Lipid metabolism and resistin gene expression in insulin-resistant Fischer 344 rats

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Levy, James R., Byrd Davenport, John N. Clore, and Wayne Stevens. Lipid metabolism and resistin gene expression in insulin-resistant Fischer 344 rats. Am J Physiol Endocrinol Metab 282: E626–E633, 2002; 10.1152/ajpendo.00346.2001.—The interrelationship between insulin and leptin resistance in young Fischer 344 (F344) rats was studied. Young F344 and Sprague-Dawley (SD) rats were fed regular chow. F344 animals had two- to threefold higher insulin and triglyceride concentrations and increased stores of triglycerides within liver and muscle. F344 animals gained more body fat. Both acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase I gene expression were 20–50% less in F344 animals than in age-matched SD animals. Peroxisome proliferator-activated receptor-α gene expression was reduced in 70-day-old F344 animals. Finally, resistin gene expression was similar in 70-day-old SD and F344 animals. Resistin gene expression increased fivefold in F344 animals and two-fold in SD animals from 70 to 130 days, without a change in insulin sensitivity. We conclude that young F344 animals have both insulin and leptin resistance, which may lead to diminished fatty oxidation and accumulation of triglycerides in insulin-sensitive target tissues. We did not detect a role for resistin in the etiology of insulin resistance in F344 animals.

Fischer 344 rats; acyl-coenzyme A oxidase; carnitine palmitoyltransferase I; peroxisome proliferator-activated receptor-α

INSULIN AND LEPTIN, intimately involved with intermediary metabolism and body weight homeostasis, appear to be physiologically linked. Insulin and leptin are secreted in the peripheral circulation by pancreatic islets and adipocytes, respectively, and they cross the blood-brain barrier (19). Both hormones have receptors within appetite centers in the hypothalamus and inhibit feeding (22). Finally, the secretion of both hormones appears to be regulated by glucose and the generation of intracellular energy (14). A close relationship also exists between insulin and leptin resistance. Nearly all individuals with type 2 diabetes are markedly insulin resistant, and the majority of them are obese and leptin resistant (2, 17). Most animal models of insulin resistance and type 2 diabetes mellitus have defects either in leptin secretion (e.g., as in the db/db mouse, Zucker fa/fa rat, diet-induced obesity; see Ref. 10). Morbid obesity and glucose intolerance are noted early in the life span of the animals that display these monogenetic mutations; therefore, they do not adequately represent the majority of human type 2 diabetes subjects in whom these diseases develop later in life.

The Fischer 344 (F344) rat has been used as a model of aging-induced insulin resistance (3). This animal strain was believed to become insulin resistant with aging, but it did not appear to gain appreciable amounts of weight or of body fat. However, recent studies in our laboratory disclosed that F344 animals gained more body fat than did a commonly studied insulin-sensitive animal strain [Sprague-Dawley (SD); see Ref. 15]. Not only did F344 rats ingest more calories per gram body weight but also they stored more calories as weight and they converted ingested calories into fat more efficiently than did the SD rats. The propensity of the F344 rats to gain body fat occurred at young ages (70 days), and it occurred despite higher fasting and meal-induced concentrations of leptin. These studies suggested to us that the F344 animals were resistant to the two main biological responses to leptin, namely the inhibition of appetite and the enhancement of energy expenditure.

Therefore, it appears that the F344 strain may be a good model of human diabetes mellitus. These F344 animals have leptin resistance without developing morbid obesity, and they develop diabetes late in their life span. The purpose of the present study was to define more rigorously the insulin sensitivity of young Fischer rats and to study the interrelationship between insulin resistance and leptin resistance. Several studies have suggested that physiological and cellular mechanisms that lead to the accumulation of lipids in insulin-sensitive target tissues (16, 18, 5) and in the pancreatic islets (12) may produce a clinical syndrome of insulin resistance and declining insulin secretory capacity; this syndrome is typical of type 2 diabetes mellitus. Leptin reduces intracellular accumulation of lipids in liver, muscle, and in the pancreatic islet (1, 8, 13, 20). Therefore, leptin resistance may exaggerate
the lipid accumulation in adipose and nonadipose tissue and worsen insulin resistance.

