TNF-α impairs endothelial function in adipose tissue resistance arteries of mice with diet-induced obesity

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As of 2008, an estimated 1.5 billion adults worldwide are overweight. This is more than double in the incidence of overweight since 1980. Of these individuals, over 500 million are clinically obese. Where being overweight/obesity was once a problem only of developed Western countries, a striking 65% of the world’s population now live in countries where the consequences of being overweight/obesity are more deadly than the consequences of underfeeding (27). The consequences of being overweight/obesity include insulin resistance, type 2 diabetes, and cardiovascular disease, all of which have been associated with dysfunction in the adipose tissue (3).

Tissue-specific blood flow is an important determinant of metabolism and lipid handling in the adipose tissue (35, 36) with epinephrine-induced increases in tissue blood flow associated with increases in fatty acid release from the adipose (36). A similar dependence on tissue blood flow can be observed in skeletal muscle where the vasodilatory effects of insulin increase nutritive blood flow (7). The direct effects of insulin on muscle glucose uptake are significantly augmented by its effects to increase limb blood flow (2), as shown by euglycemic-hyperinsulinemic clamp studies in the absence and presence of nitric oxide (NO) synthase inhibition. Although impaired adipose tissue blood flow is associated with obesity (5, 18, 37, 38), currently, the mechanism for this impairment in blood flow is unclear. A reduced vasodilator capacity of the adipose tissue resistance vasculature may be a cause. Previous studies have demonstrated adipose tissue blood flow to be modulated by both local and systemic factors such as NO bioavailability, free fatty acids, and circulating catecholamines (1, 13). Blood flow may also be impacted by inflammation in the adipose tissue microenvironment. These factors may control blood flow in the adipose tissue by modulating vasodilation in the resistance arteries. Thus, because visceral, in contrast with subcutaneous, adiposity is associated with elevated disease risk (3, 16, 28), a direct examination of the effects of high-fat (HF) feeding on the function of the resistance arteries is of critical importance.

Chronic low-grade inflammation associated with diet-induced obesity contributes to increased cardiovascular and metabolic disease risk and is characterized by increased circulating and adipose tissue cytokines and adhesion molecules as well as increased infiltration of macrophages in the adipose tissue (3, 24). Chief among the cytokines is TNF-α. When compared with subcutaneous adipose tissue, visceral adipose tissue of obese patients demonstrates a proinflammatory adipose tissue phenotype characterized by elevated TNF-α gene expression and macrophage infiltration concomitant with endothelial dysfunction in excised resistance arteries (12). Although a direct effect of this proinflammatory cytokine on vasodilator capacity...
of the adipose tissue resistance arteries is not known, TNF-α impairs endothelium-dependent dilation (EDD) and NO bioavailability in the coronary circulation of obese and type 2 diabetic rats (14, 31). Taken together, these studies provide evidence in support of our hypothesis that diet-induced obesity-mediated increases in TNF-α in the adipose tissue artery microenvironment contribute to impaired endothelial function in adipose tissue resistance arteries. Here, we demonstrate that TNF-α impairs EDD and NO bioavailability in resistance arteries excised from the epididymal white adipose tissue (eWAT), a visceral adipose depot of lean mice, but this in vitro effect of TNF-α is absent in the arteries isolated from the inflamed adipose tissue of glucose-intolerant insulin-resistant HF-fed mice.

METHODS

Ethical approval. All animal procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996) and were approved by the University of Utah and Veteran’s Affairs Medical Center–Salt Lake City (VAMC-SLC) Animal Care and Use Committees.

