 29.8 ± 3.7]. Isoenergetic- and macronutrient-matched diets that were high or low in AGE content were alternately consumed for 2 wk and separated by a 4-wk washout period. At the beginning and end of each dietary period, a hyperinsulinemic-euglycemic clamp and an intravenous glucose tolerance test were performed. Dietary, plasma and urinary AGEs N^{ϵ}-(carboxymethyl) lysine (CML), N^{ϵ}-(carboxyethyl)lysin (CEL), and methylglyoxal-derived hydroimadazolidine (MG-H1) were measured with the use of mass spectrometry.

Results: Participants consumed less CML, CEL, and MG-H1 during the low-AGE dietary period than during the high-AGE period (all $P < 0.05$), which was confirmed by changes in urinary AGE excretion. There was an overall difference in insulin sensitivity of -2.1 mg · kg^{-1} · min^{-1} between diets ($P = 0.001$). Insulin sensitivity increased by 1.3 mg · kg^{-1} · min^{-1} after the low-AGE diet ($P = 0.004$), whereas it showed a tendency to decrease by 0.8 mg · kg^{-1} · min^{-1} after the high-AGE diet ($P = 0.086$). There was no difference in body weight or insulin secretion between diets (P = NS).

Conclusions: A diet that is low in AGEs may reduce the risk of type 2 diabetes by increasing insulin sensitivity. Hence, a restriction in dietary AGE content may be an effective strategy to decrease diabetes and cardiovascular disease risks in overweight individuals. This trial was registered at clinicaltrials.gov as NCT00422253.
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Keywords: glycotoxin, insulin resistance, insulin secretion, obesity, receptors for AGEs

INTRODUCTION

In both developed and developing countries, globalization and industrialized methods of food processing have dramatically altered diets (1), thereby increasing exposure to advanced glycation end products (AGEs),[11] which are used in foods to impart desirable properties such as a longer shelf life, sterility, flavor, and color (2). Advanced glycation is the nonenzymatic posttranslational modification of amino groups on proteins and peptides by reducing sugars and reactive carbonyls. The cooking temperature is one of the important factors with baking, and roasting, frying, and grilling produce more AGEs such as N^{ϵ}-(carboxymethyl)lysine (CML) and methylglyoxal-derived hydroimadazolidines (MG-H1s) in foods than are produced by boiling or steaming (3). Foods that are high in lipids such as meat also contain advanced lipoxidation end

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[2] No funder had any role in the study design, data collection, data analysis or interpretation, or writing of the manuscript.

[3] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[11] Abbreviations used: AGE, advanced glycation end product; CEL, N^{ϵ}-(carboxyethyl)lysin; CML, N^{ϵ}-(carboxymethyl)lysine; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; IVGTT, intravenous-glucose-tolerance test; MG-H1 methylglyoxal-derived hydroimadazolidine; OGTT, oral-glucose-tolerance test; sRAGE, soluble receptor for advanced glycation end product; UPLC-MS/MS, ultraperformance liquid chromatography–tandem mass spectrometry.

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products, notably N^{ϵ}-(carboxyethyl)lysine (CEL) (4). Therefore, high amounts of AGEs and intermediate Maillard products are present in many commonly consumed foods, particularly many highly processed foods but also in some healthier options such as heated milk, baked breads, biscuits and cookies, toasted breakfast cereals, grilled steak, brewed beer, and roasted coffee beans. Several animal and human studies have shown that increased dietary consumption of CML and AGE precursors, such as the reactive carbonyl methyglyoxal, results in an increased tissue accumulation of AGEs (5–8).

In rodents, the chronic consumption of high- or low-AGE diets has been shown to affect insulin sensitivity and signaling and the development of type 2 diabetes (9–12). Impairments in insulin secretion and islet function, after long-term exposure to AGEs (13, 14), can be prevented with AGE-lowering therapy (15).

There has been some modest evidence that a modification of dietary AGE intake has positive effects of glucose metabolism in humans. Three trials have reported changes in the HOMA-IR in response to low-AGE dietary consumption in patients with type 2 diabetes (16) and in healthy, obese individuals without diabetes (17, 18). The limitation of these studies was that they used the HOMA-IR, which is an indirect measure of insulin sensitivity and is unable to clearly differentiate the relative contribution of insulin sensitivity and secretion. In addition, all trials did not match the diets for energy (i.e., diets were not isocaloric), or authors did not specify that they achieved a similar macronutrient content across test diets, which has confounded the interpretation of these trials.

To our knowledge, there have been no well-controlled human trials that have investigated the impact of dietary AGEs on insulin sensitivity and secretion. Therefore, our aim was to determine the impact of 2 wk of consumption of a high- compared with a low-AGE–containing diet on insulin sensitivity as the primary outcome in overweight healthy individuals. The effects of diet on the secondary outcomes of insulin secretion, circulating and urinary AGE concentrations, and concentrations of circulating AGE receptors were also examined.

