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Effects of tumor necrosis factor alpha on leptin secretion and gene expression: relationship to changes of glucose metabolism in isolated rat adipocytes

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OBJECTIVE: Our objective was to determine the effects of prolonged exposure to tumor necrosis factor-alpha (TNF-α) on leptin secretion from and leptin (OB) gene expression in isolated adipocytes. Because glucose uptake and the metabolism of glucose beyond lactate are important determinants of leptin production in adipocytes, we examined the effects of TNF-α on glucose uptake and lactate production and their relationship to leptin secretion.
DESIGN AND METHODS: Isolated rat adipocytes were anchored in a defined matrix of basement membrane components and cultured with media containing 5 mM glucose, 0.16 nM insulin and several concentrations of TNF-α. Leptin secretion, steady-state levels of leptin mRNA levels, glucose uptake, and lactate production were assessed over 96 h.
RESULTS: TNF-α at concentrations of 0.024, 0.24, 2.4 and 24 ng/ml did not affect leptin secretion over 24 h. TNF-α at concentrations of 0.24 to 24 ng/ml significantly inhibited leptin secretion over 96 h by 19–60%. TNF-α at concentrations of 0.024 to 24 ng/ml significantly decreased steady-state levels of leptin mRNA after 96 h by 32–95%. In addition, TNF-α at concentrations of 2.4 and 24 ng/ml significantly increased glucose uptake and lactate production over 96 h by 30–57%. TNF-α at a concentration of 0.024 ng/ml did not affect leptin secretion, glucose uptake or lactate production. Overall, for the TNF-α concentrations tested, leptin secretion was inversely related to the percent of glucose carbon released as lactate; however, TNF-α did not induce a proportional increase of lactate production from glucose.
CONCLUSION: Short-term (24 h) exposure of isolated adipocytes to TNF-α does not affect leptin secretion. Prolonged exposure to TNF-α produces a concentration-dependent inhibition of leptin secretion and gene expression. This suggests that the acute effect of TNF-α to increase circulating leptin levels *in vivo* may be indirect. TNF-α at higher concentrations increases glucose uptake, but does not increase the conversion of glucose to lactate. Therefore, TNF-α appears to induce a dissociation between adipocyte glucose metabolism and leptin production.

Keywords: leptin; TNF-α; glucose metabolism; lactate production; adipocytes

Introduction

Tumor necrosis factor-alpha (TNF-α) is a cytokine known for its effects on tumor cells as well as its various other functions in host immunity: it is a key mediator of inflammation.[1] TNF-α can also significantly affect whole-body metabolism as demonstrated by its ability to induce hyperlipidaemia, hyperglycaemia, and insulin resistance in chronic inflammation, cancer and when it is infused.[2,3] Several studies have shown that adipose tissue TNF-α expression increases with increasing adiposity in rodents and humans.[4–6] It has also been reported that decreases in body weight, which results in improved insulin sensitivity, are associated with decreased TNF-α expression.[5] Primarily based on those observations, TNF-α has been implicated in the insulin resistance of obesity. The possible causal role of TNF-α in the insulin resistance of obesity is further supported by recent evidence that obese mice with a targeted null mutation in the gene encoding TNF-α and its receptors show significantly improved insulin sensitivity.[7]

Circulating concentrations of leptin, the recently discovered protein product of the leptin (OB) gene implicated in the regulation of appetite and body adipose stores, are also correlated with adiposity in humans[8,9] and animals.[9–12] TNF-α has been reported to acutely increase leptin gene expression and circulating leptin concentrations in rodents and humans.[13–15] Lipopolysaccharide and interleukin-1 also increase circulating leptin concentrations;[13,14,16] however, of those factors, only TNF-α is reported to be elevated in obesity.[17] To date, few studies have assessed the effects of TNF-α on leptin production in cultured adipocytes. One report showed that TNF-α

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can acutely stimulate leptin release from 3T3-L1 adipocytes.[18] Two studies that used primary adipocytes reported conflicting results.[19,20] One demonstrated that TNF-α stimulates leptin secretion after one day;[19] the other did not report a stimulation of leptin secretion by TNF-α but showed that exposure for 2 days inhibits leptin production.[20] Thus, the effects of prolonged exposure of adipocytes to TNF-α on leptin production remain unclear.

