High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass

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Kim, Jong-Yeon, Lorraine A. Nolte, Polly A. Hansen, Dong-Ho Han, Kevin Ferguson, Paul A. Thompson, and John O. Holloszy. High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass. Am J Physiol Regulatory Integrative Comp Physiol 279: R2057–R2065, 2000.—It has been variously hypothesized that the insulin resistance induced in rodents by a high-fat diet is due to increased visceral fat accumulation, to an increase in muscle triglyceride (TG) content, or to an effect of diet composition. In this study we used a number of interventions: fish oil, leptin, caloric restriction, and shorter duration of fat feeding, to try to dissociate an increase in visceral fat from muscle insulin resistance. Substituting fish oil (18% of calories) for corn oil in the high-fat diet partially protected against both the increase in visceral fat and muscle insulin resistance without affecting muscle TG content. Injections of leptin during the last 4 days of a 4-wk period on the high-fat diet partially reversed the increase in visceral fat and muscle insulin resistance, while completely normalizing muscle TG. Restricting intake of the high-fat diet to 75% of ad libitum completely prevented the increase in visceral fat and muscle insulin resistance. Maximally insulin-stimulated glucose transport was negatively correlated with visceral fat mass (P < 0.001) in both the soleus and epitrochlearis muscles and with muscle TG concentration in the soleus (P < 0.05) but not in the epitrochlearis. Thus we were unable to dissociate the increase in visceral fat from muscle insulin resistance using a variety of approaches. These results support the hypothesis that an increase in visceral fat is associated with development of muscle insulin resistance.

fish oil; food restriction; glucose transport; leptin; muscle triglycerides

THE ABDOMINAL OBESITY SYNDROME consists of central/visceral obesity, insulin resistance, hyperinsulinemia, and in its later stages, impaired or diabetic glucose tolerance (3, 4, 26, 27). This syndrome is the major cause of type 2 diabetes and greatly increases the risk of developing coronary heart disease (3, 4, 26, 27). Numerous studies have shown that insulin resistance correlates strongly with central/visceral obesity but not with lower body (i.e., hip, lower extremity) obesity. On the basis of these findings, it has been postulated that the insulin-resistance syndrome is caused by excessive accumulation of fat in intra-abdominal adipocytes (3, 12, 26). If this hypothesis is correct, then 1) interventions that prevent or reverse increased visceral fat accumulation should prevent or reverse insulin resistance, and 2) interventions known to protect against development of insulin resistance might be expected to do so by protecting against visceral fat accumulation. It has also been reported that muscle triglyceride content is increased in insulin-resistant humans and rats; this finding has led to the alternative hypothesis that an increased muscle triglyceride content is responsible for the insulin resistance (24, 28, 33, 35, 38). If this hypothesis is correct, muscle triglyceride content and insulin action on muscle should change in concert. The purpose of the present study was to evaluate these possibilities using rats fed a high-fat diet, which develop an insulin resistance syndrome that appears to be the rodent equivalent of the abdominal visceral obesity syndrome (17, 19). Rats and mice fed a high-fat diet have increased visceral fat accumulation, whole body and muscle insulin resistance, and hyperinsulinemia within 4 wk (16, 17, 29, 43). A high-fat diet also results in an increase in muscle triglyceride content in rats (35). If the high-fat feeding is continued for a sufficiently long period of time (6–8 mo), rats and mice develop severe visceral obesity, diabetes or impaired glucose tolerance, and plasma lipid and lipoprotein abnormalities (17, 39).

In this study, we addressed the following questions. Does fish oil, which has been reported to protect against the insulin resistance induced in rats by a high-fat diet (36), also protect against visceral fat accumulation? Does leptin administration, which has been reported to selectively decrease visceral fat (2), reverse the muscle insulin resistance that results from feeding a high-fat diet? Does caloric restriction, which protects against development of obesity, prevent the insulin resistance induced by a high-fat diet, or does fat feeding per se cause muscle insulin resistance? Do increased visceral fat accumulation and muscle insulin resistance develop in parallel, or does the insulin resistance precede an increase in visceral fat, as has been reported recently (1)? Does muscle glucose responsive-
ness to insulin vary in concert with muscle triglyceride content?

