Ciliary Neurotrophic Factor$_{Ax15}$ Alters Energy Homeostasis, Decreases Body Weight, and Improves Metabolic Control in Diet-Induced Obese and UCP1-DTA Mice

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Ciliary neurotrophic factor (CNTF) potently reduces appetite and body weight in rodents and humans. We studied the short- and long-term effects of CNTF$_{Ax15}$, a second-generation CNTF analog, in diet-induced obese C57BL/6J mice and brown adipose tissue (BAT)-deficient obese UCP1-DTA (uncoupling protein 1–diphtheria toxin A) mice. CNTF$_{Ax15}$ administration (0.1, 0.3, or 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ s.c.) for 3 or 7 days reduced food intake and body weight (mainly body fat mass). The effect of CNTF$_{Ax15}$ on food intake and body weight was more pronounced in CNTF$_{Ax15}$-treated diet-induced obese C57BL/6J mice compared with pair-fed controls and was associated with suppressed expression of hypothalamic neuropeptide Y and agouti gene–related protein. Moreover, CNTF$_{Ax15}$ increased uncoupling protein 1 mRNA expression in BAT and energy expenditure in diet-induced obese C57BL/6J mice. Longitudinal observations revealed a sustained reduction in body weight for several days post-CNTF$_{Ax15}$ treatment of CNTF$_{Ax15}$-treated but not pair-fed mice, followed by a gradual regain in body weight over 28 days. Finally, CNTF$_{Ax15}$ administration improved the metabolic profile in both diet-induced obese C57BL/6J and UCP1-DTA mice and resulted in a significantly improved glycemic response to oral glucose tolerance tests in CNTF$_{Ax15}$-treated UCP1-DTA compared with pair-fed mice of similar body weight. These data suggest that CNTF$_{Ax15}$ may act through a pathway downstream of the putative point responsible for leptin resistance in diet-induced obese C57BL/6J and UCP1-DTA mice to alter food intake, body weight, body composition, and metabolism. CNTF$_{Ax15}$ has delayed and persistent effects in diet-induced obese C57BL/6J mice, which account for a reduction in body weight over and above what would be expected based on decreased foot intake alone. *Diabetes* 53:2787–2796, 2004

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AgRP, agouti gene–related protein; BAT, brown adipose tissue; CNTF, ciliary neurotrophic factor; NPY, neuropeptide Y; OGTT, oral glucose tolerance test; POMC, proopiomelanocortin; UCP1, uncoupling protein 1.

Administration of ciliary neurotrophic factor (CNTF), a neuronal growth factor studied in amyotrophic lateral sclerosis, results in anorexia and weight loss in humans (1,2) and mice (3,4). CNTF acts via both leptin-like and -independent mechanisms, with previous studies showing that CNTF decreases body weight and food intake in leptin-deficient *ob/ob* (4) and leptin-resistant *db/db* and diet-induced obese AKR/J mice (5). These findings suggest that CNTF acts, at least in part, downstream of the abnormally spliced leptin receptor of *db/db* mice and of the putative point of leptin resistance in mouse models of leptin resistance and insulin resistance. We studied the short- and long-term actions of CNTF$_{Ax15}$ (Axokine, a second-generation CNTF analog) in insulin- and leptin-resistant diet-induced obese C57BL/6J and UCP1-DTA (uncoupling protein 1–diphtheria toxin A) mice. Diet-induced obese C57BL/6 mice are the closest mouse model to human obesity (6), whereas UCP1-DTA mice develop deficits in energy expenditure and thermogenesis caused by ablation of brown adipose tissue (BAT) (7–9).

In this study, we investigated whether obese hyperleptinemic and hyperinsulinemic diet-induced obese C57BL/6J and UCP1-DTA mice, which are resistant to the effects of endogenous or exogenous leptin on body weight, food intake, and glucose and insulin levels (9,10), would also be resistant to the effects of CNTF$_{Ax15}$. We also sought to investigate whether *1*) the effect of CNTF$_{Ax15}$ is primarily mediated by decreased food intake and/or by an increase in energy expenditure, *2*) CNTF$_{Ax15}$ improves insulin resistance beyond what would be expected on the basis of weight loss alone, and *3*) the effects of CNTF$_{Ax15}$ persist after discontinuation of treatment.

RESEARCH DESIGN AND METHODS

Male C57BL/6J and female UCP1-DTA mice (The Jackson Laboratories, Bar Harbor, ME) were individually caged, maintained in a room with an automatically controlled 12-h lights-on (7:00 A.M.)/lights-off (7:00 P.M.) cycle with a temperature of 69–74°F and humidity of 40–60%. All animals were handled in accordance with the principles and guidelines established by the National Institutes of Health (6,10–12). Regular mouse chow (Purina Rodent Chow 5008, caloric content 3.48 kcal/g, with 17.3% of calories from fat, 55.1% from carbohydrate, and 27.6% from protein; Ralston-Purina, St. Louis, MO) and water were available to all animals ad libitum, unless noted otherwise. To obtain diet-induced obese mice, C57BL/6J mice received a western diet (TD

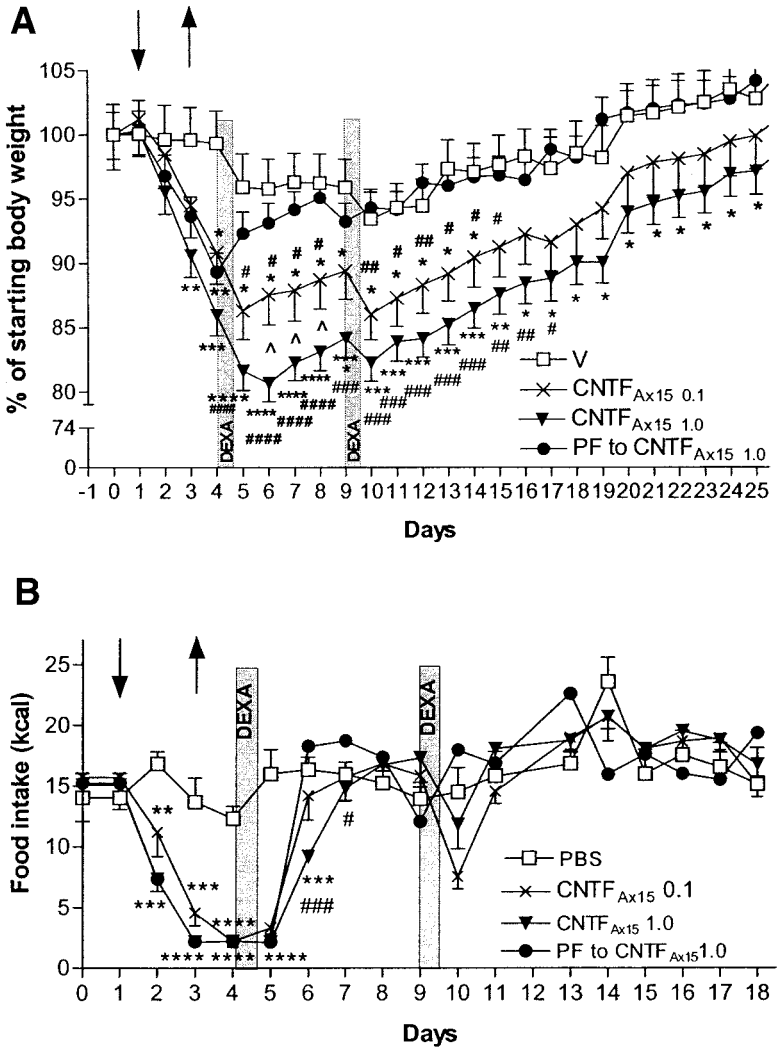

FIG. 1. The effect of short-term administration of $CNTF_{Ax15}$ to reduce food intake and body weight in diet-induced obese C57BL/6J mice. Diet-induced obese C57BL/6J mice were administered the indicated doses of $CNTF_{Ax15}$ for 3 consecutive days (arrows indicate beginning and end of treatment period), and food intake and body weight were assessed daily. Pair-feeding was discontinued 24 h after the last injection. Dual-energy X-ray absorptiometry (DEXA) scans were performed at baseline as well as 24 h and 6 days after the last injection as shown. *A*: Percent change in body weight (from starting body weight) compared with baseline levels. *B*: Caloric intake over time. Analyses were performed using unpaired *t* test and are expressed as the means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs. the vehicle-treated group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, and ####$P < 0.0001$ vs. the pair-fed group; $\wedge P < 0.05$ between both $CNTF_{Ax15}$-treated groups. PF, pair-fed group (n = 10); V, vehicle (PBS) treated.

