Central Infusion of Histamine Reduces Fat Accumulation and Upregulates UCP Family in Leptin-Resistant Obese Mice

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Leptin resistance has recently been confirmed not only in animal obese models but in human obesity. Evidence is rapidly emerging that suggests that activation of histamine signaling in the hypothalamus may have substantial anti-obesity and antidiabetic actions, particularly in leptin-resistant states. To address this issue, effects of central, chronic treatment with histamine on food intake, adiposity, and energy expenditure were examined using leptin-resistant obese and diabetic mice. Infusion of histamine (0.05 µmol · g body wt^{-1} · day^{-1}) into the lateral cerebroventricle (i.c.v.) for 7 successive days reduced food intake and body weight significantly in both diet-induced obesity (DIO) and *db/db* mice. Histamine treatment reduced body fat weight, *ob* gene expression, and serum leptin concentration more in the model mice than in pair-fed controls. The suppressive effect on fat deposition was significant in visceral fat but not in subcutaneous fat. Serum concentrations of glucose and/or insulin were reduced, and tests for glucose and insulin tolerance showed improvement of insulin sensitivity in those mice treated with histamine compared with pair-fed controls. On the other hand, gene expression of uncoupling protein (UCP)-1 in brown adipose tissue and UCP-3 expression in white adipose tissue were upregulated more in mice with i.c.v. histamine infusion than in the pair-fed controls. These upregulating effects of histamine were attenuated by targeted disruption of the H1-receptor in DIO and *db/db* mice. Sustained i.c.v. treatment with histamine thus makes it possible to partially restore the distorted energy intake and expenditure in leptin-resistant mice. Together, i.c.v. treatment with histamine contributes to improvement of energy balance even in leptin-resistant DIO and *db/db* mice. *Diabetes* 50:376–384, 2001

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BAT, brown adipose tissue; Epi, epididymal; FFA, free fatty acid; H_1-R, H_1-receptor; i.c.v., intracerebroventricularly; i.p., intraperitoneally; Mes, mesenteric; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; Ret, retroperitoneal; UCP, uncoupling protein; WAT, white adipose tissue.

Obesity, a common metabolic disorder characterized by chronic imbalance in energy intake and energy expenditure, is a serious risk factor for type 2 diabetes, coronary artery disease, hypertension, hyperlipidemia, and other common diseases (1). The pathophysiological basis of obesity, however, is poorly understood. Since discovery of the *ob* gene and its encoded protein leptin (2), it has been understood that leptin acts as a hormone at the level of the hypothalamus to inhibit food intake and favor energy expenditure (3–5). The uncoupling protein (UCP) family, consisting of inner mitochondrial proteins (6–9), is known to contribute to improvement of energy imbalance resulting from energy insufficiency or excess (7,10). Gene expression of the UCP family is highly responsive to neural and humoral factors (10–14), particularly leptin (14). Serum leptin thus reflects energy stores in adipose tissue and serves to signal the brain (15). To improve understanding of the leptin signaling pathway, a number of approaches have been tried to clarify the roles of leptin-modulated hypothalamic neuropeptides in the regulation of feeding behavior and energy homeostasis (16–18).

Leptin negatively regulates orexigenic neuropeptide Y (16) and agouti gene–related protein (17) and positively regulates anorexigenic proopio-melanocortin–derived peptide through leptin receptors on neurons in the hypothalamic arcuate nucleus (18). Anorexigenic corticotropin-releasing hormone in the paraventricular nucleus, which negatively affects neuropeptide Y neurons in the arcuate nucleus (19), is also positively regulated by leptin (20). The signals from such sites in the mediobasal hypothalamus thus communicate the neural underpinning of hunger and satiety with the orexigenic mediators orexin (21) and melanocortin concentrating hormone (22), both of which originate in the lateral hypothalamus.

Serum concentration of leptin is known to be increased in the great majority of obese humans as well as in most rodent models, indicating that most obesity is leptin resistant (23,24). Although the details and molecular basis of the mechanisms are unknown, important factors are indicated by the following findings. First, hyperleptinemia commonly develops along with the progress of obesity (25,26). Second, the high concentration of serum leptin in obesity is not paralleled by a proportional rise in cerebrospinal fluid leptin (23,24). Third, exogenous application of leptin is relatively ineffective for weight reduction of obese subjects (27). *Ob/ob* mice that either lack the ability to produce leptin or produce a truncated inactive form are highly sensitive to leptin, and treatment

with leptin markedly decreases food intake and increases energy expenditure (3–5). In contrast, *db/db* mice, an obese model with a hypothalamic leptin long-form receptor mutation (28), are severely leptin resistant (3–5). The leptin receptor mutation, although present in humans (29), occurs rarely. Diet-induced obesity (DIO) mice, in which obesity is acquired by the environmental factor of excessive energy intake, are mildly leptin resistant (30). In these contexts, such mice are useful models for analysis of human obesity.