METHODS

Participants

The sample-size calculation was reported in the published protocol (19). Sixty-five overweight and obese individuals were assessed for eligibility, and 28 adults were enrolled and randomly assigned within the study. Six randomly assigned patients withdrew from the trial and 2 did not receive an allocated intervention (**Figure 1**), which left 20 participants who completed the protocol. Recruitment took place from January 2006 to December 2010.

Participants did not have diabetes as indicated by a 75-g oral-glucose-tolerance test (OGTT) (WHO 1999 criteria). All participants were nonsmokers at the time of the study and were healthy according to a detailed physical examination by a physician and routine blood analyses. No participant had clinical or laboratory signs of an acute or chronic infection or took any medication or illicit drugs at the time of the study.

The trial took place at the Baker IDI Heart and Diabetes Institute, Melbourne, Australia, and participants were recruited from the general community. The protocol was approved by the Alfred Hospital Ethics Committee, Melbourne, Australia, and complied with the Declaration of Helsinki. All participants provided written informed consent before participation. This trial was registered at clinicaltrials.gov as NCT00422253.

Study design

The study used a 2-period randomized, crossover, double-blind design. All participants underwent both diets; one diet had a low content of AGEs, and the other diet had a high content of AGEs (typical of a modern Western diet). Participants commenced the study after a 2-wk run-in period during which they consumed their habitual diets but with the limitation of alcohol, fast food, and coffee intakes. Test diets were consumed for 2 wk and were separated by a 4-wk washout period (habitual diet). Ten participants received the low-AGE diet first (group 1) followed by the high-AGE diet, and 10 participants received the high-AGE diet first followed by the low-AGE diet (group 2).

Random assignment and masking

The allocation sequence was generated by the dietitian with the use of a computer-generated random code and placed into sealed envelopes. Random assignment occurred for 7 blocks of 4 subjects that were locked in equal numbers of men and women and in the diet order. Participants were masked to the allocation of the diet type and to how the diet might affect glucose metabolism. Clinical and laboratory investigators who performed clamps, the intravenous-glucose-tolerance test (IVGTT), and the collection of were also masked to the diet allocation.

Study procedures

All participants underwent medical and laboratory screening including a 75-g OGTT (19). Before metabolic testing, participants were asked to abstain from strenuous exercise and caffeine intake for 3 d. All metabolic testing was performed after a 12-h overnight fast. The first metabolic testing day in women took place in their follicular phase. Before and after each diet, a hyperinsulinemic-euglycemic glucose clamp and an IVGTT were performed, and midstream spot urine and serum samples were taken.

Body composition was estimated with the use of a bioelectrical impedance analysis (Body Composition Analyzer, Model BC-418MA; Tanita). Physical activity was measured with the use of the international Physical Activity Questionnaire.

On 2 separate days as previously described (19), insulin sensitivity was assessed during the hyperinsulinemic-euglycemic clamp, and the acute insulin response was assessed during the IVGTT. Briefly, a primed continuous intravenous insulin infusion (9 mU/kg) was administered for 120 min at a constant rate of 40 mU $\cdot$ m^{-2} body-surface area $\cdot$ min^{-1}. The acute insulin secretory response during the IVGTT was calculated as the AUC from the plasma insulin increment between minutes 3 and 5 after a 25-g intravenous glucose bolus (19).

Dietary intervention

Before starting the run-in period to the first diet, participants kept a 3-d diet record of their habitual diets (2 weekdays and 1 weekend day) on the basis of household measures. Nutrient contents were analyzed with the use of the SERVE Nutrition

