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# High-fat diets promote insulin resistance through cytokine gene expression in growing female rats☆

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**Abstract**

To determine if tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6). IL-6 gene expression is influenced by amount and source of dietary fat, 30 weanling female rats were randomly assigned to a moderate-fat soybean oil (MFS; 22% of total energy fed as fat), high-fat (HF) soybean oil (HFS; 39% of total energy fed as fat), or HF tallow (HFT; 39% of total energy fed as fat) diet treatments. Oral glucose tolerance tests (OGTT) were conducted serially over 10 weeks of treatment. HFT and HFS rats gained more weight and had greater body fat than the MFS rats fed similar amounts of energy. Both groups of HF-fed rats had greater ($P<.05$) insulin resistance (homeostasis model assessment) than MFS-fed rats. TNF-α mRNA abundance quantified by real-time reverse transcriptase-polymerase chain reaction was greater ($P<.05$) in liver and lower ($P<.05$) in adipose tissue in HFT compared to HFS and MFS rats. There were positive correlations ($P<.05$) between hepatic TNF-α mRNA and insulin resistance, and negative correlations between insulin sensitivity and hepatic TNF-α mRNA and hepatic IL-6 mRNA. During Week 3 and Week 6 OGTTs, hyperinsulinemic responses were observed in the HFT group, after which, on Week 9, insulin secretion was diminished in response to the OGTT, suggesting impaired pancreatic insulin secretion. HFS rats exhibited insulin resistance on Week 9 OGTT. In summary, an HFT diet fed to growing female rats caused insulin resistance associated with increased hepatic TNF-α mRNA leading to pancreatic insufficiency. Early-onset insulin resistance related to the inflammatory process in obesity is influenced by the amount and type of fat in the diet.


*Keywords:* Insulin resistance; Adipose; Liver; Muscle; Interleukin-6; Tumor necrosis factor-α

## 1. Background

In humans, consuming high-fat (HF) diets causes an increase in body fat deposition and a decrease in insulin sensitivity, which ultimately leads to an increased risk of developing type 2 diabetes mellitus [1–3]. In particular, Mayer-Davis et al. [2] observed an inverse relationship between total fat intake and insulin sensitivity in adult obese subjects. Lovejoy et al. [3] observed significant improvement in insulin sensitivity in adult obese subjects after they crossed over from HF (50% of total energy coming from fat) to low-fat (i.e., 20% of total calories from fat) diets. Unfortunately, the incidence of obesity continues to increase in children and adolescents, as well as adults, resulting in less than optimal quality of life and increased health care costs [4–6].

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The mechanism underlying the cause of obesity-induced insulin resistance involves numerous body systems, organs, and tissues including tissue derived inflammatory factors. Studies in mature rodents and humans indicate that tumor necrosis factor-α (TNF-α) may be involved in insulin resistance associated with adiposity [7–9]. The mechanism of TNF-α induced insulin resistance involves inhibition of insulin receptor signaling and glucose transport within insulin-sensitive cells [8,9]. More recently, other cytokines such as interleukin-6 (IL-6) have been implicated in obesity-induced insulin resistance [10,11]. The source of these cytokines is unclear but may include adipose tissue, skeletal muscle, and liver in addition to immune cells [10–13]. A majority of these studies used mature animals and compared diets formulated with extremely low (<20% of daily energy) or extremely high (>40% of daily energy) concentrations of dietary fat which are far from the US Dietary Guideline recommendation of 30% energy from fat daily. More common fat consumption levels in human diets contain 34–37% of total energy from fat. Thus, additional research is needed to evaluate effects of dietary fat consumption at levels more typical of humans as well as to evaluate these dietary effects during peripubertal growth.

In addition to cytokines, dietary lipid content and source may influence metabolic responses to hormones such as insulin [14,15]. Epidemiological studies provide evidence linking high saturated fat intake with hyperinsulinemia and the increased risk of diabetes. In Japanese children, the increase in the rate of Type 2 diabetes was associated with an increased intake of animal protein and fat without significant changes in total energy intake [16]. In the Zutphen Elderly Study [17], serum insulin concentrations were inversely associated with the intake of dietary fiber and polyunsaturated fatty acids and increased with increasing intake of saturated fatty acids and alcohol. In obese non-insulin-dependent diabetes mellitus patients, postprandial serum insulin, and C-peptide responses were greater in subjects who consumed high transmonounsaturated fatty acid or high saturated fatty acid diets compared to baseline or *cis*-monounsaturated fatty acid diets [18]. Utilizing the hyperinsulinemic euglycemic clamp to study insulin sensitivity, rats fed diets high in saturated fat required less glucose infusion to maintain euglycemia compared to rats fed high (i.e., >50% energy as fat) monounsaturated or polyunsaturated fats, indicating decreased insulin sensitivity [19]. Although studies clearly indicate that HF diets increase insulin resistance in humans [1–3] and rats [20–25], further study is needed to evaluate the effect of the type and quantity of fat in diets on endocrine and cytokine responses.