In parallel with neuropeptides regulated by leptin, it has been found that histamine neurons are involved in leptin-induced feeding suppression as a target in the hypothalamus (31). Histamine neurons originating from the tuberomammillary nucleus of the posterior hypothalamus project diffusely to almost all the brain areas that contribute to maintenance of energy homeostasis (32). Histaminergic neurons have been particularly implicated in the neural regulation of appetite through the postsynaptic histamine H_1-receptor (H_1-R) (33,34). Indeed, histamine neuron activation suppresses food consumption in rats (34). Thermoregulation, a major factor involved in energy homeostasis, is mediated in part by brain histamine neurons (35). Energy deficiency in the brain, i.e., neural glucoprivation, activates histamine neurons in the hypothalamus (36) and augments glycogenolysis in the brain (37). Histamine neurons also accelerate lipolysis in adipose tissues to supply energy to the brain through activation of the sympathetic nervous system (38). These findings regarding functional roles of histamine neurons show that such systems are related to nutritional status and energy storage across a broad range, from starvation to hyperglycemia (32). Evidence is thus rapidly emerging to suggest that hypothalamic histamine neurons may play essential roles in the regulation of feeding, fat accumulation, energy expenditure, and metabolism.

The aim of the present study was to examine central effects of chronic histamine infusion on regulation of food intake, fat accumulation, energy expenditure, and metabolism in leptin-resistant mice. To address this issue more precisely, targeted disruption of the H_1-R was introduced in leptin-resistant mice.

RESEARCH DESIGN AND METHODS

Subjects. Mature male C57Bl/6J (C57Bl6), C57Bl/KsJ-*misty/misty* (C57Ksj), C57Bl/KsJ-*db/db (db/db)* obese (Seac Yoshitomi, Fukuoka, Japan) and histamine H_1-R knockout (H1KO) mice were used at 12–14 weeks of age. They were housed in a room illuminated daily from 0700 to 1900 (a 12:12 h light-dark cycle) at a temperature of 21 ± 1°C and humidity at 55 ± 5%. The mice were allowed free access to standard mouse powder diet (CLEA Japan, Tokyo) and tap water. In each experiment, mice were housed individually in an accustomed cage at least 2 weeks before the start of each measurement. The animals used were treated in accordance with the Oita Medical University Guidelines for the Care and Use of Laboratory Animals.

Production and supply of H1KO mice. Male and female H1KO mice were produced at the Medical Institute of Bioregulation (Kyushu University, Fukuoka, Japan). The methods used to produce these mice are reported in detail elsewhere (39). Backcrossing H_1-R –/– mice to the C57Bl6 strain for five generations resulted in incipient congenic N4 mice of three genotypes (H_1-R +/+, H_1-R +/–, and H_1-R –/–) used here. All genotypes were confirmed by Southern blotting.

Preparation of mice with diet-induced obesity. For preparation of DIO-C57Bl6 and DIO-H1KO mice, the mice were fed a high-energy diet. Matched on the basis of body weight at 8 weeks of age, H1KO and C57Bl6 mice were placed on a high-fat diet (n = 6 for each subgroup). The high-fat diet consisted of 45% fat, 35% carbohydrate, and 20% protein with an energy density of 4.73 kcal/g. The standard diet consisted of 10% fat, 70% carbohydrate, and 20% protein, with an energy density of 3.85 kcal/g. DIO mice were fed a high-energy diet for 6 weeks. DIO H_1-R null (DIO:–/–) and DIO wild (DIO:+/+) mice were used in the experiment.

Cross-breeding of H1KO and *db/db* mice. H_1-R heterozygous gene (+/–) and *db/db* breeder pairs were cross-bred to create *db/db* H1KO models (*db*/+:+/–). H_1-R gene carriers were identified by Southern blotting analysis with genomic DNA followed by allele-specific hybridization to identify presence or absence of the H_1-R mutation. *db/db* H_1-R null (*db/db*: –/–) and *db/db* (*db/db*: +/+) mice were used in the experiment.