88137, caloric content 4.53 kcal/g, with 42.2% of calories from fat, 42.8% from carbohydrates, and 15.0% from protein; Teklad, Madison, WI) for 12 weeks. Before the experiments, mice from each strain were divided into groups of equal mean body weight.

$CNTF_{Ax15}$ (Axokine), provided by Regeneron Pharmaceuticals (Tarrytown, NY), is cleared by glomerular filtration: C_{max} is ~45 min after subcutaneous injection in mice. Thus, negligible, if any, amounts of the medication are present in circulation 24 h after injection (~30 half-lives after C_{max}) (unpublished data, Regeneron, Tarrytown, NY).

Experimental procedures

Effects of $CNTF_{Ax15}$ administration for 3 days in diet-induced obese C57BL/6J mice. Food intake and body weights were measured daily in diet-induced obese C57BL/6J mice injected subcutaneously with $CNTF_{Ax15}$ at 0.1 or 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ between 8:00 and 9:00 A.M. for 3 days, mice pair-fed to $CNTF_{Ax15}$ 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$, and vehicle-treated controls (n = 10/group). For pair-feeding, the amount of food consumed by the $CNTF_{Ax15}$-treated group during the past 24 h was measured at 8:00 A.M. the following morning, and a corresponding amount of pellets was given to the pair-fed group during a 24-h period.

As we sought to investigate potential long-term effects of $CNTF_{Ax15}$, including differences in posttreatment body weight regain, measurements were taken for 28 days after treatment was discontinued. Dual-energy X-ray absorptiometry scans were performed at baseline (day −3), day 1, and day 6 of $CNTF_{Ax15}$ treatment to study the effect of $CNTF_{Ax15}$ on in vivo body composition. In a separate cohort of diet-induced obese C57BL/6J mice treated with the same doses of $CNTF_{Ax15}$ (0.1 or 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ for 3 days), including the respective pair-fed or PBS-treated control groups (n = 10/group), mice were killed, serum was collected, and tissues were isolated 4 days after the last injection.

Effects of $CNTF_{Ax15}$ administration for 7 days in diet-induced obese C57BL/6J mice. Two cohorts of diet-induced obese C57BL/6J mice treated with $CNTF_{Ax15}$ at 0.1 and 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$ s.c. for 7 days were killed either 24 h after the last injection (cohort 1) or 4 days after the last injection (cohort 2), and outcomes were compared with the effect of PBS treatment or pair-feeding as described above (n = 5/group). To investigate a potential effect of $CNTF_{Ax15}$ on energy expenditure and activity levels during treatment and in the posttreatment period, indirect calorimetry was performed during treatment (days 2–4, cohort 1) and after discontinuation of treatment (days 8–10, cohort 2). Blood and tissues were collected at the time of death.

Effects of $CNTF_{Ax15}$ administration in UCP1-DTA mice. To study the potential role of uncoupling protein 1 (UCP1) in mediating the effect of $CNTF_{Ax15}$ on food intake, body weight, and serum hormone concentrations, UCP1-DTA mice were injected with $CNTF_{Ax15}$ (0.1 or 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$ s.c.) and compared with PBS-treated and pair-fed control groups as described above (n = 5/group).

Effects of $CNTF_{Ax15}$ administration on insulin sensitivity and glucose tolerance in UCP1-DTA mice. To investigate whether insulin sensitivity and glucose tolerance improves with $CNTF_{Ax15}$ administration to UCP1-DTA mice, intraperitoneal insulin tolerance tests and oral glucose tolerance tests (OGTTs) were performed in these mice (n = 5/group) and compared with their control heterozygote littermates, which were either vehicle treated or pair-fed as described above. $CNTF_{Ax15}$ (0.1 g/g s.c.) was injected daily for 10 days. The OGTTs were conducted starting at 10:00 A.M. in the morning after mice had been fasted for 16–18 h before gavaging with a standard glucose bolus, as previously outlined (5). In nonfasted mice, intraperitoneal insulin tolerance tests were performed with 0.75 units insulin/kg body wt and results were expressed as a percentage of starting blood glucose.

Physiological characterization. Individual body weights and total amount of food per cage were measured daily between 8:00 and 10:00 A.M. with an analytical balance, with the differences in food between two measurements assumed to represent the amount of food consumed (6,11,12). For whole-body composition analysis, mice were anesthetized with ketamine (90 mg/kg) and

TABLE 1
In vivo body composition in diet-induced obese mice at baseline, 24 h, and 6 days after $CNTF_{Ax15}$ administration for 3 days (0.1 and 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$, respectively)

Groups	Total fat tissue mass (g)	Total lean tissue mass (g)	Total body fat (%)	Abdominal fat tissue mass (g)	Abdominal lean tissue mass (g)	Abdominal body fat (%)
Baseline						
PBS	21.7 ± 1.1	22.0 ± 0.7	49.5 ± 1.4	9.9 ± 0.4	9.4 ± 0.4	51.2 ± 0.9
C-0.1	21.5 ± 1.2	21.2 ± 0.6	49.3 ± 1.1	9.0 ± 0.4	8.9 ± 0.3	50.0 ± 1.0
C-1.0	20.1 ± 0.6	21.9 ± 0.5	47.7 ± 0.8	9.5 ± 0.4	9.8 ± 0.3	49.2 ± 0.7
PF to C-1.0	21.1 ± 0.7	20.9 ± 0.5	50.2 ± 0.9	10.0 ± 0.4	9.2 ± 0.3	51.4 ± 1.0
24 h after last treatment						
PBS	18.6 ± 0.7	19.8 ± 0.5	48.3 ± 1.2	8.3 ± 0.3	8.7 ± 0.3	49.0 ± 0.9
C-0.1	17.0 ± 0.9	18.7 ± 0.5	47.3 ± 1.8	7.9 ± 0.5	7.9 ± 0.4	49.7 ± 1.5
C-1.0	15.7 ± 0.7*	17.8 ± 0.4†‡	46.8 ± 1.4	7.2 ± 0.2*§	7.3 ± 0.2†‡	49.5 ± 0.9
PF to C-1.0	17.2 ± 0.6	19.6 ± 0.4	46.7 ± 0.9	8.4 ± 0.3	8.8 ± 0.2	48.7 ± 1.0
6 days after last treatment						
PBS	16.9 ± 0.6	22.0 ± 0.6	48.5 ± 1.0	8.3 ± 0.3	9.9 ± 0.5	50.9 ± 0.8
C-0.1	14.7 ± 0.8†‖	20.6 ± 0.4§‖	46.6 ± 1.6¶	6.4 ± 0.4*#	8.8 ± 0.2	48.3 ± 1.4¶
C-1.0	12.2 ± 0.6**††	22.2 ± 0.4	40.3 ± 1.2**§	5.9 ± 0.3**††	10.2 ± 0.3	41.6 ± 1.1**††
PF to C 1.0	16.2 ± 0.6	22.8 ± 0.3	46.4 ± 1.0	7.8 ± 0.3	10.2 ± 0.4	48.2 ± 0.9†

Data are means ± SE, analyzed by unpaired *t* test. *$P < 0.01$, †$P < 0.05$ vs. the PBS-treated group; ‡$P < 0.05$, §$P < 0.01$ vs. the pair-fed group; ‖$P < 0.05$, ¶$P < 0.01$ vs. the 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$-treated group; #$0.07 < P < 0.05$, **$P < 0.0001$ vs. the PBS-treated group; ††$P <$ 0.0001 vs. the pair-fed group. C-0.1, 0.1 $\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$-treated group; C-1.0, 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$-treated group; PF, pair-fed.

xylazine (10 mg/kg) and scanned with a PIXImus densitometer (Lunar, Madison, WI) (13).