In the present study, we set out to determine the effects of HF soybean (HFS) oil diets, HF tallow (HFT) diets, and moderate-fat soybean (MFS) oil diets on insulin resistance and TNF-α and IL-6 gene expression in adipocytes, muscle, and liver tissues of growing female rats. The HF diets were designed to enable comparisons between amounts of dietary fat (i.e., 20–40% of total energy from fat) more commonly consumed by humans [3]. Thus, the objective of the current study was to compare the effects of amount and source of dietary fat on TNF-α and IL-6 gene expression in various tissues, glucose disposal, and insulin and leptin secretion during the peripubertal growth period. We hypothesized that rats fed HFT diets will have higher levels of TNF-α and IL-6 gene expression in adipose, muscle, and liver tissue and have decreased insulin sensitivity compared to rats fed HFS and MFS diets.

## 2. Methods

Thirty weanling Sprague–Dawley female rats were randomly assigned to HFT (39% of total energy fed as fat, 20% fat by weight; $n$=10), HFS (39% of total energy fed as fat, 20% fat by weight; $n$=10), or MFS (22% of total energy fed as fat, 10% fat by weight; $n$=10) diet treatments in an incomplete 2×2 factorial experimental design for 10 weeks (Table 1). The soybean oil used for the HFS diet contained 14% saturated and 59% polyunsaturated fats, and the tallow used for the HFT diet contained 50% saturated and 9% polyunsaturated fats. Diets were prepared to provide equal vitamins and minerals per unit of energy and contain equal percentages by weight of protein and fiber providing 0.01 g protein per kilojoule (J). For the first 2 weeks of the study, rats consumed an average of 222 J (53 kcal) per day, and for

Table 1
Composition and energy values of experimental diets

| | g/kg diet | | |
|---|---|---|---|
| | MFS | HFS | HFT |
| Casein | 200 | 200 | 200 |
| Cornstarch | 100 | 100 | 100 |
| Sucrose | 500 | 400 | 400 |
| Cellulose | 50 | 50 | 50 |
| Soybean oil | 100 | 200 | 0 |
| Beef tallow | 0 | 0 | 200 |
| Mineral mix [a] | 35 | 39.3 [b] | 39.3 [b] |
| Vitamin mix [c] | 10 | 11.2 [b] | 11.2 [b] |
| L-Cysteine | 3 | 3 | 3 |
| Choline bitartrate | 2 | 2 | 2 |
| *% Total energy* | | | |
| Carbohydrates | 58 | 43 | 43 |
| Fat | 22 | 39 | 39 |
| Protein | 20 | 17 | 17 |
| *kJ/g Diet* | | | |
| Energy density | 17 | 19 | 19 |

[a] Mineral mix composition, g/kg mix: $CaCO_3$, 357; $KH_2PO_4$,196; potassium citrate, 70.78; NaCl, 74; $K_2SO_4$, 46.6; MgO, 24; $FeCl_2$–$6H_2O$, 3.6; $ZnCO_3$, 1.65; $MnCO_3$, 0.63; $CuCO_3$, 0.3; $KIO_3$, 0.01; $Na_2SeO_4$, 0.01; $NH_4MoO_4$–$H_2O$, 0.008; $Na_2SiO_2$, 1.45; $LiCl_2$, 0.017; $H_3BO_3$, 0.08; NaF, 0.064; $NiCO_3$, 0.032; $NH_4VO_3$, 0.0066.

[b] Mineral mix and vitamin mix were adjusted to provide equal amounts of micronutrients per unit of energy, resulting in a 0.55% increase in the total weight of the mixed HF diets (1.0055 kg instead of 1 kg).

[c] Vitamin mix obtained from Teklad, Madison, WI, USA, catalog #40060.

the last 8 weeks, rats consumed an average of 272 J (65 kcal) per day. Feed intake was measured for the 24-h period preceding each body weight measurement and adjusted as needed so that all rats consumed approximately equal amounts of energy. The rats were weighed weekly.

Oral glucose tolerance tests were administered after 3, 6, and 9 weeks of feeding experimental diets. The rats were fasted overnight before the oral glucose tolerance test of 2 g glucose per kilogram of body weight, performed as previously described [20]. Blood samples were collected at fasting (0) and at 30, 60, and 120 min after glucose administration then analyzed for concentrations of glucose, insulin, and leptin by previously validated assays [20]. One week following the last glucose tolerance test, after 10 weeks of treatment, the rats were anesthetized, and body composition was determined by a Dual X-ray Absorptiometry (Hologic, Walham, MA, USA), after which blood was collected at necropsy and analyzed for total cholesterol and triglycerides (Roche Reagent Kits, Roche Cobas Fara Centrifugal Analyzer) as well as serum TNF-α concentrations, using a rat-specific sandwich enzyme immunoassay kit (R&D Systems, Minneapolis, MN, USA). The sensitivity of the TNF-α immunoassay was 6.25 pg/ml using 50 μl of serum. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) equation by Matthews et al. [26], and insulin sensitivity was determined using the insulin sensitivity index (ISI) equation by Gutt et al. [27]. Adipose, muscle, and liver tissues were collected at 10 weeks of treatment and analyzed for IL-6 and TNF-α gene expression using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), as described below. All protocols were approved by the Oklahoma State University Animal Care and Use Committee.