MATERIALS AND METHODS

Materials. 2-[1, 2-3H]deoxy-D-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO), and d-[1-14C]mannitol was obtained from NEN Life Science Products (Boston, MA). Insulin (Novolin) was purchased from Novo Nordisk (Princeton, NJ). All other reagents were obtained from Sigma Chemical (St. Louis, MO).

Treatment of animals. Male (~50 g) Wistar rats were obtained from Charles River and placed on either a high-fat or a rat chow diet for 4 wk. The high-fat diet was prepared using lard, corn oil, sucrose, and casein (32, 18, 27, and 23% respectively, of total calories), supplemented with vitamins (22 g/kg Teklad vitamin mix no. 40077), minerals (51 g/kg Teklad mineral mix no. 170915), and methionine (4.4 g/kg). The fish oil diet had the same composition as the high-fat diet except that 100 g menhaden oil (18% of total calories) was substituted for corn oil. The rat chow, Constant-Formula Purina Redent Chow no. 5001, was obtained from Purina Mills (St. Louis, MO); it contained as percentage of calories, 58.9% carbohydrate, 12.4% fat, and 28.7% protein. The energy content of the high-fat and high-fat/fish oil diets was 5.1 kcal/g, whereas that of the rat chow was 3.3 kcal/g. The rats were provided the diets and water ad libitum. This research was approved by the Animals Studies Committee of Washington University.

Leptin administration. Rats fed the high-fat diet were separated into two groups matched for body weight after 24 days on the diet. The animals in one group were given a daily injection of rat leptin (Research Diagnostics, NJ), 1 mg/kg body wt, subcutaneously, between 9:00 AM and 10:00 AM for the 4 days before the experiment. The control animals were given daily injections of phosphate buffer.

Tissue collection. Food was removed after 6:00 PM the day before the experiment. The following morning, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and blood samples for measurement of plasma glucose, insulin, and triglycerides were drawn from a tail vain, and the epididymal and soleus muscles were removed. Before incubation, the soleus muscle was split longitudinally into strips with an average weight of 20–25 mg.

Fat pad weights. After the muscle dissection was completed, the abdominal cavity was opened, and the epididymal, mesenteric, and retroperitoneal fat pads were removed and weighed.

Muscle incubations: effects of insulin. To allow recovery from the dissection and splitting procedures, muscles were incubated for 30 min at 35°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% bovine serum albumin (BSA). After the 30-min recovery period, epididymal muscles and soleus strips were incubated for 60 min at 35°C in 2 ml of KHB containing 8 mM glucose, 32 mM mannitol, and 0.1% BSA in the presence or absence of a maximally effective concentration of insulin (2 mU/ml) before measurement of 2-DG transport activity. Muscles were then washed for 10 min at 30°C in KHB containing 40 mM mannitol and 0.1% BSA, with or without insulin, to remove glucose from the extracellular space. The flasks were gassed with 95% O2-5% CO2 and shaken continuously in a Dubnoff incubator (Precision Scientific, Chicago, IL) during the incubations.

Measurement of 2-DG transport activity. Glucose transport activity was measured using 2-DG, as described previously (18). Muscles were incubated for 20 min at 30°C in 2 ml KHB containing 4 mM 2-[1,2-3H]DG (1.5 μCi/ml), 36 mM [1-14C]mannitol (0.2 μCi/ml), 0.1% BSA, and insulin if it was present in the previous incubation. Extracellular space and intracellular 2-DG concentration were determined as previously described (41).

Analytical procedures. Plasma glucose concentrations were determined using the glucose oxidase method, with a Beckman Glucose Analyzer II (Beckman Instrument, Fullerton, CA). Plasma insulin was measured by radioimmunoassay. Serum triglycerides concentration was measured using a kit obtained from Sigma Chemical. Muscle triglyceride concentration was determined by extracting total lipids from clamp-frozen muscle samples with chloroform-methanol (2:1 vol/vol) as described by Folch et al. (14), separating the chloroform and methanol-water phases, removing phospholipids, and further processing the sample using Frayn and Maycock’s (15) modification of the method of Denton and Randle (11). Triglycerides were then quantified spectrophotometrically as glycerol using an enzymatic assay kit (Sigma Chemical).