Animals. Male B6D2F1 mice were obtained from Charles River. All mice were housed in an animal care facility at the VAMC-SLC on a 12-h:12-h light:dark cycle. Young (5.7 ± 0.3 mo, n = 17) mice were fed normal rodent chow (NC, 8640 Harlan Teklad 22/5 Standard Rodent Chow; protein, 29%; carbohydrate, 55%; fat, 16% by kcal) or a HF (Harlan Teklad custom diet TD.96132, adjusted fat diet; protein, 29%; carbohydrate, 40.7%; fat, 40.7% by kcal) or rodent chow (8640 Harlan Teklad 22/5 Standard Rodent Chow; protein, 29%; carbohydrate, 55%; fat, 16% by kcal) (24) diet ad libitum for 9 to 10 mo.

Metabolic testing. Glucose tolerance was assessed by an intraperitoneal glucose tolerance test (GTT) as described previously (24). Briefly, mice were fasted 2 h in the morning before baseline blood glucose was measured using a Precision Xceed Pro Glucose Analyzer (Abbott, Chicago, IL). Glucose (2,000 mg/kg) was administered by intraperitoneal injection, and blood glucose was monitored at 15, 30, 45, 60, and 90 min after the injection. As a control, saline (2 ml/kg) was injected intraperitoneally. To assess differences in glucose tolerance, plasma insulin during the GTT, and endothelium-dependent and -independent dilation, repeated-measures ANOVA tests were performed with least significant difference post hoc tests were performed. Data are presented as means ± SE. Significance was set at P < 0.05.

RESULTS

At euthanasia, total body mass was greater in HF- compared with NC-fed mice (Table 1). Daily caloric intake in kilocalories per day did not differ between NC- and HF-fed mice (Table 1). HF was associated with a large increase in adiposity such that the mass of the eWAT was doubled after HF feeding and accounted for ~4% of total body mass compared with only ~2% in the NC-fed mice (Table 1). Soleus muscle mass was also greater in HF- compared with NC-fed mice (Table 1). No other differences in tissue masses were found after HF diet (gastrocnemius muscle, heart, liver; all P > 0.24 vs. NC). Glucose intolerance and impaired insulin sensitivity after HF feeding (Table 1).

Fasted blood glucose (Fig. 1A; P < 0.01), but not plasma insulin (Fig. 1B), was higher in HF- compared with NC-fed mice. Mice were glucose intolerant after HF feeding (Fig. 1A; P < 0.01 main effect for diet) and had elevated plasma insulin concentrations throughout a GTT (Fig. 1B; P < 0.05 main effect for diet), suggestive of impaired insulin sensitivity after HF feeding. Indeed, estimating insulin sensitivity from fasted
glucose and insulin concentrations with HOMA, we found insulin sensitivity (HOMA-S, 64.1 ± 4.3 vs. 85.7 ± 6.4; P = 0.05), but not β-cell function (HOMA-B, 37.8 ± 3.0 vs. 33.3 ± 5.3), to be lower in HF- compared with NC-fed mice.

This metabolic dysfunction was accompanied by elevated tissue inflammation, but not circulating inflammation. This was demonstrated by greater macrophage infiltration (Fig. 2A; P = 0.01) and elevated TNF-α concentrations (Fig. 2B; P < 0.05) in the eWAT of HF- compared with NC-fed mice, and the absence of an increase in plasma TNF-α (Fig. 2B) after HF feeding.

There was no difference in the maximal diameter or preconstriction to phenylephrine of eWAT resistance arteries from NC- and HF-fed mice (Table 2). Incubation with the NO synthase inhibitor L-NAME did not change phenylephrine-mediated preconstriction in either the NC- or HF-fed mice (Table 2). After in vitro treatment with rTNF, preconstriction to phenylephrine was lower in eWAT resistance arteries from HF- compared with NC-fed mice, and this was true in both the absence and presence of L-NAME (both P < 0.05; Table 2). There was no effect of L-NAME on preconstriction in rTNF-treated eWAT resistance arteries from NC- or HF-fed mice (Table 2). In vitro treatment with abTNF did not affect preconstriction to phenylephrine in NC- or HF-fed mice in either the absence or presence of L-NAME (Table 2).

