Metabolic Remodeling in Adipocytes Promotes Ciliary Neurotrophic Factor-Mediated Fat Loss in Obesity

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Obesity is characterized by an expanded adipose tissue mass, and reversing obesity reduces the risk of insulin resistance and cardiovascular disease. Ciliary neurotrophic factor (CNTF) reverses obesity by promoting the preferential loss of white adipose tissue. We evaluated the cellular and molecular mechanisms by which CNTF regulates adiposity. Obese mice fed a high-fat diet were treated with saline or recombinant CNTF for 10 d, and adipose tissue was removed for analysis. Another group fed a high-fat diet was pair fed to CNTF mice. In separate experiments, 3T3-L1 adipocytes were treated with CNTF to examine metabolic responses and signaling. CNTF reduced adipose mass that resulted from reductions in adipocyte area and triglyceride content. CNTF treatment did not affect lipolysis but resulted in decreases in fat esterification and lipogenesis and enhanced fatty acid oxidation. The enhanced fat oxidation was associated with the expression of peroxisome proliferator-activated receptor coactivator-1α (PGC1α) and nuclear respiratory factor 1 and increases in oxidative phosphorylation subunits and mitochondrial biogenesis as determined by electron microscopy. Studies in cultured adipocytes revealed that CNTF activates p38 MAPK and AMP-activated protein kinase. Inhibiting p38 activation prevented the CNTF-induced increase in PGC1α but not AMP-activated protein kinase activation. Diminished food intake with pair feeding induced similar decreases in fat mass, but this was related to increased expression of uncoupling protein 1. We conclude that CNTF reprograms adipose tissue to promote mitochondrial biogenesis, enhancing oxidative capacity and reducing lipogenic capacity, thereby resulting in triglyceride loss.

Materials and Methods

Cell culture

3T3-L1 preadipocytes were grown in DMEM supplemented with 25 mmol/liter glucose and 10% fetal bovine serum (FBS). Fibroblasts were grown to confluence, and differentiation was induced by changing the media to a adipogenic serum-free medium containing 0.5 mmol/liter insulin, 1 μmol/liter 3-isobutyl-1-methylxanthine, and 1 μmol/liter dexamethasone, with the addition of 2 mmol/liter glycercol and 1 μmol/liter 3-β-hydroxybutyrate at day 3. Finally, the adipogenic medium was removed, and the adipocytes were grown in DMEM containing 25 mmol/liter glucose and 10% FBS.
culture medium to DMEM and 2% FBS containing Actrapid insulin (0.5 mU/ml; Novo Nordisk, Baulkham Hills, New South Wales, Australia), 0.1 μg/ml dexamethasone, and 25 μg/ml 3-isobutyl-1-methyloxanthine. Differentiating medium was removed after 3 d and replaced with DMEM in 5% FBS with 0.5 μM Actrapid insulin. The medium was then replaced every 2 d until cells were loaded with lipid droplets, usually after 8–10 d. For acute experiments, the cells were incubated overnight in DMEM and 0.5% FBS (no insulin) before stimulation with 10 ng/ml recombinant CNTF (CNTF 245, kindly provided by Regeneron Pharmaceuticals, Tarrytown, NY). This dose does not induce inflammation in adipocytes (see Fig. 6, A–C). For chronic treatment with CNTF (10 ng/ml), adipocyte medium was replaced with fresh medium every 24 h. For experiments involving p38α/β inhibition, cells were pretreated with 10 μM SB202190 (Sigma Aldrich, St. Louis, MO) before the addition of 10 ng/ml CNTF. cDNAs encoding wild-type p38 MAPK and kinase-deficient p38 MAPK with a glutamate to alanine mutation at residue 168 (D168A) were kindly provided by Dr. Gregory Steinberg (St. Vincent’s Institute). For infection studies, differentiated 3T3-L1 adipocytes (d 7) were infected with adenovirus for 40 h and then incubated overnight in DMEM plus 0.5% serum before experiments. Oil Red O was used to quantify triglyceride mass as described (14).

Animals and animal care

Male C57BL/6 mice were purchased from Monash Animal Services (Clayton, Victoria, Australia) at 8 wk of age and placed on a standard chow diet consisting of 5% calories from fat or a high-fat diet (HFD) consisting of 45% of calories from fat for 10 wk. This experimental design was used to induce obesity in HFD mice. Animals were housed in a pathogen-free facility with a 12-h light, 12-h dark cycle and were given free access to food and water. All procedures were approved by the St. Vincent’s Hospital Animal Experimentation Ethics Committee and were conducted in accordance with the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation.