Indirect calorimetry and activity. To assess potential changes in energy expenditure, in vivo indirect open-circuit calorimetry using a comprehensive laboratory animal monitoring system (CLAMS; Columbus Instruments, Columbus, OH) was performed in three groups of diet-induced obese C57BL/6J mice (PBS, $CNTF_{Ax15}$ 0.3, and pair-fed; $n = 5$/group) during and after treatment with $CNTF_{Ax15}$, as described above. All animals were individually housed in metabolic cages over a 48-h period and had free access to food and water, except the pair-fed-group. After a 6-h acclimation period, oxygen and carbon dioxide gas fractions were monitored (two 12-h dark and two 12-h light phases), and oxygen consumption (V_{O_2}) and respiratory quotient (ratio of V_{CO_2} to V_{O_2}) were calculated as described previously (14,15). Locomotor activity was measured using passive infrared motion detectors (Columbus Instruments, Columbus, OH). Each movement of the animals was recorded as a 3-s impulse, which was regarded as one event. For all indirect calorimetry measurements, no changes in diurnal rhythmicity were noted.

Tissue collection and expression analysis. Animals were killed with carbon dioxide asphyxiation, and tissues (epididymal white adipose tissue, BAT and hypothalami) were collected, immediately frozen in liquid nitrogen, and stored at −80°C. Total tissue RNA was purified using the RNA STAT-60 total RNA/mRNA isolation reagent according to the manufacturer's instructions (Tel-Test, Friendswood, TX). Hormone levels were measured, and hypothalamic neuropeptide mRNA expression (proopiomelanocortin [POMC], neuropeptide Y [NPY], agouti gene–related protein [AgRP], orexin) was analyzed using mouse-specific primers, as previously described (6,10,11,14). UCP1 mRNA was amplified in a 20-l reaction mixture using SYBR-Green master mix (Applied Biosystems). The following mouse specific primers were used for the amplification of UCP1-DTA: 5′-*ttgcctggcagatatcatca*-3′, 5′*tgcattctgaccttcacgac*-3′. All samples were quantified in triplicate, and averages were used for statistical analyses.

Statistics. Data are expressed as the means ± SE. Statistical significance was assessed by standard Student's *t* tests or two-tailed *t* tests as well as ANOVA with post hoc corrections, at the conventional $P < 0.05$ value. All statistical analyses were performed using StatView (Abacus, Berkley, CA) and SPSS 8 (Texas Instruments, Chicago, IL). Graphs were created with Prism (GraphPad, San Diego, CA).

RESULTS

Effects of $CNTF_{Ax15}$ on body weight and food intake in diet-induced obese C57BL/6J mice

Short-term experiment. We investigated the effects of short- and long-term treatment of $CNTF_{Ax15}$ on food intake and body weight in diet-induced obese C57BL/6J mice, particularly focusing on the posttreatment period. Consistent with studies in other strains of mice, $CNTF_{Ax15}$ treatment for 3 days resulted in significantly reduced food intake and body weight (starting body weights: PBS group: 44.1 ± 1.0 g; $CNTF_{Ax15}$ 0.1 group: 44.1 ± 1.2 g; $CNTF_{Ax15}$ 1.0 group: 44.1 ± 0.8 g; pair-fed group: 44.1 ± 0.8 g) (Fig. 1*A*). Caloric intake was significantly decreased in both $CNTF_{Ax15}$-treated diet-induced obese C57BL/6J groups ($CNTF_{Ax15}$ 1.0 group: 9.7 ± 0.9 kcal; $CNTF_{Ax15}$ 0.1 group: 17.9 ± 0.9 kcal) when compared with PBS-treated controls (41.1 ± 1.6 kcal, $P < 0.01$ for both treatment groups) (Fig. 1*B*). By experimental design, food intake of the pair-fed group did not differ from the $CNTF_{Ax15}$ 1.0–treated group (9.7 ± 0.03 kcal) (Fig. 1*B*). After discontinuation of pair-feeding, food intake of mice from both control groups (PBS and pair-fed) rapidly increased above baseline (Fig. 1*B*), resulting in an attenuation of body weight loss within 4 days (Fig. 1*A*). Consistent with previous data (3), food intake of animals from both $CNTF_{Ax15}$-treated groups was significantly decreased even 3 days after discontinuation of treatment, but it eventually returned to baseline. Body weight loss of $CNTF_{Ax15}$-treated mice was maintained until the end of the observation period (Fig. 1*A*). More importantly, body weight reduction was more pronounced in $CNTF_{Ax15}$-treated than pair-fed mice (Fig. 1*A*), an effect not previously observed in other diet-induced obese mouse models (e.g., AKR/J) (3–5). Consistent with results from other strains, administration of $CNTF_{Ax15}$ for 3 days to diet-induced obese C57BL/6J mice significantly decreased body fat mass (3), an effect still evident 6 days after discontinuation of treatment, when food intake had already returned to baseline (Table 1). Moreover, $CNTF_{Ax15}$ treatment significantly reduces abdominal fat mass bordered cranially by the xiphoid apophysis and caudally by the sacrum ($P < 0.01$ vs. PBS-treated mice) (Table 1),