Statistical analysis. Values are expressed as means ± SE. The significance of differences among groups was evaluated using a one-way analysis of variance (ANOVA). When ANOVA showed significant differences, post hoc analysis was performed with the Newman-Keuls multiple range test. ANCOVA tests were performed using PROC GLM in SAS (SAS Institute, 1999).

RESULTS

Effect of the high-fat diet on insulin-stimulated muscle glucose transport. As in our previous studies (17, 19, 20), feeding rats a high-fat diet (50% of calories) resulted in a marked decrease in the insulin responsiveness of glucose transport in both the epitrochlearis and soleus muscles (Fig. 1). The increase in 2-DG transport above basal induced by a maximally effective insulin stimulus (2 mU insulin/ml) was ~55% smaller in epitrochlearis muscles of the fat-fed rats than in those of the chow-fed rats (1.55 ± 0.09 μmol·ml⁻¹·20 min⁻¹ for chow-fed group vs. 0.71 ± 0.04 μmol·ml⁻¹·20 min⁻¹ for the fat-fed group; P < 0.001) after 4 wk of the high-fat diet. The insulin-induced increase in 2-DG transport was similarly reduced in the soleus muscles of the group fed the high-fat diet (3.60 ± 0.1 μmol·ml⁻¹·20 min⁻¹ for chow-fed rats vs. 1.98 ± 0.12 μmol·ml⁻¹·20 min⁻¹ for fat-fed rats; P < 0.001).

Protection against high-fat diet-induced muscle insulin resistance: effect of fish oil. Replacement of the corn oil (18% of total energy) in the diet with fish (menhaden) oil provided significant, but only partial, protection against the muscle insulin resistance induced by 4 wk on the high-fat diet (Fig. 1). The insulin-induced increase in glucose transport activity in epitrochlearis muscles of the fish oil/high-fat diet group was 20% lower than in those of the chow-fed group (1.55 ± 0.09 μmol·ml⁻¹·20 min⁻¹ for chow fed group vs. 1.24 ± 0.12 μmol·ml⁻¹·20 min⁻¹ for fish oil-fed group, P < 0.05) compared with a 55% reduction in the high-fat fed group (P < 0.001). The fish oil also partially protected against the insulin resistance of glucose transport induced by the high-fat diet in soleus muscle. The insulin-induced increase in 2-DG transport was 20%
rats. * Daily injections of leptin, 1 mg/kg body wt, for the 4 days preceding the measurement of glucose transport improved insulin responsiveness of glucose transport in muscles of rats fed the high-fat diet (Fig. 1). In epitrochlearis muscles, the insulin-induced increase in 2-DG transport was reduced by 54% in the high-fat diet group and by only 21% in the high-fat diet, leptin-treated group (P < 0.05) (1.55 ± 0.09 μmol·ml⁻¹·20 min⁻¹ for the chow-fed group, 1.22 ± 0.11 μmol·ml⁻¹·20 min⁻¹ for the leptin-treated, high-fat diet group, and 0.71 ± 0.04 μmol·ml⁻¹·20 min⁻¹ for the high-fat diet group; P < 0.05, leptin-treated vs. nonleptin-treated, high-fat diet group). In the soleus, the insulin-induced increase in 2-DG transport was reduced by 46% in the high-fat diet group and by only 20% in the leptin-treated, high-fat diet group (3.66 ± 0.17 μmol·ml⁻¹·20 min⁻¹ for the chow-fed group, 2.92 ± 0.36 μmol·ml⁻¹·20 min⁻¹ for the leptin-treated, high-fat diet group, and 1.98 μmol·ml⁻¹·20 min⁻¹ for the high-fat diet group; P < 0.05, leptin-treated vs. nonleptin-treated, high-fat diet group).

