Ciliary neurotrophic factor (CNTF) is primarily known for its roles as a lesion factor released by the ruptured glial cells that prevent neuronal degeneration. However, CNTF has also been shown to cause weight loss in a variety of rodent models of obesity/type II diabetes, whereas a modified form also causes weight loss in humans. CNTF administration can correct or improve hyperinsulinemia, hyperphagia, and hyperlipidemia associated with these models of obesity. In order to investigate the effects of CNTF on fat cells, we examined the expression of CNTF receptor complex proteins (LIFR, gp130, and CNTFRα) during adipocyte differentiation and the effects of CNTF on STAT 3, Akt, and MAPK activation. We also examined the ability of CNTF to regulate the expression of adipocyte transcription factors and other adipogenic proteins. Our studies clearly demonstrate that the expression of two of the three CNTF receptor complex components, CNTFRα and LIFR, decreases during adipocyte differentiation. In contrast, gp130 expression is relatively unaffected by differentiation. In addition, preadipocytes are more sensitive to CNTF treatment than adipocytes, as judged by both STAT 3 and Akt activation. Despite decreased levels of CNTFRα expression in fully differentiated 3T3-L1 adipocytes, CNTF treatment of these cells resulted in a time-dependent activation of STAT 3. Chronic treatment of adipocytes resulted in a substantial decrease in fatty-acid synthase and a notable decline in SREBP-1 levels but had no effect on the expression of peroxisome proliferator-activated receptor γ, aerp30, adipocyte-expressed STAT proteins, or C/EBPα. However, CNTFα resulted in a significant increase in IRS-1 expression. CNTFRα receptor expression was substantially induced in the fat pads of four rodent models of obesity/type II diabetes as compared with lean littersmates. Moreover, we demonstrated that CNTF can activate STAT 3 in adipose tissue and skeletal muscle in vivo. In summary, CNTF affects adipocyte gene expression, and the specific receptor for this cytokine is induced in rodent models of obesity/type II diabetes.
provides the first evidence that CNTFR
in rodent adipose tissue. Also this study
that two of the three CNTF receptors are down-regulated dur-
STATs and proteins by CNTF in adipocytes. The objective of
signaling (22, 25).

stress, inflammatory responses, nausea, or conditioned taste
inution during CNTF treatment does not appear to be due to
weight gain (22). Unlike cachectic cytokines, the appetite dim-
CNTF treatment does not result in overeating and rebound
ing the hypothalamic weight set point, such that cessation of
CNTF treatment did not effect the expression of
nuclei involved in the regulation of feeding (20). CNTF can also
cluded in the hypothalamus (21). It has been shown that CNTF
the development of insulin resistance in cultured adipocytes.
Moreover, acute CNTF administration resulted in increased
GLUT4 expression, whereas chronic CNTF treatment resulted
in a substantial increase in IRS-1 expression. Also, acute CNTF
Treatment resulted in an increase in insulin-induced IRS-1 and
Akt activation. In summary, the results of this study demon-
strate that both cultured and native adipocytes, as well as
skeletal muscle, are responsive to CNTF and that this cytokine
may act as an insulin-sensitizer in cultured adipocytes. These
studies support our hypothesis that the ability of CNTF to
result in weight loss is not solely mediated by the central
nerve system.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s media (DMEM) was pur-
chased from Invitrogen. Bovine and fetal bovine serum (FBS) were
obtained from Sigma and Invitrogen, respectively. Rat CN
was pur-
chased from Calbiochem. The non-phospho-STAT antibodies were
obtained from Sigma and Invitrogen, respectively. Rat CNTF was pur-
red from Promega. C/EBPα was a rabbit polyclonal from Dr. Ormond Mac-
Dougal (Ann Arbor, MI), and GLUT 4 was a rabbit polyclonal from Dr.
Flora Pilch (Boston). CNTFRα was a mouse monoclonal purchased from
BD Biosciences. IRS-1 polyclonal was a polyclonal obtained from Up-
state Biotechnology, Inc., and the phospho-specific IRS-1 polyclonal was
from BIOSOURCE International. PGNase F was obtained from New
England Biolabs.