We have measured indexes of lipid accumulation within insulin-sensitive target tissues and the gene expression of factors and enzymes involved with mitochondrial and peroxisomal fatty acid oxidation in the leptin-resistant F344 strain. Furthermore, we tested the hypothesis that the newly discovered resistin gene links obesity and insulin resistance (21). We have found that the F344 animals are both leptin and insulin resistant. We have also found that this strain accumulates lipid excessively in liver and muscle and that the gene expression of factors and enzymes involved with fatty acid oxidation are reduced. However, we did not find that resistin gene expression played a role in the insulin resistance observed in F344 animals.

METHODS

Animals and diets. All animals were humanely treated, and the experimental protocols were reviewed and accepted by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Sixty-day-old SD and F344 rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and housed in individual cages under controlled lighting conditions on a natural dark-light cycle (1800–0600). The animals were allowed free access to food and water. All animals were fed a regular chow diet (Purina).

Serum and tissue measurements. Seventy- and 130-day-old SD (n = 10 at each age) and F344 (n = 10 at each age) animals were fasted overnight. One-half of the animals was killed (fasted), and the other one-half of the animals was fed a regular powdered chow (total 8 g) for 3 h and then killed (refed). Therefore, there were five animals in four experimental groups (70-day-old SD and F344 animals, fasted; 130-day-old SD and F344 animals, refed). Invariably, the animals ingested the entire 8 g of pelleted chow that were supplied. All animals were anaesthetized with isoflurane before they were decapitated. Blood was collected and saved on ice. After being spun in a centrifuge, the serum was isolated and saved in a −80 °C freezer. Liver, abdominal muscle, and the epididymal fat pad were quickly dissected and weighed, and then they were frozen in liquid nitrogen and stored in a −80 °C freezer.

Serum glucose was measured by an automated colorimetric glucose oxidase system (Vitros 700 system; Johnson and Johnson). Serum leptin and insulin were measured by RIA kits (Linco Research, St. Charles, MO). The limit of sensitivity and linearity for the rat leptin assay was 0.5 and 50 ng/ml, respectively. The intra-assay variation for the leptin assay was 0.5 and 50 ng/ml, and the intra-assay and linearity for the rat leptin assay was 0.5 and 50 ng/ml, respectively. The intra-assay variation for the rat leptin assay was 0.5 and 50 ng/ml, respectively. The intra-assay and linearity for the rat leptin assay was 0.5 and 50 ng/ml, respectively. The intra-assay variation for the rat leptin assay was 0.5 and 50 ng/ml, respectively.

Solutions for PCR differed only by the addition of various primers for each gene of interest and a housekeeping gene. The sense and anti-sense primers for each gene are as follows: peroxisome proliferator-activated receptor-α (PPARα), sense 5′-AGGCCATCTTCCACGATGCTG-3′ and anti-sense 5′-TCAGAGGTCCCTGAAACAGTG-3′ (25); acyl-Coa oxidase (ACO), sense 5′-CCCTCCAGTATGTTTACTAC-3′ and anti-sense 5′-AGGAACTGCTCCTCACACAGC-3′ (25); carnitine palmitoyltransferase I (CPT I), sense 5′-TATGTTGAGGATGCTGCTTC-3′ and anti-sense 5′-CTCGGAGAGCTAAGCTTGTC-3′ (25); resistin, sense 5′-GGAGTTGTTGCGCTTGGGTG-3′ and anti-sense 5′-CAGCATCTGGAGGGCA-3′ (9); enolase, sense 5′-TTTCAGATGCTGCCAGG-3′ and anti-sense 5′-GGTTGCGACAAACTTAGA-3′; β-actin, sense 5′-GTGAGGAGCCCAGAGCAAG-3′ and anti-sense 5′-AGGCGGACCTCATGATC-3′.

PCR conditions were carefully chosen to optimize amplification of the experimental and housekeeping cDNA and to limit the amplification of nonspecific cDNA. As shown in Fig. 1, amplification of each duplex cDNA was relatively linear within a range of cycle numbers. The range of linearity depended on the gene of interest and the conditions of PCR. When comparing gene expression between experimental groups, cycle number was chosen so that the amplification of the cDNA pair was well within the linear portion of the cycle number-quantity of DNA curve. The conditions of each duplex PCR are as follows: PPARα-enolase, 33 cycles of melt at 92 °C for 45 s, anneal at 57 °C for 45 s, extend at 72 °C for 60 s; ACO-actin, 22 cycles of melt 92 °C for 45 s, anneal at 55.5 °C for 45 s, and extend at 72 °C for 60 s; CPT I-actin, 24 cycles of melt at 92 °C for 45 s, anneal at 54 °C for 45 s, and extend at 72 °C for 60 s.