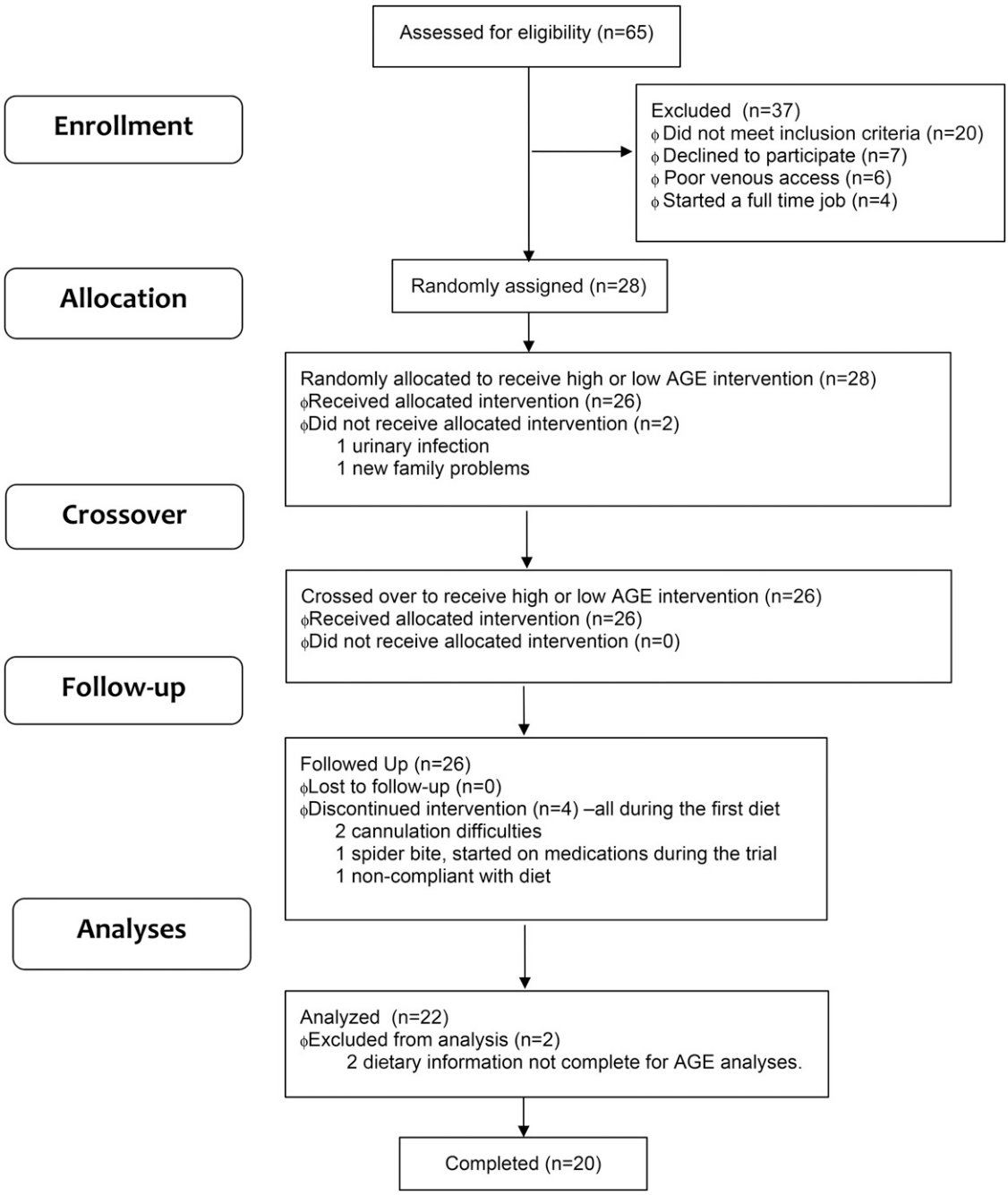

FIGURE 1 Consolidated Standards of Reporting Trials diagram of the study. AGE, advanced glycation end product.

Management System (version 5.0.012, 2004; Serve Nutrition Systems) on the basis of Australian food-composition tables. With the use of Australian food-composition data from SERVE as well as data from the United States on the CML contents of common foods, a menu of carefully matched alternative food choices (of low-AGE compared with high-AGE contents) was designed that was individualized to suit the preferences and habitual diet of each participant. The alternative food choices were matched for macronutrient contents and total energy but differed in calculated CML contents and were provided for each meal of the day including for snacks and beverages. Foods were also chemically analyzed for AGE contents. All foods for the 2 test diets were provided weekly to the participants as ready-to-eat items or as packed food portions to assist with dietary compliance. For food that required cooking, detailed instructions for storage and reheating (method, temperature, and duration) were provided. Participants were asked to eat to appetite throughout both dietary periods to maintain a constant body weight. Subjects were required to keep a detailed dietary record that indicated the cooking method and number of portions eaten for each food item supplied, for unconsumed foods, or for additional foods eaten. The dietitian made telephone contact 2 times/wk to provide support and resolve difficulties.

Measurement of dietary AGEs

To analyze the dietary AGE content, food items were obtained from local supermarkets and prepared according to the instructions provided to the participants in the study. AGE-free adducts were quantified with the use of ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) as previously described (19, 20). The measured AGE content, as distinct from the initial calculated CML content from previously published data tables (19), is shown in **Supplemental Table 1**).

Blood and urine analyses

Plasma glucose concentrations were measured with the use of the glucose oxidase method (ELM 105, Radiometer). Plasma insulin concentrations were measured with the use of a Chemiluminescent Microparticle Immunoassay performed on the Archicentre ci62000 instrument (Abbott), and plasma high-sensitivity C-reactive protein (hs-CRP) was measured with the use of an immunotubimetric assay on the Archicentre ci62000 instrument.

The concentrations of protein-bound AGEs (CML, CEL, and MG-H1) in serum and free AGEs in urine were quantified with the use of UPLC-MS/MS as previously described (21). Soluble receptor for advanced glycation end product (sRAGE) concentrations were analyzed in serum samples with the use of a human sRAGE sandwich ELISA according to the manufacturer's instructions (R&D Systems).