Total cellular RNA was isolated from adipocytes (visceral), skeletal muscle (soleus), and liver tissue with Trizol reagent (Life Technologies), as previously described [28,29]. Quantification of total RNA was established spectrophotometrically at the 260-nm reading. After determining quantification of total RNA, samples were aliquoted and stored at −80°C. Aliquoted samples were thawed on ice for 3–5 min, and fluorescent real-time quantitative PCR was used to determine IL-6 and TNF-α mRNA abundance within adipocytes, muscle, and liver tissue using one-step RT-PCR reaction of Taqman Gold RT-PCR kit (Applied Biosystems, Foster City, CA, USA), as previously described [28,29]. IL-6 forward and reverse primers were constructed from base pair (bp) 47 to 67 with a sequence of TGCCCTTCAGGAACAGCTATG and from bp 105 to 127 with a sequence of TGTCAACAACATCAGTCCCAAGA, respectively. The probe for IL-6 was constructed between bp 76 and 101 with a sequence of CTCCGCAAGAGACTTCCAGCCAGTTG. TNF-α forward and reverse primers were constructed from bp 434 to 452 with a sequence of GACAAGGCTGCCCCGACTA and from bp 501 to 479 with a sequence of CTCCTGGTATGAAGTGGCAAATC, respectively. The probe for TNF-α was constructed between bp 455 to 477 with a sequence of TGCTCCTCACCCACACCGTCAGC. A blast search (www.ncbi.nlm.nih.gov/BLAST/) was preformed to assure that no homologous regions for other proteins were present. Verification that the desired PCR product was achieved using high resolution gel electrophoresis and further confirmed through sequence analysis.

A total reaction volume of 25 μl consisted of 200 nM forward primer (IL-6 and TNF-α), 200 nM reverse primer (IL-6 and TNF-α), 100 nM fluorescent probe for IL-6 and TNF-α, 12.5 μl Taqman Master Mix, 0.625 μl Multiscribe and RNase inhibitor mix (Applied Biosystems), and 100 ng of total RNA diluted in RNase-free water. One-step RT-PCR amplification was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Thermal cycling parameters were as follows: 30 min at 48°C for reverse transcription, 10 min at 95°C for AmpliTaq Gold Activation, and 40 cycles at 95°C for 15 sec for denaturing and 60°C for 1 min for annealing and extension. Ribosomal 18S rRNA control kit (Applied Biosystems) was used as the housekeeping gene to normalize samples for variations in RNA loading as previously described [28,29]. 18S rRNA was verified as a valid housekeeping gene by determining that decreasing amounts of 18S rRNA (500, 100, 50, 10, 5, or 1 pg) were parallel to decreasing amounts of IL-6 and TNF-α mRNA (500, 100, 50, 10, 5 or 1 ng) (data not shown). The relative quantification of IL-6 and TNF-α mRNA was accomplished using the comparative threshold cycle method [30,31], as previously described [28,29]. Data for IL-6 and TNF-α mRNA were expressed as fold of the lowest treatment-group mean gene expression within each experiment as previously described [29].

The data are presented as the least-square means±S.E.M. Each experiment used duplicate samples of extracted IL-6 and TNF-α mRNA from adipose, muscle, and liver tissue from each rat. Diet (treatment) effects on tissue and serum variables were determined using one-way analysis of variance and the general linear models procedure of SAS [32]. Plasma glucose, insulin, and leptin levels at 30, 60, and 120 min during OGTT were also averaged for analysis and referred to as values "2 h post glucose administration." Outlier determination tests were used to detect any outliers within the data sets [33]; one value was determined to be an outlier in each of the RNA data sets. Pearson correlation coefficients were calculated to determine associations among variables [34]. Significance levels for all analyses were set at $P<.05$.

## 3. Results

### 3.1. Body measures

Although rats were fed approximately the same amount of food energy [intake averaged 247 J (59 kcal) per day for all treatment groups during the 10-week study] with different sources of fat, there was a 6% increase ($P<.05$) in body

weight and a 1.4–1.5-fold increase ($P<.0005$) in percentage of body fat (%BF) in rats fed HF diets when compared to MFS fed rats (Table 2). Positive correlations existed between body weight and %BF ($r=0.56$, $P<.01$), fasting insulin ($r=0.43$, $P<.05$), fasting leptin ($r=0.41$, $P<.05$), and HOMA-IR ($r=0.54$, $P<.001$). Likewise, %BF was positively correlated with fasting insulin ($r=0.68$, $P<.001$), fasting leptin ($r=0.570$, $P<.01$), and HOMA-IR ($r=0.62$, $P<.001$). Negative correlations were found between ISI and body weight ($r=-0.69$, $P<.001$) and %BF ($r=-0.63$, $P<.001$).