Cell Culture—Murine 3T3-L1 preadipocytes were plated and grown
to 2 days post-confluence in DMEM with 10% bovine serum. Medium
was changed every 48 h. Cells were induced to differentiate by changing
the medium to DMEM containing 10% fetal bovine serum, 0.5 mM
3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 1.7 μM insulin.
After 48 h this medium was replaced with DMEM supplemented with
10% FBS, and cells were maintained in this medium until utilized for
experiments.

Preparation of Whole Cell Extracts—Monolayers of 3T3-L1 preadip-
cytes or adipocytes were rinsed with phosphate-buffered saline and
then harvested in a non-denaturing buffer containing 150 mM NaCl, 10
mM Tris, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.5% Nonidet P-40, 1 μM phenylmethylsulfonyl fluoride, 1 μM peptatin, 50
trypsin inhibitory milliunits of aprotinin, 10 μM leupeptin, and 2
mM sodium vanadate. Samples were extracted for 30 min on ice and
centrifuged at 15,000 rpm at 4 °C for 15 min. Supernatants containing
whole cell extracts were analyzed for protein content using a BCA kit
(Pierce) according to the manufacurer’s instructions.

Preparation of Nuclear/Cytosolic Extracts—Cell monolayers were
rinsed with phosphate-buffered saline and then harvested in a nuclear
homogenization buffer (NHB): containing 20 mM Tris, pH 7.4, 10 mM
NaCl, and 3 mM MgCl2. Nonidet P-40 was added to a final concentra-
tion of 0.15%, and cells were homogenized with 16 strokes in a Dounce
homogenizer. The homogenates were centrifuged at 1500 rpm for 5 min.
Supernatants were saved as cytosolic extract, and the nuclear pellets
were resuspended in 0.5 volume of NHB and were centrifuged as before.
The pellet of intact nuclei was resuspended again in 0.5 of the original
volume of NHB and centrifuged again. A small portion of the nuclei
was used for trypsin blue staining to examine the integrity of the nuclei.
The majority of the pellet (intact nuclei) was resuspended in an extrac-
tion buffer containing 20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2,
1 mM EGTA, and 25% glycerol. Nuclei were extracted with ice
and then placed at room temperature for 10 min. Two hundred units of
DNase I was added to each sample, and tubes were inactivated and
incubated an additional 10 min at room temperature. Finally, the
sample was subjected to centrifugation at 15,000 rpm at 4 °C for 30
min. Supernatants containing nuclear extracts were analyzed for protein
content.

Gel Electrophoresis and Immunoblotting—Proteins were separated
in 5, 7.5, 10, or 12% polyacrylamide (acrylamide from National Diag-
nostics) gels containing SDS according to Laemmli (26) and transferred
to nitrocellulose (Bio-Rad) in 25 mM Tris, 192 mM glycine, and 20%
ethanol. Following transfer, the membrane was blocked in 4% milk
in 1X TBS at room temperature. Resulting blots were visualized with
diastase-peroxidase-conjugated secondary antibodies (Sigma) and enhanced
chemiluminescence (Pierce). Following chemiluminescence, some
results were quantified by scanning film, and densitometry analysis was
performed with associated analytical software (Biomax, Eastman Kodak Co.).

Determination of 2-Decoxyglucose—The assay of 2-3Hdeoxyglucose
was performed as described previously (27). Prior to the assay, fully
differentiated 3T3-L1 adipocytes were serum-deprived for 4 h. Uptake
measurements were performed in triplicate under conditions where
hexose uptake was linear, and the results were corrected for nonspecific
uptake, and absorption was determined by 2-3Hdeoxyglucose uptake
in the presence of 5 μM cytochalasin B (Sigma). Nuclear uptake and
absorption were always less than 10% of the total uptake.

