Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats

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Chalkley, Simon M., Manthinda Hettiarachchi, Donald J. Chisholm, and Edward W. Kraegen. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. Am J Physiol Endocrinol Metab 282: E1231–E1238, 2002. First published January 15, 2002; 10.1152/ajpendo.00173.2001.—Although lipid excess can impair β-cell function in vitro, short-term high-fat feeding in normal rats produces insulin resistance but not hyperglycemia. This study examines the effect of long-term (10-mo) high polyunsaturated fat feeding on glucose tolerance in Wistar rats. The high-fat-fed compared with the Chow-fed group was 30% heavier and 60% fatter, with approximately doubled fasting hyperinsulinemia (P < 0.001) but only marginal fasting hyperglycemia (7.5 ± 0.1 vs. 7.2 ± 0.1 mmol/l, P < 0.01). Insulin sensitivity was ~67% lower in the high-fat group (P < 0.01). The acute insulin response to intravenous arginine was approximately double in the insulin-resistant high-fat group (P < 0.001), but that to intravenous glucose was similar in the two groups. After the intravenous glucose bolus, plasma glucose decline was slower in the high-fat group, confirming mild glucose intolerance. Therefore, despite severe insulin resistance, there was only a mildly elevated fasting glucose level and a relative deficiency in glucose-stimulated insulin secretion; this suggests that a genetic or congenital susceptibility to β-cell impairment is required for overt hyperglycemia to develop in the presence of severe insulin resistance.

Diabetes factors such as high-fat feeding can induce insulin resistance, but it is generally assumed that, without a genetic predisposition, diet alone will not suffice to induce diabetes mellitus. This notion has been challenged by recent work suggesting a toxic effect of excess lipid on β-cell function. Although short-term exposure (<6 h) to elevated fatty acids potentiates glucose-stimulated insulin secretion, longer-term exposure (24–48 h) can inhibit insulin secretion in rat β-cells in vitro (41), in perfused pancreata (32), and in humans (6, 28).

Previous studies from our institution with short-term (3 wk-1 mo) high polyunsaturated fat-fed rats showed no metabolically significant hyperglycemia despite the induction of insulin resistance (23, 26, 37). One other study of long-term (32 wk) high-fat feeding produced a wider variation in fasting plasma glucose levels with some hyperglycemia (15). However, that study differed in method from our usual model of high-fat feeding, in that there was a greater proportion of saturated fat and a longer period of fasting (24 h).

This current study extends our previous short-term high-fat feeding in normal rats for a 10-mo period to determine whether long-term high-fat feeding adversely affects glucose tolerance. We examine this in the context of lipid levels, insulin sensitivity, and body composition.

Materials and Methods

Experimental animals. Male Wistar rats obtained from an in-house breeding colony were randomized at ~2 mo of age into two groups; one group received standard laboratory chow (Allied Feeds, Rhodes, NSW, Australia) ad libitum and the other a safflower oil-based high-fat diet ad libitum, as previously described (23). The high-fat diet supplied 59% of the calories as fat and 20% of the calories as carbohydrate comprised of cornstarch and sucrose (2:1 wt/wt). The Chow diet provided 10% of the calories as fat and 65% as carbohydrate. The Chow or high-fat diets were continued for a further 9–10 mo. All animals had free access to water and were subjected to controlled temperature (22 ± 1°C) and lighting (lights on 0600–1800). When larger, the animals were housed separately and rested on a regularly changed sawdust bed. All of the surgical and experimental procedures performed were approved by the Garvan Institute of Medical Research/St Vincent’s Hospital Animal Experimentation Ethics Committee and were in accordance with National Health and Medical Research Council of Australia guidelines for the use of animals in research.

Surgical procedures. A detailed description of surgical procedures and preparation of animals has been given previously (23). Briefly, canulas were inserted into the right jugular vein and left carotid artery and exteriorized at the back of the neck, with animals under isoflurane (Fortane; Abbott, Kurnell, NSW, Australia) anesthesia administered via a Fluotec 3 anesthetic machine (Easy Veterinary Equipment, Sydney, NSW, Australia). Rats were subsequently studied when they were again eating their full diet and regaining weight.