Because leptin can have an appetite-suppressing effect that we wanted to avoid, and because rats eat most of their food during the night, we gave the leptin injections at 9 AM with the hope that the appetite-suppressing effect would have worn off by the following night. That this approach was reasonably successful is evidenced by the finding that the body weights of the rats fed the high-fat diet treated with leptin were not different from those not given leptin (Table 1), and food intake for the 4 days of leptin injections was reduced by only 8% (19.8 ± 0.8 g/day for high-fat diet group vs. 18.3 ± 0.5 g/day for the high-fat diet + leptin group).

**Body weights and visceral fat weights.** As shown in Table 1, there were no significant differences in body weight among the chow diet, high-fat diet, high-fat/fish oil diet, and high-fat diet + leptin treatment groups after 4 wk on the diets. [We have previously shown that body weights start to diverge, with the high-fat diet group becoming significantly heavier, after ~8 wk on the diet (17).] Total visceral fat mass was ~55% greater in the rats fed the high-fat diet than in the rats fed the regular chow after 4 wk (Table 1). Each of three visceral fat depots, mesenteric, epididymal, and retroperitoneal, was heavier in the fat-fed animals than in the chow-fed animals (data not shown). Inclusion of fish oil in the high-fat diet slowed the increase in visceral fat, so that at the end of the 4-wk diet period, visceral fat mass, although greater than in the chow-fed group, was smaller in the fish oil/high-fat diet group than in the high-fat diet group (Table 1). Daily injections of leptin for the last 4 days in rats on the high-fat diet caused a significant reduction in visceral fat mass, resulting in a total visceral fat mass that was intermediate between those of the chow-fed and the untreated high-fat diet groups (Table 1).

**Effect of caloric restriction.** There has been some uncertainty regarding the role of diet composition per se in the etiology of high-fat diet-induced muscle insulin resistance. As an approach to distinguish between an effect of diet composition per se and of visceral fat accumulation as factors in the development of muscle insulin resistance induced by a high-fat diet, we examined the effect of moderate caloric restriction. A group of rats was given 75% as much of the high-fat diet each
day as was eaten by an ad libitum fed high-fat diet group. As shown in Table 1, the calorie-restricted, high-fat diet group had a significantly lower body weight and total visceral fat mass than either the ad libitum chow-fed or high-fat diet fed rats. As shown in Fig. 2, the caloric restriction completely protected against the high-fat diet-induced development of insulin resistance of skeletal muscle glucose transport. It also lowered plasma triglyceride level and protected against the increase in plasma insulin (Table 2).

Plasma glucose, insulin, leptin, and triglyceride levels. As shown in Table 2, the high-fat diet resulted in a significant increase in plasma insulin concentration, which was not significantly affected by either the fish oil or leptin treatments. The high-fat diet had no significant effect on fasting plasma glucose concentrations after 4 wk. [Previous studies have shown that more prolonged high-fat feeding does result in the development of hyperglycemia in rodents (17, 19, 23, 39).] The high-fat diet did not significantly affect plasma triglyceride concentration. However, the fish oil had a triglyceride-lowering effect.

All of the groups on the high-fat diet had significantly elevated plasma leptin concentrations compared with the chow-fed group. The finding that the calorically restricted animals on the high-fat diet had an elevated leptin level is particularly interesting, because it provides evidence for an effect of diet per se, independent of an increase in body fat.

Effect of 2 wk on the high-fat diet. It has been reported that feeding rats a high-fat diet for 2 wk results in development of insulin resistance of muscle glucose transport before increased visceral fat accumulation (1). We, therefore, examined the effect of 2 wk of the high-fat diet to try to dissociate the effect of the high-fat diet on muscle insulin resistance from the increase in visceral fat. We found that 2 wk of high-fat feeding was too short a time period to result in a significant increase in visceral fat mass in rats fed the high-fat diet compared with control rats fed the regular chow. Total visceral fat averaged 2.7 ± 0.3 g in the chow-fed group and 3.1 ± 0.3 g in the high-fat diet group (means ± SE for 8 rats/group). However, 2 wk of eating the high-fat diet also had no significant effect on the magnitude of the increase in glucose transport induced by insulin in either the epitrochlearis or soleus muscle (Fig. 3).