While the factors that regulate leptin production are not completely understood, several studies indicate that insulin stimulates leptin secretion and gene expression *in vivo*[21,22] and *in vitro*.[23,24] For example, decreases of circulating leptin correlate with decreases of insulin after weight loss, fasting or energy restriction[8,10,25,26] independent of changes of body weight or adiposity. In addition, plasma leptin decreases markedly and rapidly after induction of insulin-deficient diabetes in rats and increases in proportion to the degree of glucose lowering after insulin treatment.[27] These observations led us to hypothesize that adipocyte glucose metabolism is involved in the regulation of leptin secretion. Our laboratory recently reported that insulin-stimulated leptin secretion is likely to be mediated by increases of adipocyte glucose utilization and that increased conversion of glucose to lactate by adipocytes is associated with decreased leptin secretion.[23,28] These *in vitro* studies, and the present one, utilized a culture system in which isolated rat adipocytes are anchored in a defined mixture of extracellular matrix components that mimics the normal basement membrane attachment of cells; adherent culture of adipocytes maintains differentiation[29] and enables long-term studies of leptin production.[23,28]

Because adipose tissue TNF-α expression is chronically elevated in obesity,[4–6] it is possible that TNF-α has a chronic influence on leptin production and other cytokine-sensitive functions of adipocytes such as glucose and lipid metabolism.[30] Studies that assess the effects of long-term exposure of adipocytes to TNF-α, on leptin production and metabolism, may clarify mechanisms of dysregulation that play a role in obesity or its pathogenesis. Therefore, the aim of the present study was to determine the effects of prolonged exposure to TNF-α on leptin secretion from and leptin gene expression in cultured rat adipocytes. In addition, because glucose uptake and the metabolism of glucose beyond lactate may be important determinants of leptin production, we also examined the effects of TNF-α on glucose uptake and lactate production and their relationship to leptin secretion.

Materials and methods

Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Life Technologies (Grand Island, NY). The media were supplemented with 6 ml each of MEM non-essential amino acids, penicillin/streptomycin (5000 U/ml/5000 μg/ml), and nystatin (10,000 U/ml; all from Life Technologies) per 500 ml DMEM. BSA fraction V, HEPES, collagenase (*Clostridium histolyticum;* type II; specific activity 456 U/mg), and insulin were purchased from Sigma Chemical Co., (St. Louis, MO). Matrigel matrix was purchased from Becton-Dickinson (Franklin Lakes, NJ). Recombinant mouse TNF-α was purchased from Genzyme (Cambridge, MA). Six-well Falcon tissue culture plates were purchased from Fisher Scientific (Pittsburgh, PA). Nylon filters were purchased from Tetko (Kansas City, MO).

Animals

Male Sprague-Dawley rats were obtained from Charles River (Wilmington, MA). Animals were housed in hanging wire cages in temperature controlled rooms, fed Purina chow (Ralston-Purina, St. Louis, MO) diet, and given deionized water *ad libitum.* The study protocol was approved by the University of California, Davis Animal Care and Use Committee.

Cell isolation/preparation

Adipocytes were prepared from epididymal fat pads obtained from halothane-anaesthetized rats (350–550 g). Epididymal fat depots were resected under sterile conditions and prepared by collagenase digestion with minor modification to the procedure by Rodbell[31] as described below. The fat pads were minced in Krebs-Ringer HEPES buffer with 2% BSA.[23] Adipose tissue fragments were digested in the same buffer in the presence of type II collagenase (2.5 mg/2 ml Buffer/g tissue) at 37°C with gentle shaking for 45 min. The resulting cell suspension was diluted in cold HEPES-phosphate buffer and isolated adipocytes were separated from undigested tissue by filtration through a 400 μm nylon mesh and washed. For washing, cells were centrifuged and the adipocytes resuspended in Krebs-Ringer HEPES buffer. The final media were 5 mM glucose culture media (DMEM plus antibiotics, non-essential amino acids, and 1% FBS). The isolated adipocytes were then incubated for 30 min at 37°C before being plated on Matrigel-coated culture plates.