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2-deoxy-D-[2,6-3H]glucose was given at an equivalent to 1,500 pmol. It was maintained with a variable-rate 20% 0.9% saline and returned to the animal. Euglycemia was estimated of plasma glucose, insulin, nonesterified (NEFA), and triglyceride (TG) levels. Similar blood sample of 600 μl was collected at time 0 for estimation of plasma glucose and insulin levels. Red blood cells were resuspended in 200 μl of normal saline and returned to the animal.

**Study protocol.** Animals were randomly allocated to have intravenous glucose and arginine tolerance tests on the same day (n = 9 each group), a basal study (n = 6 each group), or a euglycemic hyperinsulinemic clamp study (n = 6 each group). All animals were deprived of food for 5 h before studies. Body composition analysis was performed on the 12 animals that had a basal or clamp study. During the studies, blood sampling lines were kept patent by heparinized saline. Blood samples taken during all the studies were placed in heparinized (5 μl of 1,000 U/ml) tubes and centrifuged. Plasma samples were stored in a −10°C freezer until analyzed.

**Intravenous glucose and arginine tolerance tests.** To achieve similar elevations of plasma glucose and arginine levels during the tolerance tests and similar levels of insulin during the hyperinsulinemic clamp, body surface area was used to calculate the doses required. Body surface area was chosen rather than weight, because there were considerable differences in body weight and degree of fatness between groups. Body surface area was calculated according to the formula of Meeh (see Dubois, Ref. 10), where body surface area (cm²) = k × body wt (g)0.385, with k being 10.4 for rats (34).

The calculated doses for the intravenous tolerance tests were for glucose (mg) = 0.355 × cm² of body surface area, and for arginine (mg) = 0.213 × cm² of body surface area, which for a 400-g rat represents 500 and 300 mg/kg of glucose and arginine, respectively. The intravenous bolus of glucose and arginine were separated by 4 h. The boluses were given over 30 s, and 200-μl blood samples were collected at 0, 2, 5, 10, 20, and 50 min in heparinized tubes and centrifuged for estimation of plasma glucose and insulin levels. Red blood cells were resuspended in 200 μl of normal saline and returned to the animal.

**Basal study.** A bolus injection of D-[U-14C]glucose and 2-deoxy-D-[2,6-3H]glucose was given at time 0, after which plasma samples were collected at 2, 5, 10, 15, 20, and 45 min. An initial blood sample of 600 μl was collected at time 0 for estimation of plasma glucose, insulin, nonesterified fatty acid (NEFA), and triglyceride (TG) levels, and a similar sample was also taken at 45 min.

**Hyperinsulinemic euglycemic clamp.** Physiological hyperinsulinemia was achieved with an infusion of human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at a constant rate of 1.06 pmol·cm⁻² of body surface area⁻¹·h⁻¹, which is equivalent to 1,500 pmol·kg⁻¹·h⁻¹ for a 400-g rat. An initial blood sample of 600 μl was collected at time 0 for estimation of plasma glucose, insulin, NEFA, and TG levels. Similar samples were taken at 75 and 120 min. For the 0- and 75-min samples, the red blood cells were resuspended in 200 μl of 0.9% saline and returned to the animal. Euglycemia was maintained with a variable-rate 20–30% (wt/vol) dextrose infusion adjusted every 10–15 min. After ~75 min, a bolus injection of D-[U-14C]glucose and 2-deoxy-D-[2,6-3H]glucose (Amersham) was given, and 200 μl of blood were collected at 2, 5, 10, 15, 20, and 45 min after the bolus for determination of plasma glucose and tracer concentrations.

**Intravenous glucose tolerance tests.** An estimate of insulin clearance during the euglycemic hyperinsulinemic clamp studies was performed by dividing human insulin levels during the clamp by the infusion rate and expressing them per unit of body surface area.

**Tissue samples and body composition.** At the end of the basal or clamp period (i.e., 45 min after the tracer bolus), the rats were killed with a lethal dose (150 mg/kg) of intravenous pentobarbitone sodium (Nembutal; Abbott Laboratories, Sydney, Australia). Soleus, red portions of gastrocnemius and quadriceps muscles, and liver were rapidly removed, freeze-clamped in liquid nitrogen, and then stored at −70°C for subsequent analyses. After this, body composition was estimated by underwater weighing. Before underwater weighing, the viscera were removed from the abdominal and thoracic cavities and weighed, and the fur was closely shaved so as to avoid the effects of air trapping in any of these structures. The assumed density of fat was 0.9 g/cm³ and for lean tissue 1.1 g/cm³. Fat mass and fat-free mass were calculated according to the equations of Siri (33). Twenty grams of lean tissue (skeletal muscle) were assumed to have been dissected and freeze-clamped. Subsequently, 20 g were added to the values obtained from the underwater weighing for lean tissue. Intra-abdominal fat weight was determined via dissection of epididymal, retroperitoneal, and mesenteric fat depots.