When we compared the gene expression between experimental groups, we carefully prepared the master mixes. All PCR solutions were pipetted at the same time, were amplified in the same run in the thermocycler (MJ Research thermocycler), and were analyzed on the same agarose gel. Solutions for PCR differed only by the addition of various cDNAs.

Forty percent of the PCR reaction (total volume = 50 μl) was analyzed on a 1.5% agarose gel with ethidium bromide. The gel was placed in a Luminescent Image Analyzer (LAS-1000; Fujifilm), and the densities (light arbitrary units/mm²) of the amplified DNA were assessed by the Advanced Image Data Analyzer software (AIDA version 2.0). The abundance of the expressed gene was calculated by dividing the density...
of the amplified experimental gene by the density of the amplified housekeeping gene in the same sample.

Statistical analysis. We performed a two-way ANOVA (GraphPad Prism Version 3.0; GraphPad Software) to compare the effects of the fed state (fasting vs. refeeding) or strain (SD vs. F344) on various indexes of weight, body composition, and insulin sensitivity. We performed an unpaired t-test (GraphPad Prism version 3.0) when an experimental parameter in one strain of animal was compared with the other strain of animal.

RESULTS

Seventy-day-old SD and F344 animals were fed regular rat chow ad libitum for 60 days. Indexes of weight and body composition are shown before (Table 1) and after (Table 2) the 60-day feeding period. In one-half of the animals, measurements were obtained in animals fasted overnight (fast). In the other one-half of the animals, measurements were obtained in animals that were fasted overnight and then refed regular chow for 3 h (refed). Age-matched SD rats are larger than F344 rats, and both strains gain ~1.5-fold their body weight in 60 days when eating regular chow. However, the F344 animals are fatter than the SD animals. At 60 days, the epididymal fat pad is ~15% larger in F344 animals than for the SD, and body composition, and glucose and leptin sensitivity in 70-day-old animals

Table 1. Effect of fasting and refeeding on indexes of weight, body composition, and glucose and leptin sensitivity in 70-day-old animals

<table>
<thead>
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<th>SD</th>
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<tr>
<td></td>
<td>Fast</td>
<td>Refed</td>
<td>Fast</td>
<td>Refed</td>
<td></td>
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<tr>
<td>Weight, g</td>
<td>283 ± 3.8</td>
<td>279 ± 4.6</td>
<td>199 ± 1.8*</td>
<td>207 ± 1.6</td>
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</tr>
<tr>
<td>Fat pad, g</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.5 ± 0.05*</td>
<td>2.6 ± 0.1</td>
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<tr>
<td>TG, mg/mg</td>
<td>64.5 ± 6.2</td>
<td>NA</td>
<td>59.7 ± 8.9</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>199.9 ± 43</td>
<td>NA</td>
<td>300 ± 22†</td>
<td>NA</td>
<td></td>
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<tr>
<td>Liver</td>
<td>59 ± 3.1</td>
<td>156 ± 5.1</td>
<td>119 ± 2.4*</td>
<td>164 ± 2.2‡</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>0.2 ± 0.02</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.03*</td>
<td>1.3 ± 0.3‡</td>
<td></td>
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<tr>
<td>FFA, meq/l</td>
<td>0.6 ± 0.03</td>
<td>0.3 ± 0.02</td>
<td>0.48 ± 0.04*</td>
<td>0.28 ± 0.02‡</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>ND</td>
<td>ND</td>
<td>0.63 ± 0.04</td>
<td>1.25 ± 0.2</td>
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<tr>
<td>TG in serum, mg/dl</td>
<td>39 ± 2.6</td>
<td>35.8 ± 1.9</td>
<td>47.8 ± 3.5*</td>
<td>82.4 ± 3.9‡</td>
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</table>

Values are means ± SE; n = 5 rats in each group. SD, Sprague-Dawley; F344, Fischer 344; TG, triglyceride; FFA, free fatty acid. Seventy-day-old SD or F344 animals were either killed after fasting overnight or after refeeding for 3 h as specified in METHODS. Total body weight, epididymal fat pad weight, triglycerides in muscle and liver, serum glucose, insulin, free fatty acids, leptin, and triglycerides were measured as described in METHODS. In a separate experiment, leptin concentrations were measured in 3 age-matched SD animals that were fasted overnight. The serum was concentrated by lyophilization before the leptin concentration was assessed. Correcting for the concentration factor, the leptin level in fasting SD animals was estimated to be 0.64 ± 0.01 ng/ml, a value close to the lower detection limit of the leptin assay. NA, not assessed; ND, none detected. P < 0.001, strain difference, 2-way ANOVA (*), unpaired t-test, compared to SD fasted (†), fed state difference, 2-way ANOVA ‡, and unpaired t-test, compared to SD refed (§); interaction in 2-way ANOVA analysis.