### 3.2. Blood hormones and metabolites

Fasting serum insulin levels were greater in the HFT treatment group when compared to the HFS- ($P<.05$) and MFS- ($P<.0005$) fed rats (Table 2). The HFS-fed rats also had greater ($P<.05$) fasting serum insulin levels than MFS-fed rats. Serum leptin concentrations during the 2-h post glucose administration (i.e., 30, 60, and 120 min values averaged) were greater ($P<.05$) in rats fed HFT and HFS compared to those fed MFS diets (Table 2). However, no significant differences were observed in 2-h insulin, fasting leptin, fasting glucose, or 2-h glucose levels among dietary treatments (Table 2). Fasting leptin levels were positively correlated with fasting insulin ($r=0.41$, $P<.05$) and HOMA-IR ($r=0.40$, $P<.05$). Fasting leptin also had a significant negative correlation with ISI ($r=0.45$, $P<.05$). All serum samples collected at Week 10 had TNF-α levels that were below the detectable limit of the TNF-α assay (i.e.,<6.25 pg/ml).

Insulin responses to the OGTT at Weeks 3, 6, and 9 are shown in Fig. 1. Feeding the HFT diet caused increased ($P<.05$) insulin responses at Weeks 3 and 6, whereas feeding the HFS diet caused increased insulin response at Week 9 only (Fig. 1). There was no insulin response at Week 9 to the OGTT for rats fed the HFT diet, but fasting insulin levels were significantly elevated (Fig. 1C).

Table 2
Variables associated with insulin resistance in weaning female Sprague–Dawley rats fed MFS, HFS, or HFT diets

| Variable | MFS | HFS | HFT | S.E.M. |
|---|---|---|---|---|
| Body weight (g) | 203.2[a] | 218.4[b] | 211.9[b] | 4.54 |
| %BF | 8.83[a] | 13.03[b] | 12.50[b] | 0.70 |
| Fasting glucose (mmol/l) | 5.9 | 6.4 | 6.4 | 0.2 |
| Mean glucose (mmol/l) * | 10.2 | 10.1 | 9.9 | 0.5 |
| Fasting insulin (pmol/l) | 0.09[a] | 0.18[b] | 0.25[c] | 0.02 |
| Mean insulin (pmol/l) * | 0.21[a] | 0.26[a] | 0.17[b] | 0.02 |
| Fasting leptin (pmol/l) | 73.6 | 102.5 | 100.6 | 12.5 |
| Mean leptin (pmol/l) * | 78.1[a] | 133.7[b] | 144.4[b] | 12.9 |
| HOMA-IR | 5.76[a] | 7.03[b] | 7.45[b] | 0.26 |
| ISI | 4.70[a] | 2.43[b] | 2.43[b] | 0.46 |
| Total serum cholesterol (mmol/l) | 2.20 | 2.08 | 2.02 | 0.09 |
| Serum triglycerides (mmol/l) | 0.68 | 0.58 | 0.68 | 0.05 |

Values represent least square means±S.E.M. ($n=7–10$; NS=$P>.10$). At 9 weeks of age, the rats were fasted 24 h prior to bolus of 2 g glucose per kilogram of body weight. Within a row, means without a common letter are significantly different ($P<.05$).

* Mean values are averages of values measured at 30, 60, and 120 min post-glucose administration.