Experimental design

Mice were assigned to one of four experimental groups for the 10-d experimental period. Control chow-fed and high-fat-fed mice were allowed ad libitum access to food. Another group of HFD animals was injected at 0800 daily with 0.3 mg/kg CNTF, which was previously shown to induce weight loss without induction of an inflammatory response (7, 9). A third group of HFD were pair-fed (PF) to mice in the HFD CNTF group to account for the suppression of food intake observed with CNTF administration. Animals were fasted for 4 h before experiments and were anesthetized by ip injection of sodium pentobarbital (60 mg/kg body mass). The epididymal fat pad was excised and weighed and then rinsed in PBS containing 0.1% BSA and dissected into 20- to 40-mg pieces for immediate functional analysis. The remaining tissue was fixed for morphological assessment or rapidly frozen for protein and mRNA analysis. A venous blood sample was obtained from the pleural cavity, and the plasma was frozen for later analysis. Animals were killed by lethal injection of sodium pentobarbital.

Adipose tissue lipolysis, oxidation, and lipogenesis

For all experiments, a modified Kreb’s-Henseleit buffer was gassed for 40 min with 9% O2/5% CO2. Glucose (5 mM) and fatty acid-free BSA (4%) was added to the buffer immediately before experiments. All experiments were conducted in a shaking water bath at 30 C.

Lipogenesis. [3-3H]Glucose (TRK239; Amersham, Rydalmere, New South Wales, Australia) was added to the buffer to give a final concentration of 0.5 μCi/ml. Adipose tissue explants were incubated for 2 h, and the medium was removed. The tissue was washed in PBS and then homogenized in 1 ml PBS. The lipids were extracted in 2:1 chloroform-methanol, a 1-ml aliquot of the organic phase was removed, scintillation fluid was added, and radioactivity was counted in a liquid scintillation analyzer.

Lipolysis. Adipose tissue explants were placed in 2 ml buffer. The medium was collected after 2 h for later determination of glycerol by an enzymatic colorimetric method (Sigma). Oxidation. Palmitate oxidation was assessed over 5 h by radiometric methods as described previously for skeletal muscle (15).

Adipocyte area

Adipose tissue was immersed in Bouin’s solution overnight and then transferred to 70% ethanol and stored at 4 C. Tissues were fixed and embedded with a random orientation in paraffin, and 10-μm sections were stained with hematoxylin and counterstained with eosin. For each sample, area was determined in 190 × 9 adipocytes (obtained from three independent sections of tissue) at ×400 magnification using an Olympus BX50 microscope (Olympus, Tokyo, Japan) and AB software (Ontario, Canada).

Electron microscopy and determination of mitochondria size and density

Adipose tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 2 h and postfixed in 2% osmium tetroxide solution for 1 h. After dehydration in graded acetone, tissue was embedded in araldite/epon resin. Thick sections (90 nm) were cut using the same microtome mounted on copper/palladium 200 mesh grids and then stained with 3% aqueous solution of uranyl acetate and lead citrate. Grids were examined in a Siemens Elmskop 102 electron microscope at 60 kV.

Immunoblotting

Adipose tissue was lysed, and the homogenate was centrifuged at 14,000 × g for 30 min at 4 C. The infranatant containing cytosolic proteins was carefully removed, and proteins were solubilized, subjected to SDS-PAGE, and transferred onto polyvinylidene difluoride membranes. Membranes were blocked in 5% skim milk and incubated for 1 h in primary antibody, washed and incubated with protein G-horseradish peroxidase. Primary antibodies for cytochrome c and OxPhos complexes were obtained from BioVision (Mountain View, CA) and Mito Sciences (Eugene, OR). The phospho-p38 MAPK antibody was from Cell Signaling (Beverly, MA) and α-actin from Sigma-Aldrich. The immunoreactive proteins were detected by enhanced chemiluminescence and quantified by densitometry.

