

Improved Insulin Sensitivity Is Associated With Restricted Intake of Dietary Glycoxidation Products in the *db/db* Mouse

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Advanced glycation end products (AGEs), known promoters of diabetic complications, form abundantly in heated foods and are ingested in bioreactive forms. To test whether dietary AGEs play a role in the progression of insulin resistance, C57/BL/KsJ *db/db* mice were randomly placed for 20 weeks on a diet with either a low AGE content (LAD) or a 3.4-fold higher content of AGE (high AGE diet [HAD]), including ^εN-carboxymethyllysine (CML) and methylglyoxal (MG). LAD-fed mice showed lower fasting plasma insulin levels throughout the study ($P = 0.01$). Body weight was reduced by ~13% compared with HAD-fed mice ($P = 0.04$) despite equal food intake. LAD-fed mice exhibited significantly improved responses to both glucose (at 40 min, $P = 0.003$) and insulin (at 60 min, $P = 0.007$) tolerance tests, which correlated with a twofold higher glucose uptake by adipose tissue ($P = 0.02$). Compared with the severe hypertrophy and morphological disorganization of islets from HAD-fed mice, LAD-fed mice presented a better-preserved structure of the islets. LAD-fed mice demonstrated significantly increased plasma HDL concentrations ($P < 0.0001$). Consistent with these observations, LAD-fed mice exhibited twofold lower serum CML and MG concentrations compared with HAD-fed mice ($P = 0.02$). These results demonstrate that reduced AGE intake leads to lower levels of circulating AGE and to improved insulin sensitivity in *db/db* mice. *Diabetes* 51:2082–2089, 2002

D diabetes is a major cause of morbidity and mortality in industrialized societies (1). Type 2 diabetes, the most prevalent form of diabetes, is characterized by resistance to the action of insulin in peripheral tissues and deficiency in insulin

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²-[³H]DG, [1,2-³H]deoxy-D-glucose; AGE, advanced glycation end product; CML, ^εN-carboxymethyllysine; ELISA, enzyme-linked immunosorbent assay; FPLC, fast-performance liquid chromatography; HAD, high AGE diet; IR, insulin resistance; ITT, insulin tolerance test; IVGTT, intravenous glucose tolerance test; LAD, low AGE diet; MG, methylglyoxal.

secretion by the pancreatic islet β -cell (2). Insulin resistance (IR) is associated with progressive increases in fasting serum insulin levels and visceral adiposity in humans and in animal models (3). Nutrients, such as certain types of fat and carbohydrates, play a major role in the etiology of IR (3,4). Epidemiologic studies in Japanese and Pima populations demonstrate that populations with the same genetic background develop increased incidence of IR and type 2 diabetes whenever a “Western” lifestyle and diet is adopted (1,5). Furthermore, recent evidence suggests that nutrients can directly modulate insulin signaling and thus contribute to cellular IR independently from associated factors, such as physical activity, dyslipidemia, hypertension, and diabetes (4,6,7).

Prolonged hyperglycemia leads to complications, such as vascular and renal disease (8). Under hyperglycemic conditions, endogenous nonenzymatic glycoxidation of proteins and lipids leads to the formation of heterogeneous products, collectively termed advanced glycation end products (AGEs) (9). Many of the tissue changes observed in diabetic complications are attributed to their chemical, pro-oxidant, and inflammatory actions (10).

In addition to those endogenously formed, AGEs are abundant in exogenous sources such as foods, especially when prepared under elevated temperatures (11,12). After ingestion, 10% of preformed AGEs are absorbed into the human or rodent circulation (13,14), of which two-thirds are retained in tissues. Among them are tissue-reactive α,β -dicarbonyl-containing intermediate products, such as methylglyoxal (MG), and terminal products, such as ^εN-carboxymethyllysine (CML) (15,16). MG, which has been linked to cellular oxidant stress and apoptosis (17), and CML, which is formed by glycoxidation as well as by lipoxidation (18), have both been identified in vivo and are linked to tissue toxicity (10,16,19). The enhanced chemical modification of proteins and lipids or “carbonyl stress” leads to oxidant stress and tissue damage (16), illustrating their role in the pathogenesis of diabetic complications. Other conditions, such as renal insufficiency (13,20), dyslipidemia (21), and aging (22,23) are also associated with increased serum AGE levels.

Among the multiple targets of bioactive AGEs are such diverse tissues as the vascular endothelium (9,10) and the pancreatic islet (24). Pharmacological inhibition of glycoxidation protects against damage to either tissue (10,25). Also, reduced intake of dietary AGEs has been recently shown to decrease the incidence of type 1 diabe-

TABLE 1
Characteristics of HAD and LAD

Nutrients	HAD	LAD	Requirements*
Protein (%)	20.0	18.1	18
Fat (%)	4.5	7.2	5
Carbohydrate (%)	54.8	58.6	65
Fiber (%)	6.0	5.1	
Thiamin hydrochloride (mg/kg)	18	5.0	5
Riboflavin (mg/kg)	8	6	7
Niacin (mg/kg)	93	30	15
Pantothenic acid (mg/kg)	17	15	16
Choline chloride (g/kg)	1.8	1.02	2
Folic acid (mg/kg)	2.9	2.0	0.5
Pyridoxine (mg/kg)	7.4	6.0	8
Biotin (mg/kg)	0.1	0.2	0.2
B ₁₂ (μg/kg)	18	25	10
Vitamin A (IU/kg)	2,500	4,000	2,400
Vitamin D ₃ (IU/kg)	2,200	1,000	1,000
Vitamin E (IU/kg)	99	75	32
Caloric profile (kcal/g)	3.4	3.7	