Measurement of body composition and food intake. DIO, *db/db*, and their corresponding controls of C57Bl6 and C57Ksj mice (12 in each) were equally divided into histamine-treatment and phosphate-buffered saline (PBS)–treatment groups, respectively. To evaluate parameters regarding adipose tissues, DIO and *db/db* mice (18 in each) were equally divided into histamine, PBS, and pair-fed control groups. DIO and *db/db* mice with and without H1KO (12 in each) were equally divided into the histamine and PBS control groups. These groupings at the start of experiment were made to avoid any difference in body weight between the groups. After the mice were killed, total fat pads were surgically removed and separated into brown adipose tissue (BAT), subcutaneous white adipose tissue (WAT), and visceral WAT, including mesenteric (Mes), retroperitoneal (Ret) and epididymal (Epi) fat. These samples were immediately weighed, and all the tissues were then frozen in liquid nitrogen and stored at –80°C. Epi WAT and BAT were thawed, and RNA was extracted for measurement of gene expression of *ob* and UCP families. To exclude differences in food consumption between the histamine and control groups, the mice used in each histamine infusion study were also pair-fed using standard powdered mouse food (pair-fed groups were restricted to histamine-treated levels). In addition, evaluation of regional fat accumulation was assessed by analytical balance for small animals (Metler.Tolado, Osaka, Japan).

Chronic implantation of a cannula and infusion methods. Before surgery, mice were anesthetized with nembutal (1 mg/kg) intraperitoneally (i.p.). Mice used were placed in a stereotactic device and implanted with a 29-gauge stainless steel cannula into the left lateral cerebroventricle (0.5 mm posterior, 1.0 mm lateral, and 2.0 mm ventral to the bregma). To prevent the cannula from being blocked by blood coagulation, a 30-gauge wire plug remained inserted into each cannula until use. Subjects were allowed 1 week of postoperative recovery, during which they were handled daily to equilibrate their arousal levels. Cannula placement was verified on each brain slice at the end of each experiment by injecting 1.0 µl of 1% Indiana green. The test solution was infused by a 30-gauge stainless steel injector that projected 1.0 mm below the tip of the cannula. Histamine (Sigma, St. Louis, MO) was freshly dissolved in PBS on the infusion day. Histamine was infused intracerebroventricularly (i.c.v.) at a dose of 0.05 µmol/g body wt daily for 7 successive days. Intracerebroventricular infusion volume of histamine and PBS solutions was limited to a total volume of 1.0 µl.

Blood sampling procedures and methods for assay. Blood samples were collected from timed treatment mice at 1000–1030. The samples were separated into serum, immediately frozen at –20°C, and briefly stored until measurement. For serum glucose, insulin, and free fatty acid (FFA) measurement, DIO, *db/db*, and their appropriate controls of C57Bl6 and C57Ksj mice (18 in each) were equally divided into three groups, i.e., histamine-treated, pair-fed, and PBS control. To measure serum leptin, DIO and *db/db* mice (18 in each) were equally divided into histamine, PBS, and pair-fed control groups. Serum glucose, insulin, FFA (Eiken Chemical, Tokyo, Japan), and leptin (sandwich enzyme immunoassay; Immune Biological Laboratory, Gunma, Japan) were measured by commercially available kits. To test glucose tolerance, each mouse was injected i.p. with glucose at a dose of 1.0 mg/g body wt after an 8-h fast. For the insulin tolerance test, each mouse was injected with human regular insulin (Nobolin R; Novo Nordisk, Bagsvaerd, Denmark) at a dose of 0.5 mU/g body wt after a 2-h fast. Procedures for histamine treatment were the same as those in the forgoing methods. Blood sampling for these tolerance tests was carried out succeeding the 7-day treatment with histamine.

Preparation of the probes and Northern blotting analysis. Polymerase chain reaction (PCR) primers of 5′-AGTGCCACTGTTGTCTTCAG-3′ and 5′-TTCTCCAAGTCGCCTATGTG-3′ were designed for the coding region of the mouse *UCP-1* gene, and primers of 5′-GTTACCTTTCCACTGGACAC-3′ and 5′-CCGTTTCAGCTGCTCATAGG-3′ were designed for the *UCP-3* gene. Reverse transcription of 10 µg total RNA from C57Bl6 mice was performed using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD). PCR was carried out with Taq DNA polymerase (Amersham International, Buckinghamshire, England) and 20 pmol of each primer. The reaction profiles were as follows: denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, for 30 cycles. The PCR fragment was subcloned into pCRTM2.1 vector (TA cloning kit; Invitrogen, San Diego, CA), and the nucleotide sequence of amplified cDNA was confirmed by sequencing. The nucleotide sequences were determined by the dideoxynu-