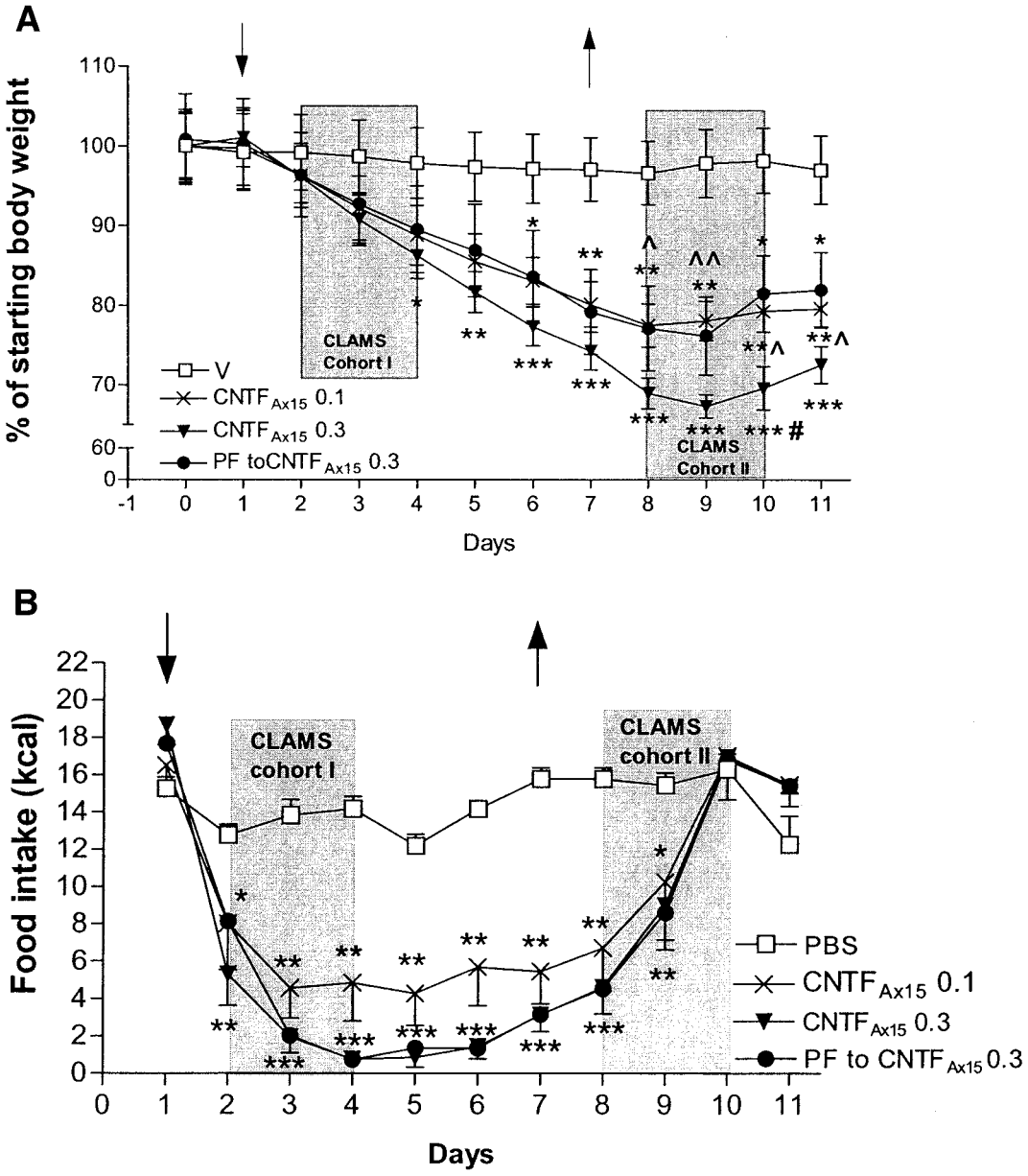

FIG. 2. The effect of long-term administration of CNTF$_{Ax15}$ to reduce food intake and body weight in diet-induced obese C57BL/6J mice. Diet-induced obese C57BL/6J mice were administered the indicated doses of CNTF$_{Ax15}$ for 7 consecutive days (arrows indicate beginning and end of treatment period), and food intake and body weight was assessed daily. Pair-feeding was continued until the end of the study. Indirect calorimetry using a comprehensive laboratory animal monitoring system (CLAMS) was performed on days 2–4 and 8–10. *A*: Percent change in body weight (from starting body weight) compared with baseline levels. *B*: Caloric intake over time. Analyses were performed using an unpaired *t* test and are expressed as means ± SE. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. the vehicle-treated group; $\wedge P < 0.05$ and $\wedge\wedge P < 0.01$ between both CNTF$_{Ax15}$-treated groups; #$P = 0.06$ vs. the pair-fed group. PF, pair-fed group ($n = 5$); V, vehicle (PBS) treated.

even when total fat mass was not significantly decreased (Table 1).

Long-term experiment. To expand the above observations in a more prolonged treatment regimen and using an intermediate CNTF$_{Ax15}$ dose, a cohort of diet-induced obese C57BL/6J mice were treated with CNTF$_{Ax15}$ for 7 days (0.1 and 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$), and food intake and body weights were measured (starting body weights: PBS group: 44.0 ± 2.3 g; CNTF$_{Ax15}$ 0.1 group: 44.2 ± 2.2 g; CNTF$_{Ax15}$ 0.3 group: 44.2 ± 1.8 g; pair-fed group: 44.3 ± 2.6 g) (Fig. 2*A* and *B*). Importantly, pair-feeding was continued beyond the treatment period and until death to directly differentiate between an effect of reduced food intake versus increased energy expenditure. Similar to the short-term studies, after 7 days of treatment, cumulative food intake was significantly reduced in both treatment groups compared with PBS-treated mice ($P < 0.01$ for both treatment groups vs. PBS-treated mice) (Fig. 2*B*), whereas weight loss was significantly reduced in the CNTF$_{Ax15}$ 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$–treated group compared with the pair-fed group (Fig. 2*A*). Interestingly, gains in body weight were still significantly more pronounced in mice that continued to be pair-fed the same amount of food that the formerly CNTF$_{Ax15}$-treated group consumed ($P < 0.05$ on day 10 vs. the pair-fed group) (Fig. 2*A*), indicating an effect of CNTF$_{Ax15}$ to increase energy expenditure.

Effects of CNTF$_{Ax15}$ on energy expenditure and metabolic rate. To address the effects of CNTF$_{Ax15}$ adminis-