*"Nutrient Requirements of Laboratory Animals," The National Academy of Sciences, Washington, DC, National Academy Press, 1995.

tes in NOD mice (26) as well as the formation of atherosclerotic lesions in diabetic apolipoprotein E-deficient mice (27). Based on these findings and taking into account the interrelationships among IR, type 2 diabetes, and excessive food intake in humans or animals, we investigated whether dietary AGEs influence the progression of IR in C57/BL/KsJ *db/db* mice, a well-established animal model for obesity and type 2 diabetes (28).

RESEARCH DESIGN AND METHODS

Animals and treatment. Female 4-week-old *db/db* (+/+) C57/BL/KsJ ($n = 20$) and control mice ($n = 16$) were purchased from The Jackson Laboratories (Bar Harbor, MN). Mice were given pelleted food and water ad libitum in a specific pathogen-free environment at the Center for Laboratory Animal Science, Mount Sinai School of Medicine. Animal care and experimental procedures conformed with the "Guide for the Care and Use of Laboratory Animals" (Department of Health, Education, and Welfare, publication no. NIH 78-23, 1996). After 1 week of adjustment, *db/db* and control mice were randomly divided into two groups and placed for 20 weeks on two commercially available standard rodent diets that were similar in nutritional and caloric content (Table 1) but had different AGE content based on enzyme-linked immunosorbent assay (ELISA) (see below). AIN-93G (Bio-Serv, Frenchtown, NJ) was used for the low-AGE diet (LAD), and PicoLab Rodent Diet 20 (Labdiet; Purina Mills, St. Louis, MO), with a 3.4-fold higher AGE content, was used for the high-AGE diet (HAD) (Table 2). Fasting blood glucose, insulin levels, and body weights were monitored biweekly. After 1 week of adjust-

TABLE 2
AGE content in diets (LAD and HAD) and in mouse sera of *db/db* and controls on LAD and HAD

Diet AGE	n	Serum AGE			
		Control mice (U/ml)		<i>db/db</i> mice (U/ml)	
		Baseline	14 weeks	Baseline	14 weeks
LAD	209.7 U/mg	52 ± 4	83 ± 6*	53 ± 4	93 ± 14†
HAD	718.5 U/mg	54 ± 3	116 ± 8	57 ± 5	194 ± 43

Data were obtained by ELISA using 4G9 monoclonal antibody (29,30) and are means ± SE. * $P < 0.05$ (* P calculated for LAD-fed versus HAD-fed control mice; † P calculated for LAD-fed versus HAD-fed *db/db* mice).

ment, food intake (in grams of food) of individual mice was recorded daily for 1 week. Intravenous assays were performed in all mice in groups of five. Because statistical significant difference was reached with five mice per group, we report our results with this number of animals.

AGE assays. AGE concentrations in diets (LAD and HAD) and in mouse serum were determined by ELISA using a monoclonal antibody raised against AGE-KLH (4G9; Alton, Ramsey, NJ), as previously described (29–31). The antibody is highly reactive with BSA modified by CML (32), an established biomarker for AGEs (10,18,22,33). We also assessed MG derivatives in the diets as well as in pooled mouse serum by ELISA using a monoclonal anti-MG-BSA antibody (MG3D11) raised against MG-modified BSA (21.8 MG-modified Arg/mol BSA, by HPLC [high-performance liquid chromatography]; obtained from Dr. Y. Al-Abed, The Picower Institute, Manhasset, NY) (34). MG3D11 was found to be strongly immunoreactive against MG-ovalbumin and AGE-BSA, but not with CML-BSA or unmodified BSA.

Metabolite assays. Blood glucose was determined with an Elite Glucometer (Bayer, Mishawaka, IN). Serum insulin levels were measured with the Ultra-Sensitive Rat Insulin ELISA Kit (Alpco Diagnostics, Windham, NH) using rat standards (100% cross-reactivity with mouse insulin). Intravenous glucose tolerance test (IVGTT) was performed by tail vein injection of glucose (1 g/kg i.v.) after an overnight fast (13–15 h). Blood samples were taken before and at intervals between 2 and 60 min after glucose infusion. Insulin tolerance test (ITT) was performed by intraperitoneal injection of human regular insulin (0.75 units/kg) (Novolin R; Novo Nordisk, Clayton, NC) after a 6-h fast. Blood samples were collected before and 15, 30, 60, 90, and 120 min after insulin administration.

Serum leptin was measured using the mouse leptin ELISA kit (Crystal Chemical, Chicago). Serum free fatty acids were measured using the NEFA (nonesterified fatty acid) C kit (Wako Chemicals, Neuss, Germany) with oleic acid as standard. Serum triglycerides and glycerol were measured with the GPO-Trinder colorimetric assay kit (Sigma Diagnostics, St. Louis, MO). Total plasma cholesterol and HDL cholesterol were determined using the infinity cholesterol reagent colorimetric assay kit (Sigma Diagnostics). For plasma HDL cholesterol measurement, plasma samples (60 μl) were fractionated by two sequential steps of ultracentrifugation using a Beckman TLA-100 rotor at 70,000 rpm for 3 h at 4°C followed by centrifugation with KBr (1.12 density) at 70,000 rpm at 4°C overnight. After centrifugation, the lower portion of the samples was used for the determination of HDL cholesterol.