3 h (refed). Age-matched SD rats are larger than F344 rats, and both strains gain ~1.5-fold their body weight in 60 days when eating regular chow. However, the F344 animals are fatter than the SD animals. At 60 days, the epididymal fat pad is ~15% larger in F344 animals than for the SD, and body composition, and glucose and leptin sensitivity in 130-day-old animals

Table 2. Effect of fasting and refeeding on indexes of weight, body composition, and glucose and leptin sensitivity in 130-day-old animals

<table>
<thead>
<tr>
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<th>SD</th>
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<tbody>
<tr>
<td></td>
<td>Fast</td>
<td>Refed</td>
<td>Fast</td>
<td>Refed</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>424 ± 6.0</td>
<td>422.8 ± 7.6</td>
<td>291 ± 5.9*</td>
<td>288 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>Fat pad, g</td>
<td>4.3 ± 0.6</td>
<td>4.1 ± 0.3</td>
<td>6.8 ± 0.2*</td>
<td>5.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>TG, mg/mg</td>
<td>48.1 ± 5</td>
<td>NA</td>
<td>99.2 ± 11†</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>204 ± 16</td>
<td>NA</td>
<td>367.7 ± 22†</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>120 ± 3.5</td>
<td>156 ± 5.2</td>
<td>124 ± 3.1</td>
<td>159 ± 2.0‡</td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>0.2 ± 0.02</td>
<td>0.7 ± 0.07</td>
<td>0.7 ± 0.07*</td>
<td>1.3 ± 0.1‡</td>
<td></td>
</tr>
<tr>
<td>FFA, meq/l</td>
<td>0.42 ± 0.04</td>
<td>0.22 ± 0.01</td>
<td>0.4 ± 0.03</td>
<td>0.26 ± 0.02‡</td>
<td></td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>ND</td>
<td>ND</td>
<td>1.6 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>TG in serum, mg/dl</td>
<td>38.2 ± 4.4</td>
<td>37.4 ± 4.0</td>
<td>66.5 ± 4.0</td>
<td>102 ± 11‡</td>
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</table>

Values are means ± SE; n = 5 rats in each group. One-hundred thirty-day-old SD or F344 animals were either killed after fasting overnight or after refeeding for 3 h as specified in METHODS. Total body weight, epididymal fat pad weight, triglycerides in muscle and liver, serum glucose, insulin, free fatty acids, leptin, and triglycerides were measured as described in METHODS. P < 0.001, strain difference, 2-way ANOVA (*), unpaired t-test, compared to SD fasted (†), and fed state difference, 2-way ANOVA ‡.
animals compared with SD animals (Table 1). In the
60-day feeding period, the fat pad weight in SD animals
increased by ~1.9-fold, whereas the fat pad weight in
F344 animals increased between 2.2- and 2.7-fold (Table
2). As previously demonstrated in F344 animals by our
laboratory, epididymal fat pad weight correlates well
with total body adiposity, as measured by dual-energy
X-ray absorptiometry scanning (15). Fat storage in
nonadipocytic tissue was also higher in F344 animals
than in SD animals. Liver triglyceride content was
higher in 70-day-old F344 animals than in age-
matched SD animals (Table 1); after 60 days, the mus-
cle and liver triglyceride content increased fractionally
more in F344 animals than in SD animals. Muscle and
liver triglyceride content were both higher in 130-day-
old F344 animals than in SD animals (Table 2).