**Analytical methods.** Whole body glucose turnover. For the determination of tracer activity, plasma samples were immediately deproteinized with 2.8% ZnSO4 and saturated Ba(OH)2 before the counting of radioactivity. The disappearance kinetics of 2-deoxy-D-[2,6-3H]glucose were used to calculate peripheral glucose disposal (Rg) as previously described (18). Hepatic glucose output (HGO) was calculated as Rg minus the glucose infusion rate.

**Tissue-specific analyses.** The rate of individual tissue glucose uptake (Rg) was calculated as previously described (24). The net rate of glycogen synthesis in red skeletal muscle and liver was calculated from the accumulation of 14C from [14C]glucose into glycogen. Tissue glycogen levels were determined as described by Handel (16).

**Tissue TG was extracted according to the method of Polch et al. (12) and quantified by the method of Carr et al. (7). Red skeletal muscle and liver long-chain acyl (LCA)-CoA content was measured as previously described (17). Essentially this method involves extraction, phase separation, and purification of LCA-CoA from tissues and injection into a Novapak C18 reverse-phase HPLC column (Waters Millipore). Under ultraviolet detection, individual LCA-CoA species were identified.

**Table 1. Body composition after 10 mo of diet**

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total weight</td>
<td>676 ± 14</td>
<td>872 ± 41*</td>
</tr>
<tr>
<td>Total fat</td>
<td>162 ± 8</td>
<td>330 ± 29*</td>
</tr>
<tr>
<td>Lean tissue</td>
<td>399 ± 7</td>
<td>412 ± 11</td>
</tr>
<tr>
<td>Intra-abdominal fat</td>
<td>53 ± 4</td>
<td>90 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SE in g; n = 12/group. *P < 0.001 vs. chow-fed group.

**Table 2. Fasting plasma parameters**

<table>
<thead>
<tr>
<th>Plasma Concentration</th>
<th>Chow</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>7.2 ± 0.1</td>
<td>7.5 ± 0.1*</td>
</tr>
<tr>
<td>Fructosamines, μmol/l</td>
<td>285 ± 17</td>
<td>301 ± 16</td>
</tr>
<tr>
<td>Hemoglobin A1, %</td>
<td>7.2 ± 0.3</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>270 ± 40</td>
<td>600 ± 50‡</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>1,150 ± 180</td>
<td>730 ± 90*</td>
</tr>
<tr>
<td>TG, μmol/l</td>
<td>1,060 ± 110</td>
<td>750 ± 70*</td>
</tr>
</tbody>
</table>

Values are results (means ± SE of 15 rats/group) after 10 mo of diet. NEFA, nonesterified fatty acids; TG, triglycerides. *P < 0.05, †P < 0.01, and ‡P < 0.001 ns. chow group.
Table 3. Glucose fluxes per whole body and lean tissue

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Clamp</td>
<td>Basal Clamp</td>
</tr>
<tr>
<td>GIR</td>
<td>86 ± 8</td>
<td>28 ± 6†</td>
</tr>
<tr>
<td>Rₐ</td>
<td>51 ± 5</td>
<td>31 ± 6‡</td>
</tr>
<tr>
<td>HGO</td>
<td>51 ± 5</td>
<td>24 ± 5‡</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Clamp</td>
<td>Basal Clamp</td>
</tr>
<tr>
<td>GIR</td>
<td>142 ± 12</td>
<td>55 ± 11†</td>
</tr>
<tr>
<td>Rₐ</td>
<td>143 ± 13†</td>
<td>60 ± 9‡</td>
</tr>
<tr>
<td>HGO</td>
<td>1 ± 11†</td>
<td>48 ± 14‡</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats/group expressed in μmol·kg⁻¹·min⁻¹. GIR, glucose infusion rate; Rₐ, peripheral glucose disposal; HGO, hepatic glucose output. *P < 0.05 and †P < 0.01 vs. basal parameter in same group; ‡ P < 0.05 and §P < 0.01 vs. corresponding parameter in Chow group.

dified according to their respective retention times. Peaks corresponding to palmitoyl (16:0), palmitoleoyl (16:1), linolenoyl (18:3), linoleoyl (18:2), oleoyl (18:1), and stearoyl (18:0) CoAs were identified and individually quantified with respect to a known amount of internal standard (lauryl-CoA, 12:0).