For monitoring insulin levels, 40-μl whole-blood samples were taken from the retroorbital sinus by a capillary pipette containing heparin. Serum was separated from blood cells by centrifugation (4,000 rpm for 15 min) and stored at –20°C until measurement. During the IVGTT and ITT, blood was collected from the tail vein. For the lipid profile, 200 μl whole blood was collected from the retroorbital sinus and 5 μl of 0.5 mol/l EDTA, pH 8, was immediately added. Plasma was separated by centrifugation (4,000 rpm for 15 min.) and stored at 4°C; measurements were completed within 2 days of blood withdrawal. In all the assays described above, samples were not pooled but processed and analyzed individually. However, for lipoprotein separation, it was necessary to pool samples (0.5 ml) from eight animals per group. The pooled plasma from each group was subjected to fast-performance liquid chromatography (FPLC) gel filtration on two Superose 6 columns. Ninety-five fractions were collected, and the total cholesterol concentration of each fraction was assessed enzymatically using the Sigma kit.

Measurement of [1,2-³H]deoxy-D-glucose uptake by adipose tissue and liver. After a 5-h fast, [1,2-³H]deoxy-D-glucose (2-[³H]DG) (NEN Life Science Products, Boston, MA) (0.5 μCi/g body wt) was administered as an intravenous bolus. Sixty minutes after 2-[³H]DG administration, the animals ($n = 5$) were killed. Abdominal adipose tissue and the livers were removed, weighed, and solubilized with NCS^{II} Tissue Solubilizer (Amersham, Arlington Heights, IL). Radioactivity was counted in the samples using a liquid scintillation counter (Model 1219; Perkin Elmer, Gaithersburg, MD).

Histological analysis. After the animals were killed, pancreata were removed and fixed overnight in 10% formalin. Fixed tissues were processed for paraffin embedding. Sections were cut at a thickness of 4 μm, mounted on glass slides, and stained with hematoxylin and eosin. For detection of insulin, 10 consecutive paraffin-embedded sections of the pancreata were stained with a guinea pig polyclonal anti-swine insulin antibody (Dako, Carpinteria, CA). The immunoreactivity was detected by the Multi-link-HRP Supersensitive System (BioGenex, San Ramon, CA).

Renal function analysis. Twenty-four-hour urine was collected from all mice, which were housed singularly in metabolic cages. Total urine glucose was measured by Elite Glucometer (Bayer, Mishawaka, IN), total urinary creatinine content was assessed with the Reflotron creatinine reagents in the Reflotron system (Roche, Indianapolis, IN), and urinary albumin was measured by an ELISA kit (Alpco Diagnostics, Windham, NH).

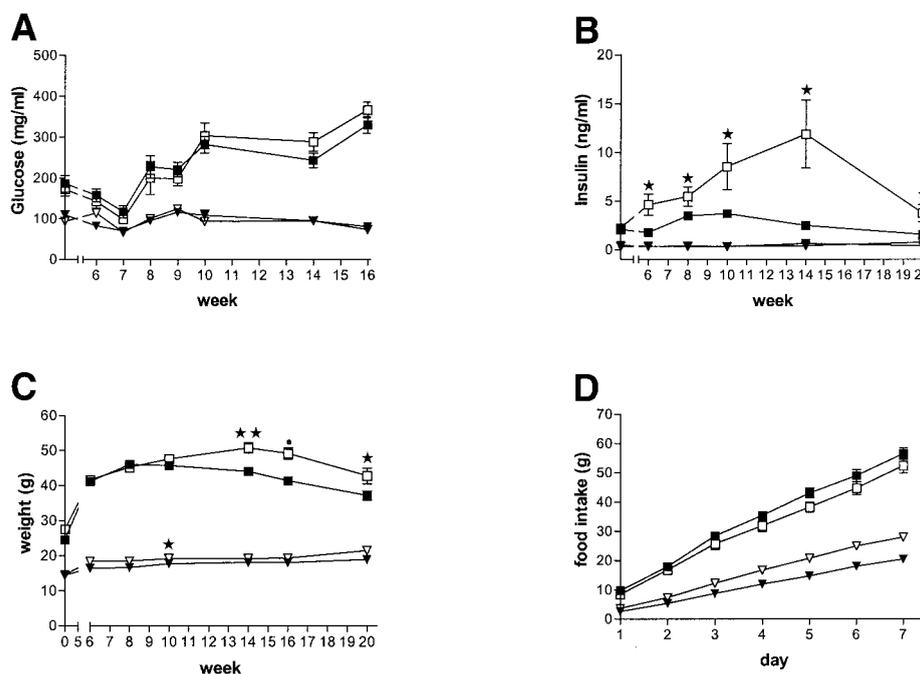


FIG. 1. Fasting glucose (A), serum insulin levels (B), body weights (C), and individual daily food intake (D) of *db/db* mice on LAD (■) and HAD (□) and control mice on LAD (▼) and HAD (▽). Data are expressed as means ± SE of values for 10 (*db/db*) and 8 (control) mice per group. **P* < 0.05; ***P* < 0.005; **P* < 0.0005.