Statistical analysis

Statistical analyses were performed with the use of SAS JMP 12.1 Statistics Software (SAS). The values for plasma and urinary AGEs, insulin sensitivity, and secretion were logarithmically transformed before analysis to approximate normal distributions. All statistical tests were 2 sided with $\alpha = 0.05$, and paired t tests were performed to assess the changes at the beginning and end of each dietary period. A 2-factor ANOVA with Bonferroni correction was used for analyses of group 1 compared with group 2 to determine differences in the diet order and carryover effects after the washout period. Student's t tests were used to compare baseline variables between groups 1 and 2. A linear regression was performed to determine associations between covariates, and Pearson's coefficients were reported. The sample size was determined as previously described in the clinical trial protocol (19).

RESULTS

Anthropometric and metabolic characteristics of the study population are summarized in **Table 1**. All participants were healthy according to their physical examinations and had normal full blood counts (mean ± SD: 6.0 ± 1.2 × 10^9 cells/L), liver-function tests, plasma hs-CRP concentrations (2.1 ± 2.1 mg/L), and renal function [eGFR-Chronic Kidney Disease Epidemiology Collaboration equation for the estimated glomerular filtration rate (eGFR)] (Table 1) at study commencement. Participants in groups 1 and 2 were of a similar age, sex, body weight, insulin sensitivity, and secretion at study commencement (all $P > 0.2$). Baseline values for insulin sensitivity, secretion, serum and urinary AGEs, sRAGE, and related anthropometric variables were not different between groups 1 and 2 before each test diet (all P = NS).

At baseline, higher circulating CML concentrations were associated with greater insulin sensitivity, decreased insulin secretion during the IVGTT, and lower 2-h plasma glucose concentrations during the OGTT (Table 1). Baseline lower serum MG-H1 concentrations were related to increases in indexes of adiposity and decreased insulin sensitivity (Table 1). Serum CEL concentrations were not related to the baseline characteristics that were measured. There was an inverse relation between circulating AGE concentrations and their corresponding free-AGE adducts in the urine (Table 1).

Both diets were well tolerated, and no side effects of either interventions were reported. Total CML, CEL, and MG-H1 intakes (20) during the low-AGE dietary period were significantly lower than during the high-AGE dietary period as determined with the use of UPLC-MS/MS (all $P < 0.002$) (**Table 2**). The mean consumption of CML, CEL, and MG-H1 decreased by 27%, 38%, and 21%, respectively, while subjects consumed the low-AGE diet compared with when they consumed the high-AGE diet (Table 2, **Supplemental Table 2**). During the low-AGE and high-AGE dietary periods, participants consumed similar energy contents, and proportions of total energy (percentage of energy) from protein, fat, and carbohydrate were matched (all $P >$ NS) (Table 2). On the last day of each dietary period, the urinary excretion of both free-CML ($r = 0.43$; $P = 0.017$) and MG-H1 adducts ($r = 0.66$, $P = 0.017$) were associated with their cumulative dietary intakes. There was no relation between the cumulative dietary intake and urinary excretion of CEL at the end of the dietary periods ($P = 0.42$). No significant differences between the 2 dietary periods were seen for serum concentrations of any AGE or for serum sRAGE concentrations (**Table 3**). Both urinary MG-H1 (+562.1 nmol/mmol creatine) ($P = 0.003$) (Table 3) and CEL (−45.4 nmol/mmol creatine) ($P = 0.03$) (Table 3) differed between diets, whereas urinary CML excretion between diets was NS (+82.0 nmol/mmol creatine; $P = 0.12$ (Table 3). However, dietary CML intake was associated with the urinary excretion of MG-H1 ($r = 0.58$, $P = 0.0006$). In addition, there were no significant changes in the inflammatory marker hs-CRP, self-reported exercise, or nuclear translocation of the p65 subunit of nuclear transcription factor κB in peripheral blood mononuclear cells after either dietary period (data not shown).

As expected, baseline insulin sensitivity was related to a lower chronological age, 2-h glucose during the OGTT, insulin secretion, eGFR, and self-reported physical activity (Table 1). A comparison of insulin sensitivity after the low- compared with high-AGE dietary periods showed a net difference of 2.1 ± 1.9 mg · kg^{-1} · min^{-1} between diets ($P = 0.001$) (**Figure 2**A, B; Table 3). This differential effect of the diets on insulin sensitivity was apparent during both diet-intervention periods and in both groups 1 and 2 (Figure 2A, B; Table 3). The low-AGE diet significantly increased insulin sensitivity from baseline by 1.28 mg · kg^{-1} · min^{-1} (Figure 2A, B; Table 3). Insulin sensitivity tended to decrease (−0.8 mg · kg^{-1} · min^{-1}) after the high-AGE diet period (Figure 2A, B; Table 3). There was a modest but significant difference in the eGFR determined with the use of the Chronic Kidney Disease Epidemiology Collaboration equation for eGFR between high- and low-AGE dietary groups (Table 3). There was a trend for an effect of dietary AGE intake on insulin secretion ($P = 0.07$) (Figure 2C, D; Table 3). There was no overall difference in body weight between the 2

TABLE 1
Baseline characteristics of participants (n = 20)[1]