Animals and Adipose Tissue Isolation—Seven-week-old ob/ob
and ob/ob mice were purchased from The Jackson Laboratories. Eight-week-
old fa/+/ and fa/fa rats were purchased from Harlan. C57Bl/6J mice
were obtained from The Jackson Laboratories at 3–5 weeks of age, and
ob/ob mice were placed on a high fat/high sucrose diet (Research Diet
12331, Surwit diet) or a low fat/high sucrose diet (Research Diet
12392). In each experiment, at least five animals were used for each
condition. Twelve-week-old transgenic mice expressing agouti under
the control of the β-actin promoter were obtained from a colony at the
Pennington Biomedical Research Center. Rodents were euthanized by
carbon dioxide asphyxiation, and adipose tissue was quickly removed, homogenized,
and frozen in liquid nitrogen. Frozen fat pads were homogenized in a buffer
containing 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EGTA, 1 mM
EDTA, 1% Triton X-100, 0.5% Nonidet P-40, 1 μM phenylmethylsulfonyl
fluoride, 1 μM peptatin, 50 trypsin inhibitory milliunits of aprotinin,
and 10 μM leupeptin, and 2 mM sodium vanadate. Homogenates were centrifuged for 10 min at 5,000 rpm to remove any debris and insoluble material and then analyzed for protein content. All animal studies were carried out with protocols that were reviewed and approved by the institutional animal care and use committee. Adipocytes were isolated from the epididymal fat pads of male C57Bl/6J mice (35g) by collagenase digestion.

RESULTS

The sensitivity of 3T3-L1 cells to cytokine treatment was examined by treating undifferentiated preadipocytes and fully differentiated 3T3-L1 adipocytes with an acute treatment of 0.8 nM CNTF or 0.8 nM LIF. As shown in Fig. 1, immunoblotting of whole cell extracts demonstrated that both preadipocytes and adipocytes express STAT 3. Treatment of both preadipocytes and adipocytes with LIF or CNTF resulted in the rapid activation of STAT 3, as evident by increased tyrosine phosphorylation. However, treatment of preadipocytes resulted in a greater stimulation of STAT 3 activation, relative to adipocytes, despite equivalent expression of STAT 3 protein. In addition, CNTF and LIF treatment caused a robust activation of Akt in preadipocytes, whereas the same treatment of adipocytes did not result in a detectable activation of Akt. The expression of two CNTF receptor complex proteins, LIFR and gp130, was also examined. LIFR was expressed at a substantially higher level in preadipocytes than in adipocytes, whereas the expression of gp130 protein was not differentially expressed in these two cell types.

The expression of CNTF receptor complex proteins was also examined during a time course of adipocyte differentiation. As shown in Fig. 2, the expression of CNTF receptor protein decreases notably after 15 min of induction of differentiation, and this lower level of expression is maintained for 48 h. However, there were no detectable levels of CNTFRα 72 h after the initiation of differentiation in whole cell extracts. Yet we did observe the presence of CNTFRα in the media at 72, 96, and 120 h at lower levels (data not shown). As indicated in Fig. 1, the expression of LIFR decreased during adipogenesis, and there was a slight modulation of gp130 expression. The expression of STAT 5A is known to be induced during adipocyte differentiation and is shown as a positive control for adipogenesis.

Because the expression of two CNTF receptor complex proteins was reduced during adipogenesis, we wanted to determine whether these proteins were expressed in adipose tissue and to compare the expression levels to other tissues. Whole cell extracts were isolated from the various tissues indicated in Fig. 3. Western blot analysis revealed lung, stomach, epididymal fat, spleen, heart, brain, testes, and skeletal muscle as tissues expressing both CNTFRα and LIFR. All of these tissues had comparable receptor expression levels, except for the brain, which had significantly higher levels of CNTFRα expression. Also the molecular weight of CNTFRα in stomach and brain was greater than in other tissues. In agreement with our earlier observations (Fig. 2), the expression of CNTFRα was abundant in preadipocytes and undetectable in 3T3-L1 adipocytes. We also observed that the expression of CNTFRα was up-regulated in the epididymal fat pad of an obese Zucker rat as compared with a lean littermate.

To determine whether the altered mobility of CNTF receptor was due to glycosylation, tissue extracts were incubated with PNGaseF. As shown in Fig. 4, treatment with PNGaseF resulted in the deglycosylation of CNTF receptor and LIFR. In particular, the CNTF receptor bands of larger molecular weights (brain and stomach) co-migrated with the CNTFRα from other tissues following digestion, indicating that the size difference between CNTFRα in these tissues was due to different glycosylation patterns. Also all LIFR bands migrated at the same molecular weight following PNGaseF treatment.

