Insulin resistance, alone or as part of the metabolic syndrome, is becoming an increasingly common problem in modern society (1,2). Epidemiological studies (3,4) have demonstrated that populations with the same genetic background exhibit increased incidence of insulin resistance and type 2 diabetes whenever a western lifestyle and diet is adopted. Western diets are characterized by high fat and protein content (5). Nutrients, including fatty acids, have been shown to directly modulate insulin signaling and thus contribute to insulin resistance (6,7).

Advanced glycation end products (AGEs) as well as advanced lipoxidation end products (ALEs) are prooxidant and proinflammatory compounds that have recently been linked to impaired insulin sensitivity (8). These compounds continuously form in the body from the reaction of reducing sugars and reactive carbonyls with free amino groups (9), while amine-containing lipids are also generators of lipid peroxidation products (10–12). AGEs/ALEs can also originate exogenously, during heat processing of food (13–16), and become incorporated in body components after intestinal absorption (17). It has now become apparent that dietary AGEs represent a significant source of circulating and tissue AGEs, manifesting similar pathogenic properties to their endogenous counterparts (17–24). The restriction of the AGE content in standard mouse diets was found, among other effects, to markedly improve insulin resistance in obese db/db (8).

Because fat-rich foods are also particularly rich in AGEs/ALEs (16), we postulated that the insulin resistance observed after chronic high-fat feeding (25) is related to the obligatory intake of large amounts of AGEs inherent in these diets. To test this hypothesis, we evaluated glucose and insulin responses, visceral adiposity, pancreatic islet morphology, and type 2 diabetes incidence in mice subjected to long-term feeding on high-fat diets but with either high or low AGE/ALE content. We also measured plasma 8-isoprostane as an index of systemic oxidant stress and plasma adiponectin as a molecule that has been found to be inversely correlated with insulin resistance.
TABLE 1.
Characteristics of mouse diets*  

<table>
<thead>
<tr>
<th></th>
<th>Regular</th>
<th>LAGE-HF</th>
<th>HAGE-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g%)</td>
<td>25.6</td>
<td>25.6</td>
<td>25.6</td>
</tr>
<tr>
<td>Carbohydrate (g%)</td>
<td>50.3</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Fat (g%)</td>
<td>5.1</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Calories (kcal/g)</td>
<td>3.4</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Total AGE (units/mg)</td>
<td>117.4</td>
<td>329.6</td>
<td>995.4</td>
</tr>
<tr>
<td>Fat-associated AGE (units/mg)</td>
<td>1.4</td>
<td>167</td>
<td>341.9</td>
</tr>
<tr>
<td>Protein-associated AGE (units/mg)</td>
<td>116</td>
<td>167.6</td>
<td>653.5</td>
</tr>
<tr>
<td>Casein (mg%)</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L-cystine (g%)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch (g%)</td>
<td>315</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maltodextrin (g%)</td>
<td>25</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Sucrose (g%)</td>
<td>350</td>
<td>68.8</td>
<td>68.8</td>
</tr>
<tr>
<td>Soybean oil (g%)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lard (g%)</td>
<td>20</td>
<td>245</td>
<td>245</td>
</tr>
</tbody>
</table>

Regular and high-fat diets were obtained from Labdiet (Purina Mills) and prepared by standard procedures. The HAGE-HF diet was exposed to an additional step of autoclaving at 120°C for 30 min. All micronutrients were within the range established by the Nutrient Requirements of Laboratory Animals, The National Academy of Sciences, Washington, DC, National Academy Press, 1995. There were no differences in mineral and vitamin content between regular and high-fat diets. A total of 100 g lard contains 39.2 g saturated 45.1 g monounsaturated, and 11.2 g polyunsaturated fat, while 100 g soybean contains 15.0 g saturated, 59.0 g monounsaturated, and 24.5 g polyunsaturated fat.