Statistical analysis. Numerical data are expressed as means ± SE. An unpaired two-tailed Student's *t* test was used for statistical analysis. *P* values < 0.05 were considered significant.

RESULTS

Effect of dietary AGEs on mouse serum AGE levels.

Based on published daily requirements, both standard diets (LAD and HAD) were nutritionally equivalent and well balanced with supplemental micronutrients against possible antioxidant vitamin losses (Table 1). As shown in Table 2, they differed by 3.4-fold in AGE content, assessed as CML-like immunopeptides. Because the daily food intake was equal between HAD- and LAD-fed *db/db* mice (Fig. 1D), HAD-fed *db/db* mice ingested ~3.4-fold more CML than the LAD-fed *db/db* mice. This was reflected in part in the fasting serum CML levels, which were twofold lower in LAD-fed animals than in HAD-fed animals (*P* = 0.02) (Table 2). A similar pattern between dietary and pooled mouse serum MG levels was obtained using a MG-specific ELISA (LAD 0.7 μg/mg vs. HAD 2.3 μg/mg; LAD-fed *db/db* mice 22.2 nmol/ml vs. HAD-fed *db/db* mice 34.4 nmol/ml; LAD-fed control mice 9.3 nmol/ml vs. HAD-fed control mice 27.6 nmol/ml; *n* = 8–10/group). Thus, steady-state serum levels of two distinct AGEs, CML and MG, were consistent with the estimated content of the respective AGEs in the dietary formulas (Table 2).

Effect of dietary AGEs on fasting blood glucose and serum insulin levels.

As expected, all *db/db* mice exhibited marked hyperinsulinemia compared with controls throughout the study. Within 6 weeks of treatment, LAD-fed *db/db* mice displayed significantly lower fasting serum insulin levels, which, by 14 weeks, remained one-quarter of those in HAD-fed *db/db* mice (*P* = 0.01) (Fig. 1B). In the control mice of either diet group, fasting glucose and fasting serum insulin levels remained similar regardless of

diet (Fig. 1A and B). After 14 weeks, fasting insulin levels in HAD-fed *db/db* mice began to decline, possibly due to the expected pancreatic islet atrophy in the *db/db* (+/+) C57/BL/KsJ strain (28).

Effect of dietary AGEs on body weight and food intake.

No differences in body weight and growth rate were noted up to week 8, when a plateau was reached in the LAD-fed *db/db* mice. Thereafter, LAD-fed *db/db* mice stopped gaining and began to lose weight, reaching significantly lower levels compared with the HAD-fed *db/db* mice (*P* = 0.001) (Fig. 1C) by week 14. In addition, at the end of the study, despite identical food intake, serum leptin levels were significantly lower in LAD-fed versus HAD-fed *db/db* mice (36 ± 2.5 vs. 48 ± 4 ng/ml; *P* = 0.02). Control mice from either diet group showed no significant differences in body weight (Fig. 1C) or in serum leptin concentrations (2.1 ± 0.4 vs. 2.7 ± 0.4 ng/ml). After week 15, body weight began to decline in both *db/db* diet groups, consistent with previous observations (28).

Effect of diet AGEs on IVGTT and ITT.

To determine possible changes in insulin sensitivity versus IR, we performed an ITT at week 11. As shown in Fig. 2A, LAD-fed *db/db* mice revealed a significant improvement in glycemic response compared with that of HAD-fed *db/db* mice (at 60 min, *P* = 0.007). Of interest, ITT data from the control LAD-fed mice were significantly different, with a better glucose disposal rate than that of the HAD-fed control mice (at 60 min, *P* = 0.0003) (Fig. 2B).

Based on the above findings, the effect of LAD on glucose tolerance was tested by IVGTT in all groups. At week 18 of the study, glucose tolerance was significantly improved in LAD-fed *db/db* mice compared with HAD-fed *db/db* mice (at 40 min, *P* = 0.004) (Fig. 2C). A similar tendency (but not statistically significant) in glucose tolerance was obtained in control mice (Fig. 2D).