Other analytical methods. Plasma glucose levels were analyzed by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured by radioimmunoassay (Linco Research, St Charles, MO) with rat and human antibody kits. Plasma NEFA levels were determined by an acyl-CoA oxidase-based colorimetric kit (Wako Pure Chemicals Industries, Osaka, Japan), and plasma TG levels were determined by an enzymatic colorimetric assay (TG, INT) (Procedure no. 336, Sigma Diagnostics, St. Louis, MO). NEFA and TG assays were performed within 24 h of sample collection. Glycated proteins were also separated from unbound hemoglobin with an affinity resin (Quik-Sep; Isolab, Barberton, OH) after separation on unbound hemoglobin with an affinity resin (1, 19).

Statistical analysis. Data are expressed as means ± SE. Comparisons between the groups were made using Student’s t-test for unpaired data (Macintosh Statview SE + Graphics program, Abacus Concepts; Brain Power, Berkeley, CA).

RESULTS

Age and body composition. The Chow (C) and high fat-fed (HFF) groups were matched for age (C 48 ± 1 vs. HFF 50 ± 1 wk). The HFF rats were significantly heavier, fatter, and with more central abdominal fat than the C-fed rats. The amount of lean tissue was similar in the two groups (Table 1).

Fasting parameters. The fasting plasma glucose levels were slightly but significantly raised in HFF compared with C rats (Table 2). No rat became overtly diabetic, and the range of fasting glucose levels was 6.8–7.6 (C) and 7.0–8.1 (HFF) mmol/l. Furthermore, glycated protein levels were not significantly different between groups. The fasting plasma insulin level in HFF rats was approximately double that of C rats. Fasting NEFA and TG levels were lower in HFF rats (Table 2).

Whole body glucose fluxes in the basal state. Basal glucose turnover (Rₐ and HGO) was lower in the HFF compared with the C group (Table 3).

Hyperinsulinemic euglycemic clamp. The clamp glucose infusion rate was significantly lower in the HFF group, as was the peripheral glucose disposal. With physiological hyperinsulinemia, the hepatic glucose output in the C-fed group was well suppressed, whereas the HFF group showed no significant suppression compared with its basal state (Table 3). The plasma glucose, insulin, NEFA, and TG levels before and during the euglycemic hyperinsulinemic clamp are shown in Table 4. The percent fall in mean NEFA levels during the clamp was greater in the C group than in the HFF group (34 vs. 2% reduction, P < 0.05). The clamp insulin levels were higher in the HFF group (Table 4), perhaps due to reduced clearance or because of higher basal levels, making the differences in insulin sensitivity more significant if expressed per unit of plasma insulin concentration. The amount of central abdominal fat correlated significantly with Rₐ (Fig. 1).

Peripheral glucose disposal. Under conditions of hyperinsulinemia, Rₐ in red quadriceps muscle and the net rate of glycogen synthesis in red gastrocnemius muscle were lower in HFF compared with C rats (Table 5).

Muscle TG and glycogen levels. The differences between the two groups in the basal or clamp state for TG levels in red gastrocnemius muscle were not significant. Glycogen stores were similar in red gastrocnemius muscle for the two groups in the basal and hyperinsulinemic states (Table 5).

Table 4. Plasma parameters before and during euglycemic hyperinsulinemic clamp

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preclamp</td>
<td>Clamp</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>7.0 ± 0.1</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>350 ± 130</td>
<td>770 ± 100**</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>1,015 ± 210</td>
<td>525 ± 70*</td>
</tr>
<tr>
<td>TG, μmol/l</td>
<td>1,230 ± 200</td>
<td>930 ± 270</td>
</tr>
</tbody>
</table>

Clamp values are means of 75- and 120-min samples. *P < 0.05 and †P < 0.01 vs. preclamp parameter in same group by Student’s paired t-test; ‡P < 0.05 vs. corresponding parameter in Chow group. There were 6 rats in each group.
Liver TG and glycogen levels. The liver had markedly increased TG levels in the HFF rat. Glycogen levels were reduced in the HFF rat but in part could be related to the excessive accumulation of TG (Table 6).