ACh-induced dilation was impaired in resistance arteries from the eWAT of HF- compared with NC-fed mice (Fig. 3A; P < 0.01). Sensitivity (IC50) to ACh did not differ between groups (Table 2). Although L-NAME reduced ACh-induced dilation in arteries from both NC- and HF-fed mice (Fig. 3A; both P < 0.01), L-NAME eliminated the differences in dilation previously observed between NC- and HF-fed mice, indicating that impaired dilation observed after HF feeding resulted from reduced NO bioavailability (Fig. 3A). Endothelium-independent dilation (Fig. 3B) and sensitivity (Table 2) to SNP did not differ between groups.

In vitro incubation of eWAT resistance arteries from NC-fed mice with rTNF resulted in an impaired dilation (Fig. 4; P = <0.01) but no change in sensitivity (Table 2) to ACh. Although incubation with L-NAME reduced dilation to ACh in rTNF-treated arteries from NC-fed mice (Fig. 4A; both P < 0.01), L-NAME eliminated the rTNF-mediated differences in dilation (Fig. 4A and Table 2). Thus impaired dilation after rTNF treatment results from a reduction in NO bioavailability, similar to what was observed after HF feeding (Fig. 4A).

Vasodilation to ACh was not impaired by incubation with rTNF in eWAT arteries from HF-fed mice, and L-NAME failed to reduce ACh dilation in these arteries (Fig. 4B). No differences in sensitivity to ACh were observed in eWAT arteries from HF-fed mice after L-NAME, rTNF, or combined L-NAME and rTNF incubation (Table 2). These results suggest that the effect of rTNF to impair eWAT artery EDD and NO bioavailability is lost after HF feeding, a state during which the eWAT arteries are chronically exposed to elevations in this cytokine in vivo (Fig. 2B). rTNF did not affect endothelium-
independent dilation (not shown) or sensitivity (Table 2) to SNP in arteries from NC- or HF-fed mice.

Although not reduced at maximal dilation, the dose response to ACh in eWAT resistance arteries from NC-fed mice was reduced (shifted rightward) after in vitro treatment with abTNF (Fig. 5A; P < 0.05). However, this rightward shift was insufficient to reduce sensitivity to ACh (Table 2). L-NAME reduced dilation (Fig. 5A; P < 0.01) but not sensitivity (Table 2) to ACh in abTNF-treated arteries from NC-fed mice.

Table 2. White adipose tissue artery maximal luminal diameter, percent preconstriction to PE, and sensitivity (IC50) to ACh in the absence or presence of the l-NAME and rTNF or abTNF as well as sensitivity to SNP

<table>
<thead>
<tr>
<th></th>
<th>Normal Chow</th>
<th>High-Fat Diet</th>
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<tbody>
<tr>
<td>Maximal diameter, μm</td>
<td>179 ± 9</td>
<td>183 ± 6</td>
</tr>
<tr>
<td>Preconstriction (% max diameter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>47 ± 4</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>PE + l-NAME</td>
<td>60 ± 6</td>
<td>42 ± 10</td>
</tr>
<tr>
<td>PE + rTNF</td>
<td>57 ± 4</td>
<td>42 ± 7*</td>
</tr>
<tr>
<td>PE + rTNF + l-NAME</td>
<td>62 ± 8</td>
<td>42 ± 7*</td>
</tr>
<tr>
<td>PE + abTNF</td>
<td>45 ± 5</td>
<td>40 ± 11</td>
</tr>
<tr>
<td>PE + abTNF + l-NAME</td>
<td>48 ± 7</td>
<td>44 ± 9</td>
</tr>
<tr>
<td>IC50 (log M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>-7.66 ± 0.27</td>
<td>-6.94 ± 0.43</td>
</tr>
<tr>
<td>ACh + l-NAME</td>
<td>-6.94 ± 0.43</td>
<td>-7.57 ± 0.40</td>
</tr>
<tr>
<td>rTNF + ACh</td>
<td>-6.78 ± 0.49</td>
<td>-7.65 ± 0.55</td>
</tr>
<tr>
<td>rTNF + ACh + l-NAME</td>
<td>-7.49 ± 0.62</td>
<td>-6.89 ± 0.68</td>
</tr>
<tr>
<td>abTNF + ACh</td>
<td>-6.80 ± 0.24</td>
<td>-6.87 ± 0.58</td>
</tr>
<tr>
<td>abTNF + ACh + l-NAME</td>
<td>-6.69 ± 0.39</td>
<td>-6.42 ± 0.74</td>
</tr>
<tr>
<td>SNP</td>
<td>-8.21 ± 0.33</td>
<td>-7.87 ± 0.47</td>
</tr>
<tr>
<td>rTNF + SNP</td>
<td>-8.64 ± 0.36</td>
<td>-7.59 ± 0.46</td>
</tr>
<tr>
<td>abTNF + SNP</td>
<td>-8.04 ± 0.43</td>
<td>-7.34 ± 0.14</td>
</tr>
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</table>