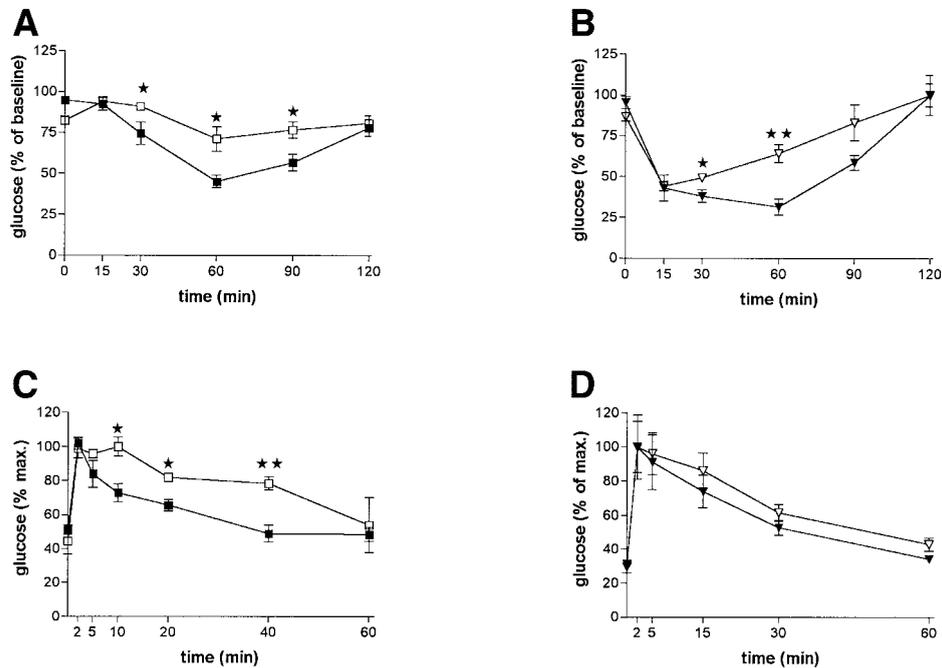


FIG. 2. Insulin and glucose tolerance tests and IR in *db/db* mice on LAD (■) and HAD (□) and control mice on LAD (▼) and HAD (▽). **A and B:** Intraperitoneal ITT (0.75 units/kg) of *db/db* (A) and control mice (B) was performed at week 11 of the study ($n = 8-10/\text{group}$). **C and D:** IVGTT (1 g/kg) of *db/db* (C) and control mice (D) was performed at week 18 of the study ($n = 5/\text{group}$). Data are plotted as percent of maximal blood glucose concentration after glucose injection. Values are expressed as means \pm SE. * $P < 0.05$; ** $P < 0.005$.

In vivo glucose uptake. To investigate insulin action on peripheral tissues, glucose uptake by white adipose tissue and liver was compared in the two diet groups at week 20. 2-[^3H]DG uptake in adipose tissue from LAD-fed *db/db* mice increased by twofold compared with HAD-fed *db/db* mice ($P = 0.02$) (Fig. 3A). Adipose tissue 2-[^3H]DG uptake in HAD-fed *db/db* mice was 6.2-fold lower than in age-matched control animals. However, LAD-fed *db/db* mice showed a threefold lower 2-[^3H]DG uptake than the respective control group. 2-[^3H]DG uptake by adipose tissue was similar in control animals on either diet. In comparison, liver tissue 2-[^3H]DG uptake in LAD- and HAD-fed *db/db* mice showed no discernible difference (Fig. 3B). Of note, age-matched HAD-fed control mice exhibited a significantly lower liver tissue 2-[^3H]DG uptake than LAD-fed control mice ($P = 0.05$) (Fig. 3B).

Plasma lipids and lipoproteins. Plasma lipids were measured after an overnight fast (13–15 h) at week 17. Total cholesterol levels in *db/db* mice from both dietary groups were significantly higher than in control animals (Table 3). In LAD-fed *db/db* mice, they were significantly higher than in HAD-fed *db/db* mice ($P < 0.0001$); a minor

but significant difference related to dietary AGE intake was seen in the control groups ($P = 0.02$) (Table 3). Although *db/db* mice exhibited higher plasma triglyceride and FFA levels than the control groups (by 1.5-fold, $P < 0.001$), no difference was noted between the LAD and HAD groups (Table 3).

Lipoprotein fractions in pooled plasma from each group of mice were resolved by FPLC (35). Mouse plasma appeared to contain the majority of cholesterol in the form of HDL (Fig. 4, fraction 58–70). As expected, the HDL peak was higher in *db/db* mice than in the corresponding control mice, regardless of diet (36). Most notable, however, was a marked rise in the HDL peak of LAD-fed mice (Fig. 4). In addition, in these animals, we detected significant peaks in the intermediate-density lipoprotein/LDL range (Fig. 4, fraction 18–21). The distribution of plasma cholesterol in the various lipoprotein fractions was also examined by sequential ultracentrifugation of HDL. By this method, consistent with the FPLC profile, HDL cholesterol levels were increased in all *db/db* mice compared with the respective control animals. However, in LAD-fed *db/db* mice, HDL cholesterol was twofold higher than in the HAD-fed

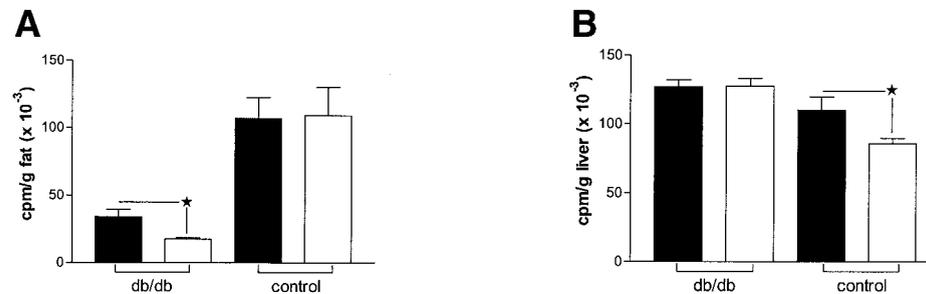


FIG. 3. 2-[^3H]DG uptake by adipose tissue (A) and liver (B) of *db/db* and control mice on LAD (■) and HAD (□) 60 min after intravenous administration at week 20 of the study. Data are expressed as means \pm SE of values of five mice per group. * $P < 0.05$.