Although our results demonstrate that fully differentiated 3T3-L1 adipocytes do not express CNTFRα, it has been demonstrated previously (15, 16) that CNTF can signal via gp130 and LIFR in the absence of CNTFRα. Therefore, we examined the ability of CNTF to activate STATs in a time-dependent manner in 3T3-L1 adipocytes. Serum-deprived fully differentiated 3T3-L1 adipocytes were exposed to CNTF and examined over an 8-h period. Cells were harvested at the times indicated at the top of Fig. 5 and fractionated into cytosolic and nuclear extracts. As shown in Fig. 5A, CNTF administration to 3T3-L1 adipocytes resulted in the nuclear translocation of STAT 3. STAT 3 was present in the nucleus after a 10- or 30-min treatment with CNTF, and the amount of STAT 3 nuclear protein was decreased after a 1-h treatment. After 2 h, there was little STAT 3 present in the nucleus. CNTF treatment did not result in the activation/nuclear translocation of STAT 1 or STAT 5A.
The dose-dependent effects of CNTF on 3T3-L1 adipocytes were also examined by treating adipocytes for 10 min with varying concentrations of CNTF. As shown in Fig. 5B, CNTF had no effect on STATs 1 or 5A but resulted in the tyrosine phosphorylation and nuclear translocation of STAT 3. In addition, CNTF treatment resulted in a dose-dependent activation of MAPK (ERKs 1 and 2). To assess the dose effects of CNTF on STAT 3 activation, we compared STAT 3 activation in preadipocytes and adipocytes. As shown in Fig. 5C, CNTF results in a dose-dependent effect on STAT 3 activation in preadipocytes but not in adipocytes.

To characterize further the effects of CNTF, we treated fully differentiated 3T3-L1 adipocytes for a 12-h period, and we isolated whole cell extracts at the times indicated at the top of Fig. 6. As indicated previously, acute CNTF treatment resulted in a time-dependent activation of STAT 3 and MAPK but was unable to activate Akt. A positive control for Akt activation (10 min of treatment of 3T3-L1 adipocytes with 50 nM insulin) is shown in the bottom panel of Fig. 6. Acute CNTF treatment did not affect the expression levels of STATs 1, 3, or 5A. There were also no observable differences in the expression of SREBP-1 protein, as indicated by the levels of the cleaved 67-kDa form of the protein.

Next, we examined the effects of chronic CNTF administration on the expression of adipocyte transcription factors and other adipocyte proteins. Fully differentiated 3T3-L1 adipocytes were exposed to CNTF over a 96-h period. A fresh bolus of CNTF was added to the cells every 24 h. Whole cell extracts were isolated at the times indicated at the top of Fig. 7 and subjected to Western blot analysis. Chronic administration of CNTF did not alter the expression of adipocyte expressed...
STATs, PPARγ, or C/EBPα. There were no notable differences in the levels of gp130 and LIFR expression, and chronic CNTF treatment was insufficient to induce the expression of CNTFRα. A positive control of confluent preadipocytes is shown for CNTFRα expression. We also observed that CNTF treatment did not alter the expression of acrp 30 in 3T3-L1 adipocytes. Moreover, CNTF had no effect on the expression or secretion of leptin from 3T3-L1 adipocytes (data not shown). Interestingly, CNTF treatment resulted in a decrease of FAS expression and a substantial increase in the expression of IRS-1. Also, the levels of the 67-kDa SREBP-1 protein were slightly decreased by CNTF treatment after 72 h.