Adipocyte culture

Matrigel was thawed on ice to a liquid and uniformly applied to the surface of the culture dish. 150 μl of the packed adipocyte suspension (2:1 ratio of packed cells to media) were plated on the liquid matrix, effectively anchoring them to the culture dish. After a 30 min incubation at 37°C, 2 ml of warm culture medium with or without insulin and/or TNF-α was added. The cells were maintained in an incubator at 37°C for 96 h with 6% CO_2. Isolated adipocytes from each animal

were divided into wells, with a control well for each concentration of TNF-α. Adipocytes were incubated with 5 mM glucose and concentrations of TNF-α at 0.024, 0.24, 2.4 or 24 ng/ml plus 0.16 nM insulin. Insulin at 0.16 nM was utilized because it is a low concentration which, we have previously found, increases leptin secretion above basal levels.[23] Aliquots of media (300 µl) were collected at 24, 48, 72 and 96 h with fresh media replacement after sampling. After 96 h, culture plates were frozen at −85°C until analyzed for leptin mRNA levels by Northern blot. Adipocytes from 9 different animals were tested (9 experiments).

Assays

Leptin concentrations in the media were determined with a radioimmunoassay for murine leptin (Linco Research, St. Louis, MO).[8] The intra- and interassay coefficients of variation are 4.0 and 11.2%, respectively. Leptin concentrations in the media from cultured rat adipocytes measured with this assay are very similar to those obtained with a more recently developed assay specific for rat leptin.[32] With the rat-specific assay, measured leptin concentrations are highly correlated with results obtained with the murine leptin assay. Therefore, measurements of rat leptin made with the mouse assay are a valid measurement of leptin concentrations. The antibody used in the assay does not cross-react with insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin.[10] Glucose and lactate were measured with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Northern blot analysis

At the end of the 96 h incubation, total RNA was isolated by adding 3 ml of TRIzol (Life Technologies) directly to the wells in which the cells were incubated, and the RNA then extracted by standard ethanol precipitation as suggested by the manufacturer. 1.2 µg of total RNA were loaded into each lane. Northern blot analyses were as previously described.[33] After leptin mRNA was quantified by assessing the intensity of the leptin mRNA band, the blots were stripped and probed for 18S ribosomal RNA, which was then also similarly quantified for each lane. The final results were normalized as leptin mRNA/18S rRNA for each lane and then expressed as a percentage of control.

Data analysis

Glucose uptake by adipocytes was assessed by measuring the concentration in the medium before and after 96 h of incubation and calculating the decrease over 96 h. The amount of carbon released as lactate per amount of carbon taken up as glucose over 96 h was calculated as $\Delta(\text{lactate})/\Delta(\text{glucose})\times 100$. Changes of leptin secretion and expression, glucose uptake, and lactate production in response to TNF-α were determined by paired t test. To examine the relationships between the media concentrations of TNF-α, glucose uptake, lactate production, leptin secretion and leptin expression, simple and multiple linear regression analyses were performed (StatView, Abacus Concepts, Inc., Berkeley, CA). Data are expressed as mean ± s.e.m.

Results

Effects of TNF-α on leptin secretion

The effects of prolonged exposure to TNF-α on leptin secretion were examined. TNF-α at concentrations of 0.024, 0.24, 2.4 and 24.0 ng/ml did not significantly affect leptin secretion from adipocytes cultured in media containing glucose and insulin over 24 h (Figure 1). TNF-α at concentrations of 2.4 and 24.0 ng/ml inhibited leptin secretion over 96 h by 60.1 ± 5.5 and 58.8 ± 8.2%, respectively (both $P < 0.0005$). TNF-α at 0.24 ng/ml inhibited leptin secretion by 18.6 ± 6.1% ($P < 0.01$). A ten fold lower concentration (0.024 ng/ml) did not significantly inhibit leptin secretion (5.4 ± 9.9%, NS). Overall, TNF-α concentration was inversely correlated with leptin secretion ($r = -0.51$; $P < 0.0005$). The effect of TNF-α to inhibit leptin secretion was similar when adipocytes were incubated without insulin (data not shown). For example, in three experiments, adipocytes cultured with 24.0 ng/ml of TNF-α inhibited leptin secretion by 49.5 ± 10.8% ($P < 0.025$).