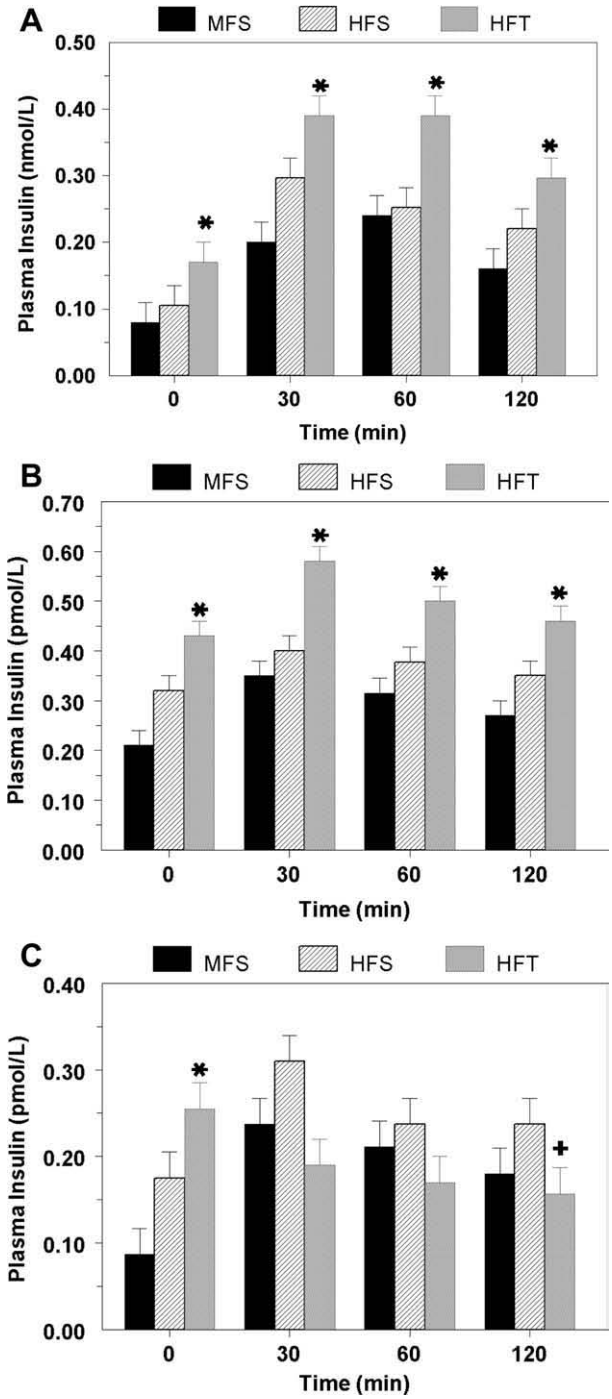


Fig. 1. Plasma insulin response to OGTT after Week 3 (OGTT1; A), Week 6 (OGTT2; B) and Week 9 (OGTT3; C) of feeding female rats an MFS, HFS, or HFT diet. Asterisk indicates mean differs ($P<.0005$) from its respective MFS control mean ($n=7–9$ rats per group). Plus sign indicates mean differs ($P<.0001$) from fasting mean in the same treatment group.

Leptin responses to the OGTT at Weeks 3, 6, and 9 are shown in Fig. 2. The HFS and HFT rats had significantly greater leptin concentrations compared to the MFS rats 120 min after the glucose challenge after 3 weeks of treatment

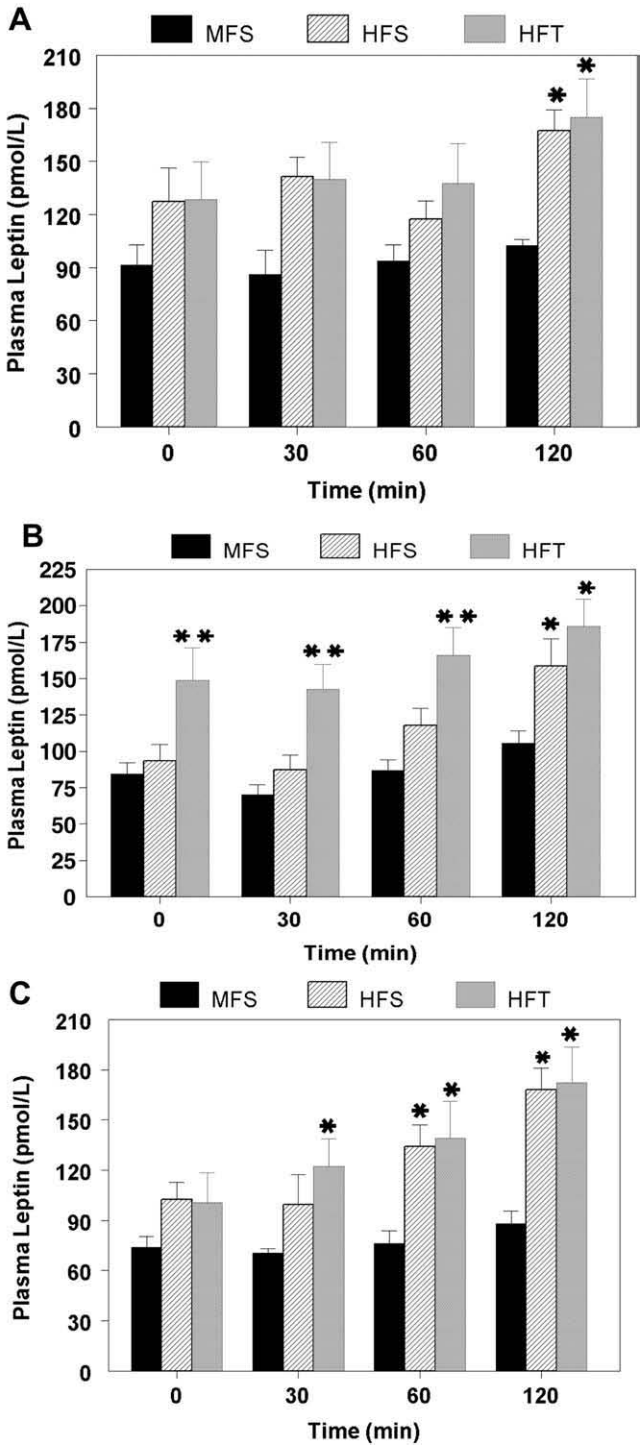


Fig. 2. Plasma leptin response to OGTT after week 3 (OGTT1; A), Week 6 (OGTT2; B), and Week 9 (OGTT3; C) of feeding female rats an MFS, HFS, or HFT diet. Asterisk indicates mean differs ($P<.05$) from its respective MFS control mean ($n=7–9$ rats per group). Double asterisk indicates mean differs ($P<.05$) from both MFS and HFS treatment groups.

(Fig. 2A). After 6 weeks of treatment, the HFT-fed rats had significantly higher leptin concentrations compared to the soybean oil fed rats regardless of fat content of the diet at fasting and at 30 and 60 min after the glucose challenge (Fig. 2B). But at 120 min after the glucose challenge, both the HFS- and HFT-fed rats had significantly greater leptin concentrations compared to the MFS-fed rats. After 9 weeks of treatment, rats fed the HFT and HFS diets had significantly greater leptin responses compared to the MFS rats 60 and 120 min after the glucose challenge (Fig. 2C). The HFT fed rats also exhibited significantly higher leptin concentrations than the two other groups 30 min after the challenge.