Although we did not observe substantial levels of CNTFRα in cultured 3T3-L1 adipocytes (Figs. 2, 3, and 5), we were able to detect the expression of CNTFRα in rodent adipose tissue from an obese Zucker rat, and the levels of this receptor appeared to be up-regulated in conditions of obesity (Fig. 3). Hence, we examined the expression of CNTF receptors in adipose tissue of additional rodent models of obesity/type II diabetes. Whole cell extracts were prepared from epididymal fat pads of five ob/ob and five ob/+ lean littermates. As shown in Fig. 8A, we observed very little expression of CNTFRα in the adipose tissue of lean mice, but we observed a substantial increase in the expression of this receptor in three of the five obese insulin-resistant ob/ob littermates. In the other two ob/ob mice, there was a modest increase in CNTFRα expression. In addition, we observed increased LIFR expression in all five ob/ob mice compared with lean littermates, but we did not observe any substantial changes in gp130 expression. We also examined the expression of these proteins in the epididymal fat pads of fa/+ and fa/.fa rats. As shown in Fig. 8B, the expression of CNTFRα was also substantially up-regulated in this rodent model of obesity/type II diabetes. However, we did not observe an increase in LIFR levels in the fa/fa rats as compared with their lean littermates, although there was a modest increase in gp130 levels in adipose tissue from fa/fa rats. We also examined the expression of these receptors in transgenic mice that over express agouti under the control of the β-actin promoter, a condition that causes obesity and type II diabetes (29). We observed a substantial increase in CNTFRα levels in the epididymal fat pads of three obese transgenic mice (Tg/+) compared with wild-type lean (+/+) mice. There was also a modest decrease in LIFR and gp130 in the fat pads of mice with agouti-induced obesity. Finally, we examined the expression of CNTF receptors after low fat or high fat feeding in C57Bl/6J mice. Seven mice from each condition were analyzed for CNTF receptor expression. The results in Fig. 8D only include three animals per condition. However, this pattern of regulation was observed for all seven animals examined for each condition (data not shown). In C57Bl/6J mice, we observed an increase in CNTFRα levels with high fat feeding after 12 weeks. A similar pattern was also observed after 7 weeks (data not shown). Overall, there was no modulation of LIFR or gp130 with high fat feeding in the C57Bl/6J mice.

We have shown that cultured adipocytes do not express CNTFRα, but rodent adipose tissues express detectable levels of the receptor. Therefore, we examined the ability of CNTF to activate STAT 3 in vitro. C57Bl/6J mice were given an intraperitoneal injection of CNTF (33.3 μg/kg) or vehicle (saline) control. Fifteen minutes after the injection, the mice were sacrificed, and epididymal adipose tissue, brains, and skeletal muscle were immediately removed and frozen in liquid nitrogen. Whole cell extracts were prepared from these tissues and...
analyzed for STAT 3 phosphorylation by Western blot analysis. As shown in Fig. 9, acute CNTF treatment resulted in the activation of STAT 3 in epididymal adipose tissue. We were unable to detect STAT 3 phosphorylation in the adipose tissue of five saline-injected mice, but four of the five CNTF injected mice had readily detectable levels of phosphorylated STAT 3. The increase in STAT 3 phosphorylation was not due to increased STAT 3 expression. Also, the expression of LIFR was not changed, and the levels of CNTFR were variable in the 10 mice. The results in Fig. 9B demonstrate constitutive STAT 3 phosphorylation in brain, which was unresponsive to exogenous CNTF. Moreover, we observed an increase in STAT 3 phosphorylation in the skeletal muscle of CNTF-treated animals, as compared with saline controls. As indicated previously (Fig. 2), the levels of CNTFR in the brain are substantially greater than the levels in skeletal muscle.

Our results demonstrate that CNTFR receptor expression was decreased during the adipogenesis of 3T3-L1 cells but expressed in the fat pads of rodents. Therefore, we fractionated epididymal fat pads from C57Bl/6J mice and fractionated into adipocyte and stromovascular fractions. Seventy-five µg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed, and results were visualized as described in Fig. 1 legend.

Fig. 8. The expression of CNTF receptor components in the adipose tissue of lean and obese rodents. Whole cell extracts were isolated from the epididymal fat pads of 7-week-old ob/+ (lean) and ob/ob (obese) littermate mice (A), 8-week-old fa/+ (lean) and fa/fa (obese) littermate rats (B), 12-week-old lean mice or obese agouti (Tg+/+) littermates (C), and 17-week-old C57Bl/6J mice fed a low or high fat diet for 12 weeks (D). In each panel, 75 µg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in Fig. 1 legend.