Effects of TNF-α on leptin mRNA

The effects of TNF-α on steady-state levels of leptin mRNA were examined. TNF-α at concentrations of

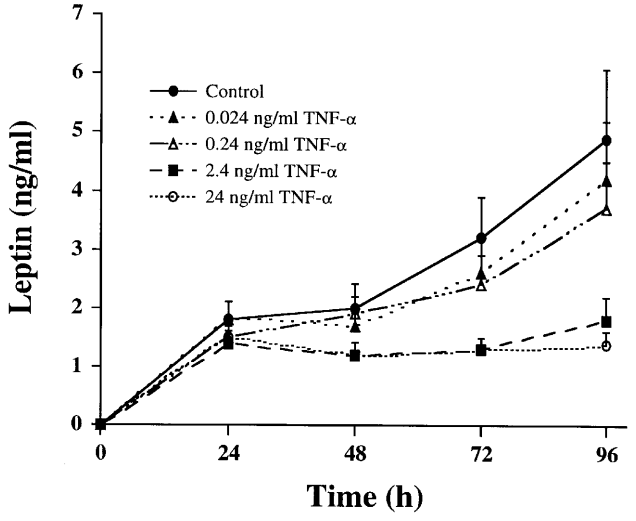

Figure 1 Effect of TNF-α on leptin secretion. Isolated rat adipocytes were cultured in media containing 5.0 mM glucose and 0.16 nM insulin. Cultures were incubated for 96 h with 0.024 to 24 ng/ml TNF-α or without TNF-α (control). Samples were collected at 24, 48, 72 and 96 h and leptin concentrations assessed by RIA. Values are the mean ± s.e.m. from nine experiments.

2.4 and 24.0 ng/ml decreased steady-state levels of leptin mRNA in adipocytes cultured in media containing glucose and insulin after 96 h, by 87.6 ± 2.3 and 95.2 ± 3.1%, respectively (both $P < 0.001$) (Figure 2). TNF-α at 0.024 and 0.24 ng/ml decreased leptin mRNA levels by 31.5 ± 8.1 ($P < 0.01$) and 40.6 ± 1.9% ($P < 0.05$), respectively. Overall, TNF-α concentration was inversely correlated with steady-state levels of leptin mRNA ($r = -0.70$; $P < 0.05$). The effect of TNF-α to decrease leptin mRNA levels was similar when adipocytes were incubated in the absence of insulin (data not shown). For example, in two experiments, adipocytes cultured with 2.4 ng/ml and 24.0 ng/ml of TNF-α decreased leptin mRNA levels by 89.7 ± 1.3 and 93.0 ± 9.3%, respectively (both $P < 0.025$).