RNA extraction and real-time quantitative PCR

Total RNA was isolated from adipose tissue by QiAzel mini kit (QiAGEN, Doncaster, Australia). RT of mRNA was performed using the transcriptor RT-PCR system (Invitrogen, Mount Waverly, Australia) with random hexamer priming. Quantitative real-time PCR was performed on the Stratagene MX3000P using Assay-on-Demand gene expression kits for mouse NRF-1, PGC1α, PGC1β, UCP1, UCP2, UCP3, GPAT, C6D8, F4/80, and IGS (Applied Biosystems, Foster City, CA). cDNA was amplified using 20 μl TaqMan PCR containing 3 mM MgCl2, 200 mM dNTPs, 100 nM primers, 50 nM TaqMan probe, 1× Gold reaction buffer, and 0.5 U AmpliTaq Gold (Applied Biosystems). Assays were performed in triplicate and normalized using 18S rRNA (Applied Biosystems). The relative quantities of each transcript were calculated using the comparative critical threshold (Ct) method.

DNA microarray

Affymetrix GeneChip technology was used (Affymetrix Inc., Santa Clara, CA) to determine gene expression changes at the genome level. RNA quality was assessed on an Agilent Bioanalyzer PicoChip. Samples were labeled and hybridized to the GeneChip Mouse Expression Set 430 2.0 following the manufacturer’s protocols. The arrays were scanned on an Affymetrix GeneArray scanner. Data analysis was performed using Spotfire (Spotfire Inc., Somerville, MA) and Ingenuity Pathways Analysis 5.5. Expression values for each gene were calculated using multi-array average. The false discovery rate was adjusted to P < 0.05.

Statistical analysis

Results are presented as the mean ± sem. Data were analyzed for differences by one-way ANOVA with specific differences located with a Tukey’s post hoc test. Statistical significance was set a priori at P < 0.05.
Results

**CNTF decreases adiposity and adipocyte size in mice**

CNTF and PF mice consumed less food (~40%) when compared with HFD mice (Table 1). Body mass was decreased by 14.9 ± 1.1 and 16.7 ± 1.4% in CNTF and PF after 10 d treatment (Table 1). Epididymal fat pad mass was increased in HFD vs. chow (Fig. 1A). The epididymal fat mass was reduced by 32 and 43% in CNTF and PF, respectively, but remained heavier than chow. Similar reductions occurred in retroperitoneal and sc tissue (data not shown). The average adipocyte area was greater in HFD vs. chow (Fig. 1B) and was reduced in CNTF and PF. A representative picture of epididymal adipose tissue is shown in Fig. 1E. Consistent with the enlarged adipocyte area, triglyceride content was increased in HFD vs. PF and reduced by PF to a greater degree in CNTF (Fig. 1C). To explain the apparent mismatch in fat mass, adipocyte area, and triglyceride content, we examined adipocyte number in a subset of animals (n = 3 per group). Adipocyte number was higher in CNTF vs. PF and chow (2.49 ± 0.23 × 10^6 vs. 2.13 ± 0.29 × 10^6 vs. 1.88 ± 0.12 cells/mg tissue, respectively). Leptin is exclusively produced by adipocytes and was markedly reduced in CNTF and PF mice, which is consistent with the reduced adiposity and smaller adipocytes (Fig. 1D).

The frequency distribution of adipocytes by area was similar among chow, CNTF, and PF, whereas adipose tissue from HFD possessed a greater abundance of larger adipocytes (Fig. 1E). Close inspection revealed a greater number of smaller adipocytes in CNTF (i.e. <1000 μm^2), indicating the possibility of adipogenesis. Accordingly, we assessed known markers of adipocyte differentiation by quantitative RT-PCR (qRT-PCR). PPARY (1.8-fold) and C/EBPα (1.6-fold) were elevated in CNTF compared with chow but were not different from other groups (Table 1).