Fig. 9. In vivo effect of acute CNTF administration in rodents. Six-week-old C57Bl/6J mice were given an intraperitoneal injection of CNTF (33.3 µg/kg) or vehicle (saline) control. Fifteen minutes after the injection, the mice were sacrificed, and epididymal fat pads, brains, and skeletal muscle were immediately removed and frozen in liquid nitrogen. Tissue extracts were analyzed from epididymal fat pads (A) and brain and skeletal muscle (B). In each panel, 75 µg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed, and results were visualized as described in Fig. 1 legend. This is a representative experiment independently performed two times.

Fig. 10. In vivo expression of CNTFRα in epididymal fat pads. Epididymal fat pads were extracted from 6-week-old lean C57Bl/6J mice and fractionated into adipocyte and stromovascular fractions. Seventy-five µg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed, and results were visualized as described in Fig. 1 legend.
Because CNTF administration of ob/ob, db/db, and diet-induced obesity mice has been shown to improve insulin sensitivity in vivo, we examined the ability of CNTF to regulate insulin-sensitive glucose uptake in 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were treated for 24 h with CNTF. As shown in Fig. 1A, CNTF treatment resulted in a notable increase (25–50%) in GLUT4 levels. However, additional treatments of CNTF did not result in a further increase in GLUT4 levels as chronic CNTF treatment did not substantially increase GLUT4 mRNA or protein levels (data not shown). Therefore, we examined the ability of CNTF to affect glucose uptake. Fully differentiated adipocytes were treated for 72 h with CNTF. Every 24 h, cells were treated with a fresh bolus of CNTF. Acute insulin treatment (50 nM, 7 min) resulted in a 5-fold increase in insulin-stimulated glucose uptake and was relatively unaffected by chronic CNTF treatment (Fig. 1B). In addition, CNTF had no effect on basal glucose uptake. Because CNTF treatment resulted in an increase in IRS-1 expression levels (Fig. 7), we examined the ability of this cytokine to induce IRS-1 activation, as judged by tyrosine phosphorylation at residue 896. As shown in Fig. 12, acute insulin treatment (15 min) results in the activation of IRS-1 and Akt, whereas acute CNTF treatment does not. However, CNTF pretreatment (30 min) prior to insulin stimulation resulted in an increased IRS-1 activation (>20%) and increased Akt phosphorylation (>25%). The efficacy of the CNTF is demonstrated by the activation of STAT 3.

DISCUSSION

In the light of recent findings demonstrating that CNTF administration results in weight loss and correction of many other obesity/type II diabetes-related symptoms (19, 22, 24, 30), we hypothesized that the effects of this cytokine may not be limited to the central nervous system and that CNTF may also have effects on peripheral tissues such as adipose tissue. Our in vitro studies using 3T3-L1 preadipocytes and adipocytes have shown that CNTF indeed has significant, yet different, effects on these two cell types. In 3T3-L1 adipocytes we observed that CNTF was a potent activator of the Jak/STAT pathway, in particular STAT 3, as well as an activator of a MAPK signaling cascade that resulted in activation of ERKs 1 and 2. In preadipocytes CNTF elicited similar effects but also resulted in the activation of Akt. Our studies revealed that two of the three CNTF receptor components, LIFR and CNTFRa, were down-regulated during the adipogenesis of 3T3-L1 cells. Our study clearly demonstrates that the expression of CNTFRa is substantially decreased during the course of adipocyte differentiation. Other studies (16) have shown that CNTFRa is down-regulated during astrocyte differentiation. A previous investigation had also indicated a decrease in LIFR during adipogenesis (31), but this is the first investigation to demonstrate a decrease in CNTFRa during adipogenesis. We hypothesize that decreased expression of CNTF receptors upon differentiation accounts for cultured adipocytes being less sensitive to CNTF treatment than preadipocytes, as judged by STAT 3 or Akt activation.