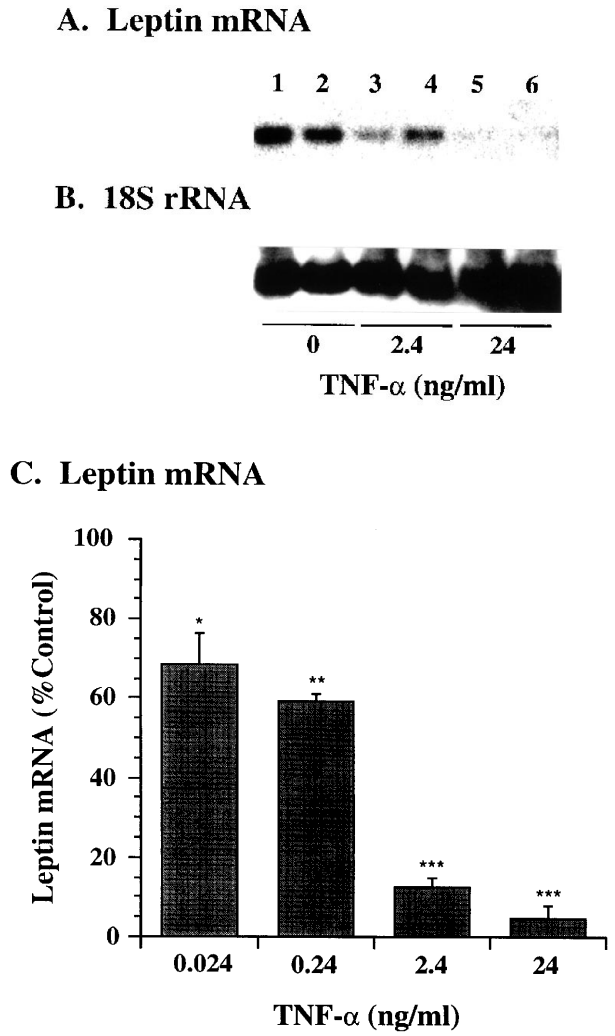

Figure 2 Effect of TNF-α on leptin gene expression. Isolated rat adipocytes were cultured in media containing 5.0 mM glucose and 0.16 nM insulin. Cultures were incubated for 96 h with 0.024 to 24 ng/ml TNF-α or without TNF-α (control). After 96 h plates were frozen and then analyzed for leptin mRNA by Northern blot. Leptin mRNA (A) and 18S rRNA (B) on a representative Northern blot. Total RNA (1.2 μg) from cultures incubated with 0, 2.4 and 24.0 ng/ml TNF-α from two experiments (not pooled) were loaded into each lane. Lanes 1–2: adipocytes incubated with no TNF-α. Lanes 3–4: adipocytes incubated with 2.4 ng/ml TNF-α. Lanes 5–6: adipocytes incubated with 24 ng/ml TNF-α. (C) Quantification of leptin mRNA levels. The leptin mRNA level for each culture was normalized to the amount of 18S rRNA as described under Materials and methods. Values are the mean ± SEM from three experiments. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$ *vs* control.

Effects of TNF-α on glucose uptake

We recently showed that leptin secretion in primary cultures of isolated rat adipocytes is related to glucose uptake by adipocytes.[23] Thus, we assessed the effects of TNF-α on glucose uptake over 96 h by adipocytes cultured in media containing insulin and glucose. Glucose uptake by adipocytes is reflected by the depletion of glucose in the culture media over time. TNF-α at concentrations of 2.4 and 24.0 ng/ml, increased glucose uptake by 33.1 ± 8.4 and 54.5 ± 8.7%, respectively (both $P < 0.0025$) (Figure 3). TNF-α increased absolute glucose uptake at 2.4 ng/ml ($\Delta = 1.5 \pm 0.4$ μmoles; $P < 0.005$) and at 24.0 ng/ml ($\Delta = 2.5 \pm 0.3$ μmoles; $P < 0.0005$) (Table 1). TNF-α at 0.24 and 0.024 ng/ml did not significantly increase glucose uptake (Δ over

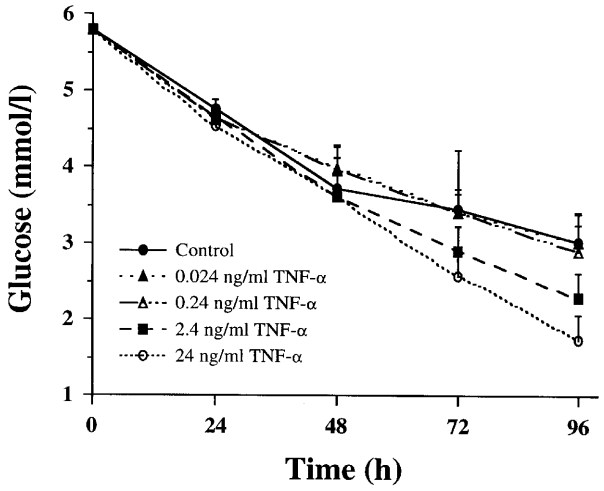

Figure 3 Effect of TNF-α on glucose uptake. Isolated rat adipocytes were cultured in media containing 5.0 mM glucose and 0.16 nM insulin. Cultures were incubated for 96 h with 0.024 to 24 ng/ml TNF-α or without TNF-α (control). Samples were collected at 24, 48, 72 and 96 h and glucose concentrations measured. Values are the mean ± s.e.m. from nine experiments.