Values are means ± SE. PE, phenylephrine; l-NAME, Nω-nitro-l-arginine methyl ester; rTNF, recombinant TNF-α; abTNF, neutralizing antibody against TNF-α; SNP, sodium nitroprusside. *Difference, NC vs. HF, P < 0.05.

In vitro incubation of eWAT resistance arteries from HF-fed mice with abTNF resulted in an improved dilation (Fig. 5B; P = 0.05) to ACh. Incubation with l-NAME reduced dilation to ACh in abTNF-treated arteries from HF-fed mice (Fig. 5B; P < 0.01). Thus impaired dilation and reduced NO bioavailability observed with HF feeding in eWAT resistance arteries can be restored by abTNF treatment. There were no differences in sensitivity to ACh with or without l-NAME treatment in abTNF-treated arteries (Table 2). Neither dilation (not shown) nor sensitivity (Table 2) to SNP differed between NC- and HF-fed mice after abTNF treatment.

**DISCUSSION**

The novel findings of the present study are that HF diet-induced glucose intolerance and insulin resistance is concomitant with endothelial dysfunction and reduced NO bioavailability in visceral eWAT resistance arteries. We extend these observations by demonstrating that exogenous administration of TNF-α can impair function in eWAT arteries from NC-, but not HF-fed, mice and that the chronic in vivo exposure of eWAT arteries to elevated TNF-α resulting from HF feeding underlies the observed endothelial dysfunction. Although the causal role of adipose tissue dysfunction and inflammation in obesity-associated metabolic and cardiovascular disease risk is well established (3, 15, 24, 32), there is surprisingly little information regarding the role of vascular function per se in the overall function/phenotype of the adipose tissue (30), or how this vascular function is affected by obesity (12). Here, we provide direct evidence for a HF-diet-mediated, TNF-α-induced, impairment in adipose tissue arterial function that may have important implications for tissue-specific and systemic metabolic and vascular health.

The findings presented here extend our knowledge of the consequences of obesity on vascular function. Our results
circulations (14, 29), and this dysfunction has been attributed, the skeletal muscle (11, 22), mesenteric, (6, 34) and coronary
ated with arterial dysfunction in various vascular beds such as

tissue blood flow is the consequence of a reduction in vasodi-
determine whether a putative impairment in visceral adipose
effect of obesity on visceral adipose blood flow, as well as

dysfunction on impairments in adipose tissue blood flow in
not provide direct evidence for a causal effect of endothelial
contributes to macrophage infiltration (40). Although, we can-

tissue inflammatory phenotype by en-
nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester
from the eWAT of NC (N = 10)- and HF (N = 9)-fed mice. Endothelium-

dilation is simply the result of tissue specificity, as has been
observed in resistance arteries from other closely related tis-
sues like white and red skeletal muscle (26). The authors
recent study by Farb et al. (12) demonstrated a reduced
dilation in the resistance arteries from the visceral compared
with subcutaneous adipose tissue of obese humans. However,
this study lacked samples from lean patients, and thus cannot
exclude the possibility that the reduced visceral adipose artery
dilation is simply the result of tissue specificity, as has been
observed in resistance arteries from other closely related tis-
uose stores.