Metabolic variable (n = 20)	Mean ± SD (range)	Baseline associations, P, r			
		M	Serum CML	Serum MG-H1	Serum CEL
Age, y	34 ± 10 (21–50)	0.039, 0.47	NS	0.07, −0.40	NS
Weight, kg	94 ± 15 (74–121)	NS	NS	0.09, −0.35	NS
BMI, kg/m^2	31.3 ± 3.8 (26.7–41.0)	NS	NS	0.09, −0.35	NS
Body fat, %	31.1 ± 6.7 (20.1–44.7)	NS	NS	NS	NS
WHR	0.9 ± 0.1 (0.8–1.3)	NS	NS	0.047, −0.40	NS
Fasting plasma glucose, mmol/L	5.0 ± 0.5 (4.2–5.9)	NS	NS	NS	NS
2-h OGTT glucose, mmol/L	5.6 ± 1.5 (3.1–8.6)	0.06, −0.43	0.028, −0.49	NS	NS
Insulin sensitivity (M), mg · kg^{-1} · min^{-1}	7.0 ± 2.5 (2.5–10.8)	—	0.07, 0.41	0.02, −0.51	NS
Acute insulin secretion, mU/L	128.6 ± 68 (27.2–289.6)	0.014, −0.58	0.04, −0.49	NS	NS
eGFR (CKD-EPI), mL · min^{-1} · 1.73 m^{-2}	108.2 ± 18.2 (75–129)	0.0002, −0.74		0.017, 0.40	NS
Urinary Alb:Cr, mg/mmol	1.6 ± 2.7 (0.1–11.2)	NS		NS	NS
LDL cholesterol, mmol/L	3.1 ± 0.8 (0.6–3.9)	NS	NS	NS	NS
Mean SBP, mm Hg	123 ± 12 (98–150)	NS	NS	NS	NS
Physical activity, MET-h/d	2351 ± 2029 (362–6676)	0.06, 0.42	NS	NS	NS
Serum CML, μmol/mol lysine	77.6 ± 14.0 (48.1–102.0)	0.07, 0.41	—	NS	NS
Serum MG-H1, μmol/mol lysine	273.8 ± 60.6 (164.0–373.2)	0.02, −0.51	NS	—	NS
Serum CEL, μmol/mol lysine	10.0 ± 2.9 (6.0–16.1)	NS	NS	NS	—
Urinary CML, nmol/mmol creatine	657 ± 367 (197–1297)	NS	0.06, −0.46	NS	NS
Urinary MG-H1, nmol/mmol creatine	1534 ± 1482 (347–6322)	NS	NS	0.091, −0.46	NS
Urinary CEL, nmol/mmol creatine	5543 ± 4010 (543–13,523)	NS	NS	0.041, −0.44	0.008, −0.63
Serum sRAGE, pg/mL	807 ± 309 (557–1651)	NS	0.07, −0.41	NS	NS

[1]Associations between variables were assessed with the use of Pearson's correlation, and corresponding P values are shown. Nonparametric data were logarithmically transformed to approximate normal distributions. Alb:Cr, albumin-creatine ratio; CEL, N^{ϵ} (carboxyethyl)lysine; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation for the estimated glomerular filtration rate; CML, N^{ϵ}-(carboxymethyl)lysine; eGFR, estimated glomerular filtration rate; M, insulin sensitivity during use of the hyperinsulinemic-euglycemic clamp; MET-h, metabolic equivalent task-hours; MG-H1, methylglyoxal-derived hydroimadazolidine, OGTT, oral-glucose-tolerance test; SBP, systolic blood pressure; sRAGE, soluble receptor for advanced glycation end products; WHR, waist-hip ratio.

interventions (P = 0.14) (Table 3). No effect of the diet order was seen on any of the variables tested ($P > 0.1$).

After the dietary interventions, the changes in insulin sensitivity were associated with the cumulative dietary intake of CML, MG-H1, and CEL (**Table 4**). Insulin sensitivity postdiet was positively associated with serum CML concentrations (Table 4) and urinary MG-H1 concentrations (Table 4). Serum CML concentrations were also negatively associated with insulin secretion (Table 4).

DISCUSSION

In the current clinical trial, we showed that the consumption of a low-AGE diet for 2-wk resulted in lower urinary AGE concentrations and improved insulin sensitivity than did a diet high in AGEs in healthy, overweight individuals. There were no significant changes in anthropometric variables or insulin secretion between the diets.

