Diet-Induced Obesity and Acute Hyperlipidemia Reduce \textit{IkB\alpha} Levels in Rat Skeletal Muscle in a Fiber-Type Dependent Manner.

by

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Running Title: Inflammatory/stress pathway activity in skeletal muscle in obesity and hyperlipidemia.

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ABSTRACT

Increased activity of pro-inflammatory/stress pathways has been implicated in the pathogenesis of insulin resistance in obesity. However, the effects of obesity on the activity of these pathways in skeletal muscle, the major insulin sensitive tissue by mass, are poorly understood. Furthermore, the mechanisms that activate pro-inflammatory/stress pathways in obesity are unknown. The current study addressed in rats the effects of diet-induced obesity (DIO, 6 weeks of high fat feeding) and acute (6h) hyperlipidemia (HL) on activity of IKK/\(\kappa B/NF\kappa B\), JNK, and p38 MAPK in three skeletal muscles differing in fiber type [superficial vastus (VAS, fast twitch-glycolytic), soleus (SOL, slow twitch-oxidative) and gastrocnemius (GAS, mixed)]. DIO decreased the levels of the NF\(\kappa B\) inhibitor, \(\kappa B\alpha\), in VAS (24\pm3\%, \(P=0.001\), \(n=8\)) but not in SOL or GAS compared to standard chow fed controls. Similar to DIO, HL decreased \(\kappa B\alpha\) levels in VAS (26\pm5\%, \(P=0.006\), \(n=6\)) and in GAS (15\pm4\%, \(P=0.01\), \(n=7\)) but not in SOL compared to saline-infused controls. Importantly, the fiber-type dependent effects on \(\kappa B\alpha\) levels could not be explained by differential accumulation of triglyceride in SOL and VAS. HL, but not DIO, decreased phospho-p38 MAPK levels in VAS (41\pm7\% \(P=0.004\), \(n=6\)) but not in SOL or GAS. Finally, skeletal muscle JNK activity was unchanged by DIO or HL. We conclude that diet-induced obesity and acute hyperlipidemia reduce \(\kappa B\alpha\) levels in rat skeletal muscle in a fiber-type dependent manner.
INTRODUCTION

Recently, it has been proposed that elevated activity of pro-inflammatory/stress signaling pathways play an important role in the pathogenesis of insulin resistance in obesity, and perhaps type II diabetes. This hypothesis is supported by the observations that obesity and type II diabetes are states of chronic, low-grade inflammation (9, 12, 35), that increased expression of pro-inflammatory factors such as TNF-\(\alpha\) (13, 26, 27) and IL-6 (21) decrease insulin sensitivity, and more recent studies demonstrating that decreased activity of pro-inflammatory/stress signaling pathways improves, or prevents the development of insulin resistance (1, 5, 14, 17, 20, 32, 38).

Cell responses to inflammatory and stress signals are mediated by a number of ubiquitously expressed signaling cascades. Of these, the IKK/I\(\kappa\)B/NF\(\kappa\)B and mitogen activated protein kinase (MAPK) pathways, specifically c-Jun N-terminal kinase (JNK) and p38 MAPK, are best described. Altered activity of each of these pathways has been implicated in changing insulin action. Thus, reduced IKK/I\(\kappa\)B/NF\(\kappa\)B activity (1, 5, 17, 20, 32, 38) prevents the development of lipid-induced and/or obesity-induced insulin resistance in cells (32) and in rodents (1, 5, 20, 38) and improves insulin sensitivity in T2DM (17). Increased activity of IKK/I\(\kappa\)B/NF\(\kappa\)B in mouse skeletal muscle induces dramatic tissue wastage, but insulin action appears to be unaltered (4), while increased IKK/I\(\kappa\)B/NF\(\kappa\)B activity in liver (5) results in the development of insulin resistance. JNK deficient mice are partially resistant to the development of insulin resistance resulting from a high fat diet (14) while basal p38 MAPK phosphorylation is increased and responsiveness to insulin is lost (22) in skeletal muscle in T2DM. Finally, studies in
mouse skeletal muscle and L6 myotubes suggests that increased activity of p38 MAPK may be a negative regulator of glucose uptake (15).