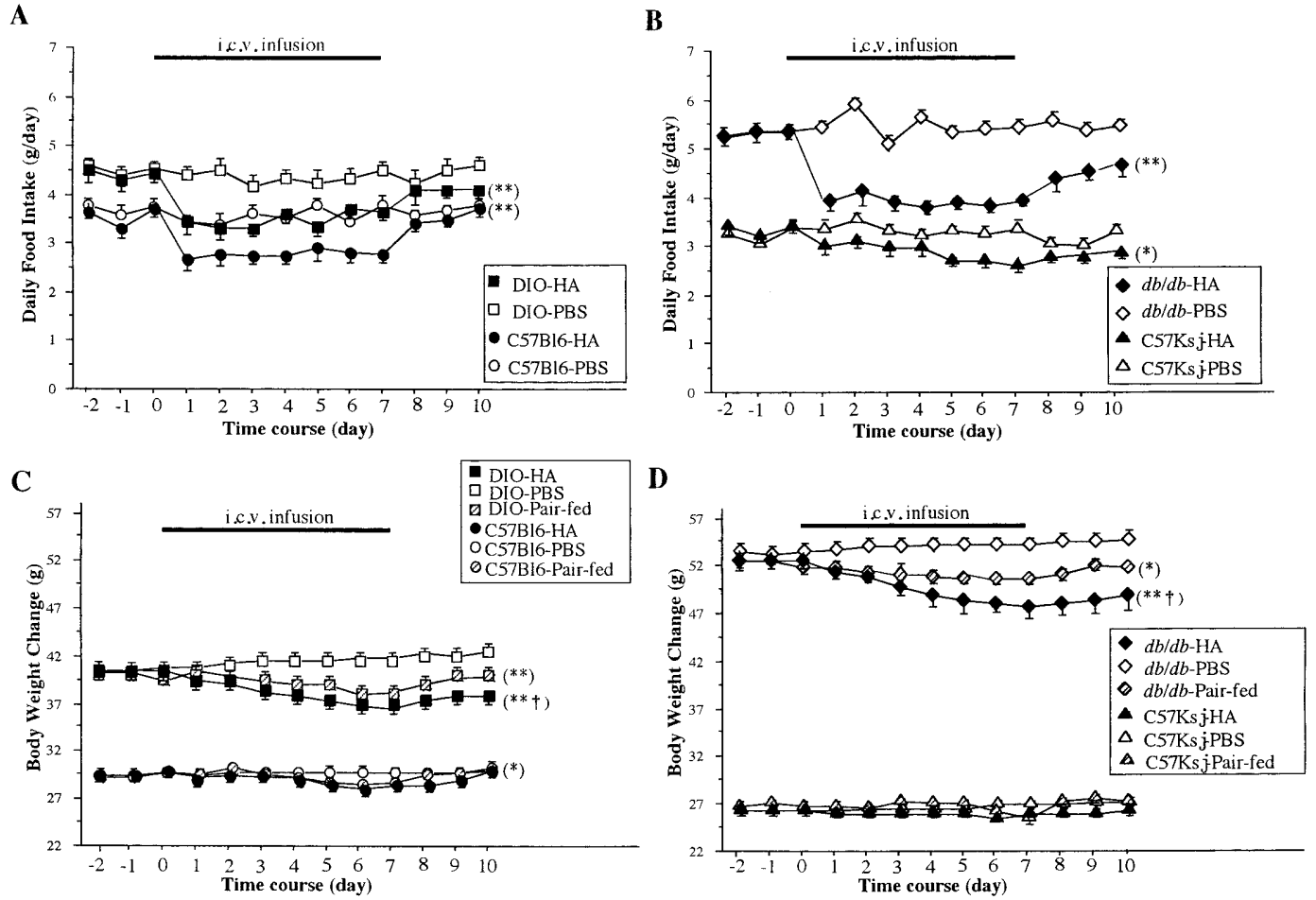

FIG. 1. Central effects of chronic histamine (HA) infusion on food intake (*A*, *B*) and body weight (*C*, *D*) in DIO (*A*, *C*) and *db/db* (*B*, *D*) obese mice. Each pair-fed group in *C* and *D* was pair-fed with the corresponding HA-treated mice. HA in this and succeeding figures was infused intracerebroventricularly (i.c.v.) at a dose of 0.05 µmol/g body wt daily for 7 successive days. Values and vertical bars are means ± SE (*n* = 6 for each). Each horizontal bar shows the infusion period of test solution i.c.v. C57Bl/6J (C57Bl6)-HA, C57Bl6 control mice with HA; C57Bl6-PBS, C57Bl6 control mice with PBS; C57Bl/KsJ (C57Ksj)-HA, C57Ksj control mice with HA; C57Ksj-PBS, C57Ksj control mice with PBS; *db/db*-HA, *db/db* mice with HA; *db/db*-PBS, *db/db* control mice with PBS; DIO-HA, DIO mice treated with HA; DIO-PBS, DIO control mice with PBS. Statistical significance marked in parentheses: **P* < 0.05, ***P* < 0.01 vs. the corresponding PBS controls; †*P* < 0.01 vs. the corresponding pair-fed PBS controls.

cleotide chain termination method, using synthetic oligonucleotide primers, which were complementary to the vector sequence and ABI373A, automated DNA Sequencing System (Perkin-Elmer, Norwalk, CT). All DNA sequences were confirmed by reading both DNA strands. The *ob* probe was generated in an analogous fashion (Genbank accession No. U18812). Total cellular RNA was prepared from various rat tissues with the use of Isogen (Nippon gene, Toyama, Japan) according to the manufacturer's protocol. Total RNA (20 µg) was electrophoresed on 1.2% formaldehyde-agarose gel. The separated RNA was transferred onto a Biodyne B membrane (Pall Canada, Toronto, ON, Canada) in 20× sodium chloride–sodium citrate by capillary blotting and immobilized by exposure to ultraviolet light (0.80 J). Prehybridization and hybridization were carried out according to the manufacturer's protocol. Membranes were washed under high-stringency conditions. After washing the membranes, the hybridization signals were analyzed with the BIO-image analyzer BAS 2000 (Fuji Film Institution, Tokyo). The membranes were stripped by exposure to boiling 0.1% SDS, and ethidium bromide staining was used to quantify the amounts of RNA species on the blots.