suggest that endothelial dysfunction in the resistance vascula-
ture of the adipose tissue per se may 1) underlie the previously
reported impairments in adipose tissue blood flow and 2) con-
tribute to the adipose tissue inflammatory phenotype by enhan-
cing adipose artery adhesion molecule expression that then

serves to macrophage infiltration (40). Although, we can-
not provide direct evidence for a causal effect of endothelial
dysfunction on impairments in adipose tissue blood flow in
obese humans (1, 18), future studies should directly examine
the effect of obesity on visceral adipose blood flow, as well as
determine whether a putative impairment in visceral adipose
tissue blood flow is the consequence of a reduction in vasodi-
ulator capacity of its resistance vasculature.

Previous studies have demonstrated that obesity is associ-
ated with arterial dysfunction in various vascular beds such as
the skeletal muscle (11, 22), mesenteric, (6, 34) and coronary
circulations (14, 29), and this dysfunction has been attributed,
at least in part, to the effects of adipose-derived factors (29, 
43). Although subcutaneous adipose tissue blood flow has been
shown to be impaired in obese humans in response to feeding
or β-adrenergic stimulation (1, 17, 18, 38), little is known
about how vascular function is modified by obesity in the
visceral adipose stores.

A recent study by Farb et al. (12) demonstrated a reduced
dilation in the resistance arteries from the visceral compared
with subcutaneous adipose tissue of obese humans. However,
this study lacked samples from lean patients, and thus cannot
exclude the possibility that the reduced visceral adipose artery
dilation is simply the result of tissue specificity, as has been
observed in resistance arteries from other closely related tis-
uose like white and red skeletal muscle (26). The authors
demonstrated that the reduced dilation in the visceral adipose

Fig. 3. Endothelium-dependent dilation (A) to ACh in the absence or presence
of the nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester
(L-NAME), measured by pressure myography in resistance arteries excised
from the eWAT of NC (N = 11)- and HF (N = 10)-fed mice. Endothelium-
dependent dilation (B) to sodium nitroprusside in resistance arteries excised
from the eWAT of NC (N = 11)- and HF (N = 6)-fed mice is also shown.
Significance marks in legend denote a time by group interaction or main effect
for diet during repeated-measures ANOVA. Marks above curves indicate
difference between groups and treatment conditions at specific time points by
1-way ANOVA with least significant difference (LSD) post hoc tests. *Dif-
fences between NC- and HF-diet groups; †differences in ACh responses in
the absence and presence of L-NAME. Data are means ± SE (P ≤ 0.05).

Fig. 4. Endothelium-dependent dilation (A) to ACh in the absence or presence
of the NOS inhibitor L-NAME and recombinant TNF-α (rTNF) measured by
pressure myography in resistance arteries excised from the eWAT of NC (N = 
7)-fed mice. Endothelium-dependent dilation (B) to ACh in the absence or
presence of the L-NAME and/or rTNF measured in resistance arteries excised
from the eWAT of HF (N = 9)-fed mice is also shown. Significance marks in
legend denote a time by group interaction or main effect for diet during
repeated-measures ANOVA. Marks above curves indicate difference between
groups and treatment conditions at specific time points by 1-way ANOVA with
LSD post hoc tests. †Differences in ACh responses in the absence and presence
of L-NAME; ††differences in ACh responses in the absence and presence of
rTNF. Data are means ± SE (P ≤ 0.05).
Here, we demonstrate that adipose tissue arteries from NC- but not HF-fed mice are sensitive to TNF-α exposure in vitro, resulting in impaired EDD and reduced NO bioavailability in these “naïve” arteries. With HF feeding, adipose arteries are chronically exposed to a proinflammatory microenvironment, characterized by increased macrophage infiltration and increased adipose tissue TNF-α. Under these conditions, the in vitro effect of TNF-α to impair endothelial function was lost in the already dysfunctional HF-fed mouse arteries. To establish a causal role for TNF-α in the HF diet-induced endothelial dysfunction observed in the eWAT arteries, we examined whether inhibition of TNF-α could restore EDD and NO bioavailability in arteries from HF-fed mice. Here we found that incubation with a neutralizing antibody against TNF-α restored EDD and NO bioavailability in eWAT arteries from HF-fed mice and was without effect in arteries from NC-fed mice. These results suggest that although other proinflammatory mediators such as IL-1β may be elevated in obesity and have the capacity to impair endothelial function (19, 42), in the adipose tissue circulation, TNF-α is a critical mediator of impaired function after HF feeding.