Dietary intakes of the previously characterized AGEs, CML, CEL, and MG-H1 in our study were altered according to the

TABLE 2
Cumulative dietary AGE and energy intakes over 14 d[1]

	Low-AGE dietary period (n = 20)	High-AGE dietary period (n = 20)	P
CML intake, mg	43 (36–51)	59 (49–68)	<0.0001
MG-H1 intake, mg	253 (217–289)	344 (292–396)	0.001
CEL intake, mg	28 (22–33)	44 (36–52)	0.002
Energy intake, MJ	10.3 (6.0–14.3)	10.3 (6.7–15.2)	NS
Protein, E%	20.9 (15.6–25.9)	20.8 (16.5–24.6)	NS
Fat, E%	33.9 (21.9–43.2)	34.9 (28.4–42.0)	NS
Carbohydrate, E%	43.8 (30.3–61.3)	44.1 (35.5–55.2)	NS

[1]All values are means; ranges in parentheses. AGEs were assessed retrospectively with the use of ultraperformance liquid chromatography–tandem mass spectrometry analyses, and energy intakes of macronutrients were calculated according to the 14 d dietary records of each participant. The urinary excretion of both free CML (r = 0.43; P = 0.017) and MG-H1 (r = 0.66; P = 0.017) at day 14 of each dietary period were associated with their cumulative dietary intake. There was no relation between the cumulative dietary intake and urinary excretion of CEL at the end of the dietary periods (P = 0.42). Associations between variables were assessed with the use of Pearson's correlation, and corresponding P values are shown. Nonparametric data were logarithmically transformed to approximate normal distributions P values were determined with the use of paired t tests for dietary AGEs. AGE, advanced glycation end product; CEL, N^{ϵ} (carboxyethyl)lysine; CML, N^{ϵ}-(carboxymethyl)lysine; E%, percentage of energy; MG-H1, methylglyoxal-derived hydroimadazolidine.

TABLE 3
Effects of 2-wk dietary AGE interventions on glucose homeostasis and AGE concentrations in serum and urine[1]

Variable	Change from baseline, low-AGE diet	*P*	Change from baseline, high-AGE diet	*P*	Overall difference between low- compared with high-AGE diets	*P*
Body weight, kg	+0.69 ± 1.3	0.03	+0.16 ± 1.2	0.54	−0.53 ± 1.5	0.14
Insulin sensitivity (*M*), mg · kg^{-1} · min^{-1}	+1.3 ± 1.8	0.004	−0.8 ± 2.0	0.086	−2.1 ± 1.9	0.001
Insulin secretion, mU/L	−17.9 ± 66.9	0.27	+23.6 ± 77.7	0.17	+41.5 ± 96.8	0.07
Urinary CML, nmol/mmol creatine	−74.3 ± 407.5	0.44	−114.1 ± 720.6	0.56	−82.0 ± 900.8	0.12
Urinary MG-H1, nmol/mmol creatine	−766.3 ± 1709.4	0.08	−204.2 ± 1185.2	0.28	+562.1 ± 1996.4	0.003
Urinary CEL, nmol/mmol creatine	−92.1 ± 345.8	0.29	−88.8 ± 611.7	0.60	−45.4 ± 702.7	0.03
Serum CML, μmol/mol lysine	+1.0 ± 9.9	0.77	−2.8 ± 8.7	0.43	+4.4 ± 15.4	0.55
Serum MG-H1, μmol/mol lysine	+16.7 ± 52.6	0.17	−0.7 ± 49.2	0.70	+15.8 ± 75.8	0.29
Serum CEL, μmol/mol lysine	−1.0 ± 2.1	0.11	+0.5 ± 3.5	0.66	+1.5 ± 4.7	0.35
Serum sRAGE, pg/mL	+5.3 ± 168.1	0.89	−47.8 ± 159.0	0.20	+53.0 ± 286.5	0.42
eGFR (CKD-EPI), mL · min^{-1} · 1.73 m^{-2}	+1.3 ± 1.8	0.94	−0.8 ± 2.0	0.49	+2.1 ± 10.8	0.001

[1]All values are means ± SDs. Differences between variables at the beginning and end of each test diet are shown. The overall difference column shows the differences between variables at the end of the low-AGE dietary period compared with at the end of the high-AGE dietary period. Serum and urinary free and protein bound advanced glycation end products CML, CEL, and MG-H1 were measured with the use of ultraperformance liquid chromatography–tandem mass spectrometry. n = 20/dietary arm. P values were calculated with the use of 2-sided paired t tests to assess changes at the beginning and end of each dietary period with testing for the diet order. AGE, advanced glycation end product; CEL, N^{ϵ} (carboxyethyl)lysine; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation for the estimated glomerular filtration rate; CML, N^{ϵ}-(carboxymethyl)lysine; eGFR, estimated glomerular filtration rate; MG-H1, methylglyoxal-derived hydroimadazolidine, sRAGE, soluble receptor for advanced glycation end products.

dietary random assignment, and the changes were temporally related to the changes in insulin sensitivity in participants as previously reported (17, 22). We report that the changes in insulin sensitivity were likely due to changes in the dietary AGE content because diets were matched for both energy and macronutrient contents. However, we could not rule out that heat-related effects on micronutrient contents may have also contributed to the observed changes in insulin sensitivity. However, strengths of our