**Acute exposure to CNTF increases fatty acid oxidation in white adipose tissue**

We next assessed the effects of acute CNTF administration on lipid metabolism in epididymal fat explants ex vivo. Under conditions where isoproterenol and insulin stimulated lipolysis and lipogenesis, respectively, CNTF did not affect lipolysis (Fig. 2A) or lipogenesis (Fig. 2B) but increased fatty acid oxidation by 48% (Fig. 2C). We also repeated these experiments in 3T3-L1 adipocytes because adipose tissue contains several cell types in the stromovascular compartment, including macrophages. CNTF administration increased fatty acid oxidation by 20% in cultured adipocytes, an increment comparable to that seen when cells were treated with the AMPK pharmacological activator 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR) (Fig. 2D). The increase in fatty acid oxidation coincided with an increase in AMPK Thr172 (Fig. 2E) and acetyl coenzyme A (CoA) carboxylase Ser221 phosphorylation (Fig. 2F), indicating activation of AMPK with CNTF. Because CNTF weight loss effects persist after cessation of treatment (6), we hypothesized that CNTF induces transcriptional responses that stably up-regulate fat oxidation. To test whether prolonged CNTF treatment could enhance the fat oxidative capacity of the adipocyte independent of AMPK activation, 3T3-L1 adipocytes were treated with CNTF for 3 d, and the medium was removed 16 h before experiments. Fatty acid oxidation was increased by 23% in CNTF-treated cells (Fig. 2D). To test whether CNTF induces triglyceride depletion independent of caloric intake and humoral factors, we incubated 3T3-L1 adipocytes (d 7) in CNTF-containing media and assessed triglyceride content by Oil Red O staining. Cells treated with CNTF exhibited a 12% reduction in triglyceride content after only 3 d (Fig. 2G). These data suggest that CNTF increases fat oxidation in adipocytes acutely by AMPK activation and chronically by enhanced fat oxidative capacity (e.g., transcriptional effects) to reduce fat mass.

**Chronic exposure to CNTF increases fatty acid oxidation and decreases lipogenesis in white adipose tissue**

To examine whether these CNTF effects are maintained in vivo, we examined fatty acid metabolism in epididymal adipose explants obtained from mice treated for 10 d with daily CNTF injections. Adipose tissue was dissected 16 h after the last CNTF injection, thereby eliminating acute CNTF effects because the half-life of CNTF is about 45 min (6). Lipolysis was unaffected by HFD and CNTF but was decreased with PF (Fig. 3A). Lipogenesis was decreased in CNTF compared...
with all groups without (Fig. 3B) and with insulin stimulation (insulin-stimulated lipogenesis fold change over basal: chow, 4.9; HFD, 3.8; PF, 4.1; CNTF, 4.4). Thus, sensitivity to insulin was maintained in the CNTF-treated animals. The incorporation of exogenous \([14C]\)palmitate into triglycerides (i.e. esterification) was decreased in CNTF compared with PF (caloric restricted) and tended to be lower than HFD (P < 0.05 vs. HFD PF). D. Plasma leptin. *, P < 0.05 vs. HFD; **, P < 0.05 vs. chow and HFD. E. Distribution of adipocytes in epididymal fat pads with a representative cross-section shown above. n = 10 (chow and CNTF), n = 9 (HFD), and n = 7 (HFD PF).

**Fig. 1.** CNTF reduces adipose tissue mass in obese mice. A, Epididymal fat pad mass. *, P < 0.05 vs. all other groups; ***, P < 0.05 vs. HFD. B, Mean adipocyte area. *, P < 0.05 vs. HFD. C, Triglyceride content in adipose tissue. *, P < 0.05 vs. HFD; #, P < 0.05 vs. HFD PF. D, Plasma leptin. *, P < 0.05 vs. HFD; **, P < 0.05 vs. chow and HFD. E, Distribution of adipocytes in epididymal fat pads with a representative cross-section shown above. n = 10 (chow and CNTF), n = 9 (HFD), and n = 7 (HFD PF).
capacity. UCP1 expression was low in white adipose tissue for all groups. Despite increasing PGC1α/H9251mRNA, CNTF treatment did not enhance UCP1. UCP1 was elevated 2-fold in adipose of PF mice compared with all groups (Table 1). UCP2 and -3 were not different between groups (Table 1). Collectively, these data demonstrate that CNTF induces mitochondrial biogenesis in adipose tissue of mice but does not enhance the capacity for uncoupled respiration. Conversely, pair feeding appears to induce adipose tissue loss, at least partially, via increased uncoupling.