Despite these observations, the effects of obesity on the activity of pro-inflammatory/stress pathways in skeletal muscle, the major insulin-sensitive tissue by mass in the body, remain unclear. Furthermore, the mechanisms of activation of pro-inflammatory/stress pathways in obesity are poorly understood. The current study was undertaken to address each of these issues. Specifically, we determined the effects of DIO and acute hyperlipidemia on levels of $I\kappa B\alpha$ and p38 MAPK phosphorylation (commonly used indicators of activity of NF$\kappa$B and p38 MAPK, respectively) and JNK activity in three skeletal muscles differing in fiber-type composition and insulin sensitivity. The data demonstrate that DIO and acute hyperlipidemia (HL) decrease skeletal muscle $I\kappa B\alpha$, and that the effects of DIO and HL on $I\kappa B\alpha$ levels are skeletal muscle fiber-type dependent. Furthermore, HL, but not DIO, decreases p38 MAPK activity in a fiber-type dependent manner. However, DIO and HL have no effect on skeletal muscle JNK activity.
MATERIALS AND METHODS

Animal care and maintenance. Male Wistar rats were purchased from Charles River (Madison, WI) at a weight of 175-275g and maintained on a constant 12:12-h light-dark cycle, with free access to water. For obesity studies, rats (starting weight 175-200g) were ad libitum fed either a standard rat chow (11% of calories from fat) or a high-fat diet (Harlan Teklad, Madison WI. TD 96001, 45% of calories from fat), for 6 weeks. For lipid infusion studies all animals (starting weight 250-275g) were ad libitum fed standard rat chow prior to the study. All experimental procedures were approved by the institutional animal care and use committee (IACUC) of the University of Pittsburgh, and were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Obesity Studies. A 4-6 week high fat diet is sufficient to induce obesity and insulin resistance in rats (3, 16, 29, 33, 34). In the current study, 6 week high fat fed animals and standard chow fed control animals were fasted overnight (18h), anesthetized with pentobarbital and three skeletal muscles differing in fiber-type [superficial vastus, fast twitch-glycolytic; soleus, slow twitch-oxidative, and gastrocnemius, mixed fast twitch-glycolytic and slow twitch-oxidative] were extracted and flash frozen in liquid nitrogen. Tissues were stored at -70°C until analysis.

Lipid Infusion Studies. Approximately 8 days prior to the study, catheters were inserted into the carotid artery and the contralateral jugular vein as described previously (7, 28, 30). When animals had recovered to >90% of pre-surgery weight, they were fasted overnight. The following day, the indwelling cannulas were cleared with saline and extended with silastic tubing after which the animals were infused with Liposyn
II/heparin (5 ml/kg/hr and 6 units/hr, respectively, Abbott Laboratories, Chicago, IL) or saline (5 ml/kg/hr) for six hours. In preliminary studies we confirmed that this intervention induced insulin resistance, as assessed by a hyperinsulinemic-euglycemic clamp (see Results). In all other studies, after completion of the lipid or saline infusions, the animals were anesthetized with pentobarbital, and skeletal muscles were extracted and flash frozen in liquid nitrogen. Tissues were stored at -70°C until analysis.