Insulin sensitivity index (ISI) was greater ($P<.005$) in the MFS treatment group than the HFT and HFS groups (Table 2). Additionally, HOMA-IR was elevated in HFT- ($P<.005$) and HFS- ($P<.0001$) fed rats compared to the MFS fed rats (Table 2).

Total serum cholesterol and triglycerides did not differ among the treatment groups (Table 2). Total serum cholesterol averaged 2.1±0.09 mmol/l, and serum triglycerides averaged 0.65±0.05 mmol/l (Table 2).

### 3.3. Tissue mRNA measures

Liver TNF-α mRNA levels were threefold greater ($P<.05$) in HFT-fed rats compared to MFS-fed rats but did not differ between HFT and HFS-fed rats (Fig. 3A). In contrast, rats fed HFT had 90% less ($P<.05$) TNF-α mRNA abundance in adipose tissue than rats fed MFS or HFS diets (Fig. 4A). TNF-α gene expression in liver tissue was

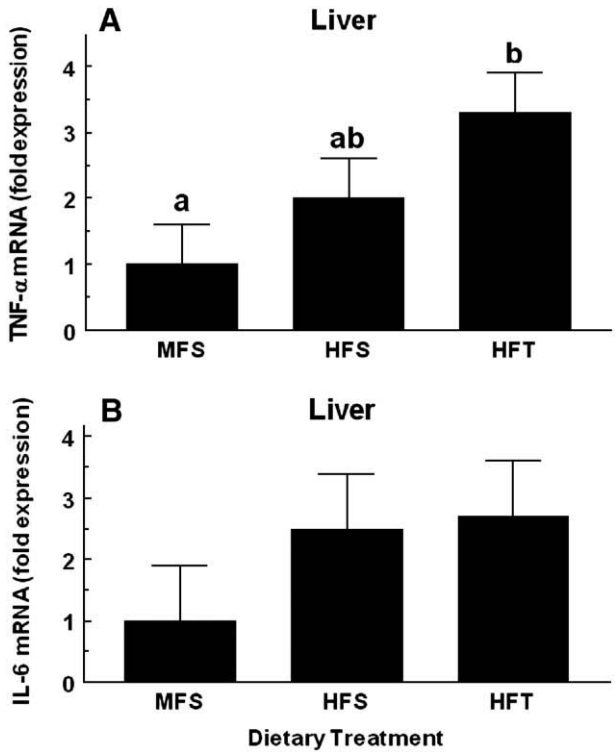


Fig. 3. TNF-α mRNA (A) and IL-6 mRNA (B) abundance in liver tissue of female rats fed an MFS, HFS, or HFT diets. (A) Means without a common letter differ ($P<.05$) ($n=7–9$ rats per group). (B) No significant differences in IL-6 fold expression existed between groups ($n=6–8$ rats per group). Abundance of mRNA is expressed as fold of MFS group and is relative target gene expression corrected for 18S rRNA abundance in each sample.

positively correlated with fasting glucose ($r$=0.41, $P$<.05), fasting insulin ($r$=0.49, $P$<.05), 2-h leptin ($r$=0.43, $P$<.05), and HOMA-IR ($r$=0.54, $P$<.05) and negatively correlated with ISI ($r$=−0.48, $P$<.05). Adipose TNF-α abundance was not significantly correlated with any of the metabolic measures. Although no significant differences in muscle TNF-α mRNA abundance were observed among treatment groups (Fig. 4B), muscle TNF-α mRNA levels were positively correlated with fasting leptin levels ($r$=0.56, $P$<.01).

IL-6 mRNA was not detectable in adipose tissue (data not shown). Only 0%, 25%, and 20% of MFS, HFS, and HFT rats, respectively, had detectable muscle IL-6 mRNA, and these proportions did not differ ($P$>.10; chi-square analysis). In contrast, IL-6 mRNA was detected in 70% to 80% of the liver samples from each of the groups of rats. Specifically, HFT- and HFS-fed rats had 2.5-to 2.7-fold greater ($P$>.10) hepatic IL-6 mRNA abundance than MFS-fed rats (Fig. 3B). Liver IL-6 mRNA abundance was positively correlated with 2-h serum glucose ($r$=0.48, $P$<.05) and negatively correlated with ISI ($r$=−0.39, $P$<.10).