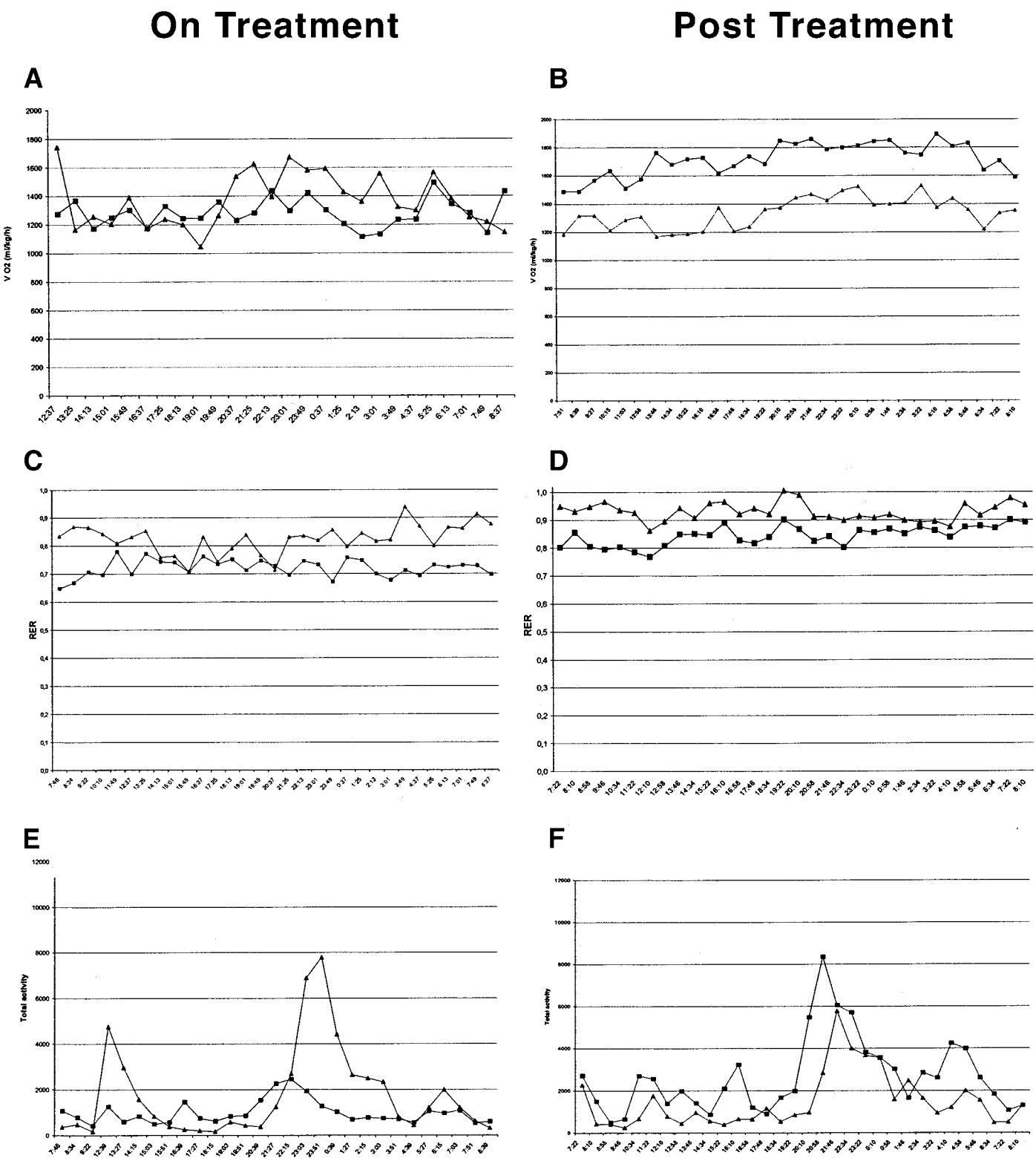

FIG. 3. Indirect calorimetry in diet-induced obese C57BL/6J mice immediately after initiating or terminating $CNTF_{Ax15}$ for 7 days. Indirect calorimetry, using a comprehensive laboratory animal monitoring system, was performed on separate cohorts on days 2–4 of $CNTF_{Ax15}$ treatment ($0.3\ \mu g \cdot g^{-1} \cdot day^{-1}$) and on days 1–3 after completing 7 consecutive days of $CNTF_{Ax15}$ treatment ($0.3\ \mu g \cdot g^{-1} \cdot day^{-1}$), presented as single time points, measured over a 24-h period. *A*: Energy expenditure (Vo_2) on treatment. *B*: Energy expenditure (Vo_2) after discontinuation of treatment. *C*: Respiratory ratio (respiratory quotient) on treatment. *D*: Respiratory ratio (respiratory quotient) after discontinuation of treatment. *E*: Locomotor activity on treatment. *F*: Locomotor activity after discontinuation of treatment. RER, respiratory quotient. ▲, PBS group; ■, $CNTF_{Ax15}$-treated group.

tration on energy expenditure, we analyzed diet-induced obese C57BL/6J mice in metabolic chambers during treatment and in the posttreatment period. Interestingly, there were no changes in total energy expenditure while on treatment (Fig. 3*A*). After discontinuation of treatment, however, formerly $CNTF_{Ax15}$-treated animals display a

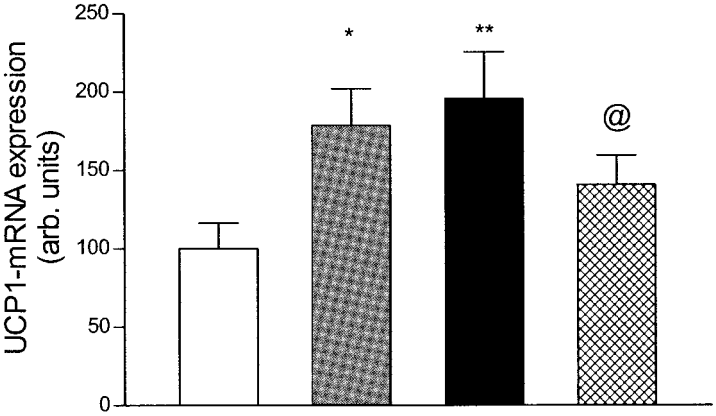

FIG. 4. UCP1 mRNA expression (in arbitrary units) in BAT of diet-induced obese C57BL/6J mice 4 days after discontinuation of $CNTF_{Ax15}$ treatment for 3 days. Values were analyzed by ANOVA and are expressed as the means ± SE. *$P < 0.05$ and **$P < 0.01$ vs. the PBS-treated control group; @$P = 0.09$ vs. the higher-dose-treated $CNTF_{Ax15}$ group. □, PBS group; ▨, group treated with $CNTF_{Ax15}$ at 0.1 $\mu g \cdot kg^{-1} \cdot day^{-1}$; ■, group treated with $CNTF_{Ax15}$ at 1.0 $\mu g \cdot kg^{-1} \cdot day^{-1}$; ▩, pair-fed group.

significant increase in energy expenditure versus PBS-treated and pair-fed controls, with V_{O_2} consumption in the $CNTF_{Ax15}$-treated group being significantly increased during both the day and night periods compared with both control groups ($P < 0.01$ vs. pair-fed and PBS-treated mice) (Fig. 3*B* and online appendix). Consistent with observed reductions in abdominal fat, we observed a significant reduction in respiratory quotient in $CNTF_{Ax15}$-treated mice, which remained reduced after discontinuation of treatment (Fig. 3*C* and *D* and online appendix [available from http://diabetes.diabetesjournals.org]). The reduction in respiratory quotient in $CNTF_{Ax15}$-treated mice was even more pronounced when data were analyzed separately for the light and dark phases (Fig. 3 and online appendix).

Assessment of total activity revealed a significant increase in the pair-fed group, a result consistent with a state of "food-seeking behavior" in response to food deprivation. This was evident in both the treatment and posttreatment periods (activity in the pair-fed group increased by 223.7 ± 47.2% of baseline on treatment and remained elevated at 159.3 ± 18.6% after cessation of treatment; $P <$ 0.05 vs. the PBS-treated group) (Fig. 3 and online appendix). In contrast, total activity remained remarkably stable in the $CNTF_{Ax15}$-treated mice, suggesting that $CNTF_{Ax15}$-treated mice do not demonstrate a comparable drive to seek food, despite decreased caloric intake (Fig. 3*E*). There is only a minor effect of $CNTF_{Ax15}$ on V_{O_2} consumption in diet-induced obese C57BL/6J mice (cohort 1, day 3 of $CNTF_{Ax15}$ treatment), suggesting that there is probably a reduction in the basic metabolic rate. In addition, there is only a small difference in V_{O_2} consumption between 0.3-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated mice and pair-fed mice for the first 24 h after the last treatment (cohort 2, days 7–8) (Fig. 3 and online appendix). After this period of time (24 h after discontinuation of treatment or pair-feeding, i.e., after $CNTF_{Ax15}$ washes out), however, there is a dramatic separation in V_{O_2}, with a significant increase in the formerly $CNTF_{Ax15}$-treated group. Similarly, when resting V_{O_2} was computed, we did not detect any significant difference between the PBS- and $CNTF_{Ax15}$-treated groups while on treatment, and resting V_{O_2} was significantly higher in the formerly $CNTF_{Ax15}$-treated group ($P < 0.001$ unpaired two-tailed t test). Based on these data, it is reasonable to assume that thermogenesis might be increased in the long term after $CNTF_{Ax15}$ treatment; therefore, an assessment of UCP-1 expression in BAT as well as in vivo studies using UCP1-DTA mice were performed.