Tissue LCA-CoA levels. The total levels of LCA-CoA in red gastrocnemius muscle were significantly elevated in HFF in the basal state. Also, the total levels of LCA-CoA remained higher in HFF than in C at the end of the hyperinsulinemic clamp and were not significantly suppressed below C basal levels (Table 5). There was a significant negative correlation between red gastrocnemius LCA-CoA content and the net rate of glycogen synthesis (Fig. 2). The predominant species increased in red gastrocnemius LCA-CoA in HFF was linoleoyl CoA (18:2), corresponding with the predominant fatty acid in the HFF diet (safflower oil).

Liver LCA-CoA levels were also elevated in the HFF-fed compared with the C-fed rat and showed no significant suppression with hyperinsulinemia (Table 6).

Insulin clearance. The clearance rate of infused human insulin during the euglycemic hyperinsulinemic clamp was ~40% lower in HFF compared with C rats (16 ± 2 vs. 28 ± 4 ml·min⁻¹·m⁻² of body surface area⁻¹·min⁻¹, P < 0.05).

Intravenous glucose and arginine tolerance tests. The peak level of plasma glucose after the glucose bolus was slightly higher in the HFF rats (C: 22.9 ± 0.8 vs. HFF: 25.1 ± 0.5 mmol/l, P < 0.05). However, the peak rise in glucose above baseline was similar in the two groups (16.1 ± 0.8 vs. 17.5 ± 0.6 mmol/l, for C and HFF, P = 0.2). The log of the declining plasma glucose slope at 2–20 min was −3.2 ± 0.1 and −2.2 ± 0.1 for C and HFF rats, respectively (P < 0.001; Fig. 3), indicating glucose intolerance in the HFF group. The peak insulin response to glucose above baseline was similar in the two groups (2,160 ± 240 vs. 2,760 ± 360 pmol/l, P = 0.2, Fig. 4), as was the incremental area under the curve for insulin levels at 0–10 min (Fig. 5). However, the peak insulin response to arginine above baseline was more than doubled in the HFF group (2,580 ± 240 vs. 5,520 ± 480 pmol/l, P < 0.001, Fig. 4). Previously, the acute insulin response to arginine has been shown to have a positive linear correlation to the prestimulus plasma glucose level (30). Therefore, we reanalyzed arginine-induced insulin levels by adjusting the incremental area under the curve proportionately by the difference in each rat’s prestimulus glucose level; there was still a significantly increased insulin response in the HFF group (50% increase, Fig. 5).

**DISCUSSION**

Our study showed that Wistar rats developed glucose intolerance with mild fasting hyperglycemia, but not overt diabetes, with long-term (~10 mo) high-fat feeding. In the HFF group, the insulin response to intravenous glucose appears comparable to C-fed rats. However, the acute insulin response to arginine in the HFF group is nearly double that in the C-fed group. The enhanced insulin response to arginine in the high-fat group would be expected, because the insulin response in other studies, albeit where glucose tolerance is normal, suggests that the acute insulin response to a secretagogue increases with decreasing insulin sensitivity (3, 20). The HFF group in our study is quite insulin resistant, with glucose infusion rates during the hyperinsulinemic euglycemic clamp being 60–70% reduced per whole body or per lean tissue compared with the C-fed group. Thus the acute insulin response to glucose in the high-fat group could be considered as being deficient relative to the arginine response. The insulin clearance during the clamp studies is reduced in the HFF group by ~40% compared with the C group. Because insulin levels were higher due to lower clearance in the high-fat group, the insulin response in the HFF group could be regarded as even more impaired.