**Tissue Measurements.** IκBα, phospho-p38 and p38 MAPK levels were measured using a standard Western blotting procedure. Briefly, 50 mg of tissue was homogenized in lysis buffer, containing as final concentrations, 20 mM TRIS-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Tween-20, 2.5 mM pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium vanadate, and 1 µg/mL leupeptin. Equal amounts of protein were resolved by SDS-PAGE on a 10% Tris-HCl gel (Biorad, Hercules, CA) and then transferred to a PVDF membrane (Immobilon Corporation, Bedford, MA). After blocking for an hour in 5% non-fat dried milk in 1% TBST at room temperature, membranes were incubated for one hour with α-IκBα (1:1000, Santa Cruz, Santa Cruz, CA), α-phospho-p38 MAPK (1:1000, Cell Signaling, Beverly, MA) or α-p38 MAPK (1:1000, Cell Signaling, Beverly, MA) at room temperature. After washing, the membrane was incubated with HRP-linked goat anti-rabbit IgG antibody for one hour at room temperature. The membrane bound antibodies were then detected by luminol chemiluminescence (Lumiglo, Cell Signaling Technology, Beverly, MA). The membrane was exposed to autoradiographic film and bands scanned, and optical density determined (NIH Image J, v 1.62). JNK activity was measured by an adaptation of the method of Hirosumi et al. (14). Briefly, 50 mg of tissue was homogenized in 0.5 mL of a lysis buffer containing as final
concentrations, 25 mM Tris-HCl pH 7.4, 10 mM Na₃VO₄, 100 mM NaF, 50 mM Na₄P₂O₇, 10 mM EGTA, 10 mM EDTA, 1% IGEPAL, 20 nM okadaic acid, 10 µg/mL leupeptin, 10 µg/mL aprotonin, 2 mM PMSF. Five hundred micrograms of protein, 2.5 µg of α-JNK antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and 22 mg of protein A sepharose beads (Amersham Biosciences, Piscataway, NJ) were mixed and incubated overnight at 4°C. After centrifugation at 1,000 rpm at 4°C, the beads were washed 3 times in lysis buffer. The pellet was then resuspended in kinase buffer (25 mM HEPES pH 7.4, 20 mM MgCl₂, 20 mM β-glycerophosphate, 0.5 mM EGTA, 0.5 mM NaF, 0.5 mM Na₃VO₄, 1 mM PMSF, 1 mM DTT, 10 mM ATP). The reaction was incubated for 20 minutes at 30°C in the presence of 8 µg of c-jun fusion protein (Cell Signaling, Beverly, MA) and 1 µL (5 mCi/mL) of γ³₂P-ATP. The reaction was terminated by adding SDS and subsequent boiling of samples. Equal amounts of reaction product were resolved by SDS-PAGE and transferred to PVDF membrane. The membrane was exposed to autoradiographic film, and then scanned and quantified. Tissue triglycerides were measured as described previously with modifications. Briefly, ~30 mg of tissue was crushed while under liquid nitrogen and homogenized in ice-cold (80%) methanol (MeOH)/H₂O containing butylated-hydroxytoluene (5 µg/mL) as an antioxidant. The MeOH/H₂O homogenate was mixed with chloroform (CHCl₃) (2:1 v/v CHCl₃:MeOH) to extract lipids as described by Folch et al (10). The extracted lipid sample was dried under vacuum and lipids resuspended in 500 µL of CHCl₃. A 200 µL aliquot of extracted lipids was placed into a glass tube and allowed to air-dry overnight. The dried sample was dissolved in 60 µl of tert-butanol and 40 µl of Triton X-114-methanol (2:1) mixture. The triglycerides were then measured spectrophotometrically (Beckman DU 530) using the
GPO-triglyceride kit (Sigma) and Lintrol lipids (Sigma) as standard (3).

**Plasma Measurements.** Plasma triglycerides were measured using the GPO-triglyceride kit (Sigma, Atlanta, GA) with Lintrol lipids as standards (Sigma, Atlanta, GA). Plasma free fatty acids were measured using the free fatty acid, half-micro test kit (Roche Diagnostics, Penzberg, Germany).

**Statistics.** All results are expressed as means ± se. Statistical significance was determined by unpaired t-test using the Systat statistical programme (Evanston, IL). Significance was assumed at $P < 0.05$. 
RESULTS

*Skeletal muscle IκBα levels are decreased by DIO in a fiber-type dependent manner.*

In previous studies we (3, 16) and others (29, 33, 34) have demonstrated in rats that a 4-6 week high fat diet is sufficient to induce obesity and insulin resistance. Thus, we first determined the effects of diet-induced obesity (DIO) on levels of skeletal muscle IκBα, a commonly used indicator of activity of the IKK/IκB/NFκB pathway (24). Body weight, adiposity, and plasma FFA and triglyceride concentrations were increased in DIO compared to standard chow fed (LEAN) controls (Table 1). In superficial vastus (VAS), a muscle composed predominantly of fast twitch, glycolytic fibers, IκBα levels were decreased by 24±3% \((P=0.001)\) in DIO compared to LEAN (Figure 1). Interestingly, these decreases did not occur in soleus (SOL), a slow-twitch, oxidative fiber type muscle, or in gastrocnemius (GAS), a mixed fiber-type muscle (Figure 1). Thus, decreases in IκBα levels induced by DIO in skeletal muscle are fiber-type dependent.