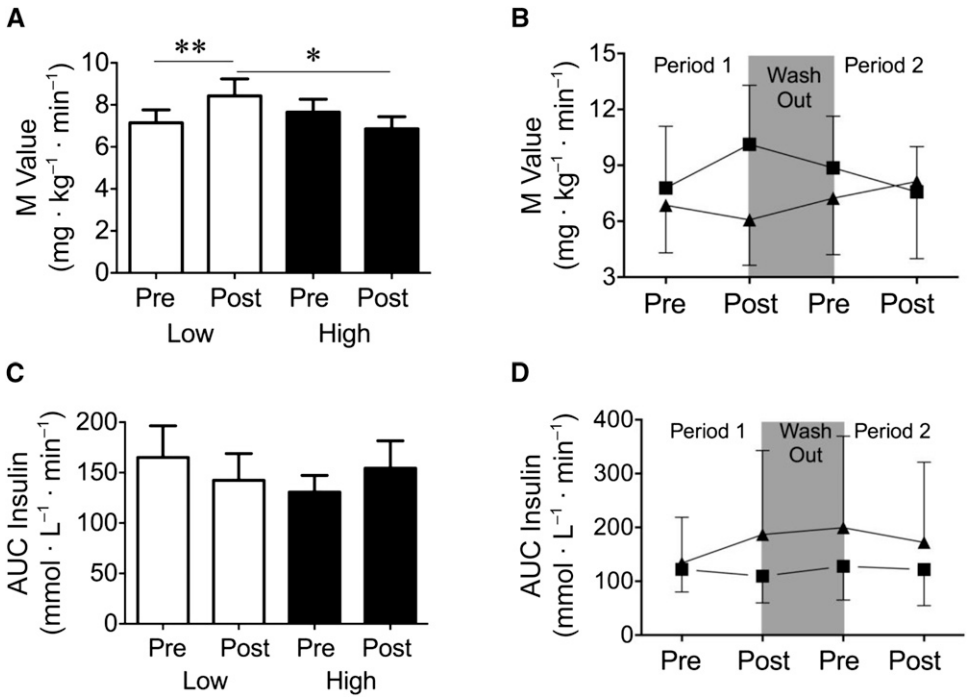

FIGURE 2 Mean ± SD values of insulin sensitivity and the AUC for insulin after interventions to modulate dietary AGEs. Insulin sensitivity assessed with the use of a hyperinsulinemic-euglycemic clamp separated by dietary intervention (A) and shown in the order as groups 1 and 2 as randomly assigned in the study (B). White bars represent the low-AGE diet (n = 20), whereas black bars represent the high-AGE diet (n = 20). (C and D) AUC for insulin separated by dietary intervention (C) and shown in the order completed as groups 1 and 2 as randomly assigned in the study (D). Group 1 (n = 10; squares) consumed the low-AGE diet (period 1) and then high-AGE diet (period 2). Group 2 (n = 10; inverted triangles) consumed the high-AGE diet (period 1) and then the low-AGE diet (period 2). The acute insulin secretory response was calculated as the AUC from the plasma insulin increment between minutes 3 and 5 after a 25-g intravenous glucose bolus (mmol · L^{-1} · min^{-1}). M values denote insulin sensitivity during the use of the hyperinsulinemic-euglycemic clamp. *After low-AGE–diet intervention compared with after a high-AGE–diet intervention, P = 0.001. Before the low-AGE–diet intervention compared with after the low-AGE–diet intervention, **P = 0.004. AGE, advanced glycation end product.

TABLE 4
Postdietary associations in insulin sensitivity (*M*) during use of a hyperinsulinemic-euglycemic clamp, insulin secretion during an IVGTT, and AGE measures[1]

	Cluster analysis	
Variable and covariates	β (95% CI)	*P*
ΔInsulin sensitivity (*M*)		
Total dietary CML intake	0.048 (0.006–0.09)	0.028
Total dietary MG-H1 intake	0.011 (0.003–0.018)	0.006
Total dietary CEL intake	0.049 (0.003–0.094)	0.037
Insulin sensitivity		
Serum CML	0.074 (0.026–0.12)	0.004
Insulin secretion	−0.11 (−0.016 to −0.005)	<0.001
eGFR (CKD-EPI)	−0.07 (−0.15–0.12)	0.09
Body weight	−0.08 (−0.16 to −0.001)	0.046
Urinary MG-H1:creatine	0.002 (0.0003–0.003)	0.022
Insulin secretion		
Serum CML	−4.9 (−9.40 to −0.32)	0.04

[1]Associations between the outcome variable and covariates were assessed with the use of linear regression that was adjusted for within-participant clustering ($n = 40$; 20 participants and 2 dietary periods). AGE, advanced glycation end product; CEL, N^{ϵ} (carboxyethyl)lysine; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration equation for the estimated glomerular filtration rate; CML, N^{ϵ}-(carboxymethyl)lysine; eGFR, estimated glomerular filtration rate; IVGTT, intravenous-glucose-tolerance test; MG-H1, methylglyoxal-derived hydroimadazolidine; sRAGE, soluble receptor for advanced glycation end products.