### Table 5. Skeletal muscle content of glycogen, TG, and LCA-CoA, and glucose fluxes

<table>
<thead>
<tr>
<th></th>
<th>Chow Basal</th>
<th>Chow Clamp</th>
<th>High Fat Basal</th>
<th>High Fat Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen, μmol/g</td>
<td>39.4 ± 2.2</td>
<td>44.4 ± 3.7</td>
<td>35.4 ± 2.4</td>
<td>39.8 ± 4.9</td>
</tr>
<tr>
<td>Triglyceride, μmol/g</td>
<td>13.4 ± 3.2</td>
<td>11.5 ± 1.4</td>
<td>20.0 ± 3.2</td>
<td>13.6 ± 2.2</td>
</tr>
<tr>
<td>LCA-CoA, nmol/g</td>
<td>10.9 ± 1.6</td>
<td>4.8 ± 0.9*</td>
<td>21.9 ± 2.5†</td>
<td>16.5 ± 2.0†</td>
</tr>
<tr>
<td>R_E, μmol·kg⁻¹·min⁻¹</td>
<td>158 ± 16</td>
<td>224 ± 8*</td>
<td>88 ± 11</td>
<td>109 ± 15†</td>
</tr>
<tr>
<td>Net GS, μmol·kg⁻¹·min⁻¹</td>
<td>11 ± 3</td>
<td>42 ± 7*</td>
<td>8 ± 1</td>
<td>15 ± 4†</td>
</tr>
</tbody>
</table>

Results (means ± SE; n = 6/group) are for red gastrocnemius muscle, except R_E, which is for red quadriceps muscle. LCA-CoA, sum total of long-chain acyl-CoA species; R_E, glucose uptake; net GS, net rate of glycogen synthesis. *P < 0.01 vs. basal parameter in same group; †P < 0.01 vs. corresponding study in chow group.
The impairment in the acute insulin response to glucose in the HFF group may be due to reduction in glucose transport or a proximal metabolic defect. High-fat feeding has been shown to decrease GLUT2 mRNA levels and glucokinase mRNA (21). Consistent with a defect in glucose transport/proximal metabolism, the acute insulin response to arginine is relatively preserved in the HFF group. Several mechanisms of arginine-induced insulin secretion have been suggested, such as metabolism to ATP and generation of nitric oxide, although the balance of evidence would suggest an electrogenic effect of arginine, leading to membrane depolarization and influx of calcium into β-cells (38).

Although we did not find development of significant hyperglycemia with our high-fat feeding, other high-fat diets may have greater effects on glucose intolerance. One other long-term (32-wk) study of high-fat feeding with a greater proportion of saturated fatty acids (32% of calories as lard oil, 18% corn oil) reported some animals with hyperglycemia after a 24-h fast (15). Saturated fatty acids have been shown to be more potent stimulators of insulin secretion than unsaturated fatty acids (36), although we are not aware of any studies suggesting that saturated fatty acids are more potent at inducing an impairment of glucose-induced insulin secretion in the long term.

Obesity and type 2 diabetes mellitus are associated with elevated NEFA and TG levels. The long-term HFF group in our study had lower fasting NEFA and TG levels. This is not necessarily an unexpected finding, as a previous study from our group showed lower NEFA levels with short-term high-fat (safflower oil) feeding (27) and, in another study (23), a trend toward decreased fasting TG levels. It is well recognized that high dietary carbohydrate content can elevate plasma TG levels, and in one study in rats this was shown relative to high-fat feeding (Boivin and Deshaies (5a)). In our study, the carbohydrate content of the chow diet was 65% of calories vs. 25% in the high-fat diet, and this may have led to a relative elevation of TG levels in the C group. Despite lower fasting serum NEFA and TG levels in the HFF group, tissue content of lipids (TG and LCA-CoA) is increased. This may be due to greater postprandial hypertriglyceridemia or to an increase in peripheral tissue uptake of fatty acids in the HFF group, for which there is recent evidence (13).

The NEFA levels were incompletely suppressed in the C-fed group. It is difficult to attribute this to aging; however, a similar level of NEFA levels during a physiologically hyperinsulinemic clamp has been found after 3 wk of high-fat feeding (23). Despite precautions taken to prevent it, it is possible that the NEFA levels could have been spuriously elevated if some degree of in vitro lipolysis occurred from heparin contamination.

In a previous short-term (1-mo) study of high-fat feeding comparing saturated fat, polyunsaturated fat, and fish oil, there was not a significantly greater degree of insulin resistance in the saturated fat diet.

