Leptin selectively reduces white adipose tissue in mice via a UCP1-dependent mechanism in brown adipose tissue

SCOTT P. COMMINS,1 PATRICIA M. WATSON,2 ISABEL C. FRAMPTON,2
AND THOMAS W. GETTYS1,2
Division of Gastroenterology and Hepatology, Department of 1Medicine and 2Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina 29425

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Commins, Scott P., Patricia M. Watson, Isabell C. Frampton, and Thomas W. Gettys. Leptin selectively reduces white adipose tissue in mice via a UCP1-dependent mechanism in brown adipose tissue. Am J Physiol Endocrinol Metab 280: E372–E377, 2001.—We tested the hypothesis that leptin, in addition to reducing body fat by restraining food intake, reduces body fat through a peripheral mechanism requiring uncoupling protein 1 (UCP1). Leptin was administered to wild-type (WT) mice and mice with a targeted disruption of the UCP1 gene (UCP1 deficient), while vehicle-injected control animals of each genotype were pair-fed to each leptin-treated group. Leptin reduced the size of white adipose tissue (WAT) depots in WT mice but not in UCP1-deficient animals. This was accompanied by a threefold increase in the amount of UCP1 protein and mRNA in the brown adipose tissue (BAT) of WT mice. Leptin also increased UCP2 mRNA in WAT of both WT and UCP1-deficient mice but increased UCP2 and UCP3 mRNA only in BAT from UCP1-deficient mice. These results indicate that leptin reduces WAT through a peripheral mechanism requiring the presence of UCP1, with little or no involvement of UCP2 or UCP3.

MITOCHONDRIAL OXIDATION of fatty acids creates a proton electrochemical gradient that is used to drive the conversion of ADP to ATP via ATP synthase (22). Brown adipose tissue (BAT) mitochondria possess an alternative pathway that allows protons to reenter the mitochondrial matrix without coupling to ATP synthesis (2), and this pathway is mediated by uncoupling protein 1 (UCP1). UCP1 transforms electrochemical energy into heat (24), enabling small mammals to tolerate cold exposure (9, 15). The sympathetic nervous system and UCP1 are essential components of this thermogenic response system (8, 31).

Recent work illustrates that thermogenesis is also induced by increased caloric intake, suggesting a potential role for BAT in maintaining energy balance during dietary challenges (26, 29). This concept was originally supported by the finding that mice with toxigenic-mediated ablation of BAT were obese and more prone to morbid obesity when fed high-calorie diets (13, 20). In view of these findings and the established role of UCP1 in thermogenesis, it was surprising to find that mice with a targeted disruption of the Ucp1 gene (UCP1 deficient) were not obese and no more prone to obesity than control mice when fed high-fat diets (8). Some evidence suggested that compensatory thermogenic mechanisms may have been induced in these mice (8), so we sought to determine whether the absence of UCP1 would compromise the ability of leptin to target and reduce white adipose tissue depots.

This approach is based on the hypothesis that leptin regulates in vivo rates of energy utilization through modulation of one or more of the uncoupling proteins. Using pair-fed wild-type (WT) and UCP1-deficient mice injected with vehicle or leptin, we show that UCP1 is required for leptin to decrease adipose tissue mass beyond the amount produced by its effect on food intake.

MATERIALS AND METHODS

Materials. All reagents, except where noted, were obtained from Sigma and were of the highest reagent grade. T1-RNase and TRIzol LS reagent were from Life Technologies (Gaithersburg, MD). The T7-Megashortscript and RNALater kits were purchased from Ambion (Austin, TX). α-[32P]CTP and 125I-labeled NaI were purchased from Du Pont-NEN Radiochemicals (Boston, MA). Immobilon-P polyvinylidene difluoride membranes were from Millipore (Bedford, MA). Recom-

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binant methionyl mouse leptin was kindly provided by Amgen (Thousand Oaks, CA).