Although LIFR and CNTFRa protein levels are reduced in cultured adipocytes, as compared with preadipocytes, we observed that adipocytes were still responsive to CNTF. It has been demonstrated previously (15, 16) that CNTF can induce signaling in the absence of CNTFRa, solely by binding to a gp130:LIFR dimeric receptor. Acute treatment of CNTF did not alter the expression levels of any STATs or any other adipocyte transcription factors in 3T3-L1 adipocytes. Hence, we examined the chronic effects of CNTF on 3T3-L1 adipocytes, and we observed that this cytokine affected the expression of several adipocyte-enriched proteins, including SREBP-1, FAS, GLUT4, and IRS-1. The reduction in the levels of SREBP-1 and FAS is indicative of decreased biosynthesis of fatty acids that may account for some portion of weight loss and decreased fat mass observed in patients treated with CNTF (Axokine). In agreement with previous findings that this CNTF-induced weight...
loss was not due to cachexia or inflammation (22, 25), we did not observe any effect of CNTF on PPARy or C/EBPα, two transcription factors known to be down-regulated by inflammatory cytokines such as tumor necrosis factor-α and interferon-γ (32–34). Also, unlike the in vitro effects of other cytokines (32, 35–37), CNTF treatment of 3T3-L1 adipocytes did not result in the onset of insulin resistance (Fig. 10). Moreover, chronic CNTF treatment of these cells actually resulted in an increase in both GLUT4 and IRS-1 protein levels. However, we did not observe any effects of CNTF on basal or insulin-stimulated glucose uptake. Clearly, additional experiments are required to determine whether CNTF can act as an insulin sensitizer. Nonetheless, we have shown that CNTF appears to act synergistically with insulin to increase the level of IRS-1 and Akt phosphorylation in 3T3-L1 adipocytes.

Our results strongly suggest that CNTF affects adipose tissue and skeletal muscle in vivo because an acute intraperitoneal injection of CNTF resulted in STAT3 activation in both adulthood, when they develop minor loss of motor neurons (10), and as neonates, when they develop normally and appear to have no visible defects well into adulthood, when they develop minor loss of motor neurons (10).

Adipose tissue of obese/type II diabetic rodents could be an adaptive response attempting to increase insulin sensitivity. Indeed, the expression of the LIFR in the ob/ob mice, as compared with lean littermates, the expression of this receptor was not altered in the fa/fa rats or in C57Bl/6J mice with diet-induced obesity. Moreover, we have shown that CNTF acts at higher levels in the adipocytes as compared with the stromavascular portion of the fat pad (Fig. 10). Although we observed an increase in the expression of the LIFR in the ob/ob mice, as compared with lean littermates, the expression of this receptor was not altered in the fa/fa rats or in C57Bl/6J mice with diet-induced obesity.

The results of our study suggest that CNTF and CNTFα may play a role in the regulation of adipocyte metabolism and, perhaps, the control of adipose tissue mass. Our results have led us to hypothesize that CNTF can act as an insulin sensitizer in adipocytes. Therefore, the up-regulation of CNTFα in adipose tissue of obese/type II diabetic rodents could be an adaptive response attempting to increase insulin sensitivity. Interestingly, some studies suggest that CNTFα may not only act as receptor for CNTF but also as a receptor for another unknown CNTF-like factor. For example, mice lacking CNTF develop normally and appear to have no visible defects well into adulthood, when they develop minor loss of motor neurons (10). Yet mice lacking CNTFα tend to have severe motor neuron defects and die perinatally because they fail to initiate feeding behaviors (11). Also the finding that CNTF expression is undetectable in the feeding-relevant brain sites, which express high levels of CNTFα (30), further supports the notion that CNTFα may have additional ligands and/or functions.

In summary, we observed that native as well as cultured adipocytes are responsive to CNTF treatment. Interestingly, CNTFα is not highly expressed in cultured adipocytes but is readily detectable in rodent adipose tissue and furthermore highly up-regulated in multiple rodent models of obesity/type II diabetes. This is the first demonstration that this receptor is expressed in adipose tissue and that it is highly regulated in obesity/type II diabetes. Current studies are underway to determine the role of CNTFα in adipose tissue function and examine the ability of CNTF to act as insulin sensitizer in fat and muscle.

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