p38 MAPK is required for CNTF-induced PGC1α expression

To determine whether the CNTF effects on PGC1α were specific and not due to secondary events such as changes in circulating hormones/cytokines (9), we examined gene expression in cultured adipocytes after 5 h and 3 d of CNTF treatment. CNTF increased PGC1α mRNA content after 5 h, and this was maintained at 3 d (P = 0.055; Fig. 5A). No effects were seen in 18S and Stat3 expression, indicating some specificity in this response (Stat3 mRNA, P = 0.88 by one-way ANOVA). To examine the upstream signaling events in CNTF-induced mitochondrial biogenesis, we examined known signaling events that up-regulate PGC1α mRNA, including p38 MAPK (17) and AMPK (18), 16 h after the final CNTF injection and observed no differences between groups (data not shown). Because CNTF is rapidly cleared (6), we next examined the acute effects of CNTF in 3T3-L1 adipocytes. CNTF increased p38 MAPK and AMPK Thr172 phosphorylation after 30 min (Fig. 5B), and this effect was maintained at 4 h (not shown). However, in experiments where the culture medium was replaced 30 min after the addition of CNTF, the effects observed on p38 MAPK and AMPK phosphorylation were not detected at 4 h, indicating that CNTF signaling to these kinases is transient. p38 MAPK and
AMPK activation was not due an autocrine response to IL-6 because no changes in IL-6 mRNA or IL-6 release into the culture medium were detected at 8 or 24 h after CNTF administration (Fig. 6A). Proinflammatory cytokines such as IL-1α, TNFα, and IL-6 can activate p38 MAPK (19, 20); therefore, to determine whether p38 MAPK was required for the increase in PGC1α mRNA, we preincubated cells with the p38α/β inhibitor SB202190 for 30 min before the addition of CNTF. SB202190 totally blocked the CNTF-mediated increase in p38 MAPK phosphorylation (Fig. 5C) but did not affect AMPK Thr172 phosphorylation (Fig. 5C). PGC1α mRNA expression was increased 2-fold with CNTF; however, inhibiting p38 MAPK phosphorylation completely blocked these effects (Fig. 5D). These findings were largely replicated in experiments where CNTF-induced p38 MAPK activation was inhibited by using a p38 MAPK dominant-negative adenovirus (Fig. 5B), and the expected rise in PGC1α mRNA expression was suppressed (Fig. 5E). These results show that CNTF-induced PGC1α transcription depends on p38 MAPK signaling.

**Discussion**

Obesity is a major risk factor for several metabolic disorders including insulin resistance and type 2 diabetes. The use of CNTF for the treatment of obesity has been established (8–10); however, the cellular and molecular mechanisms by which CNTF induces white adipose tissue loss are not completely clear (11). We describe how CNTF induces mitochondrial biogenesis to enhance fat oxidation within adipose tissue, via a process involving p38 MAPK activation and PGC1α expression. The work presented also indicates that CNTF reduces the capacity for lipogenesis and fat reesterification. Together, CNTF converts white adipose tissue toward a partial brown adipocyte phenotype and accordingly directs fatty acids toward oxidation rather than storage.

Mitochondrial capacity is an important determinant of tissue health. Obesity and type 2 diabetes are characterized by decreased mitochondrial gene expression (22, 23), reduced mitochondrial density (24), and reduced ATP turnover (25) in skeletal muscle. This contributes to an inability to efficiently oxidize fatty acids and results in excess fat deposition in this tissue. There are now reports of defective mitochondrial function in adipose tissue in obesity/type 2 diabetes. Mitochondrial content and oxygen consumption

"CNTF reduces the lipogenic capacity of white adipose tissue"

Our *ex vivo* studies demonstrated reduced lipogenesis and esterification with CNTF, independent of reductions in food intake (Fig. 3). To broadly determine the molecular mechanisms underpinning these responses, we probed for differential gene expression using cDNA microarrays on tissues obtained from mice placed on a HFD and treated with CNTF and PF controls. Comparative analysis of gene expression revealed that genes associated with fatty acid transport (FAT/CD36 and FAS), triglyceride synthesis (GPAT and DGAT), and lipogenesis (SCD1) were markedly down-regulated with CNTF treatment (Table 2). These findings were validated by qRT-PCR for FAT/CD36, GPAT, and SCD1. When 3T3-L1 adipocytes were treated with CNTF for 5 d, there was no change in FAS (−30%, *P* = 0.42), FAT/CD36 (−38%, *P* = 0.57), GPAT (+40%, *P* = 0.76), and SCD1 (−2%, *P* = 0.96), suggesting that the suppressive effects of CNTF on lipogenic gene expression *in vivo* may be mediated by a circulating/neural factor. These *in vitro* data contrast an earlier study showing reduced fatty acid synthase (FAS) and sterol regulatory element-binding protein-1 (SREBP-1) protein expression after 96 h CNTF treatment in adipocytes (11).