TABLE 3
Plasma lipid profile of *db/db* and control mice on LAD and HAD after overnight fast

	LAD-fed control mice	HAD-fed control mice	LAD-fed <i>db/db</i> mice	HAD-fed <i>db/db</i> mice
<i>n</i>	8	8	10	10
FFAs (mmol/l)	159 ± 16	156 ± 15	241 ± 12*	241 ± 16*
Triglycerides (mg/dl)	56 ± 4	53 ± 6	137 ± 9*	144 ± 11*
Total cholesterol (mg/dl)	99 ± 2†	86 ± 4	173 ± 7*‡	110 ± 6*
HDL (mg/dl)	43 ± 4	38 ± 3	81 ± 7*‡	51 ± 7*

Data are means ± SE. Blood samples were collected at week 17 of the study. **P* < 0.001, control vs. *db/db* mice; †*P* < 0.05, LAD- vs. HAD-fed control mice; ‡*P* < 0.0001, LAD- vs. HAD-fed *db/db* mice.

db/db mice (*P* < 0.0001) (Table 3). Furthermore, control LAD-fed mice showed a tendency toward higher HDL cholesterol levels than control HAD-fed mice (Table 3).

Islet morphology. Histological examination and insulin immunostaining were performed in pancreatic sections from each group at the end of the study. Islets of HAD-fed *db/db* mice exhibited hyperplasia and hypertrophy combined with loss of islet structure and cellular homogeneity, changes seen only infrequently in islets from LAD-fed *db/db* mice. In addition, islet degeneration and insulin degranulation, including glucagon-producing cell displacement from the periphery to the center of the islets, were more evident in HAD-fed *db/db* mice than in LAD-fed *db/db* mice. Overall, LAD-fed *db/db* mice presented better preserved, compact islets with intensely positive staining for insulin (Fig. 5). Comparing 10 consecutive pancreatic sections per mouse from the LAD- (*n* = 5) and HAD-fed (*n* = 5) control mice, we could not observe a discernible difference in islet structure and insulin immunostaining.

Renal function. Total 24-h urinary glucose excretion was comparable in LAD- and HAD-fed *db/db* mice (1.6 ± 0.15 vs. 1.4 ± 0.2 mg/24 h; *n* = 8–10/group), consistent with

similar blood glucose levels, whereas the albumin/creatinine ratio revealed a clear difference (1.1 ± 0.1 vs. 2.1 ± 1.1; *n* = 8–10/group). These data, although not reaching statistical significance in the present study, were consistent with earlier observations (37).

DISCUSSION

The present study reveals a significant relationship between dietary AGE content, circulating AGEs, and insulin sensitivity in a murine model of type 2 diabetes and obesity. It furthermore illustrates the protective effects of a standard rodent diet with low levels of certain AGEs against the progression of IR and possibly against obesity in the same model.

The effects demonstrated in the present study were not attributable to quantitative differences in basic nutrients, vitamins, or energy profiles of the two diets (Table 1). Instead, the findings were consistent with the dietary content in protein/lipid glycoxidation derivatives, herein reflected in levels of CML and MG immunopeptides. In this study, serum levels of CML and MG correlated with the

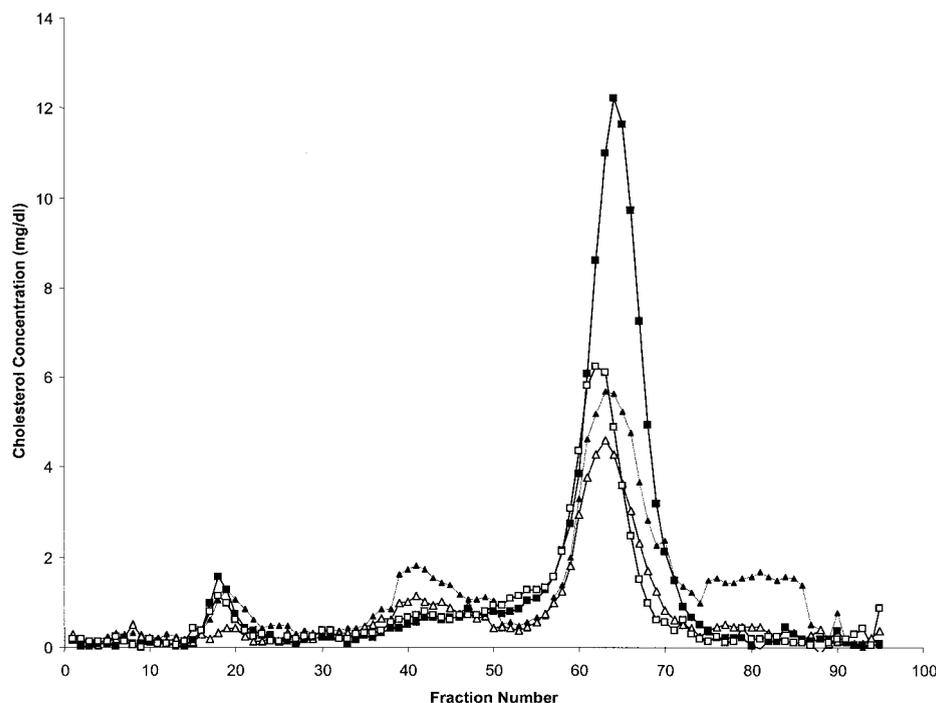


FIG. 4. FPLC fraction profile of total plasma cholesterol in *db/db* on LAD (■) and HAD (□) and control mice on LAD (▲) and HAD (△). Values are given by pooled plasma (0.5 ml) of eight mice per group. The peak at fraction 15–20 represents mainly intermediate-density lipoprotein/LDL, and the second peak (fraction 60–70) is mainly HDL.