Experimental animal protocol. Breeding pairs of mice heterozygous (+/−) for a targeted disruption of the Ucp1 gene (8) were provided by Dr. Leslie Kozak (Pennington Biomedical Research Center, Baton Rouge, LA). The mice were bred within the vivarium at the Medical University of South Carolina to establish a free-standing colony of homozygous UCP1-deficient (−/−) and WT mice (+/+) to serve as controls. Genotyping of individual offspring was performed by PCR by use of primers designed to amplify the region of exon 2, where presence of the neomycin resistance gene identifies mutant mice (5′ to 3′; exon 2 forward, ggtagtatgcaagaggtgtg, 5′ to 3′; exon 2 reverse, cttaattgactggacgtg, 5′ to 3′; neomycin reverse, ctacccgctgatcctga). Because of the temperature sensitivity of the mice, the colony was housed at 25–27°C. Mouse chow (Purina mouse chow, Ralston Purina, St. Louis, MO) and water were available ad libitum, and the lights were on a 12:12-h light-dark cycle.

Six-month-old male UCP1-deficient (−/−) and WT (+/+) mice were assigned to receive either leptin (20 μg·g body wt−1·day−1) or vehicle (100 μl sterile saline) for 8 days. The mice receiving saline were pair fed (PF) to individual leptin-injected mice of the same genotype, and each pair of mice (vehicle- and leptin-treated) was selected to be age and weight matched. The mice were housed individually in cages with raised wire flooring to facilitate recovery of spilled food. For a period of 7 days before the study began, mice were monitored daily for food intake, food spillage, and body weight. For an additional period of 7 days, all mice were given intraperitoneal injections of vehicle 2 h before the start of the dark cycle to acclimate the animals to handling and injection. After the 7th day of saline injections and for 8 days thereafter, UCP1-deficient and WT mice received either leptin (20 μg·g body wt−1·day−1) or vehicle (100 μl). All injections were given intraperitoneally and performed 2 h before the start of the dark cycle. Mice treated with leptin were provided with a preweighed amount of food known to be in excess of daily consumption. At the time of leptin injection the following day, spilled and remaining food was recovered and weighed, body weights were recorded, and preweighed food was again provided. Accounting for the amount of spilled food allowed vehicle-treated mice to be pair fed the actual amount of food consumed by the mouse receiving leptin with which it was paired. PF mice continued to receive a daily injection of saline at the time body weights and food intake were recorded. On the morning after the eighth leptin injection, UCP1-deficient and WT mice were killed. Interscapular BAT, as well as epididymal, retroperitoneal, and inguinal white adipose tissue (WAT) depots, were carefully dissected free of surrounding tissue, weighed, and processed for isolation of total RNA or preparation of mitochondria.

RNAse protection assay. RNA probes complementary to UCP1, UCP2, and UCP3 mRNA were prepared, labeled, and used as previously described to quantitate the respective mRNA species (4, 6, 32).

Mitochondrial preparation and Western blotting of UCP1. Isolation and extraction of mitochondria, followed by Western blotting of UCP1 in mitochondrial extracts, were performed on contralateral brown fat pads, as we have described in detail previously (6).

Methods of analysis. The concentrations of UCP1, UCP2, UCP3, and leptin mRNA in each sample were determined by reverse calibration from standard curves as previously described (6). One-way ANOVA was used to compare the means of each response variable. The level of protection against type I errors was set at 5%. P values for specific treatment comparisons of interest are presented in RESULTS.

RESULTS

Effect of leptin on food intake, body weight, and WAT depot weights. Exogenous leptin caused a significant (P < 0.01) decrease in food intake from 4.04 ± 0.14 to 3.19 ± 0.11 g food·day−1·g body wt−1 in WT mice and a decrease from 4.00 ± 0.09 to 3.39 ± 0.09 g food·day−1·g body wt−1 in UCP1-deficient mice. Total food consumption in the respective PF groups [WT, 24.2 ± 2.3 g; UCP1-deficient “knockout” (KO), 24.2 ± 2.7 g] was essentially identical to the leptin-injected groups (WT, 25.5 ± 1.9 g; UCP1-deficient KO, 27.2 ± 2.1 g), as intended. The initial body weights of WT (28.2 ± 3.6 g) and UCP1-deficient mice (27.7 ± 3.1 g) were similar, and comparable reductions in food consumption among the groups produced corresponding weight reductions in WT (vehicle-PF, 25.3 ± 3.4 g; leptin, 26.6 ± 2.7 g) and UCP1-deficient mice (vehicle-PF, 23.6 ± 2.4 g; leptin, 26.8 ± 3.4 g). These data provided no evidence that leptin induced weight loss in excess of that observed in the PF mice. Fat pad weights from age- and weight-matched mice of each genotype killed before the study were similar (data not shown). To assess the importance of UCP1 to leptin’s effects on fat pad size independent of leptin’s effects on food intake, the change in weight of the three WAT depots produced by leptin was expressed relative to that of the PF controls for each genotype. Figure 1 illustrates that leptin induced a significant decrease (P < 0.01) in size of all three depots examined in WT mice. In contrast, Fig. 1 shows that the same fat pads were unaffected by leptin in the UCP1-deficient mice. The lack of a change in white fat pad weights from UCP1-deficient mice indicates that the effectiveness of leptin in reducing adiposity is compromised in the absence of UCP1.