Correlation between visceral fat mass and insulin-stimulated 2-DG transport. When the values for all of the 4 wk-long treatment groups are included in the analysis, there is a significant inverse correlation between visceral fat mass and insulin-stimulated 2-DG transport for the epitrochlearis (Fig. 4). There was also a strong inverse correlation between visceral fat mass and insulin-stimulated 2-DG transport in the soleus muscle.

Table 1. Effects of fish oil, leptin, and caloric restriction on body weight and visceral fat mass in rats fed the high-fat diet

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High Fat</th>
<th>High Fat/Fish Oil</th>
<th>High Fat + Leptin</th>
<th>High Fat-Restricted Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>243 ± 3 (31)</td>
<td>247 ± 2 (31)</td>
<td>244 ± 4 (15)</td>
<td>241 ± 4 (12)</td>
<td>199 ± 2§ (8)</td>
</tr>
<tr>
<td>Total visceral fat, g</td>
<td>7.3 ± 0.7* (31)</td>
<td>11.3 ± 0.4 (31)</td>
<td>9.3 ± 0.5† (15)</td>
<td>9.5 ± 0.4‡ (12)</td>
<td>4.8 ± 0.2§ (8)</td>
</tr>
</tbody>
</table>

Values are means ± SE for the number of rats shown in parentheses. Total visceral fat is the sum of the weights of the mesenteric, epididymal, and retroperitoneal fat depots. *P < 0.01 chow vs. all other groups; †P < 0.01 vs. high-fat diet group; ‡P < 0.05 vs. high-fat diet group; §P < 0.01 vs. all other groups.

Fig. 2. Insulin responsiveness of 2-DG transport in epitrochlearis (A) and soleus muscles (B) of male rats fed either rat chow or the high-fat diet ad libitum or 75% of the average amount of the high-fat diet eaten by the ad libitum-fed rats. To determine insulin responsiveness, muscles were incubated with 2 mM/ml insulin for 60 min before, as well as during, the measurement of 2-DG transport. Values are means ± SE for 7 or 8 animals per group. *P < 0.01 vs. chow-fed and calorie-restricted groups.
Correlation between muscle triglyceride content and insulin-stimulated 2-DG transport. Figure 5 shows the relationship between muscle triglyceride concentration and insulin-stimulated 2-DG transport in the soleus

(r = 0.715, \( P < 0.0001 \); data not shown). Before computation of these correlations, heterogeneity of correlation was examined; this was not significant in either epitrochlearis or soleus. This provides evidence that the correlation is unconditionally interpretable. In addition, quadratic effects of fat were not significant in either insulin locus [epitrochlearis, \( F = 1.15, \text{df (1,85)}, P = 0.2863 \); soleus \( F = 2.80, \text{df = (1,79)}, P = 0.0981 \].

Muscle triglyceride concentration. A number of investigators have found an inverse relationship between triglyceride concentration and insulin action in skeletal muscle (24, 33, 35). This finding has led to the hypothesis that increased accumulation of triglycerides in muscle, rather than in visceral adipocytes, is responsible for development of insulin resistance in response to a high-fat diet in rodents (35) and in insulin-resistant humans (24, 33). We, therefore, also examined the effects of the high-fat diet and of the fish oil and leptin intervention on triglyceride concentrations in the epitrochlearis and soleus muscles. As shown in Table 3, the high-fat diet did not have a significant effect on triglyceride concentration in the epitrochlearis. However, the four leptin injections caused a reduction in epitrochlearis triglycerides of rats fed the high-fat diet to a concentration significantly below that found in the high-fat diet and high-fat diet, fish oil groups (Table 3). In the soleus muscle, the high-fat diet resulted in a significant increase in triglyceride concentration that was not significantly influenced by inclusion of fish oil in the high-fat diet (Table 3). The leptin injections lowered soleus muscle triglyceride concentration in the rats fed the high-fat diet to the same level as was found in the rats fed the chow (Table 3).