TABLE 2. Body weight and selected biochemical parameters  

<table>
<thead>
<tr>
<th></th>
<th>HAGE-HF</th>
<th>LAGE-HF</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>End</td>
<td>Baseline</td>
<td>End</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>15 ± 0.3</td>
<td>29.5 ± 0.1*†‡</td>
<td>16 ± 0.25</td>
</tr>
<tr>
<td>Serum AGE (units/ml)</td>
<td>46 ± 3</td>
<td>124 ± 7*†‡</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>142 ± 4</td>
<td>295 ± 6*†‡</td>
<td>147 ± 6</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>48 ± 5</td>
<td>38 ± 2</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>72 ± 8</td>
<td>181 ± 12*†‡</td>
<td>73 ± 4</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Statistically significant difference between baseline and end of study within each group. †Statistically significant difference between each group and control mice at the end of study. ‡Statistically significant difference between the HAGE-HF and LAGE-HF groups at the end of study.
1.6-fold ($P < 0.01$), respectively (Table 2). Levels of triglycerides and total cholesterol increased significantly from baseline in both groups fed the high-fat diet, while HDL cholesterol remained unchanged (Table 2). Interestingly, plasma insulin levels in the HAGE-HF group increased by 7.5-fold above baseline ($P < 0.0001$), but in the LAGE-HF group, these remained unchanged and similar to the control mice (Fig. 1B). A similar trend was observed with fasting blood glucose levels (Fig. 1A).

**Effect of diet on glucose tolerance tests and glycemic clamps.** HAGE-HF mice displayed impaired glucose response closely resembling that of db/db mice during glucose tolerance tests, which was markedly different from the LAGE-HF ($P < 0.02$) and control groups ($P < 0.01$). In contrast, the glucose tolerance test profiles of the LAGE-HF group closely paralleled those of the control mice (Fig. 2A).

During euglycemic clamp, glucose infusion rate in the HAGE-HF mice was as low as 50% that of the control mice ($P < 0.001$). In contrast, in the LAGE-HF group, glucose infusion rate showed no significant difference from the control group but differed significantly from the glucose infusion rate of both the HAGE-HF ($P < 0.001$) and the db/db ($P < 0.001$) groups (Fig. 2B). During hyperglycemic clamp, plasma insulin in HAGE-HF mice reached levels approximately threefold higher than those of control mice ($P < 0.001$), while in LAGE-HF mice, despite a similar body weight gain and dietary fat intake, plasma insulin remained close to the control levels ($P = NS$) (Fig. 2C).

**Effect of diet on visceral fat, fat-AGE content, and circulating 8-isoprostane and adiponectin levels.** As expected, significant differences were observed in total visceral fat content between the two high-fat–fed groups and the regular diet–fed controls (Fig. 3A). Of note, however, the LAGE-HF group exhibited a significantly lower (~50%) amount of visceral fat compared with that found in HAGE-HF mice ($P < 0.001$) (Fig. 3A). In addition, the amount of AGE-modified fat per gram of fat associated with the LAGE-HF group was significantly smaller compared with that of HAGE-HF mice ($P < 0.001$) (Fig. 3B). Consequently, the estimated amount of total AGE content present in visceral fat in the LAGE-HF mice was fourfold lower than that in the HAGE-HF mice ($P = 0.000$).

At the end of the study, plasma 8-isoprostane levels, a
measurement of systemic oxidant stress, were significantly elevated in the HAGE-HF and the db/db groups compared with the controls (P < 0.01). In the LAGE-HF group, 8-isoprostane levels were more modestly elevated above the control (P < 0.05) (Fig. 4A).

As in the db/db mice, serum adiponectin levels were found markedly suppressed in the HAGE-HF group compared with controls (P < 0.001), while in the LAGE-HF group, these were reduced by only 25% compared with controls (P < 0.05) (Fig. 4B).