study are that many of the same foods were used in each diet, and our diets were matched for energy and macronutrient contents as provided by us, which has not been the case in most of the previous studies. One randomized crossover trial in healthy men reported an improvement of the HOMA-IR after consumption of a low-AGE diet. In this trial, energy intake as well as carbohydrate and fat intakes were all higher with the high-AGE diet than with the low-AGE diet (18). Another parallel trial showed change in the HOMA-IR in response to low-AGE–diet patients with type 2 diabetes but not in healthy individuals (16). Caloric and macronutrient contents of the diets used were not reported. In addition, some but not all patients with type 2 diabetes were taking insulin-sensitizing medications. We previously reported an improved HOMA-IR in healthy, obese women without diabetes (17). In this case, the dietary trial was matched for energy but not for the macronutrient content and resulted in the weight loss, which could have been responsible for the changes in insulin sensitivity (17). Moreover, unlike our study that was based on gold-standard techniques, previous trials have used the HOMA-IR, which is an indirect measure of insulin sensitivity and is unable to differentiate the relative contribution of insulin sensitivity and secretion.

Over the short intervention period of our study, there were no significant changes in serum concentrations of AGEs although serum protein-bound CML remained positively related to insulin sensitivity after each AGE dietary period. These results are in contrast with those of other parallel cohort studies in healthy overweight individuals, which have either not provided serum AGE data or have shown decreases in serum CML concentrations with a low-AGE dietary intervention (17, 22). These differences may have been due to the various methodologies used to measure serum AGEs. In the current study, we examined protein-bound rather than free-CML adducts in serum and used the gold-standard UPLC-MS/MS to assess AGE concentrations. In other studies, CML ELISAs have been used so that both free and protein-bound serum AGEs may have been measured.

In addition, serum and urinary AGE concentrations, particularly of free adducts, were likely to be dependent on the glomerular filtration, a variable that differed between the 2 dietary AGE interventions. In support of our findings that lower serum CML is associated with greater insulin resistance, obese humans with evidence of very early metabolic dysfunction and insulin resistance also have lower circulating serum protein-bound AGE concentrations such as CML (6, 23, 24). The significance of this decrease in protein-bound CML before overt hyperglycemia remains to be determined. The relation between AGEs and insulin resistance is also complicated by the fact that protein-bound CML concentrations are influenced by both hyperglycemia (even postprandially) and by the absorption of AGEs in the gastrointestinal tract (16). Circulating protein-bound AGEs are also endogenously formed during oral glucose challenges in humans, although the reason for this formation is unknown (25). Hence, in the future, both the formation of AGEs in the gastrointestinal tract and the absorption of AGEs from the diet warrant further study in humans particularly because of the implications for glucose homeostasis and glomerular filtration that can be inferred from our current study.

Unlike most other studies, we used a crossover design, which controlled for many confounders. In addition, this study had adequate power with a significant change in our primary outcome of insulin sensitivity that was achieved between dietary interventions. Gold-standard measures of insulin sensitivity and secretion were also performed in contrast with other studies (3, 16–18). In addition, our diets were matched for energy and macronutrient contents, which in previous studies has confounded the interpretation of dietary AGEs (17, 18, 22). Finally, although our study was initially based on the calculated CML content for test diets, we later accurately assessed the AGE contents of foods and biological samples with the use of UPLC-MS/MS, which is a superior, highly sensitive, and quantitatively robust method. Although the UPLC-MS/MS showed that a few foods were actually lower in AGE content (Supplemental Table 1) than previously reported (19), overall these analyses confirmed the differences in AGE contents between our diets. Nevertheless, this study had some limitations. The study was not statistically powered to assess mechanistic or secondary outcomes, and therefore we could only make inferences as to the influence of certain variables that differed between dietary endpoints such as insulin sensitivity. In addition, the participants were overweight or obese but otherwise healthy. Therefore, this study did not provide evidence as to the efficacy of a low-AGE dietary intervention in a population with type 2 diabetes but inferred efficacy in individuals with impaired glucose tolerance.

In conclusion, the globalization of the food chain has resulted in increased food processing and longer periods of food storage, which have facilitated AGE formation. Our study strengthens previous evidence that has suggested that the consumption of a low-AGE diet can improve insulin sensitivity but not insulin secretion in overweight individuals. Deleterious health effects of AGEs in our diet raise important issues in relation to the current widespread chemical, physical, and heat modifications of food to impart functional properties. The current findings need to be

extended to larger-scale and longer-term clinical studies to assess whether a diet that is low in AGEs can reduce risk of developing type 2 diabetes or be used to improve glucose homeostasis in individuals who already have type 2 diabetes. In addition, the physiologic effects of dietary compared with endogenously formed protein-bound AGEs also warrant further investigation. These findings could have important public health implications such as for programs that promote sustainable low-AGE consumption, for influencing food-preparation guidelines, and for the revision of regulations for food processing for both the general and at-risk population.

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