Table 1 Effects of TNF-α on glucose uptake, lactate production and the percentage of glucose carbon taken up that was released as lactate[a]

TNF-α (ng/ml)	*Glucose Uptake (μMoles)*	*Lactate Production (μMoles)*	*Glucose to Lactate* [b] *(%)*
0 (Control)	5.5 ± 0.8	4.3 ± 0.7	44.0 ± 4.4
0.024	5.5 ± 0.8	4.8 ± 0.8*	47.8 ± 4.7
0.24	5.7 ± 0.7	4.7 ± 0.7*	44.3 ± 4.8
2.4	7.0 ± 0.7**	6.3 ± 0.9***	47.1 ± 4.1
24.0	8.0 ± 0.7***	6.5 ± 1.0***	42.2 ± 4.0

[a]Adipocytes were cultured in media containing 5.0 mM glucose and 0.16 nM insulin. Cultures were incubated for 96 h with or without TNF-α;
[b]The amount of lactate released per amount of glucose utilized over 96 h was calculated as described under Materials and methods. Values are the mean ± s.e.m. from nine experiments. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$ *vs* control.

control = 0.0 ± 0.2 μmoles and 0.2 ± 0.2 μmoles, respectively) (Figure 3, Table 1). Overall, TNF-α concentration was directly correlated with glucose uptake ($r = 0.41$; $P < 0.005$). The effect of TNF-α to increase glucose uptake was also observed when adipocytes were incubated in the absence of insulin (data not shown). For example, in two experiments, concentrations of TNF-α in the range of 0.24 to 24 ng/ml increased glucose uptake by 18.1 to 95.8%, and the TNF-α concentration was directly correlated with glucose uptake ($r = 0.70$; $P < 0.025$).

Effects of TNF-α on lactate production

We previously demonstrated that increased conversion of glucose to lactate in cultured adipocytes is associated with decreased leptin production.[23] To assess whether there was a relationship between inhibition of leptin secretion by TNF-α and increased conversion of glucose to lactate, we determined the effects of TNF-α on lactate production from adipocytes cultured in media containing glucose and insulin over 96 h. TNF-α concentrations of 0.24, 2.4, and 24.0 ng/ml increased lactate production by 10.2 ± 4.4, 47.0 ± 6.1 and 57.3 ± 11.2%, respectively (all $P < 0.025$) (Figure 4). Absolute lactate production was increased by TNF-α at all concentrations; 0.024 ng/ml (Δ = 0.5 ± 0.2 μmoles; $P < 0.05$), 0.24 ng/ml (Δ = 0.4 ± 0.2 μmoles; $P < 0.05$), 2.4 ng/ml (Δ = 2.0 ± 0.3 μmoles; $P < 0.0005$) and at 24.0 ng/ml (Δ = 2.2 ± 0.4 μmoles; $P < 0.0005$) (Table 1). Overall, TNF-α concentration was directly correlated with lactate production ($r = 0.33$; $P < 0.03$). In addition, lactate production was highly correlated with glucose uptake ($r = 0.72$; $P < 0.0001$). TNF-α did not increase the rate of conversion of glucose to lactate (Table 1).

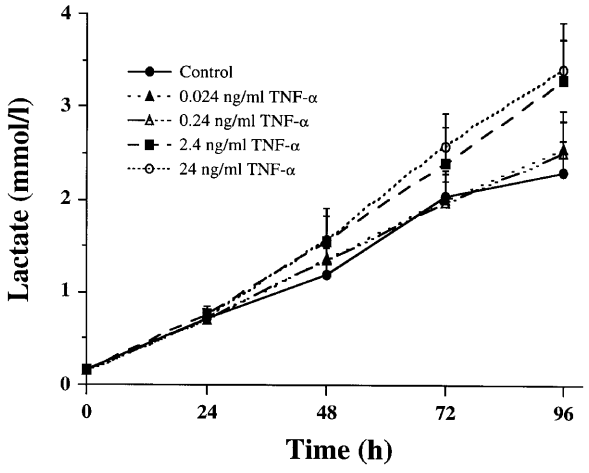

Figure 4 Effect of TNF-α on lactate production. Isolated rat adipocytes were cultured in media containing 5.0 mM glucose and 0.16 nM insulin. Cultures were incubated for 96 h with 0.024 to 24 ng/ml TNF-α or without TNF-α (control). Samples were collected at 24, 48, 72 and 96 h and lactate concentrations measured. Values are the mean ± s.e.m. from nine experiments.