**Effect of diet on islet morphology.** Consistent with previous evidence based on the use of high-fat diets, pancreatic islets from the HAGE-HF mice after 6 months exhibited hyperplasia and hypertrophy combined with loss of islet structure and cellular homogeneity. However, such changes were only seen infrequently in islets from LAGE-HF mice (Fig. 5A and B). Instead, in the latter group, islet size appeared normal and well organized, with normal architecture and abundance of insulin- and glucagon-containing cells. In addition, islet degeneration and insulin- and glucagon-producing cell displacement from the periphery to the center of the islets were only evident in islets from the HAGE-HF and not the LAGE-HF mice.

**DISCUSSION**

The studies presented demonstrate that in normal mice exposed to a high-fat diet, the metabolic changes, which lead to weight gain, glucose intolerance, insulin resistance, and type 2 diabetes are linked to the AGEs/ALEs present in the diet. In addition, the studies illustrate that visceral adiposity and systemic indicators of oxidative stress or inflammation, such as 8-isoprostane and adiponectin, can be differentially linked to the ingested AGEs beyond the excess of fat. Furthermore, pancreatic islet structure and function, which are affected negatively during prolonged exposure to a fat-rich diet, appear to be linked to the dietary content of glycoxidants and can thus be spared by a diet comparatively low in AGEs, even if it is fat rich.

These observations differ significantly from previous observations on the role of dietary AGEs in genetically type 2 diabetes–prone mice (8), the key difference here being the induction of insulin resistance and type 2 diabetes in normal mice exposed to excess fat, a dietary condition resembling that of many healthy humans.

In the present studies, high dietary fat intake by normal mice for the period between 1 and 7 months of age led to an increase in body weight of both high- and low-AGE groups, which was modest yet significantly higher in the HAGE-HF than in the LAGE-HF mice. Interestingly, a major proportion (~75%) of the mice fed a HAGE-HF diet were diabetic by the end of the study compared with none of those exposed to the LAGE-HF diet, based on a fasting blood glucose level >130 mg/dl. The AGE-rich fatty diet resulted in a pattern of profound abnormalities in glucose tolerance, glucose disposal rate, and insulin responses closely resembling those of the diabetic db/db mice (8). In contrast, exposure to the low-AGE fatty diet led to a pattern comparable with the normal metabolic profile associated with the standard (low-fat) diet. These findings suggest that dietary factors other than high fat content contribute to these metabolic changes.

Consistent with previous studies (8,18–24) and given the equal exposure to basic nutrients and energy provided by the two high-fat diets, the present evidence points to the different content in dietary AGEs/ALEs as the probable contributors to the metabolic effects described. As expected, both high-fat formulas (35% fat) contained greater ε-N-carboxymethyllysine–immuno-epitopes than did the standard diet (4.5% fat), consistent with the fact that lipid-rich food products, often rich in amino-lipids, especially phospholipids, provide an extra source of reactants for the complex series of reactions leading to the formation of AGEs/ALEs (11,12,27) as well as of oxidized fatty acids, which further enhance the formation of AGEs/ALEs (30). Furthermore, the significant variance in AGE content between the HAGE-HF and LAGE-HF diets is attributable to the AGE-promoting methods and conditions used, e.g.,
exposure to elevated temperature and not to differences in nutrient composition (8,16). This was also clearly reflected in the higher circulating AGE levels in the HAGE-HF mice, relative to the other groups.

The identity of the AGE/ALE species responsible for the metabolic effects described remains speculative given the heterogeneity of these compounds. ε-N-carboxymethyllysine, a chemically defined derivative of both glycoxidation and lipid peroxidation (27), however, is thought to contribute to oxidative stress and tissue damage and has proved a useful indicator of AGEs, correlating well with diabetes and aging (9,10,31) as well as with a range of diet-induced pathological conditions (18–22).