*Acute hyperlipidemia reproduces the effects of DIO on IκBα levels in skeletal muscle.*

Dysregulated lipid metabolism and hyperlipidemia is commonly associated with obesity and has been implicated in the pathogenesis of insulin resistance (2, 19, 25, 36). Furthermore, lipids activate IKK/IκB/NFκB in macrophages (23), endothelial cells (8), and L6 rat myotubes (32). Thus, hyperlipidemia is one possible mechanism mediating decreased skeletal muscle IκBα levels in DIO. To address this hypothesis in the absence of other confounding metabolic abnormalities of obesity, acute hyperlipidemia was induced in lean animals by an infusion of liposyn II for 6h. As previously reported (37), this intervention increased plasma FFA levels by ~20-fold (Table 1) and induced insulin
resistance as determined by the glucose clamp method (glucose infusion rate of 31.9±1.1 mg/kg/min and 38.5±0.8 mg/kg/min in lipid-infused compared to saline-infused animals, respectively, in the final 60 min of a hyperinsulinemic clamp, P=0.001 lipid vs. saline, n=6 in each group). Similar to DIO, IκBα levels were decreased in VAS (26±5%, P=0.006) in lipid-infused compared to saline-infused animals, but were unaltered in SOL, (Figure 2). However, unlike DIO, IκBα levels were also decreased in GAS (15±4%, P=0.01) compared to controls.

*Skeletal muscle triglyceride levels do not correlate with the fiber-type dependent effects of DIO and acute hyperlipidemia on IκBα levels.* Accumulation of tissue lipid (lipotoxicity) has been implicated in the pathogenesis of insulin resistance and activation of inflammatory pathways. Thus, one possible mechanism to explain the fiber-type differences in activation of the NF-κB pathway is fiber-type differences in accumulation of lipid in response to DIO and acute hyperlipidemia. Basal (lean or saline-infused groups) triglyceride levels differed by fiber-type (SOL > VAS, P<0.05, Figure 3) and by animal weight (heavier > lighter, P<0.05, Table 1 and Figure 3). DIO increased SOL triglycerides compared to lean controls by 53.7±8.9% (Figure 3, Panel A). VAS triglycerides were increased in DIO to a similar relative extent (44.2±12.4%, Figure 3, Panel A) compared to lean controls, but the absolute increase (50.6±14.9 µg/mg protein in SOL vs. 22.8±4.9 µg/mg protein in VAS) was less in VAS compared to SOL (Figure 3, Panel A, P=0.005). In response to acute hyperlipidemia there was a paradoxical decrease in SOL triglycerides, confirming previous reports (37), and no change in VAS
triglycerides. Thus, the fiber-type dependent effects of DIO and acute hyperlipidemia on IκBα levels are not correlated with differential accumulation of triglycerides.

**Skeletal muscle JNK activity and p38 MAPK phosphorylation are not changed by DIO.**

Similar to IKK/IκB/NFκB, altered JNK and p38 MAPK activity has been implicated in mediating alterations in insulin action (6, 14, 15, 22). Thus, we next determined the effects of DIO and acute hyperlipidemia on activity of these two pathways in skeletal muscle. DIO did not alter the activity of JNK or p38 MAPK in any of the skeletal muscles examined (Figures 4 and 5). Acute hyperlipidemia decreased p38 MAPK phosphorylation in VAS, but not in SOL or GAS, and had no effect on JNK activity. JNK activity was not measured in SOL because of lack of tissue.
DISCUSSION