"CNTF does not attenuate obesity-induced macrophage infiltration into adipose tissue"

It is well documented that obesity is associated with increased macrophage infiltration into adipose tissue (21). To test whether CNTF-mediated reductions in adiposity were associated with reduced macrophage infiltration, we measured the macrophage-specific markers CD68 and F4/80 by qRT-PCR. Both CD68 and F4/80 expression were increased 2-fold in adipose tissue with HFD; however, reducing adiposity with CNTF or PF did not reduce the expression of these macrophage markers (Table 1).
are reduced in the adipose tissue of genetic (3, 4, 26, 27) and fat-induced obesity (26), suggesting that this may be a primary defect underpinning these conditions. It is also evident that insulin-sensitizing therapies, such as thiazolidinedione administration (2–4, 26) and PPARα agonists (28), induce mitochondrial biogenesis and enhance fat oxidation (28) in white adipose tissue and may be implicit in the success of these strategies. In this report, we have identified two mechanisms capable of enhancing fat oxidation in adipocytes. First, CNTF promoted fat oxidation acutely via activation of AMPK, which is consistent with our previous observations in skeletal muscle (9). Second, prolonged CNTF administration induced mitochondrial biogenesis, increased the expression of fat oxidative and respiratory chain proteins, and enhanced fat oxidation, independent of acute AMPK activation. CNTF induces many of the metabolic changes seen in adenovirus-induced hyperleptinemia including up-regulation of PGC1α but not UCP-1 and UCP-2. In contrast to hyperleptinemic rats that lose almost all of their adipose triglyceride mass and develop a fatless, hypervascular fat pad remnant, CNTF-treated animals displayed smaller adipocytes and reduced triglyceride mass but retained normal morphology. Such regulated adipose loss, rather than complete adipose ablation, is important because the absence of adipose tissue (lipodystrophy) results in hepatomegaly, hy-
pertriglyceridemia, and disordered glucose metabolism leading to type 2 diabetes (29).

PGC1α is a transcriptional coactivator that is involved in the up-regulation of fatty acid oxidation genes and mitochondrial biogenesis, and its activation has been linked with insulin sensitization (30). PGC1α expression is reduced in the adipose tissue of obese (31) and type 2 diabetes patients (2), implicating PGC1α in the pathogenesis of these metabolic disorders. Several studies have indicated that the conversion of white to brown adipocytes is a viable strategy for the control of adiposity and that PGC1α is central to this approach. This is based on the rationale that brown adipose tissue possesses a large mitochondrial mass and increased UCP1 expression, which results in greater fat metabolism and uncoupled respiration. In support of this premise, stable overexpression of PGC1α in 3T3-L1 adipocytes induced mitochondrial biogenesis (16), and adenoviral-mediated PGC1α overexpression in white adipocytes induced changes consistent with brown adipocytes (32). Moreover, increased PGC1α expression occurs in several rodent models with reduced adiposity including 4E-BP1 null mice (33), transgenic mice with activated polyamide catabolism (34), and as mentioned, hyperleptinemic rats (12). Although the molecular control of adipocyte mitochondrial biogenesis is unresolved, our data indicate that PGC1α is involved in driving the CNTF-induced increase in mitochondrial biogenesis. The results also indicate that p38 MAPK signaling is involved because pharmacological and adenoviral inhibition of its activation inhibited CNTF stimulation of PGC1α expression. It is unlikely that AMPK is important for this process because its activation and PGC1α transcription were dissociated by p38 inhibition. Thus, besides CNTF inducing mitochondrial biogenesis, these studies support the intriguing possibility that an inability to efficiently oxidize fatty acids within adipose tissue may contribute to hypertrophy of adipose tissue and that enhancing fat oxidation within adipocytes may be a therapeutic strategy for obesity and related disorders.

Cytokines are known to regulate PGC1α via p38 activation and increase the expression of genes linked to mitochondrial uncoupling and energy expenditure (19). An unexpected finding of the present study was the absence of UCP1 induction with CNTF, because UCP1 is a known downstream target of PGC1α (16) and CNTF was shown previously to increase UCP1 and enhance thermogenesis in brown adipose tissue (6). Our findings of mitochondrial biogenesis and altered fuel metabolism, without UCP1 induction, indicate a partial shift from a white to brown adipose tissue phenotype. A dissociation between PGC1α control and UCP1 expression was previously reported in β-adrenergocceptor knockout brown adipocytes (35), and UCP1 expression was decreased in the absence of β3-adrenergocceptor agonism in brown adipocytes (36). Future studies are required to delineate in more detail the dissociation observed here.