Of particular interest in these studies was the differential accumulation of visceral fat in the HAGE-HF group compared with the LAGE-HF mice, despite similar dietary fat intake and body weight gain by both groups. Of further interest was the marked accumulation of total AGEs/ALEs in the visceral fat of the HAGE-HF group, reaching the total of fourfold more than that of the LAGE-HF group. This novel finding reveals a potentially important relationship between diet-related glycoxidant and lipoxidant substances and visceral adiposity, a risk factor associated with insulin resistance and the metabolic syndrome (32,33).

Visceral adipose tissue expansion has been associated with increased production of inflammatory mediators, such as tumor necrosis factor-α (34). Greater binding of AGE by proinflammatory AGE receptors, such as RAGE, could trigger oxidative stress and the release of cytokines such as tumor necrosis factor-α (35,36), which is known to inhibit insulin action (37). This and a host of autocrine/paracrine inflammatory molecules released from adipose tissue or macrophages, migrating to AGE-rich visceral fat, could contribute to the insulin resistance exhibited by HAGE-HF mice but yet not seen in the LAGE-HF mice, most likely related to the lower amount of AGE fat in the latter group.

The histology of the pancreatic islets from both experimental groups reflected a similar pattern. Nearly normal glucose and insulin responses seen in the LAGE-HF group were consistent with well-preserved pancreatic islet morphology and function in this group and contrasted with islet enlargement, structure disorganization, and sparsity of insulin production displayed by the HAGE-HF mice. The near-normal appearance of islets from the LAGE-HF mice together with the metabolic findings strongly suggest a protective role for a low-AGE diet, even if the fat content is elevated. The islet changes were consistent with those seen in db/db mice fed high-versus low-AGE diets (8) and offered further in vivo support to reports associating glycoxidation with inhibition of insulin gene transcription (38) or promotion of β-cell apoptosis in vitro (39). Thus, given the significant prooxidant and proinflammatory properties of AGEs/ALEs exhibited in the absence of chronic hyperglycemia, diet-derived AGEs/ALEs may represent diabetogenic substances worthy of further rigorous evaluation.

The significant rise in plasma 8-isoprostanes in the high-fat–fed groups, a finding consistent with a state of increased oxidant stress (40), may have also been the result of the higher supply of AGEs/ALEs in their diet (11). In agreement with this, adiponectin, a factor thought to play a role in regulating insulin sensitivity and to exert anti-inflammatory effects at large (41), correlated inversely with 8-isoprostane and with visceral adiposity and remained significantly higher only in the LAGE fat-fed mice. These findings reveal new aspects of the balance between prooxidant AGEs/ALEs and anti-inflammatory innate defense mechanisms. The positive relationship between ingested and circulating AGE levels found in the present studies has been previously also linked to acute-phase proteins (C-reactive protein) and vascular dysfunction (23,42). These properties are herein expanded to include manifestations of insulin resistance and are exemplified in the context of a diet high in fat. Thus, dietary AGE, forming at high rates in the presence of excess fat and commonly applied levels of temperature, may independently contribute to the subinflammatory state associated with insulin resistance and metabolic syndrome in humans as well. To date, AGEs/ALEs are the only food-derived compounds studied for their contribution to diabetic tissue toxicity, including insulin-producing cells (8). No other class of heat-enhanced substances has been studied for the intriguing array of cell effects exhibited by these substances, i.e., oxidant stress, nuclear factor-κB activation, cytokine (tumor necrosis factor-α) induction, inflammation, and apoptosis (15,36).

In summary, during prolonged high-fat feeding, the AGE/ALE content of food may exert significant influence on the regulation of insulin secretion and action and visceral adiposity and may ultimately lead to type 2 diabetes. These results, taken together with previous work (8) on the effects of a high-in-AGE-but-low-in-fat diet on insulin resistance support the view that in addition to the fat, the high AGE/ALE content of food is significantly linked to the insulin-resistant state. While the mechanisms linking AGES and the related deleterious metabolic effects are likely to be complex, the evidence indicates that lowering AGE/ALE content in fatty foods might be an intervention to control insulin resistance and prevent diabetes. Further long-term studies in humans are needed.

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REFERENCES