The primary goal of the current study was to determine the effects of DIO in rats on the activity of inflammatory/stress pathways in skeletal muscle and to begin to address possible mechanisms responsible for activation of these pathways. The rationale for this study was recent observations implicating altered activity of pro-inflammatory/stress pathways, specifically IKK/IκB/NFκB, JNK, and p38 MAPK in altering insulin action. A number of novel observations arise from our studies. We demonstrate that (1) DIO is associated with decreased levels of the NFκB inhibitor IκBα in skeletal muscle; (2) the effects of DIO are fiber-type dependent, since IκBα levels were decreased in fast, glycolytic fibers, but not in slow, oxidative fibers; (3) acute hyperlipidemia reduces skeletal muscle IκBα levels, suggesting one mechanism for the effects of DIO; (4) differential accumulation of skeletal muscle triglycerides do not correlate with the fiber-type dependent effects of DIO and acute hyperlipidemia on IκBα levels, and (5) DIO has no effect on p38 MAPK or JNK activity in rat skeletal muscle.

The current study demonstrates that both DIO and acute hyperlipidemia result in decreased levels of skeletal muscle IκBα, a situation that is normally associated with increased NFκB activity. These data are in good agreement with a previous observation in humans of decreased skeletal muscle IκBα levels in response to an acute lipid infusion (18). Recent studies have demonstrated that inhibition of the IKK/IκB/NFκB pathway by salicylates, an IKK inhibitor, decreases skeletal muscle insulin resistance associated with a high fat diet (38), acute hyperlipidemia (20), and type II diabetes (17). Direct evidence for a role for increased IKK/IκB/NFκB activity in the pathogenesis of skeletal muscle insulin resistance is more varied. One group reports in mice that a deficiency of IKK
activity, and hence NFκB activity, protects against the development of insulin resistance on a high fat diet (38) or in response to a lipid infusion (20), but a more recent study failed to reproduce the effects of a high fat diet (31). The reasons for these discrepant findings are unclear. Constitutively active skeletal muscle IKK activity in mouse induces severe muscle wastage but insulin sensitivity is unaltered (4). However, it is unclear how insulin sensitivity was determined in this study or if the potentially confounding variable of muscle wastage influenced insulin action in these animals. In the context of the current study, we can state that IKK/IκB/NFκB activation is associated with the development of skeletal muscle insulin resistance in two independent rat models (current study and 20). Furthermore, previous studies in L6 rat myotubes demonstrate that activation of IKK/IκB/NFκB by fatty acids induces insulin resistance, and that blocking of NFκB activation is sufficient to prevent lipid-induced insulin resistance (32). However, given the studies discussed above it cannot be stated at this time that NFκB activation in skeletal muscle is required for the development of insulin resistance in vivo.

An important conclusion arising from our study is that the effects of DIO and acute hyperlipidemia on the levels of IκBα in skeletal muscle is fiber-type dependent. Skeletal muscle fibers-types can be characterized based on differences in their oxidative capacity and insulin sensitivity. Thus, slow twitch fibers have a high oxidative capacity, and are very insulin sensitive. Conversely, fast twitch fibers have a lower oxidative capacity and insulin sensitivity. IκBα levels were decreased in DIO in superficial vastus, but not in gastrocnemius or soleus muscle. Relatively similar results were obtained with acute hyperlipidemia, although unlike DIO, IκBα levels were also decreased in gastrocnemius. In humans acute hyperlipidemia activates IKK/IκB/NFκB in a mixed
fiber-type muscle (vastus), confirming that our observations are not restricted to rodents. Perhaps the most unexpected observation was that IKK/IκB/NFκB was not activated in soleus muscle, the most insulin sensitive muscle examined, by DIO or acute hyperlipidemia. Previous studies (3, 37) have demonstrated that insulin resistance is induced in soleus muscle by DIO and acute hyperlipidemia. One possible interpretation of our data is that IKK/IκB/NFκB activity is not required for obesity/lipid-induced insulin resistance in Type I fibers. Alternatively, activation of IKK/IκB/NFκB may have occurred prior to the time-points examined in this study. In this regard, the IκBα gene is a target of NFκB activity, resulting in the replenishment of IκBα and dampening of inflammatory signals that activate NFκB. This negative feedback is commonly considered a critical mechanism for regulating the length and magnitude of inflammatory responses. Lastly, it has been demonstrated that IKK serine phosphorylates insulin receptor substrate (IRS) 1, in HepG2 cells (11), which would be expected to decrease insulin-stimulated PI-3 kinase activity independent of decreases in IκBα.