Also shown in Tables 1 and 2 are indexes of insulin
and leptin sensitivity. Serum glucose concentrations
were appropriately higher in refed animals compared
with fasted animals. However, fasting glucose concen-
trations were minimally higher in 70-day-old F344
animals than in SD animals (Table 1) but were no
different in 130-day-old animals (Table 2). However,
the differences in insulin concentration were marked.
Insulin concentrations in both fasted and refed F344
animals were approximately two- to threefold higher
than in fasted and refed SD animals in both 70 (Table
1)- and 130 (Table 2)-day-old animals. Fasting serum
triglyceride concentrations were higher in 70-day-old
(Table 1) and 130-day-old (Table 2) F344 animals than
in SD animals. In contrast to SD animals, the trigly-
ceride concentration in F344 animals increased mark-
edly with refeeding (Tables 1 and 2). In addition, fast-
ing triglycerides in F344 animals increased over the
60-day experiment, whereas fasting triglycerides in SD
animals did not change (compare Tables 1 and 2).
Fasting free fatty acids were minimally elevated in
70-day-old SD animals compared with age-matched
F344 animals (Table 1). There was no detectable dif-
fERENCE in fasting free fatty acid between 130-day-old
SD and F344 animals (Table 2). Serum free fatty acid
decreased postprandially in both strains because of
insulin-mediated inhibition of lipolysis. The serum lep-
tin concentrations were not detectable in the young SD
animals, which had small fat stores. This finding is
consistent with our previous in vivo data (15). Serum
leptin concentrations in 70-day-old F344 animals were
measurable, and they increased by ~100% with refeed-
ing (Table 1). After 60 days, the leptin concentration in
F344 animals continued to a rise in fasted animals, and
the concentration was responsive to refeeding. Note
that, despite the high concentrations of this appetite-
suppressing hormone, F344 animals still gained more
triglyceride stores in both adipose and in nonadipose
tissue than in SD animals. This observation is consist-
tent with leptin resistance in the F344 animals.

To better characterize insulin sensitivity, intrave-
nous glucose tolerance tests were performed in 70- and
130-day-old animals from both strains. The results are
shown in Fig. 2. Fasting and peak glucose concentra-
tions were similar in 70-day-old SD and F344 animals
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tissue than in SD animals. This observation is consist-
tent with leptin resistance in the F344 animals.

To better characterize insulin sensitivity, intrave-
nous glucose tolerance tests were performed in 70- and
130-day-old animals from both strains. The results are
shown in Fig. 2. Fasting and peak glucose concentra-
tions were similar in 70-day-old SD and F344 animals
(Fig. 2, top left). Glucose concentrations at 15 and 20
min were greater in 70-day-old SD than in age-
matched F344 animals (Fig. 2, top left). The area under
the curve (AUC) for glucose was ~50% higher (P <
0.05) in SD animals than in F344 animals (3,117.2 ±
94 vs. 2,125.4 ± 342 mg/dl, respectively). The insulin
response to an intravenous bolus of glucose was mark-
edly different. Fasting insulin was approximately
threefold higher in the 70-day-old F344 rats than in
age-matched SD rats (Fig. 2, bottom left). Peak insulin
concentrations were approximately fivefold higher in
the F344 than in the SD animals, and the insulin AUC
was ~2.7-fold higher (P < 0.001) in the F344 animals
than in the SD animals (66.8 ± 3.4 vs. 24.5 ± 1.9 ng/ml,
respectively). The ratio of the insulin AUC to glucose
AUC in 70-day-old F344 animals vs. SD animals was
33 ± 4 vs. 7.7 ± 0.54 × 10⁻³ (P < 0.001). Therefore,
to produce similar serum glucose concentrations after an
intravenous glucose infusion, F344 animals require
markedly higher insulin concentrations than do SD
animals. This finding is consistent with insulin resis-
tance in the F344 animals, a state that has not been
recognized previously in these young animals. There
were no statistical differences in the glucose (Fig. 2,
top right) and insulin (Fig. 2, bottom right) responses to
intravenous glucose in 130-day old animals compared
with 70-day-old animals in both strains.

A mechanistic link between the leptin and insulin
resistance observed in F344 animals may involve dys-
regulation of enzymes involved in fatty acid oxidation.
Therefore, we compared the relative gene expression of
transcription factors and enzymes involved with fatty
acid oxidation in the livers from fasted SD and F344
animals. The gene expression of the transcription fac-
tor PPARα was ~1.8-fold greater in 70-day-old SD rats
than in age-matched F344 rats (Fig. 3). PPARα stim-
ulates the gene expression of several enzymes involved
with fatty acid oxidation. These enzymes include the
rate-limiting enzyme ACO in peroxisomal lipid oxida-
tion and the protein CPT I, which transports fatty
acids for oxidation in mitochondria. The ACO gene
expression was somewhat less in 70-day-old F344 an-
imals than in SD animals. The gene expression of CPT
I in 70-day-old SD animals was 1.5-fold greater than
the CPT I gene expression in age-matched F344 an-
imals. In the 130-day-old animals, PPARα gene expres-
sion was greater in the F344 animals than in the SD
animals. However, the gene expression of ACO and
CPT I in 130-day-old SD animals was more than two
times as great as the gene expression of ACO and CPT
I in age-matched F344 animals.