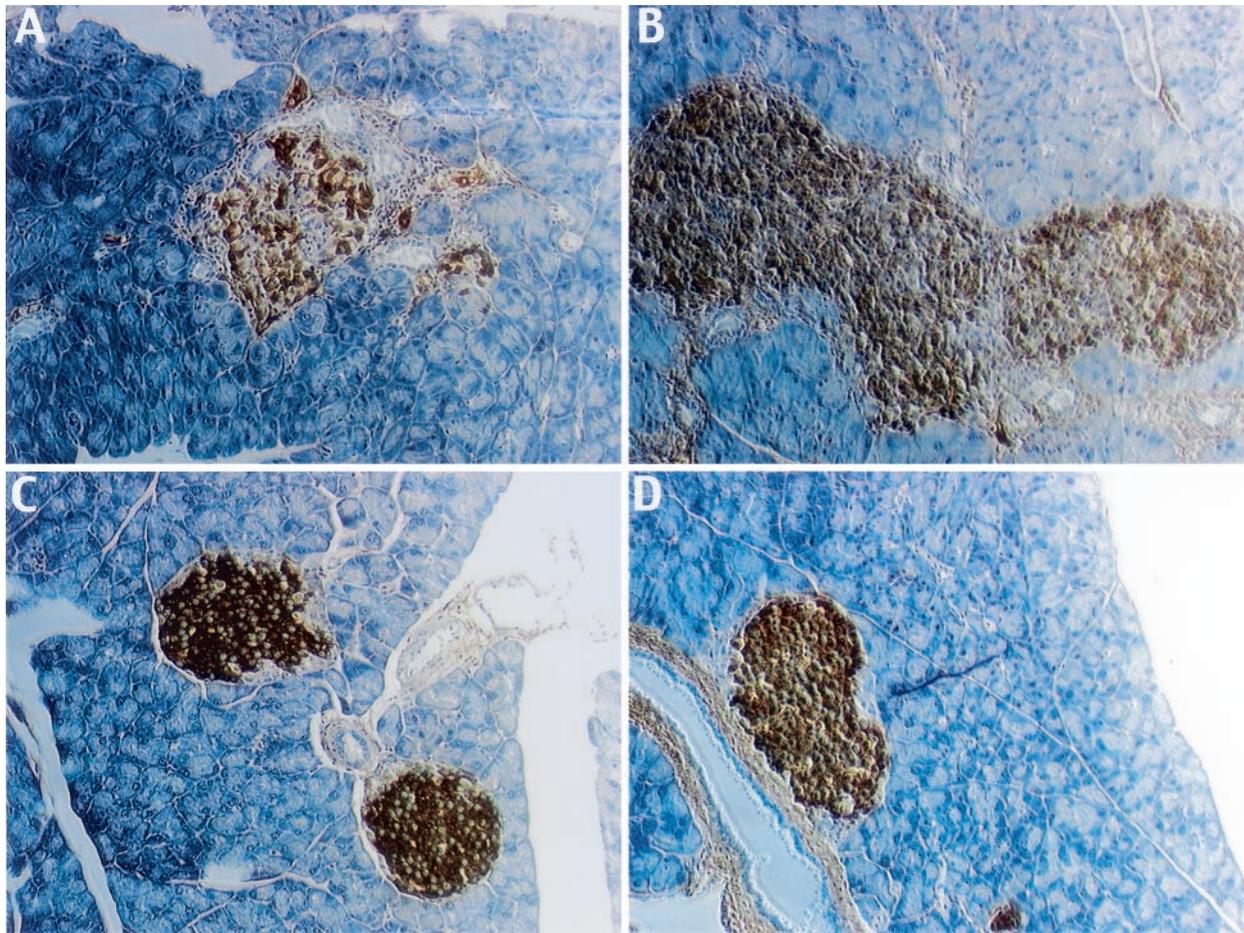


FIG. 5. Insulin immunostaining of pancreatic tissue sections in *db/db* and control mice on LAD and HAD after 20 weeks of study. *A:* *db/db* mice on LAD. *B:* *db/db* mice on HAD. *C:* Control mice on LAD. *D:* Control mice on HAD. Images shown are representative of 10 sections/mouse per group ($n = 5$).

respective LAD or HAD given to each experimental group, whether diabetic or nondiabetic.

CML and MG have been recognized as indicators of glyoxidant burden in vivo because both are well-characterized products of protein and lipid glycooxidation reactions (10,16,17), correlate with age (22,23) and with chronic hyperglycemia (9,10,16), and are thought to contribute to intracellular oxidative stress and tissue damage (16,18). Based on the vast heterogeneity of AGEs, however, the identity of the actual AGE related to the observed metabolic effects remains speculative because the pathogenic structure of the majority of these remains elusive. A similar positive relationship between ingested and circulating AGE levels has been observed previously, whether in connection to a defined oral test prepared with or without carbohydrate (14), to a single rodent diet (AIN93G) prepared under different temperatures (27,37), or to complex foods consumed by human subjects (13). The present study used two standard diets that were nutritionally equivalent despite being from different vendors—thus more closely resembling the naturally mixed human diets—and provides further support that food-based AGEs may be important contributors to the circulating AGEs.