Effects of $CNTF_{Ax15}$ on UCP-1 expression and thermogenesis. To confirm that the effects of $CNTF_{Ax15}$ are, at least in part, mediated by altered thermogenesis, we quantified UCP1 mRNA expression in BAT of diet-induced obese C57BL/6J mice treated with $CNTF_{Ax15}$ for 3 days. At 4 days after the last injection of $CNTF_{Ax15}$, when mice had already returned to baseline food intake levels, UCP1 mRNA expression in BAT was significantly and dose-dependently elevated compared with PBS-treated controls ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 4). This increase in UCP1 mRNA expression corresponds to a concomitant increase in energy expenditure in the $CNTF_{Ax15}$-treated group, which was not seen in the pair-fed group (Fig. 3*A* and 4). Although UCP1 mRNA expression was not significantly different between formerly treated and pair-fed animals, a trend was noted ($P = 0.09$, Fig. *4*). However, the observed significant increase in formerly $CNTF_{Ax15}$-treated compared with PBS-treated mice may be of physiological importance because the time point studied represents a time when food intake is already normalized in formerly pair-fed animals and is still reduced (whereas energy expenditure remains still elevated) in formerly $CNTF_{Ax15}$-treated mice.

Effects of $CNTF_{Ax15}$ on body weight and food intake in UCP1-DTA mice. To further determine the contribution $CNTF_{Ax15}$ may have on energy expenditure and thermogenesis, we performed experiments in the BAT-ablated UCP1-DTA rodent model of obesity. Similar to the diet-induced obese C57BL/6J mice studies, 7 days of $CNTF_{Ax15}$ treatment reduced caloric intake in a dose-dependent manner in UCP1-DTA mice ($P < 0.05$) (Fig. 5*B*), but more importantly, decreases in body weight in $CNTF_{Ax15}$-treated animals precisely overlapped decreases observed in pair-fed controls (starting body weights: PBS-treated group: 28.9 ± 1.8 g; $CNTF_{Ax15}$ 0.1 group: 29.0 ± 1.5g; $CNTF_{Ax15}$ 0.3 group: 28.9 ± 1.7; pair-fed group: 29.0 ± 1.8 g) (Fig. 5*A*). Similar data were obtained in an independent experiment, as seen below (initial body weights: PBS-treated group: 45.3 ± 3.2 g, $CNTF_{Ax15}$ 0.3 group: 45.9 + 1.6 g; and pair-fed group: 45.1 ± 1.4 g; final body weights: PBS group: 43.4 ± 3.2 g; $CNTF_{Ax15}$ 0.3 group: 38.6 ± 1.0 g; pair-fed group: 36.5 ± 2.8 g).

Effects of $CNTF_{Ax15}$ on metabolic control in UCP1-DTA mice. Plasma insulin and glucose concentrations were significantly and dose dependently decreased in $CNTF_{Ax15}$-treated UCP1-DTA mice compared with PBS-treated mice (Table 2), but not when compared with pair-fed mice. In the second, separate but similar experi-

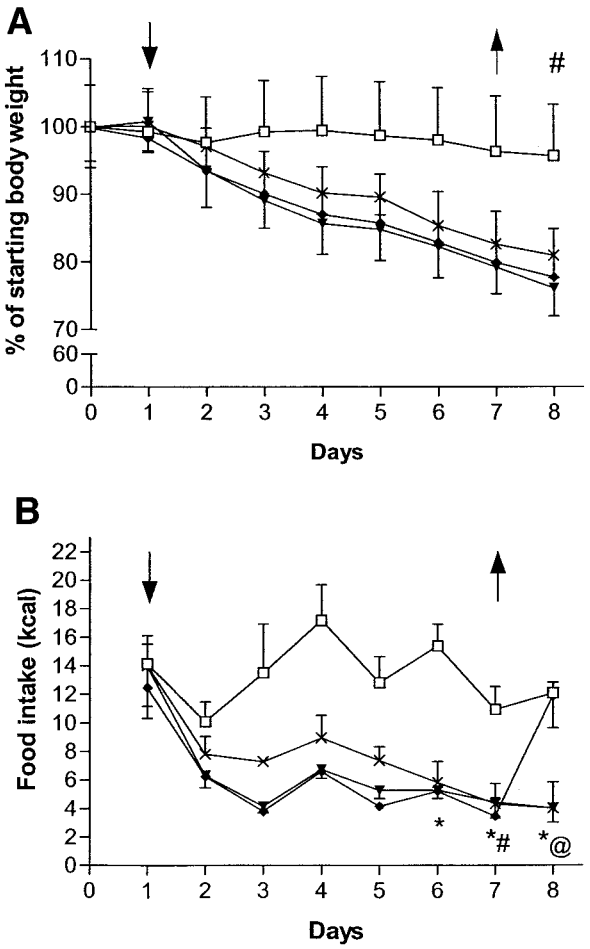

FIG. 5. $CNTF_{Ax15}$ reduces food intake and body weight in UCP1-DTA mice. In a manner similar to Fig. 1, UCP1-DTA mice were administered the indicated doses of $CNTF_{Ax15}$ for 7 consecutive days (arrows indicate treatment period), and food intake and body weight were assessed daily. Pair-feeding was continued until the end of the study. Separate cohorts of similarly treated mice were killed at day 8. *A*: Percent change in body weight compared with baseline levels. *B*: Caloric intake over time. A separate group of UCP-1-DTA mice received a daily subcutaneous injection of vehicle or $CNTF_{Ax15}$ at 0.1 $\mu g \cdot g^{-1} \cdot day^{-1}$ or were provided with the same amount of chow as that eaten by the group treated with $CNTF_{Ax15}$ at 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$. All data are expressed as the means ± SE, analyzed by unpaired *t* test ($n = 5$). *$P <$ 0.05 between the $CNTF_{Ax15}$ 0.3-$\mu g \cdot g^{-1} \cdot day^{-1}$–treated and the PBS-treated group; #$P < 0.05$ between the pair-fed and PBS-treated group; @$P < 0.05$ between the $CNTF_{Ax15}$ 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$–treated and the PBS-treated group. □, PBS group; ×, group treated with $CNTF_{Ax15}$ at 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$; ▼, group treated with $CNTF_{Ax15}$ at 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$; ♦, group pair-fed to same amount as group treated with $CNTF_{Ax15}$ at 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$.

TABLE 2
Effect of $CNTF_{Ax15}$ administration for 3 days (0.1 and 1.0 $\mu g \cdot g^{-1} \cdot day^{-1}$) or 7 days (0.1 and 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$) on circulating insulin and blood glucose concentrations in diet-induced obese C57BL/6J and UCP1-DTA mice

Groups	Insulin (ng/ml)	Glucose (mg/dl)
3-day studies		
DIO-C57 24 h after last treatment		
PBS	2.14 ± 0.39	117.2 ± 5.2
C-0.1	1.21 ± 0.29*	121.6 ± 3.3†
C-1.0	1.02 ± 0.06‡	91.3 ± 2.3§‖
PF to C-1.0	0.82 ± 0.03¶	98.3 ± 3.6¶
DIO-C57 4 days after last treatment		
PBS	2.52 ± 0.31#	NSA
C-0.1	2.16 ± 0.36#	NSA
C-1.0	2.61 ± 0.41#	NSA
PF to C-1.0	5.26 ± 1.17	NSA
7-day studies		
DIO-C57 24 h after last treatment (cohort 1)		
PBS	5.99 ± 1.09	123.6 ± 5.7
C-0.1	0.91 ± 0.23§	125.6 ± 10.4**
C-0.3	0.42 ± 0.07§	92.8 ± 5.4†††
PF to C-0.3	0.72 ± 0.16§	81.6 ± 3.7¶
DIO-C57 4 days after last treatment (cohort 2)		
PBS	4.61 ± 0.51	141.2 ± 4.8
C-0.1	1.80 ± 0.49‡	130.2 ± 5.4†
C-0.3	2.81 ± 0.79*	113.4 ± 5.7¶‡‡§§
PF to C-0.3	2.08 ± 0.43‡	96.2 ± 2.1§
UCP1-DTA 24 h after last treatment (cohort 1)		
PBS	1.95 ± 0.58	111.6 ± 6.6
C-0.1	0.84 ± 0.33	116.0 ± 8.4†
C-0.3	1.09 ± 0.30	96.0 ± 0.7*§§#
PF to C-0.3	0.49 ± 0.09	63.7 ± 5.6***