## 4. Discussion

Results of the present study demonstrate that TNF-α is a key mediator of insulin resistance through TNF-α over-expression in the liver and decreased expression in adipose tissue, and that the type of fat in a HF diet influences TNF-α production in multiple tissues involved in the early onset of type 2 diabetes. After feeding rats HF diets with different sources of fat at levels that mimic human diets, it was observed that rats fed HF diets with predominantly saturated fatty acids (tallow) had increased hepatic TNF-α mRNA and decreased adipose TNF-α mRNA abundance, as compared to the rats fed diets with soybean oil (unsaturated) diets. Insulin resistance was associated with increased hepatic IL-6 and TNF-α mRNA and decreased adipose TNF-α mRNA abundance. IL-6 gene expression was absent in adipose tissue and not often expressed in muscle tissue. TNF-α mRNA abundance in skeletal muscle was not altered by type or amount of fat fed or associated with insulin resistance. Overall indexes of HOMA-IR or ISI did not differ between rats fed high saturated diets (i.e., tallow) and those fed high unsaturated fat diets (i.e., soybean oil). However, insulin responses to OGTT revealed that high saturated fat diets (i.e., tallow) caused early and persistent insulin resistance compared to the unsaturated fat diets (i.e., soybean oil). Leptin resistance also seemed to occur especially in the HFT-fed rats. In addition, during the last OGTT, 9 weeks after treatment, the high-tallow-fed rats appeared to lack an insulin response to glucose, whereas the HFS fed rats showed a tendency for insulin resistance for the first time. Thus, the present study indicates that the high-tallow-fed rat model may be used to evaluate the effect of dietary factors on Type 2 diabetes and subsequent development of insulin-dependent diabetes.

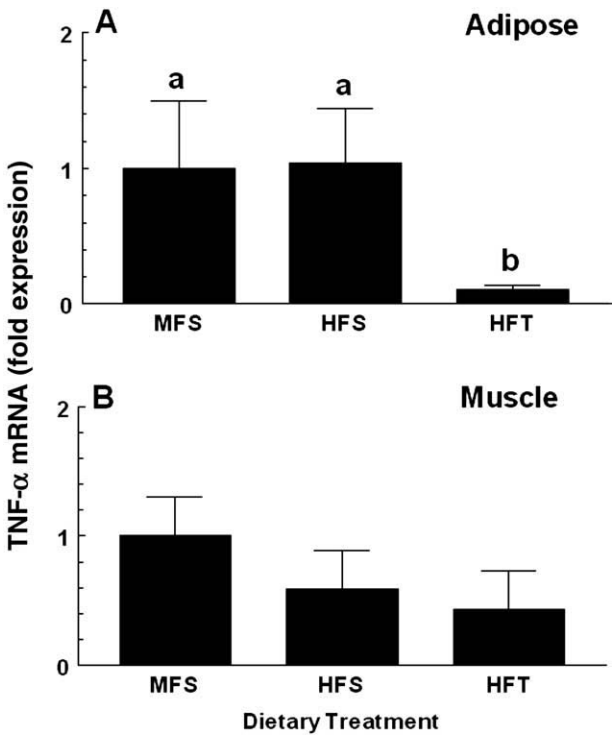


Fig. 4. TNF-α mRNA abundance in adipose (A) and muscle (B) tissue of female rats fed an MFS, HFS, HFT diets. (A) Means without a common letter differ ($P$<.05) ($n$=7–9 rats per group). (B) There were no significant differences in TNF-α fold expression between groups ($n$=7–9 rats per group). Abundance of mRNA is expressed as fold of MFS group and is relative target gene expression corrected for 18S rRNA abundance in each sample.

As in previous studies [19,20], HF diets increased body weight, %BF, insulin resistance, and impaired glucose tolerance and further indicate that HF diets may affect body fat accumulation as well as body weight, independent of the source of fat in the diet. Fasting serum insulin levels were greater in HFT-fed rats than HFS- and MFS-fed rats, and HFS-fed rats had greater serum insulin levels than MFS-fed rats. This suggests that not only will increased overall fat intake elevate serum insulin levels, but diets composed of increased saturated fat (i.e., tallow) may increase serum insulin levels above those composed of a similar level of unsaturated fats (i.e., soybean oil). It should be emphasized that difference in components in tallow and soybean oil other than the level of saturated fats may affect insulin resistance. For example, tallow is rich in cholesterol and low in vitamin E, whereas soybean oil has plant sterols and is high in Vitamin E. However, overall indexes of insulin sensitivity and resistance were found to be similar in HFT- and HFS-fed groups at the end of the study and significantly different from the MFS-fed group. Because serum cholesterol and triglycerides as well as insulin sensitivity and resistance in rats fed the HFT and HFS did not differ, types of HF diets appear to affect these early signs of Type 2 diabetes similarly.

Both hepatic TNF-α and IL-6 mRNA levels were negatively correlated with ISI. A study of adult male mice

fed HF diets (58% of total calories from unsaturated fat) demonstrated significantly elevated hepatic IL-1β, IL-6, and TNF-α mRNA levels [35]. Few studies have based the amount of dietary fat on current American dietary intakes or evaluated TNF-α and IL-6 gene expression in nonadipose tissue or in peripubertal animals. In the present study, hepatic TNF-α mRNA abundance was positively correlated with fasting glucose, fasting insulin, and HOMA-IR, whereas hepatic IL-6 mRNA abundance was positively correlated with 2-h serum glucose levels in peripubertal female rats.