**Table 2. Plasma glucose, insulin, triglyceride and leptin concentrations**

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>High Fat</th>
<th>High Fat/Fish Oil</th>
<th>High Fat + Leptin</th>
<th>High Fat-Restricted Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin, ( \mu \text{U/mL} )</strong></td>
<td>9.8 ± 0.9 (13)</td>
<td>17.2 ± 2.1† (22)</td>
<td>14.8 ± 1.7† (15)</td>
<td>16.9 ± 3.1† (8)</td>
<td>9.6 ± 2.0 (8)</td>
</tr>
<tr>
<td><strong>Glucose, mg/dL</strong></td>
<td>92 ± 3 (31)</td>
<td>96 ± 4 (31)</td>
<td>85 ± 5 (15)</td>
<td>98 ± 5 (8)</td>
<td>96 ± 3 (8)</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
<td>60 ± 3 (31)</td>
<td>63 ± 3 (31)</td>
<td>46 ± 2† (15)</td>
<td>61 ± 4 (8)</td>
<td>32 ± 3‡ (8)</td>
</tr>
<tr>
<td><strong>Leptin, ng/ml</strong></td>
<td>0.7 ± 0.1* (18)</td>
<td>2.0 ± 0.2 (22)</td>
<td>1.6 ± 0.1 (14)</td>
<td>1.6 ± 0.3 (8)</td>
<td>1.7 ± 0.3 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SE for the number of rats shown in parentheses. *\( P < 0.01 \) chow vs. all other groups. †\( P < 0.05 \) vs. chow or high-fat diet-restricted intake group. ‡\( P < 0.05 \) vs. chow, high-fat, or high-fat + leptin groups.

Fig. 3. Insulin responsiveness of 2-DG transport in epitrochlearis (A) and soleus muscles (B) of male rats fed either rat chow or the high-fat diet for 2 wk.
Table 3. Muscle triglyceride concentrations

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Chow</th>
<th>High fat</th>
<th>High fat/fish oil</th>
<th>High fat + leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epitrochlearis</td>
<td>4.3 ± 0.6</td>
<td>5.6 ± 1.0</td>
<td>5.3 ± 0.7</td>
<td>3.7 ± 0.2*</td>
</tr>
<tr>
<td>Soleus</td>
<td>7.6 ± 0.5*</td>
<td>11.5 ± 0.6</td>
<td>10.8 ± 1.0</td>
<td>7.5 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 muscles per group. *P < 0.05 vs. high-fat and high-fat/fish oil groups.

DISCUSSION

The concept that central/visceral obesity causes insulin resistance is largely based on the finding, in numerous studies, that these two phenomena are closely correlated (3, 4, 26, 27). However, the existence of a strong correlation does not prove a cause-effect relationship, and it is possible that visceral obesity develops in parallel with, and serves as a marker for, some other phenomenon that is the actual cause of the insulin resistance. Relative to this possibility, a number of investigators have found that muscle triglycerides are elevated in insulin-resistant humans and rats, leading to the alternative hypothesis that increased muscle triglyceride accumulation is responsible for the insulin resistance (24, 28, 33, 35, 38).

We have been testing the hypothesis that visceral fat accumulation is responsible for the insulin-resistance syndrome by using various interventions to try to dissociate visceral fat accumulation from insulin resistance. In support of the hypothesis, we found in a previous study (20) that dehydroepiandrosterone, which reduces fat accumulation in various rodent models of obesity (7–9, 40), largely protects against both the accumulation of visceral fat and development of muscle insulin resistance in rats fed a high-fat diet. Also in support of the hypothesis, we have shown that feeding rats a high-sucrose diet, which had been reported to cause muscle insulin resistance without increasing visceral fat (30–32, 37), does cause increases in both visceral fat and muscle insulin resistance comparable to those induced by our high-fat diet (25). The probable reason for the difference between our results (25) and those of the previous studies was that we compared our sucrose-fed animals to chow-fed controls and, because of the greater caloric density of the high-sucrose diet, the sucrose-fed rats had a higher energy intake than the chow-fed animals. In the previous studies, the control rats were fed a high-starch diet with the same high caloric density as the sucrose diet (30–32, 37).