The effects of fasting and refeeding on gene expres-
sion of proteins involved in fatty acid oxidation were
assessed in both 70-day-old (Table 3) and 130-day-old
(Table 4) animals. The gene expression of PPARα in
fasted animals was ~1.5- to 1.75-fold greater than the
gene expression of PPARα in refed animals in both
strains and ages of rat. However, the ratio of fasted to
refed ACO and CPT I gene expression was significantly
greater in SD animals than in age-matched F344 an-
imals.
Finally, recent studies in experimental animals have shown that resistin gene expression increases with adiposity and with insulin resistance (21). We therefore measured the gene expression of resistin in the insulin- and leptin-resistant F344 strain and the more insulin- and leptin-sensitive SD strain. As shown in Fig. 4, left, resistin gene expression was slightly lower in fasted, 70-day-old F344 animals than in fasted, 70-day-old SD animals. We did not detect a statistically significant difference in resistin gene expression between fasted and refed 70-day old animals. After 2 mo, the resistin gene expression increased by ~1.6-fold ($P < 0.001$) in SD animals. By contrast, in F344 animals, the resistin gene expression increased by ~5.0-fold ($P < 0.0001$) in the 130-day-old animals than in the 70-day-old animals. In 130-day-old animals, resistin gene expression in the F344 strain was significantly greater than the resistin gene expression in the SD strain in the refed state. In the fasted state, there was not a statistically significant difference in resistin gene expression ($P = 0.07$).

**DISCUSSION**

In the present study, we have characterized an inbred rodent strain that we believe represents a good model for typical type 2 diabetes in humans. We have found that young F344 animals with normal glucose tolerance have evidence of insulin resistance (Fig. 2), leptin resistance (Tables 1 and 2), dyslipidemia (fasting and postprandial hypertriglyceridemia; Tables 1 and 2), and excess triglyceride accumulation in insulin-sensitive target tissues such as liver and muscle (Tables 1 and 2). These constellations of findings are consistent with a metabolic syndrome that is observed in young animals with normal glucose tolerance, and they probably represent a genetic risk for diabetes mellitus that occurs later in life.

We also investigated the link between insulin resistance and the propensity for obesity in F344 animals. Because leptin has been shown to stimulate fatty acid oxidation and reduce triglyceride storage in nonadipose tissue (23), we hypothesized that leptin resistance
in F344 animals would diminish fatty acid oxidation and enhance triglyceride storage in nonadipose tissue. Although we did not directly measure fatty acid oxidation, we did measure the gene expression of several key regulatory proteins in fatty acid oxidation. We have demonstrated that the hepatic gene expression of ACO and CPT I is consistently less in the leptin- and insulin-resistant F344 animals than in age-matched SD animals (Fig. 3). ACO and CPT I are critical regulatory proteins in the fatty acid oxidation pathway in peroxisomes and mitochondria, respectively. The gene expression of both proteins is regulated, in part, by the transcription factor PPARα. Consistent with this observation, we have found that the gene expression of PPARα is less in 70-day-old F344 rats than in age-matched SD rats. Diminished PPARα gene expression in the leptin-resistant F344 animal supports the findings by Zhou et al. (25) that leptin normally stimulates PPARα gene expression. However, we did not find that PPARα gene expression was less in the 130-day-old F344 rats than in SD rats. The reason for this apparent discrepancy is unknown. However, despite increased

Fig. 3. Gene expression of proteins involved in fatty acid oxidation. Total RNA was extracted from liver in 70-day-old animals (left) and 130-day-old animals (right) before reverse transcription and duplex PCR as described in methods. Each bar represents the mean ± SE (n = 5 in each group). Statistical comparison performed by unpaired t-test.