Although the mechanism by which TNF-α leads to impaired EDD and reduced NO likely involves either a direct inhibition of NOS activation (20) or increases in oxidative stress (8, 45), these possibilities require direct examination. It is also possible that the lack of effect of rTNF in HF mouse arteries is the result of receptor desensitization/downregulation following chronic exposure in vitro, resulting in impaired function after HF feeding.

In the present study, we observed an increase in soleus muscle mass after HF feeding. Although the mechanism for the observed increase in soleus muscle mass is unclear, it may be related to an increase in lipid accumulation. To explore this possibility we measured lipid content in quadriceps muscle from NC- and HF-fed mice. It should be noted that ~100 mg of tissue is used for digestion and triglyceride determination, and thus the small mass of the soleus precluded us from making these measures directly in the soleus muscle. Although the triglyceride content of the quadriceps muscle was significantly increased after HF feeding (Table 1), if one assumes a similar content of lipid between the quadriceps and soleus muscles, lipid accumulation cannot completely explain the increase in soleus mass since this concentration of lipid would account for less than a milligram of the total muscle mass in the soleus after HF feeding. Hypertrophy of the muscle in response to increased body mass may explain the remaining differences in soleus mass between NC- and HF-fed mice.

**Limitations.** We acknowledge that the concentration of TNF-α used in the bath is an order of magnitude greater than what was measured in the eWAT. Although this is a limitation of the present study, we felt that due to the acute (60 min) in vitro exposure of the arteries, the higher concentration was warranted and necessary to evaluate the effects of TNF-α on vascular function. Indeed, this dose of TNF-α (1 ng/ml) has previously been demonstrated to induce endothelial dysfunction in the coronary arteries (44). Our studies cannot rule out other inflammatory mediators in the development of obesity-associated adipose tissue endothelial dysfunction, nor can we...
delineate the specific cellular source of the TNF-α, e.g., vascular or immune cell derived (8, 45). These are questions that require further study. Finally, there have been concerns raised over the use of SNP as an endothelium-independent vasodilator, since its long-term and/or high dose usage can lead to cyanide toxicity. However, the reaction has a long time course and requires the binding of the iron group in SNP to a sulfhydryl group present in vivo in erythrocytes (41) and are not present in our preparation. Thus we believe that SNP is appropriate in the present investigation.

In conclusion, HF feeding results in a decreased EDD in visceral adipose tissue arteries and appears to be mediated by their proinflammatory tissue environment. This adipose tissue arterial dysfunction may impact adipose tissue blood flow and contribute to the adipose inflammation that results in the metabolic derangements observed; however, this possibility requires direct examination. By demonstrating a HF-diet induced reduction in eWAT resistance artery vasodilator capacity, these studies raise the possibility that inflammation-mediated vascular dysfunction within the adipose tissue may be an important factor in adipose tissue dysfunction and subsequent systemic metabolic dysfunction resulting from diet-induced obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