The ability of insulin to stimulate peripheral glucose utilization and inhibit glucose production is impaired in rats infused with TNF-α [36]. In vitro, IL-6 inhibits insulin receptor signal transduction and insulin action in both primary mouse hepatocytes and human HepG2 hepatocarcinoma cells [12]. In vivo, liver insulin receptor knockout mice had severe insulin resistance, confirming that hepatic insulin signaling plays a major role in the regulation of systemic glucose levels and insulin sensitivity [37]. Recently, it was suggested that the nuclear transcription factor kappa B (NF-κB) could be involved in the etiology of insulin resistance [35,38]. In particular, increased hepatic NF-κB activity is associated with increased abundance of mRNAs for IL-1β, TNF-α, and IL-6 in liver of HF-fed mice [35], and blocking NF-κB reduces IL-6 mRNA levels [39]. Other studies indicate that increases in TNF-α mRNA levels decrease insulin signaling via disrupting insulin receptor and IRS-1 tyrosine phosphorylation [8,9].

Results of the present study indicate that the consumption of high saturated fat (i.e., tallow) diets increase hepatic production of TNF-α and IL-6, which previously have been found to be involved in the development of systemic insulin resistance [8–11]. This response to elevated TNF-α appears to be an autocrine rather than endocrine effect, since TNF-α has been found to be undetectable in serum of diet induced obese rats [40]. Feeding saturated fat (i.e., HFT) affected adipose and hepatic TNF-α gene expression more dramatically than did feeding unsaturated fat (i.e., HFS). Further studies are warranted to evaluate whether these early changes in cytokine gene expression are antecedent to longer-term changes in insulin sensitivity/resistance or disease.

In the present study, IL-6 and TNF-α mRNA levels in skeletal muscle were not significantly affected by amount or type of dietary fat, nor were they correlated with measures of insulin resistance or sensitivity except for fasting serum leptin levels. Both smooth muscle and skeletal muscle of rats [41,42] and humans [43–46] contain mRNA for IL-6 and TNF-α. Gene expression of these cytokines in muscle appears to be under hormonal and metabolite control. Rats infused with non-esterified fatty acids (NEFA) increase skeletal muscle TNF-α mRNA [24], and both palmitate and TNF-α stimulate human smooth muscle IL-6 mRNA in vitro [45]. In adult human beings, an inverse relationship exists between maximal glucose disposal rate and skeletal muscle TNF-α mRNA levels, and muscle TNF-α mRNA abundance is greater in insulin-resistant and diabetic subjects than controls [44]. Whether the role of skeletal muscle cytokine gene expression in the development of insulin resistance in humans and rodents differs will require further study.

During early onset of insulin resistance in the KKA$^y$ mouse model of obesity-linked diabetes, adipose TNF-α expression is significantly decreased [47], as was observed in HFT-fed rats of the present study. Both human [48–51] and murine [7,47,52,53] adipose tissue contain IL-6 and TNF-α mRNA but may require specific stimuli for its induction and subsequent detection [53]. Elevated, locally produced TNF-α may act directly on adipocytes to regulate release of preformed pools of leptin [54,55] and inhibit insulin receptor signaling [56–58], whereas IL-6 may regulate hormone-sensitive lipase mRNA in adipose tissue [59]. An association between adipose TNF-α mRNA and leptin secretion was not clear in the present study because weanling rats fed HFT and HFS diets both had increased 2-h leptin levels, while only HFT-fed rats, and not HFS-fed rats, had lower adipose TNF-α mRNA and elevated hepatic TNF-α mRNA. Only muscle TNF-α mRNA was positively correlated with fasting leptin levels, suggesting that nonadipose sources of TNF-α may regulate leptin secretion or vice versa. Because serum TNF-α was undetectable, it is likely the effect of muscle TNF-α on leptin secretion is indirect (e.g., via increased serum glucose due to reduced muscle insulin sensitivity). Further research will be needed to clarify the mode of action and relationship between increased leptin secretion and changes in TNF-α gene expression in adipose and muscle tissue, as well as to elucidate the differential responses to feeding similar amounts of saturated (i.e., HFT) and unsaturated fats (i.e., HFS).

## 5. Conclusions

Results from the present study indicate that changes in TNF-α production in multiple tissues due to the overconsumption of HF diets may be involved in early onset of insulin resistance and the consequent development of type 2 diabetes or metabolic syndrome. Further research will be required to elucidate the mechanisms by which the effects of feeding saturated (i.e., tallow) vs. unsaturated HF (i.e., soybean oil) diets are mediated.

In summary, this study demonstrates that involvement of the proinflammatory cytokines in the development of insulin resistance results from the overconsumption of HF diets. A high-saturated-fat (i.e., tallow) diet fed to growing female rats causes decreased adipose and increased hepatic levels of TNF-α mRNA, compared to diets containing unsaturated fat (i.e., soybean oil), without affecting muscle cytokine gene expression. These findings imply that early onset of obesity and insulin resistance due to consumption of HF diet involves adipose and hepatic-specific mechanisms that partly have inflammatory origin. A high-tallow-fed rat model may be an ideal paradigm to evaluate the effect of dietary factors on type 2 diabetes and the subsequent development of insulin-dependent diabetes mellitus.

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