While the mechanism(s) responsible for the decrease in IκBα levels in skeletal muscle in DIO are unclear, our demonstration that acute hyperlipidemia decreases IκBα in skeletal muscle in lean animals is suggestive. In support of a role for dyslipidemia in activation of inflammatory pathways are the observations of dysregulated lipid metabolism in obesity, resulting in substantially elevated plasma levels of free fatty acids, triglycerides, and lipoproteins, and elevated tissue levels of triglyceride, and other lipid metabolites (2, 19, 36). Furthermore, lipid-induced activation of IKK/IκB/NFκB has been demonstrated in L6 myotubes (32), macrophages (23), and endothelial cells (8). Finally, there is a substantial body of work supporting a role for lipids in the pathogenesis
of insulin resistance (2, 19, 36). However, it should be noted that a number of pro-
inflammatory factors that are elevated in obesity are also capable of activating
IKK/IκB/NFκB, suggesting that activation of this pathway may be mediated by a number
of mechanisms in obesity.

It has been proposed that accumulation of lipids within tissues may be a
mechanism of activation of inflammatory pathways. In the current study, triglyceride
levels were increased in both soleus and superficial vastus muscles in DIO compared to
lean controls, whereas IκBα levels were only reduced in the superficial vastus. The
effects of acute hyperlipidemia were somewhat more complex. Thus, as previously
reported by Yu et al. (37) soleus muscle triglycerides decreased in response to acute
hyperlipidemia. Superficial vastus triglycerides were unchanged. What one can
conclude from these data is that the fiber-type dependent effects of DIO and HL on IkBα
levels are not explained by differential accumulation of triglycerides in the different
fiber-types. Furthermore, in a separate study we measured the accumulation of ceramide
and diacylglycerol (two other lipid metabolites implicated in the activation of the NFκB
pathway) in response to acute hyperlipidemia and found accumulations of both
metabolites but no differences in the extent of accumulation between superficial vastus
and soleus muscles (Dube JJ et al., unpublished data).

A somewhat surprising observation in the current study is that the activity of JNK
and p38 MAPK were for the most part unaltered by DIO or acute hyperlipidemia in the
three muscles examined. A previous study (14) in mice demonstrated increased JNK
activity in skeletal muscle of DIO mice compared to lean mice, while increased basal
activity of p38 MAPK has been reported in skeletal muscle in type II diabetes (6, 22).
The reason for the differences between our current observations and those from other studies are unclear. In the case of JNK, one can propose species difference (rats vs. mice), but given the close phylogenetic relationship of the two species, this is a somewhat unsatisfactory explanation. In the case of p38 MAPK, it is more likely that the species differences do play a role, in addition to the fact that type II diabetes is a potentially confounding variable.

In conclusion, the current study demonstrates that both DIO and acute hyperlipidemia decrease skeletal muscle IκBα levels in a fiber-type dependent manner. Since the effects of DIO and acute hyperlipidemia are similar we speculate that dyslipidemia may be a mechanism for activation of inflammatory/stress pathways in obesity and type II diabetes.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

**Figure 1. Effects of diet-induced obesity on skeletal muscle IκBα levels.** Three skeletal muscles differing in fiber-type [superficial vastus, fast twitch-glycolytic; soleus, slow twitch-oxidative, and gastrocnemius, mixed fast twitch-glycolytic and slow twitch-oxidative] were isolated from overnight fasted DIO and LEAN rats. Subsequently, IκBα levels were measured using standard immunoblotting techniques as described in Materials and Methods. Representative autoradiographs of IκBα levels in the three muscles are shown. A quantification of the complete data set from each muscle, as determined by densitometry, is shown below each autoradiograph. n=a minimum of six animals for the three muscles. Results are presented as mean±se. Significant differences are indicated.