UCP1 expression in BAT and WAT. UCP1 expression was examined in BAT mitochondrial extracts from each group to confirm that leptin increased UCP1 protein levels in WT mice and to establish that UCP1 was not expressed in mice identified as UCP1 knockouts. Results presented in Fig. 2 demonstrate that UCP1 is absent in mitochondrial extracts from mice identified as UCP1 deficient. Figure 2 also shows that leptin induced a significant threefold increase in UCP1 expression in BAT from WT mice (P < 0.01). Measurements of UCP1 mRNA in the contralateral brown fat pads from each animal revealed that leptin increased UCP1 mRNA by an amount (4-fold) that was comparable to the increase in protein expression noted above (data not shown). Several reports have demonstrated that UCP1 expression can be induced in WAT from certain mouse strains under conditions that mimic chronic sympathetic stimulation (3, 6, 10, 23). Because leptin reduced the mass of all WAT depots examined, we attempted to measure UCP1 mRNA in each site to test whether leptin induced UCP1 expression as a mechanism to increase fatty acid oxidation within these sites. Using both in situ hybridization, to detect discrete groups of
cells within depots that might be expressing UCP1, and a sensitive RNase protection assay to screen total RNA preparations, we concluded that UCP1 mRNA levels were below the detection limits of both assays (data not shown). We also found no evidence that leptin increased UCP1 mRNA to detectable levels in these sites (data not shown).

Effect of leptin on UCP2 and UCP3 mRNA in BAT. The initial description of UCP1-deficient mice (8) indicated that UCP2 mRNA was upregulated in BAT. We wanted to quantitate the reported difference in UCP2 mRNA between the genotypes and test for differences in the responses of UCP2 and UCP3 to leptin between the groups. Figure 3A shows that UCP2 mRNA was significantly higher \((P < 0.01)\) in PF UCP1-deficient mice \((0.125 \pm 0.011 \text{ fmol/\mu g RNA})\) than in their PF WT controls \((0.057 \pm 0.002 \text{ fmol/\mu g RNA})\). Leptin had no effect on UCP2 mRNA in WT mice \((0.051 \pm 0.006 \text{ fmol/\mu g RNA})\) but produced a significant \((P < 0.01)\) twofold increase in UCP2 mRNA to \(0.246 \pm 0.042 \text{ fmol/\mu g RNA}\) in UCP1-deficient mice (Fig. 3A). A similar pattern was seen with UCP3 in BAT, with the notable exception that UCP3 mRNA levels were similar between PF WT \((0.109 \pm 0.017 \text{ fmol mRNA/\mu g RNA})\) and PF UCP1-deficient mice \((0.135 \pm 0.013 \text{ fmol mRNA/\mu g RNA})\). As with UCP2, leptin failed to increase UCP3 in WT mice but produced a twofold increase in UCP3 mRNA in BAT from UCP1-deficient mice \((0.217 \pm 0.032 \text{ fmol/\mu g RNA}, P < 0.01)\). These results establish that UCP2 and UCP3 mRNA levels are significantly higher in BAT from UCP1-deficient mice after leptin treatment, and they suggest that the altered regulation of UCP2 and UCP3 may be a mechanism to compensate for the absence of UCP1.