Data are means ± SE, analyzed by ANOVA. Data were obtained 24 h after the last treatment (cohort 1) or in the posttreatment period (4 days after the last treatment, cohort 2). *$P < 0.05$ vs. the PBS-treated group; †$P < 0.0001$ vs. the pair-fed group; ‡$P < 0.01$ and §$P < 0.0001$ vs. the PBS-treated group; ‖$P < 0.0001$ vs. the 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group; ¶$P < 0.001$ vs. the PBS-treated group; #$P <$ 0.01 vs. the pair-fed group; **$P < 0.001$ vs. the pair-fed group; ††$P <$ 0.01; vs. the 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group; ‡‡$P < 0.05$ vs. the pair-fed group; §§$P < 0.05$ vs. the 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group. C-0.1, 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group; C-1.0, 1.0-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group; C-0.3, 0.3- $\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$–treated group; NSA, no sample available; PF, pair-fed.

ment, we demonstrate, using more sophisticated methods to assess metabolic control, that treatment of $CNTF_{Ax15}$ for 7 days not only decreases body weight to a similar degree in treated and pair-fed mice (Fig. 5*A*) but also improves glucose tolerance (Fig. 6*A* and *B*), which is more obvious in UCP1-DTA mice than in pair-fed controls. After receiving 0.75 units/kg body wt of insulin, $CNTF_{Ax15}$-treated and pair-fed UCP1-DTA mice show an approximate 50% decrease in blood glucose levels after 30 min, whereas vehicle-treated control mice failed to significantly decrease their glucose levels for 120 min (Fig. 6*B*); however, although the effect of insulin administration was significant ($P < 0.001$), no significant difference between groups could be detected ($P = 0.3$). In addition, OGTTs demonstrate that $CNTF_{Ax15}$ treatment tended to result in improved glucose tolerance in $CNTF_{Ax15}$-treated compared with pair-fed animals (mean differences by ANOVA: effect of time $P < 0.01$, effect of group $P < 0.01$, and effect of time and group interaction $P = 0.07$; and area under the curve measurements: $CNTF_{Ax15}$ 0.3: 5.51 ± 9.2 vs. PBS-treated: 17.12 ± 6.24 [$P < 0.05$], pair-fed: 8.85 ± 2.24 [$P =$ 0.09 vs. PBS treated and $P = 0.12$ vs. 0.3 $\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$]) (Fig. 6*A*).

Effects of $CNTF_{Ax15}$ on circulating glucose and insulin levels in diet-induced obese C57BL/6J mice. $CNTF_{Ax15}$ treatment for 3 days to diet-induced obese C57BL/6J mice significantly decreased serum insulin and plasma glucose concentrations, which were similar in $CNTF_{Ax15}$-

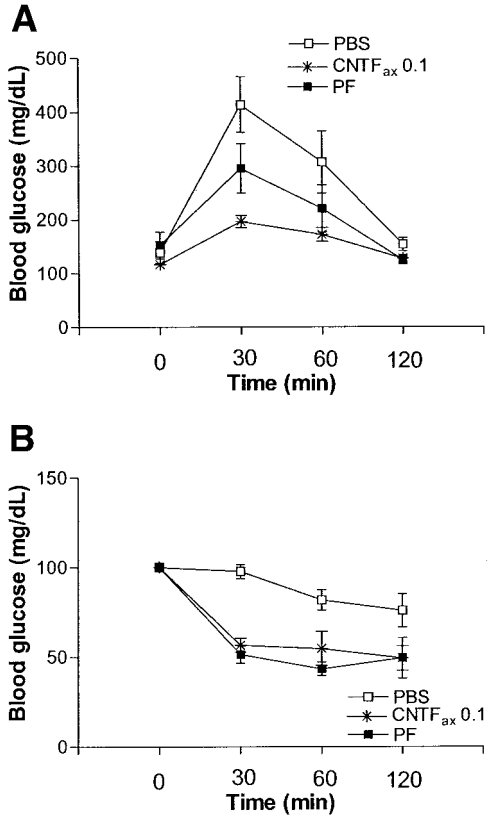

FIG. 6. Treatment with $CNTF_{Ax15}$ improves glucose tolerance. Groups of UCP1-DTA mice received a daily subcutaneous injection of vehicle, $CNTF_{Ax15}$ at 0.1 $\mu g \cdot g^{-1} \cdot day^{-1}$, or were provided with the same amount of chow eaten by the 0.1-$\mu g \cdot g^{-1} \cdot day^{-1}$ $CNTF_{Ax15}$ treatment group. *A*: An OGTT was conducted 24 h after the last treatment and serum glucose was measured at the indicated times after oral gavage. All data are expressed as the means ± SE ($n = 5$), analyzed by ANOVA. *B*: In nonfasted mice an intraperitoneal insulin tolerance test was performed with 0.75 units insulin/kg body wt. Results are expressed as a percentage of starting blood glucose (means ± SE, $n = 5$, analyzed by ANOVA with post hoc). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. the PBS-treated group; ∧$0.07 < P < 0.05$, #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ vs. the pair-fed group. PF, pair-fed group.

treated and pair-fed mice (Table 2). In accordance with these findings, $CNTF_{Ax15}$ treatment for 7 days significantly decreased serum insulin levels in diet-induced obese C57BL/6J mice, but again, the decline was similar between treated and pair-fed-mice when measured 24 h or 4 days after the last treatment (Table 2). Plasma glucose concentrations were significantly and dose-dependently lower in $CNTF_{Ax15}$-treated and pair-fed diet-induced obese C57BL/6J mice compared with PBS-treated mice (Table 2).

These data indicate that $CNTF_{Ax15}$ treatment has a favorable effect on the metabolic profile that proves to be more pronounced than pair-feeding when more sophisticated methods for assessing insulin resistance are used (as seen above). Thus, the effects of $CNTF_{Ax15}$ on improving insulin resistance and metabolic control as well as the underlying mechanisms need to be studied further.

Effects of $CNTF_{Ax15}$ on expression of hypothalamic neuropeptides regulating food intake. Given the suppressive effect of $CNTF_{Ax15}$ on food intake, the expression pattern of hypothalamic neuropeptides known to be involved in energy homeostasis (the orexigenic molecules NPY and AgRP and the anorexigenic molecules POMC and orexin) was studied. After food deprivation, NPY mRNA expression increased to 173% of baseline levels in the pair-fed group ($P < 0.05$ vs. the PBS-treated group) (data not shown). The compensatory increase of NPY normally seen with caloric restriction was also observed immediately after the 3-day treatment period with $CNTF_{Ax15}$ in diet-induced obese C57BL/6J mice. However, after a 7-day treatment period, NPY mRNA expression in $CNTF_{Ax15}$-treated mice was lower than in the pair-fed mice and comparable to levels seen in the PBS-treated controls (data not shown). The expression pattern of AgRP was not significantly different between groups in the short-term studies because AgRP expression is altered only after more prolonged food deprivation. Accordingly, after 7 days of $CNTF_{Ax15}$ treatment, AgRP mRNA expression followed the pattern of NPY and was significantly increased in the pair-fed, but not the $CNTF_{Ax15}$-treated, group compared with the PBS-treated controls (data not shown). Finally, no significant changes in POMC or orexin mRNA expression were observed, suggesting that these molecules do not play a role of major significance in mediating the effects of $CNTF_{Ax15}$ on food intake (data not shown).