### Table 6. Hepatic content of glycogen, TG, and LCA-CoA, and glucose fluxes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal Chow</th>
<th>Clamp Chow</th>
<th>Basal High Fat</th>
<th>Clamp High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen, μmol/g</td>
<td>133 ± 34</td>
<td>125 ± 28</td>
<td>44 ± 12</td>
<td>53 ± 10†</td>
</tr>
<tr>
<td>Triglyceride, μmol/g</td>
<td>14.7 ± 1.3</td>
<td>16.4 ± 4.7</td>
<td>116.4 ± 14.6§</td>
<td>91.2 ± 6.3§</td>
</tr>
<tr>
<td>LCA-CoA, nmol/g</td>
<td>15.3 ± 2.3</td>
<td>18.0 ± 1.9</td>
<td>30.6 ± 5.4‡</td>
<td>28.6 ± 1.9§</td>
</tr>
<tr>
<td>Net GS, μmol·kg⁻¹·min⁻¹</td>
<td>6 ± 1</td>
<td>13 ± 2‡</td>
<td>5 ± 1</td>
<td>14 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/groups. *P < 0.05 and †P < 0.001 vs. basal parameter in same group; ‡P < 0.05 and §P < 0.01 vs. corresponding parameter in chow group.

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**Fig. 2.** Relationship between long-chain acyl-CoA content and net rate of glycogen synthesis in red gastrocnemius muscle during hyperinsulinemic clamp. Rats were fed a chow (○) or high-fat diet (●); r² = −0.5, P < 0.05. Values are means ± SE; n = 6 for each group.

**Fig. 3.** Glucose levels from intravenous glucose tolerance test. Chow (○), and high fat (●). **P < 0.01, †P < 0.005, and ††P < 0.001. Values are means ± SE; n = 9 for each group.
group compared with the polyunsaturated fat group (37). In the same study, fasting levels of NEFA and TG were not significantly different between the polyunsaturated and saturated groups and did not correlate with insulin sensitivity, whereas skeletal muscle TG levels were inversely correlated with insulin sensitivity (37). In our study, there was a significant negative correlation between tissue measures of lipid accumulation, namely central abdominal fat and red gastrocnemius LCA-CoA levels, with parameters of insulin sensitivity: peripheral glucose disposal and net rate of glycogen synthesis. The significant negative correlation between red gastrocnemius LCA-CoA levels and the net rate of glycogen synthesis in our study is in agreement with an in vitro study showing that palmitoyl-CoA caused dissociation and inactivation of glycogen synthase, albeit in rat liver (40).

Unexpectedly, the skeletal muscle TG levels were not significantly different between the two groups, even though LCA-CoA levels were. A possible explanation of this discrepancy may be that the TG content of rat skeletal muscle in this study was overestimated because of contamination of muscle samples by adherent fat (despite careful dissection), especially when we consider that both groups are fatter than short-term high fat-fed rats. Previous estimates of LCA-CoA content in adipose tissue have shown these levels to be much less than the level of muscle (11). So if there is contamination by adherent fat to muscle samples, then LCA-CoA content should better reflect lipid availability within muscle than TG content.

Basal hepatic glucose output is lower in the high-fat group, even when expressed per lean tissue, despite greater hepatic TG content, perhaps due to lower fasting NEFA levels, basal hyperinsulinemia, and lower peripheral glucose uptake, which in turn may be due to increased peripheral fatty acid uptake. This is in keeping with a situation in which the animal is utilizing and “turning over” more fatty acids and less glucose because of the composition of the food supply. Our group has recently demonstrated increased clearance of fatty acids into muscle in HFF animals (13), which is consistent with this scenario.

High-fat or -energy feeding in some genetic rodent models of diabetes (2, 9), or in rats with reduced β-cell mass (29), can precipitate diabetes. That none of our HFF rats became overtly diabetic suggests that a susceptibility (genetic or congenital) to develop diabetes is required before overt hyperglycemia can occur. β-Cell mass is reduced by 40–60% in several type 2 diabetes human autopsy studies (8, 14, 22, 39), although there are exceptions (31, 35). In partial pancreatectomy animal models, a 90% reduction in mass is required in rodents (5) and a 50% reduction in baboons (25) for hyperglycemia to develop, implying that a functional defect in insulin secretion is also required, with lesser degrees of β-cell mass reduction, to develop diabetes. On the other hand,
there is some evidence to suggest that the adult mammal has the ability to significantly increase β-cell mass, such as during pregnancy and obesity (4). Thus the genetic/congenital susceptibility to develop diabetes may relate to how an animal compensates for metabolic alterations or insults, such as excess fat or nutritional energy availability through adaptation of β-cell mass or function.

We conclude that nutritionally obese but otherwise normal rats are relatively resistant to developing type 2 diabetes mellitus despite prolonged lipid oversupply and insulin resistance.

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