Aside from enhanced fat oxidation, CNTF reduced lipogenesis and fat esterification. These physiological changes can be explained by stable CNTF-mediated transcriptional reprogramming. Notably, the CNTF-mediated changes inhibited key proteins of several pathways involved in lipo-
genesis including FAT/CD36, which is involved in fatty acid uptake; glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT); which are key proteins involved in glycerolipid synthesis; FAS, which catalyzes de novo synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and reduced nicotinamide adenine dinucleotide phosphate (NADPH); and stearoyl-CoA desaturase 1 (SCD1), which is a rate-limiting enzyme in the synthesis of unsaturated fats. These findings extend on a previous report demonstrating decreased lipogenic gene expression in the liver with CNTF treatment (8).

Pck1 encodes phosphoenolpyruvate carboxykinase, which produces glycerol-3-phosphate as a precursor for fatty acid esterification into triglycerides. Interestingly, Pck1 was down-regulated by CNTF and is noteworthy because increasing Pck1 may underpin the adiposity observed with PPARγ agonists (37). Thus, CNTF is able to induce insulin sensitization (9, 10) without the weight gain observed with other traditional insulin sensitizers such as thiazolidinediones.

Fasting and CNTF induced a similar loss of adipose tissue. Although this suggests that the reduced caloric intake is driving the adipose loss, an important point is that adipose loss was achieved through different mechanisms. Fasting was associated with increased plasma fatty acid levels and expression of UCP1, which is likely to promote uncoupled respiration and create a negative energy balance in this tissue. Fat-specific overexpression of UCP1 is known to reduce sc fat in aP2-Ucp1 transgenic mice (38). In contrast, CNTF reduced fatty acid synthesis and promoted fat oxidation, without evidence of uncoupling.

An abundance of evidence demonstrates a close link between obesity, chronic inflammation, and insulin resistance (39). A recent advance in the understanding of obesity-induced inflammation and insulin resistance was the finding that the source of inflammatory cytokines in obesity is related to the number of resident macrophages in adipose tissue. Furthermore, the percentage of macrophages in a given adipose tissue depot is positively correlated with adiposity and adipocyte size (21, 40). However, recent studies suggest that macrophage infiltration is not necessarily a function of fat mass per se, and may be a reflection of adipose quality (41), a finding supported by the observation that macrophage infiltration is related to adipocyte death (42). The present in vivo data indicate that although CNTF reduces adiposity, it does not affect the relative expression of adipose tissue macrophages (ATM) with high-fat feeding. This may have occurred because ATM turnover is relatively slow in mice fed a HFD (43). Alternatively, CNTF is known to induce proinflammatory signaling, which would presumably enhance ATM infiltration. This is unlikely because the concentrations of CNTF used in the in vivo studies do not induce an inflammatory or febrile response (9), observations supported by our in vitro analysis (Fig. 6). Also, adipose tissue insulin sensitivity, as assessed by insulin-stimulated lipogenesis, was restored in CNTF-treated animals, suggesting that the proportion of ATMs may not be an important factor determining insulin sensitivity or that CNTF may alter the properties of ATMs.

In conclusion, these studies advance the understanding of

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FIG. 6. Low-dose CNTF does not induce proinflammatory signaling in 3T3-L1 adipocytes. A, IL-6 gene expression was not increased in adipocytes treated with 10 ng/ml CNTF. In other experiments, 3T3-L1 adipocytes were treated with various concentrations of CNTF, and the medium was collected for IL-6 (B) and TNFα (C) release. Experiments were performed in triplicate on two occasions (total n = 6). *, P < 0.05 vs. 0 ng/ml CNTF.
the treatment of obesity by identifying CNTF as a direct regulator of adipose tissue metabolism. The present study provides compelling evidence linking CNTF administration to mitochondrial biogenesis and transcriptional reprogramming resulting in enhanced fat oxidative and down-regulated fat synthesis capacities. It remains to be determined whether approaches targeting gp130 signaling will be efficacious against obesity therapies in humans.

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