Effect of leptin on UCP2 and UCP3 mRNA in epididymal WAT. The original work with UCP1-deficient mice failed to detect an upregulation of UCP2 mRNA in WAT (8). However, on the basis of the specific effects of leptin on WAT, we tested the idea that differences in the upregulation of UCP2 by leptin might explain leptin’s differential effects on adiposity between the groups. As predicted, UCP2 mRNA levels in epididymal WAT (EWAT) did not differ between PF mice of each genotype \((WT, 0.033 \pm 0.005 \text{ fmol/\mu g RNA}; UCP1-deficient, 0.036 \pm 0.004 \text{ fmol/\mu g RNA}, \text{Fig. 3B})\). The responses to leptin were similar in that leptin produced a significant \((P < 0.01)\) increase in UCP2 mRNA in both WT \((0.062 \pm 0.007 \text{ fmol/\mu g RNA})\) and UCP1-deficient mice \((0.051 \pm 0.005 \text{ fmol/\mu g RNA})\). The levels of UCP3 mRNA in EWAT were similar between control animals in each genotype and were unaffected by leptin.
Thus differences in the induction of UCP2 or UCP3 by leptin are not likely to be responsible for the differential effects of leptin on adiposity between the genotypes.

Skeletal muscle is also a significant site for UCP3 expression, so we compared UCP3 mRNA levels in skeletal muscle of control and leptin-treated animals of each genotype. As in other tissues, the aim was to test whether compensatory upregulation of UCP3 could account for differences in adiposity among the treatment groups. Figure 4 indicates that UCP3 mRNA levels did not differ between WT and UCP1-deficient mice. In addition, leptin treatment had no discernible effect on UCP3 mRNA in either group (Fig. 4). Taken together, these results indicate that group differences in basal or stimulated levels of UCP2 and/or UCP3 mRNA cannot account for the differential reductions in WAT size produced by leptin between the genotypes. And although the absence of UCP1 had no apparent effect on WAT size in control animals, its absence blocked the specific reduction in WAT mass that was produced by leptin in WT mice.

DISCUSSION

The sole known function of uncoupling proteins is to transform energy contained in the mitochondrial proton electrochemical gradient into heat (24). The metabolic consequence of this thermogenic process is decreased coupling efficiency between fatty acid oxidation and ATP formation. Given that the leak of protons across the inner mitochondrial membrane has been estimated to account for between 15 and 35% of basal metabolic rate (1, 25), modulation of UCP expression and/or function has the potential to have major effects on energy balance and fat deposition. Various approaches have been used to test this concept, including a recent study showing that overexpression of UCP1 from the aP2 gene promoter reduced genetically determined obesity in mice and conferred resistance to diet-induced obesity (18, 19). In another approach, targeted disruption of the RIIβ-subunit of protein kinase A (PKA) led to upregulation of the more readily activated RIIα-isoform in adipose tissue (7). The involvement of UCP1 in leptin reduction of white fat by 10.220.33.5 on June 25, 2017 http://ajpendo.physiology.org/ Downloaded from
creased sensitivity of the R1α isoform to cAMP induced
ectopic UCP1 expression in WAT, decreased WAT
mass, and prevented diet-induced obesity (7). Over-
expression of the β1-adrenoceptor in adipose tissue pro-
duced a similar upregulation of UCP1 expression and
resistance to obesity (28). The findings from these
studies show that direct overexpression of UCP1 or
elevation of adrenergic stimulation of adipose tis-
sue produces a common lean, obesity-resistant pheno-
type. Based on the well established observation that
leptin induces UCP1 expression, the major goal of the
present study was to determine whether UCP1 is an
essential component of the mechanism used by leptin
to reduce WAT stores. Findings from the present study
confirm the requirement for UCP1 and provide no
evidence to support the involvement of UCP2 and/or
UCP3 in the response.