Table 3. Seventy-day-old animals, fasted vs. refed

<table>
<thead>
<tr>
<th></th>
<th>Sprague-Dawley</th>
<th>Fischer 344</th>
<th>Fasted/refed</th>
<th>Fasted/refed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>1.68 ± 0.13</td>
<td>0.93 ± 0.14</td>
<td>1.62</td>
<td>1.75</td>
</tr>
<tr>
<td>ACO</td>
<td>0.9 ± 0.05</td>
<td>0.51 ± 0.04</td>
<td>3</td>
<td>1.54</td>
</tr>
<tr>
<td>CPT I</td>
<td>0.44 ± 0.03</td>
<td>0.2 ± 0.006</td>
<td>14.6</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>0.03 ± 0.01†</td>
<td>0.09 ± 0.01†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 animals in each group. PPARα, peroxisome proliferator-activated receptor-α; ACO, acyl-CoA oxidase; CPT I, carnitine palmitoyltransferase I. Experiment was performed as described in Fig. 2. *P < 0.05 and †P < 0.01 by unpaired t-test.

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PPARα gene expression, 130-day-old F344 animals had diminished downstream expression of regulatory proteins in fatty acid oxidation. Two reasons might account for an increase in PPARα but a decrease in ACO and CPT I gene expression. First, PPARα transcription activity not only depends on the amount of the gene product but also on the binding of its endogenous ligand, purportedly a fatty acid. PPARα transcriptional activity could be reduced in the 130-day-old F344 animals if the intrahepatic fatty acid composition was different and the activated PPARα gene transcribed less well than age-matched SD animals. Second, PPARα is not the only transcriptional activator of ACO and CPT I; other PPARα-independent transcriptional inhibitors may play a role in the gene expression of ACO and CPT I in F344 animals.

We believe that the reduction in gene expression of ACO and CPT I in F344 animals probably reduced hepatic fatty acid oxidation and increased the propensity to store fatty acids as triglycerides. We found that liver contains more triglycerides in F344 animals than in SD animals (Tables 1 and 2). Muscle contains more triglycerides in 130-day-old F344 animals than in age-matched SD animals (Table 2). The enhanced triglyceride storage in nonadipose tissue resulted in insulin resistance (Fig. 2) and its associated disturbance of lipid accumulation within insulin-sensitive target tissues, whereas the failure to fully suppress lipid oxidation during refeeding may be a manifestation of the relative resistance to insulin.

Finally, we have examined the role of resistin in the etiology of insulin resistance in F344 animals. Resistin gene expression and protein secretion are increased in both genetic and diet-induced models of obesity and diabetes (21). Furthermore, resistin appears to antagonize the effects of insulin both in vivo and in vitro (21). Such an antagonism might provide a link between obesity and insulin resistance. In vivo administration of resistin antiserum to diet-induced obese mice improved blood glucose levels and insulin sensitivity (21).

**Table 4. One hundred thirty-day-old animals, fasted vs. refeed**

<table>
<thead>
<tr>
<th>Sprague-Dawley</th>
<th>Fischer 344</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>Refed</td>
</tr>
<tr>
<td>PPARα</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>ACO</td>
<td>1.23 ± 0.07</td>
</tr>
<tr>
<td>CPT I</td>
<td>0.48 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 rats in each group. Experiment was performed as described in Fig. 2. *P < 0.05 and †P < 0.01 by unpaired t-test.
Conversely, administration of recombinant resistin impairs glucose tolerance and insulin action in normal mice. Resistin’s role in obesity and insulin resistance has not been confirmed by other investigators (24). We have hypothesized that resistin gene expression in the insulin-resistant, obesity-prone F344 rats would be higher than in SD rats. Our results do not support this hypothesis. The resistin gene expression in insulin-resistant, 70-day-old F344 animals was less than in age-matched SD animals (Fig. 4). At 70 days, the difference in the amount of stored fat in the epididymal fat pad (Table 1) or in total body adiposity (15) differs only slightly between the two strains. After 60 days of a low-fat diet, F344 rats gain markedly more fat, without much change in insulin resistance, as measured by the intravenous glucose tolerance test. Despite almost equal glucose tolerance between 70- and 130-day-old F344 animals, the expression of the resistin gene increases dramatically. From these observations, we must conclude that resistin gene expression is not involved in the etiology of insulin resistance in F344 animals. Resistin gene expression does increase proportionally to the gain in total body fat, but animals do not become progressively more insulin resistant in this 2-mo period. Resistin gene expression is another marker for total body adiposity in both SD and F344 strains.

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REFERENCES