It has been reported that replacement of a portion of the fat in the diet with fish oil, 12% of the total kilocalories in one study (36) and 18% of the total kilocalories in another (35), completely protected against the insulin resistance induced by feeding rats a diet that provided 59% of total energy from fat. In this context, we reasoned that if the insulin resistance induced by a high-fat diet is due to rapid visceral fat accumulation, fish oil should protect against the increase in visceral fat. Our results show that fish oil (18% of energy intake) does have a protective effect against both the visceral fat accumulation and muscle insulin resistance induced by a high-fat diet. This finding is compatible with the hypothesis that visceral fat accumulation is responsible for the insulin resistance. Our results also show that, although fish oil has a protective effect, it does not prevent, but only reduces or slows the increase in visceral fat and muscle insulin resistance.

A report that infusion of leptin for 8 days decreased visceral adiposity in 4-mo-old male rats (2) suggested another approach to testing the hypothesis that visceral fat accumulation mediates the insulin resistance that develops in fat-fed rats. This approach was made feasible by the finding that leptin has no direct effect on insulin-stimulated glucose transport in muscle (42). In the present study, four daily leptin injections induced a reduction in visceral fat mass in rats fed the high-fat diet for 4 wk to a level intermediate between those of chow-fed and high-fat diet-fed rats not given leptin. The leptin injections also partially reversed the insulin resistance of muscle glucose transport. This finding is compatible with the hypothesis that visceral fat accumulation plays a role in the development of muscle insulin resistance.

There has been some uncertainty regarding the role of diet composition per se in the etiology of high-fat diet-induced muscle insulin resistance via direct effects on cell membrane composition (5, 13, 34). As an
In contrast, Barnard et al. (1) found that insulin-stimulated glucose transport in epitrochlearis muscle, Barnard et al. measured glucose transport in a sarcolemmal vesicle preparation made from muscle homogenates by centrifugation procedures. Studies from Cartee’s laboratory have shown that food restriction of 8-mo-old (6) or 24-mo-old (10) female rats to either 75% or 52% of ad libitum intake for 20 days results in ~30% decreases in visceral fat mass and significant improvements in insulin-stimulated muscle glucose transport. In the present study, restricting intake of the high-fat diet to 75% of ad libitum resulted in a visceral fat mass that was smaller than that of the chow-fed controls and completely protected against the high-fat diet-induced decrease in insulin responsiveness of glucose transport. These findings provide evidence that a high-fat diet does not cause muscle insulin resistance unless energy intake is sufficiently high to result in increased visceral fat accumulation.

Perhaps the strongest published evidence that the muscle insulin resistance induced by a high-fat diet is mediated by the diet per se rather than by visceral fat accumulation is that of Barnard et al. (1). These investigators reported that feeding rats a high-fat diet for 2 wk caused muscle insulin resistance even though this treatment was too short to result in significantly increased visceral fat accumulation. The results of the present study do not confirm this finding. As in the study by Barnard et al. (1), 2 wk on a high-fat diet did not result in a significantly increased visceral fat mass. However, it also did not result in a significant decrease in insulin-stimulated muscle glucose transport. Thus the high-fat diet did not result in muscle insulin resistance before an increase in visceral fat accumulation. While it is impossible to be certain why our results differ from those of Barnard et al. (1), there was a major difference between the two studies. Whereas we measured insulin-stimulated glucose transport in intact muscle, Barnard et al. measured glucose transport in a sarcolemmal vesicle preparation made from muscle homogenates by centrifugation procedures. Studies of muscle glucose uptake in vivo and in intact muscles in vitro have shown that insulin resistance gets progressively worse in response to a high-fat diet (17, 19).

In contrast, Barnard et al. (1) found that insulin-stimulated glucose transport was decreased by ~20% in vesicles prepared from animals that had been on a high-fat diet for either 2 wk, 2 mo, or 2 yr. This difference, together with the present results, suggests that data obtained on the vesicle preparation do not accurately reflect the changes in insulin-stimulated glucose transport that are induced in muscle by a high-fat diet.