Peripheral or central administration of leptin regu-
lates gene expression in adipose tissue through modu-
lation of sympathetic tone and activation of β-adreno-
ceptors (5, 6, 32). The respiratory quotient is also
decreased by leptin (16, 17), and the associated in-
creases in fat oxidation, oxygen consumption, and re-
lease of free fatty acids indicate that these responses
are coordinated elements of the mechanism used to
decrease adipose tissue stores. Although not detected
in the present study, previous work has shown that
UCP1 expression can be induced in specific WAT depot
sites by leptin or other physiological cues that activate
the sympathetic nervous system (6, 12). The heritabil-
ity of this characteristic was documented by study of
recombinant inbred strains of mice produced from
backcrossing two strains with low and high expression
of the trait (12). The UCP1-deficient mice used here
were developed on the B/6 background, which has low
potential for expression of UCP1 in WAT (8, 12). The
already low expression of UCP1 in this mouse strain
was likely compounded by the slightly older mice and
higher housing temperature used in the present study
(27°C). On the basis of results from mice reared at
22–23°C, we suggested previously that increased ex-
pression of UCP1 in WAT from responsive strains
might allow significant in situ fat oxidation to occur in
response to leptin (6). However, the absence of leptin-
induced UCP1 expression in WAT from control mice
here, coupled with the uniform decreases in size among
the WAT depots, supports a different mechanism. It
seems more likely that the leptin-mediated increase in
fat oxidation documented by Hwa and colleagues (16,
17) could be occurring in BAT or muscle after mobiliz-
ation and transfer of fatty acids from WAT. However,
despite the potential significance of skeletal muscle as
a site for lipid oxidation, the present results imply that
BAT is the primary metabolic sink for mobilized lipid.

The concept that thermogenesis requires the par-
ticipation of both BAT and WAT is supported by Grujic et
al. (11), who showed that transgenic reexpression of
β3-adrenoceptors in both BAT and WAT was required
to restore a full thermogenic response in β3-KO mice.
Taken together, these findings indicate that WAT is
targeted by leptin by virtue of its role as a fuel source
for the thermogenic process.

UCP1-deficient mice are cold intolerant but do not
develop obesity when provided either standard mouse
chow or a highly palatable diet (8). This finding and a
similar one in dopamine β-hydroxylase-deficient mice
(31) suggest that the absence of UCP1 can be compen-
sated for by alternative thermogenic components.
UCP2 and UCP3 are likely candidates given their
broad tissue distribution, and it is interesting to note
that UCP2 mRNA was upregulated in BAT from both
studies (8, 31). We also found increased UCP2 mRNA
in BAT from UCP1-deficient mice, but our comparisons
of UCP3 mRNA in BAT and skeletal muscle found no
difference between control and transgenic mice. Resis-
tance to diet-induced obesity has been associated with
upregulation of UCP2 in WAT (30, 32), but it remains
to be established whether UCP2 is responsible for the
leanness of UCP1-deficient mice.

In the present experiments, the important questions
are whether leptin differentially affects UCP2 and/or
UCP3 expression between control and transgenic ani-
mals and whether UCP2 and/or UCP3 can actually
serve as uncouplers. In WAT, we found similar initial
levels and a similar twofold induction of UCP2 mRNA
in both groups. The responses in BAT were different in
the sense that UCP1-deficient mice increased both
UCP2 and UCP3 mRNA in response to leptin, whereas
leptin did not affect either variable in the control mice.
Therefore, if UCP2 and UCP3 were contributing to
increased fat oxidation, leptin should be effective in
reducing fat pad weights in the UCP1-deficient mice.
Our results indicate that group differences in neither
basal nor stimulated levels of UCP2 and/or UCP3
mRNA can account for the differential reductions in
WAT size produced by leptin between the genotypes.
An additional perspective on this issue is provided by
the recent work of Matthias et al. (21) who showed that
UCP1, but not UCP2 and UCP3, was the only uncou-
pling protein able to convey a thermogenic response to
adrenergic stimulation in BAT. On the basis of the
documented involvement of the sympathetic nervous
system in mediating effects of leptin in BAT (4, 14, 27),
the conclusion of Matthias et al. is consistent with our
conclusion that UCP1 is required for leptin action. And
although the absence of UCP1 had no apparent effect
on WAT size in vehicle-treated mice, its absence
blocked the specific reduction in WAT mass that was
produced by leptin in WT mice. Taken together, our
findings support the hypothesis that leptin stimulates
energy utilization and fat oxidation by acutely activat-
ing thermogenesis and enhancing thermogenic capac-
ity through increased UCP1 expression.

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