More importantly, the present study reveals a novel finding in that certain diet-related glyoxidant substances influence the progression of IR in C57/BL/KsJ *db/db* mice,

in which obesity and type 2 diabetes are caused by a single gene mutation (38).

Despite equal food intake in the two groups, LAD-fed *db/db* mice, already obese and diabetic, ceased to gain weight within 10 weeks of study, and, by 20 weeks, they exhibited a significant weight loss. Throughout this period, fasting serum insulin levels, a well-established indicator for the progression of IR (39), were significantly lower in LAD-fed *db/db* mice than in HAD-fed *db/db* mice, without the characteristic rise and fall typically seen in the C57/BL/KsJ *db/db* strain (36) and in the HAD-fed *db/db* mice herein. A further indication of improved peripheral glucose disposal and insulin sensitivity in the LAD-fed *db/db* mice was provided by the nearly complete restoration of glycemic response to IVGTT and ITT.

The insulin kinetics seen in the LAD-fed mice were consistent with the well-preserved pancreatic islet morphology and function demonstrated in this group at the end of the study. This may have resulted from a reduced demand for insulin production, which is attributable to improved peripheral glucose utilization in this group. In contrast, islets of age-matched HAD-fed *db/db* mice displayed the expected hypertrophy coupled with a loss of insulin-producing cells and the normal islet architecture (40). These results, together with the metabolic findings, reinforced the protective effects related to the LAD diet.

Glucose uptake in abdominal adipose tissue revealed

that LAD improved IR by partially restoring the severely impaired insulin-stimulated glucose uptake by adipocytes in LAD-fed *db/db* mice. Due to the relatively high fat content in muscle samples of the obese *db/db* mice, accurate determination of 2-[³H]DG specific uptake by skeletal muscle cells was questionable. Thus, this study relied on glucose uptake by abdominal adipose tissue, an equally important contributor to IR (41). The doubling of glucose uptake by fat tissue in LAD-fed *db/db* mice, although far from that of controls, together with the significantly improved IVGTT and ITT, supported the hypothesis that dietary AGE intake exerts direct effects at sites of peripheral insulin action; a restriction of AGE intake clearly results in improved glucose uptake. In the context of equal food intake, the body weight loss in the LAD-fed *db/db* mice is also consistent with a shift in endogenous glucose metabolism, resulting in a more efficient glucose disposal rate.

By comparison, hepatic glucose output in the *db/db* mice, assessed as fasting glycemia, was not affected by the LAD diet. Increased hepatic glucose output is primarily responsible for fasting hyperglycemia in diabetes. A relative insulin deficiency, along with an increase in the counter-regulatory hormones glucagon and catecholamines, is involved in the pathogenesis of enhanced hepatic gluconeogenesis. IR and increased hepatic gluconeogenesis are already present in young *db/db* C57/BL/KsJ mice at a time when they are still normoglycemic (42,43). A possible explanation for the enhanced hepatic gluconeogenesis, not influenced by AGE intake in this study, is the increased secretion of glucagon and the glucagon/insulin ratio in *db/db* C57/BL/KsJ mice (44,45). Further supportive evidence for increased gluconeogenesis in type 2 diabetes animal models was provided by increased liver mRNA levels of the transcription factor PGC-1 and the gluconeogenic enzyme PEPCK (46).

As expected, all *db/db* mice developed the dyslipidemia previously described in this model (36), with elevated serum FFA, tryglyceride, total plasma cholesterol, and HDL cholesterol levels. As previously noted, the increase in HDL cholesterol in *db/db* mice is in contrast to the decreased levels frequently found in humans with obesity or diabetes but is consistent with another mouse model of obesity, the *ob/ob* mouse (47). In those mice, it was found that there was a decrease in hepatic apoAI mRNA that resulted in decreased HDL production, but there was an even greater decrease in HDL plasma clearance, which explained the elevated HDL cholesterol level. In the present study, there were significantly higher total serum cholesterol levels in the LAD-fed *db/db* mice than in the control and HAD-fed mice, attributable almost exclusively to a marked increase in HDL cholesterol concentrations. This finding constitutes an additional novel observation and may be in part related to the improvement in IR found in the LAD-fed *db/db* mice, which may further elevate the normally high level of plasma HDL cholesterol in *db/db* mice. The molecular mechanism for the further increase is not known but may be related to our previous findings in diabetic rats (48). In those studies, we also found (as in the *ob/ob* mouse) decreased hepatic apoAI mRNA levels that were increased by insulin treatment and that would be expected to result in increased production of HDL. Al-

though as noted above, unlike *db/db* or *ob/ob* mice, obese and diabetic patients have depressed plasma HDL cholesterol levels but, like the *db/db* mice in the present study, they also exhibit elevations in HDL cholesterol levels as insulin sensitivity increases (49,50). In addition, treatment of type 2 diabetic patients with the insulin sensitizer rosiglitazone is thought to favorably affect the LDL and HDL cholesterol heterogeneity (51). Given the substantial clinical implications of a potentially similar effect on human HDL levels imparted by the modulation of dietary AGEs, further studies with regard to the mechanisms involved in the elevation in the LAD *db/db* mice are warranted.

From the present studies, it can be inferred that a diet rich in AGEs may constitute a critical environmental modulator of endogenous metabolism in diabetic mice.

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