In addition to a role of visceral fat in the etiology of insulin resistance, there has been considerable interest in the possibility that an increased muscle triglyceride content is causally involved in muscle insulin resistance (24, 28, 33, 35). It has been reported that the degree of muscle insulin resistance in rats fed a high-fat diet is strongly correlated with accumulation of muscle triglycerides (35). Studies on Pima Indians (33) and insulin-resistant relatives of type 2 diabetics (24) have also suggested that muscle insulin resistance correlates with muscle triglyceride content. The results of the present study relative to an association between muscle triglycerides and insulin resistance are less clear cut. In the epitrochlearis muscle, the high-fat diet induced marked insulin resistance of glucose transport without a significant increase in muscle triglycerides.

In the soleus, the high-fat diet did result in a significant increase in triglyceride content, and there was a significant inverse correlation between soleus muscles’ triglyceride concentration and insulin-stimulated glucose transport activity. However, fish oil, which partially protected against both visceral fat accumulation and muscle insulin resistance, had no significant lowering effect on muscle triglyceride content in either the epitrochlearis or soleus muscle of the rats fed the high-fat diet. In contrast, four daily leptin injections, which improved insulin-stimulated muscle glucose transport and decreased visceral fat to roughly the same extent as did the fish oil, resulted in a remarkable lowering of muscle triglyceride content in fat-fed rats to values similar to those of the chow-fed controls. The correlation between soleus muscle triglyceride and glucose transport was considerably weaker ($r = -0.291; P < 0.05$) than that between visceral fat mass and 2-DG transport ($r = -0.726; P < 0.0001$). Taken together, these findings suggest that an increased muscle triglyceride content plays a less important role in causing the muscle insulin resistance induced by a high-fat diet than does the increase in visceral fat. It is of interest in this context that like a high-fat diet, exercise training can result in an increase in muscle triglycerides (22). However, in contrast to a high-fat diet, exercise improves insulin action (21).

Whereas 4 wk is not sufficiently long to bring about the increase in blood glucose that occurs in Wistar rats fed a high-fat diet (17, 19), fasting plasma insulin concentration is already significantly elevated (17, Table 2). Somewhat surprisingly, in view of the improvements in muscle insulin resistance and the smaller visceral fat mass in the fish oil and leptin-treated groups, neither of these interventions significantly affected the increase in plasma insulin induced by the high-fat diet. This finding raises the possibility that the high-fat diet has a direct effect on the regulation of plasma insulin in addition to the roles of muscle insulin resistance and increased visceral fat; this possibility will require more specific and detailed investigation. However, restricting intake of the high-fat diet to 75% of ad libitum completely protected against the increase in fasting plasma insulin, showing that energy intake plays a major role.

**Perspectives**

Rats fed a high-fat diet develop visceral obesity, muscle insulin resistance, and, if the high-fat diet is continued for sufficiently long, impaired glucose tolerance or diabetes. The rat fed a high-fat diet appears to be the rodent equivalent of the human abdominal obesity-insulin resistance syndrome. We have been using the high-fat diet-fed rat to investigate the factors in-
volved in the development of muscle insulin resistance in the earliest stages of a process that leads to obesity and type 2 diabetes. Despite a close correlation between abdominal/visceral obesity and insulin resistance in humans, there is also support for the hypothesis that an increase in muscle triglyceride content is responsible for the insulin resistance in fat-fed rodents and humans with insulin resistance. In this and previous studies we used various approaches to try to disassociate an increase in visceral fat from development of muscle insulin resistance. In the present study, we varied the amount of visceral fat that accumulated in response to a high-fat diet using a number of interventions: fish oil, leptin, caloric restriction, and shorter duration of fat feeding. These treatments did not result in disassociation of muscle insulin resistance from visceral fat mass, and there was a consistently good negative correlation between visceral fat mass and insulin responsiveness of muscle glucose transport. In contrast, our results did not provide support for a consistent relationship between muscle triglyceride content and insulin resistance.

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