Relationships of glucose uptake, lactate production, and glucose conversion to lactate, with leptin secretion

Overall, across all TNF-α concentrations, leptin secretion was inversely related to glucose uptake ($r = -0.33$; $P < 0.03$), lactate production ($r = -0.58$; $P < 0.0001$) and the % of glucose carbon released as lactate ($r = -0.52$; $P < 0.0002$). By multiple regression analysis combining glucose uptake, lactate production, and glucose conversion to lactate where the r value for the model = 0.74 ($P < 0.0001$), leptin secretion was more closely related to the percent of glucose converted to lactate ($P < 0.0002$) than to glucose uptake ($P < 0.002$) or lactate production ($P < 0.02$).

Discussion

A previous study showed that TNF-α acutely stimulates leptin secretion from 3T3-L1 adipocytes by possibly regulating the release of leptin from preformed pools. Thus, it was hypothesized that TNF-α contributes to obesity-related hyperleptinaemia.[18] However, the precise effects of TNF-α on leptin secretion from and leptin gene expression in cultured adipocytes are uncertain. For example, the two studies that utilized floating cultures of primary mouse adipocytes to assess the effects of TNF-α yielded conflicting results.[19,20] While one of the studies demonstrated that TNF-α stimulates leptin secretion at 24 h,[19] the other study showed that exposure to TNF-α for 24 h does not stimulate leptin secretion and that exposure over a period of 6 d inhibits leptin secretion and gene expression.[20] In the present study, TNF-α did not stimulate leptin secretion from primary adipocytes anchored in an extracellular matrix after 24 h, and it inhibited leptin secretion and gene expression in a concentration-dependent manner over the next 72 h. While our data contradict the study that reported a stimulation of leptin secretion after 24 h, it agrees with the other reports. However, the study conducted in 3T3-L1 cells did show that both leptin secretion and gene expression were completely blunted by 24 h. This discrepancy with our results at 24 h may be due to differences between a cloned cell line, 3T3-L1, and primary mature adipocytes. For example, the 3T3-L1 and F442A cell lines express very low levels of leptin in comparison to mature adipocytes.[34] However, even floating cultures of primary adipocytes have been demonstrated to lose their differentiation in comparison to adipocytes that are cultured in a matrix of extracellular basement membrane components.[29,35] Those observations indicate possible advantages of an adherent primary culture system for assessing the chronic effects of TNF-α on adipocytes. Therefore, our data show that relatively short-term (24 h) exposure to TNF-α does not increase leptin secretion, and confirm that prolonged exposure inhibits leptin secretion and

gene expression. This suggests that the acute effect of TNF-α to increase circulating leptin levels *in vivo* may be indirect. These results also raise the possibility that chronic exposure to TNF-α suppresses leptin production *in vivo.* This conclusion is supported by a recent report which showed that patients with AIDS, who have chronically elevated plasma levels of TNF-α,[36] show a tendency toward having decreased circulating leptin concentrations relative to body fat.[37]