**Figure 2: Effects of acute hyperlipidemia on skeletal muscle IκBα levels.** Tissues were isolated from Liposyn II (lipid) or saline infused rats and IκBα levels were measured as described in Figure 1 and Materials and Methods. Representative autoradiographs of IκBα levels in the three muscles are shown. A quantification of the complete data set from each muscle, as determined by densitometry, is shown below each autoradiograph. n=a minimum of six animals for the three muscles. Results are presented as mean±se. Significant differences are indicated.

**Figure 3. Effects of diet-induced obesity or acute hyperlipidemia on skeletal muscle triglyceride levels.** Two skeletal muscles were isolated from overnight fasted DIO and LEAN rats or from lipid or saline infused rats as described in the legend of Figure 1. Subsequently, triglyceride levels were measured as described in Materials and Methods. Results are presented as mean±se. n=a minimum of six animals for the two muscles. Significant differences are indicated.

**Figure 4. Effects of diet-induced obesity or acute hyperlipidemia on skeletal muscle p38 MAPK phosphorylation.** Three skeletal muscles were isolated from overnight fasted DIO and LEAN rats or from lipid or saline infused rats as described in the legend of Figure 1. Subsequently, phospho-p38 and p38 levels were measured as described in
Material and Methods. Representative autoradiographs of phospho-p38 and p38 levels are shown in the three muscles (Panel A for DIO and Panel B for lipid infusion). A quantification of the entire data set from each muscle, as determined by densitometry, is shown below each radiograph. Results are presented as mean±se. n=a minimum of six animals for the three muscles. Significant differences are indicated.

Figure 5. Effects of diet-induced obesity or acute hyperlipidemia on skeletal muscle JNK activity. Two skeletal muscles (SOL and GAS) were isolated from overnight fasted DIO and LEAN rats or from lipid or saline infused rats as described in the legend of Figure 1. Subsequently, JNK activity was measured as described in Materials and Methods. Representative autoradiographs of JNK activity are shown in vastus and gastrocnemius (Panel A for DIO and Panel B for lipid infusion). A quantification of the entire data set from each muscle, as determined by densitometry, is shown below each radiograph. Results are presented as mean±se. n=a minimum of eight animals for the two muscles.
Table 1: Baseline animal characteristics.

<table>
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<th></th>
<th>Lean</th>
<th>DIO</th>
<th>P=</th>
<th>Saline</th>
<th>Lipid</th>
<th>P=</th>
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<tr>
<td>Weight (gms)</td>
<td>446±9</td>
<td>513±12</td>
<td>0.001</td>
<td>276±7</td>
<td>260±3</td>
<td>NS</td>
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<td>Epididymal Fat (gms)</td>
<td>8.99±0.2</td>
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<td>NS</td>
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<tr>
<td>Plasma TG (mg/dL)</td>
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<td>ND</td>
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<tr>
<td>Plasma FFA (mmol)</td>
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<td>0.27±0.02</td>
<td>0.012</td>
<td>0.16±0.03</td>
<td>2.75±0.66</td>
<td>0.017</td>
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</tbody>
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Values are mean±se. n=a minimum of 6/group, except for FFA where n=3/group. DIO, diet-induced obesity; ND, not determined; NS, not significant.
Figure 1

Vastus

Gastrocnemius

Soleus

Arbitrary Units

Lean DIO

Lean DIO

Lean DIO

P=0.001

P=NS

P=NS
Figure 2

Gastrocnemius

Soleus
Figure 3

Panel A

Panel B

Vastus

Soleus

µg/mg protein

µg/mg protein

Lean

DIO

Lean

DIO

Saline

Lipid

Saline

Lipid

P=0.001

P=0.02

P=NS

P=0.04

P=NS

P=0.04

P=0.001

P=0.02

P=0.04

P=0.001

P=0.02

P=0.04
Figure 4

Panel A

P-p38

 lean DIO

 Vastus

Gastrocnemius

Soleus

Panel B

P-p38

 Saline Lipid

 Vastus

Gastrocnemius

Soleus

Saline Lipid

P-p38

Lean DIO

Vastus

Gastrocnemius

Soleus

Saline Lipid
Figure 5

Panel A

Lean DIO

Vastus

Saline Lipid

P = NS

Panel B

Lean DIO

Vastus

Saline Lipid

P = NS

Gastrocnemius

Saline Lipid

P = NS