DISCUSSION

We show that $CNTF_{Ax15}$ has effects on energy homeostasis, body weight, body composition, and metabolism during active treatment and after discontinuation of treatment. Specifically, we show that $CNTF_{Ax15}$ reduces food intake and body weight through a pathway downstream of the putative point responsible for leptin resistance in both diet-induced obese C57BL/6J and UCP1-DTA mice and that it increases energy expenditure in diet-induced obese C57BL/6J mice, in part by upregulating UCP1 expression. Even more importantly, these effects persist several days after discontinuation of treatment.

Previous studies have shown that food intake is significantly reduced after peripheral CNTF administration to DIO-AKR/J or *ob/ob* mice (3) as well as central (intracerebroventricular) administration to Sprague-Dawley rats (4). We extend these findings by showing that $CNTF_{Ax15}$ effectively decreases food intake in diet-induced obese C57BL/6J and UCP1-DTA mice and that decreased caloric intake in UCP1-DTA mice is comparable to what is observed in diet-induced obese C57BL/6J mice. The resulting weight loss is less pronounced, however, probably because of the impaired thermogenesis/energy expenditure in UCP-DTA mice (16).

When food and water intake were measured using the CLAMS apparatus, water intake closely paralleled food intake, and no specific effect of the drug was observed. Activity levels of mice, oxygen consumption, carbon dioxide production, as well as respiratory quotients were measured in metabolic chambers. Behavioral effects or taste aversion phenomenon were not specifically studied; conditioned taste aversion response was previously assessed in diet-induced obese mice, and no adverse effects were found (3).

We have shown that $CNTF_{Ax15}$ promotes weight loss in obese diet-induced obese C57BL/6J and UCP1-DTA mice and that reduced body weight in diet-induced obese C57BL/6J mice is mainly caused by decreased body fat mass. More importantly, we demonstrate that weight loss

in $CNTF_{Ax15}$-treated diet-induced obese C57BL/6J mice is significantly higher than what is observed in pair-fed controls, supporting our hypothesis that $CNTF_{Ax15}$ exerts its effects not only by reducing food intake but also by increasing thermogenesis. $CNTF_{Ax15}$ administration has resulted in significant decreases of both fat and lean body mass, but changes of the latter were much less pronounced than changes in fat mass. Similar changes have been observed in response to all weight-reducing methods in the past; future studies are needed to address in more detail the $CNTF_{Ax15}$-induced alterations in body composition.

To gain a better understanding of the mechanisms driving CNTF-induced decreases in body weight and increases in thermogenesis, we investigated the effects of $CNTF_{Ax15}$ in BAT-deficient UCP1-DTA mice and directly measured UCP1 expression and energy expenditure in diet-induced obese C57BL/6J mice. BAT is responsible for nonshivering thermogenesis, a major component of facultative thermogenesis in newborns and mammals, which is mediated by UCP1. The hypothesis of BAT playing an important role in the regulation of energy expenditure has been supported by the creation of BAT-deficient mice (UCP1-DTA mice), which are characterized by marked obesity and insulin resistance (7–9). Moreover, UCP1-DTA mice raised at thermoneutrality (35°C), a temperature that would lead to total inactivation of BAT, completely prevents the development of obesity in these mice (16). UCP1-DTA mice were therefore used herein to support our hypothesis that $CNTF_{Ax15}$ acts, at least in part, by altering energy expenditure. We show that weight loss in $CNTF_{Ax15}$-treated diet-induced obese C57BL/6J mice is significantly higher than in pair-fed controls, whereas weight loss in $CNTF_{Ax15}$-treated UCP1-DTA mice precisely parallels and overlaps the weight loss seen in their pair-fed controls. Thus, the effects of $CNTF_{Ax15}$ to induce anorexia by altering hypothalamic neuropeptide expression are intact in these mice, but the peripheral effects mediating energy expenditure are disrupted.

Similar to leptin, CNTF-induced weight loss and appetite suppression is mediated through neurons in the medial arcuate nucleus, but unlike leptin, no evidence of CNTF action in other regions of the hypothalamus could be found (17,18). In addition, anorectic effects of CNTF are in part mediated by altered hypothalamic NPY expression (17,18), and CNTF induces expression of hypothalamic inhibitors of leptin signaling (19). We confirm these findings by showing that $CNTF_{Ax15}$ exerts anorectic effects on diet-induced obese C57BL/6J mice, at least in part, by altering hypothalamic NPY and AgRP mRNA expression, and we extend previous findings by showing that POMC and orexin expression do not play a role of comparable significance. The question of whether changes in those neuropeptides are also responsible for the anorectic effects of $CNTF_{Ax15}$ in UCP1-DTA mice needs to be addressed in future studies. Based on the data presented herein, we speculated that $CNTF_{Ax15}$ mediates its effects in diet-induced obese C57BL/6J mice, at least in part, by altering energy expenditure, possibly through increased thermogenesis, demonstrating directly that $CNTF_{Ax15}$ increases energy expenditure in diet-induced obese C57BL/6J mice. The fact that body fat mass and respiratory quotient are still decreased while energy consumption is significantly elevated in the posttreatment period implies that, similar to leptin, $CNTF_{Ax15}$ promotes energy expenditure and possibly fatty acid oxidation, but that, in contrast to leptin, this effect persists after cessation of treatment and beyond the half-life of the medication. In accordance, we show herein that UCP1 mRNA expression in BAT is significantly elevated in $CNTF_{Ax15}$-treated but not PBS-treated or pair-fed mice after cessation of treatment. Possibilities of posttranscriptional regulation of UCP-1 as well as the question of whether the effect of $CNTF_{Ax15}$ on UCP1 mRNA expression is specific or secondary to other metabolic changes needs to be addressed in future studies.

The $CNTF_{Ax15}$-induced weight loss significantly decreased circulating insulin and glucose levels, which were similar in $CNTF_{Ax15}$-treated and pair-fed mice 24 h after the last treatment. However, 4 days after cessation of treatment, formerly $CNTF_{Ax15}$-treated mice displayed significantly lower insulin concentrations than formerly pair-fed controls, which at that point showed an overshoot in food intake. In the long-term studies, when pair-feeding was maintained until the end of the experiments, circulating insulin levels were similar between formerly $CNTF_{Ax15}$-treated and pair-fed mice, indicating that the persistent effect of $CNTF_{Ax15}$ to reduce food intake in the long term may have beneficial effects on the metabolic profile. Conclusions derived by measuring serum insulin concentrations were further supported and extended by using more detailed methods for assessment of metabolic milieu, i.e., insulin tolerance test and OGTT in UCP1-DTA mice. $CNTF_{Ax15}$-treated mice responded with a relatively lower excursion of blood glucose levels in response to an OGTT, suggesting that $CNTF_{Ax15}$ treatment might exert a beneficial effect on metabolism of exogenously administered glucose. These observations need to be confirmed and the underlying mechanisms further studied by future experiments.

In summary, we demonstrate that $CNTF_{Ax15}$ exerts central and peripheral effects in regulating energy homeostasis, body weight, body composition, and metabolism, and that these effects persist even after discontinuation of treatment. $CNTF_{Ax15}$ reduces food intake and body weight in diet-induced obese C57BL/6J and UCP1-DTA mice through a pathway downstream of the putative point responsible for leptin and insulin resistance in these mouse models of obesity. An increase in energy expenditure is an additional mechanism driving weight loss in diet-induced obese C57BL/6J mice. In conclusion, $CNTF_{Ax15}$ may be a valuable drug target toward obesity, insulin resistance/diabetes, and associated metabolic disorders.

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