Because we found that TNF-α inhibited leptin secretion from adipocytes, and because leptin secretion has been shown to be proportional to glucose uptake by adipocytes, we expected TNF-α to decrease glucose uptake.[23] However, in this study, TNF-α increased glucose uptake by adipocytes. Thus, it appears that TNF-α induced a dissociation between adipocyte glucose utilization and leptin production. In addition, because we observed that TNF-α increased glucose uptake in the absence of insulin, it is probable that TNF-α increased GLUT1-mediated basal glucose uptake rather than induced the recruitment of insulin-regulated GLUT4 transporters. This possibility is supported by reports which showed that TNF-α decreases GLUT4 mRNA levels in human fat cells[38] and in 3T3-L1 adipocytes at doses that increased glucose uptake in the present study; TNF-α also reduces insulin-stimulated glucose uptake at those same concentrations.[38,39] The report that utilized human fat cells also demonstrated that a 72 h exposure of fat cells to TNF-α significantly increased basal glucose uptake and GLUT1 protein.[38] In addition, it has been reported that lipopolysaccharide, the bacterial endotoxin that stimulates higher serum levels of TNF-α,[40] increases noninsulin-mediated glucose uptake by adipose tissue[41] while decreasing GLUT4 and increasing GLUT1 mRNA levels.[42] Our results conflict with one recent report which showed that prolonged exposure of 3T3-L1 adipocytes to TNF-α either slightly increases or markedly decreases basal glucose transport.[43] Those effects depended on whether the cells were allowed to differentiate to maturity mostly in the absence or presence of insulin, respectively.[43] The discrepancy in results may reflect differences between the 3T3-L1 adipocyte cell line and mature adipocytes. Further studies are required to determine the levels of glucose transporter protein and mRNA expression in our culture system and their modulation by chronic exposure to TNF-α.

Our laboratory has reported that an increased conversion of glucose to lactate by adipocytes is associated with decreased leptin secretion.[23] For example, inhibition of leptin secretion by the antidiabetic drug, metformin, is related to an increased conversion of glucose to lactate. This suggests that the metabolism of glucose to pyruvate without conversion to lactate may be involved in the effect of glucose to increase leptin production.[28] In the present study, prolonged exposure to TNF-α dose-dependently increased lactate production by adipocytes; lactate production was highly correlated with glucose uptake. However, unlike metformin, the inhibition of leptin secretion by TNF-α was not related to an increased conversion of glucose to lactate. Therefore, while the amount of leptin secretion was inversely related to the conversion of glucose to lactate, it does not appear that TNF-α inhibits leptin secretion through a mechanism related to alterations of adipocyte glucose metabolism. However, because chronic exposure to TNF-α inhibited both basal and insulin-stimulated leptin secretion and gene expression, it is possible that TNF-α inhibits leptin production through a mechanism that does not involve the insulin-signaling pathway.

Because lipopolysaccharide and interleukin-1 have also been shown to increase circulating leptin concentrations,[13,14,16] TNF-α is probably one of several cytokines that is capable of modulating leptin production. This is supported by reports that adipose tissue can synthesize and secrete several factors[44] capable of modulating leptin secretion, including TGF-β.[44,45] However, research efforts have largely overlooked the fact that TNF-α and TGF-β, like leptin, are expressed in the adipose tissue of not only obese rodents, but also pre-obese, normal and lean rodents.[44] Thus, how molecules such as TNF-α, TGF-β, leptin and insulin interact to modulate adipocyte physiology in homeostasis and in the pathogenesis of obesity remains undefined. Yet, it is reasonable to speculate that a dysregulation of cytokine-leptin interactions can occur that leads to the development of obesity. For example, it has been reported that relatively low plasma leptin concentrations precede weight gain in Pima Indians, a population with high prevalence of Type II Diabetes.[46] It is possible that adipose tissue TNF-α contributes to low plasma leptin concentrations of susceptible members of that population. Such a possibility warrants exploration.

In summary, a relatively short-term (24 h) exposure to TNF-α did not affect leptin secretion from isolated rat adipocytes in primary culture. Chronic exposure to TNF-α produced a concentration-dependent inhibition of leptin secretion and gene expression. TNF-α also increased glucose uptake and lactate production. However, the inhibition of leptin secretion was not related to an increased conversion of glucose to lactate. TNF-α also increased glucose uptake and inhibited leptin secretion and gene expression in the absence of insulin. We conclude that although TNF-α increased glucose uptake, it chronically inhibited leptin secretion, and thus induced a dissociation between adipocyte glucose metabolism and leptin secretion. These results suggest that the acute effect of TNF-α to increase circulating leptin concentrations *in vivo* may be indirect. Further studies are needed to determine the mechanism by which that acute effect occurs and the mechanism by which TNF-α inhibits leptin production in isolated adipocytes.

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