Evaluation of data and statistical analysis. All the data were expressed as means ± SE. Values of parameters excluding food intake, body weight, and humoral factors were expressed as percentage of the values in normally fed controls with PBS. Unpaired *t* test or two-way analysis of variance with repeated measures assessed the statistical analysis of difference between mean values.

RESULTS

Effects of histamine treatment on food intake and body weight. Figure 1*A* and *C* show time-course changes in food intake and body weight of DIO and C57Bl6 control mice after i.c.v. infusion of histamine (0.05 µmol · g body wt^{-1} · day^{-1}) for 7 successive days. Histamine infusion into DIO mice for 7 days induced 25.5 and 11.2% decreases in cumulative food intake and body weight, respectively [$F(1,11) = 12.10$, $P < 0.01$; $F(1,11) = 16.94$, $P < 0.01$]. In the mice fed a normal diet, the suppressive effect of histamine was 18.8 and 6.8% decreases of food intake and body weight, respectively, compared with vehicle-treated C57Bl6 controls [$F(1,11) = 7.84$, $P < 0.01$; $F(1,11) = 2.19$, $P < 0.05$] (Fig. 1*A* and *C*). Histamine infusion with the same dose and same period as described in DIO mice caused 31.7 and 13.3% decreases of cumulative food intake and body weight, respectively, in *db/db* mice [$F(1,11) = 36.03$, $P < 0.01$; $F(1,11) = 29.81$, $P < 0.01$]. The decrease in cumulative food intake for C57Ksj was 16.8% after histamine treatment [$F(1,11) = 2.04$, $P < 0.05$] (Fig. 1*B* and *D*). The body

TABLE 1
Central effects of chronic histamine infusion on serum glucose, insulin, and FFA in DIO and *db/db* mice

Mice and treatment	Glucose (mg/dl)	Insulin (μU/ml)	FFA (mmol/l)
C57Bl/6J mice			
Histamine	112.8 ± 13.1	54.2 ± 5.1*	0.4 ± 0.2
PBS pair-fed	110.6 ± 12.5	58.2 ± 6.6*	0.5 ± 0.1
PBS	125.3 ± 19.7	66.1 ± 7.9	0.6 ± 0.2
DIO mice			
Histamine	152.8 ± 8.1*	56.4 ± 5.6†‡	1.0 ± 0.2*
PBS pair-fed	158.6 ± 7.5*	76.6 ± 7.1*	1.1 ± 0.1*
PBS	198.6 ± 11.7	94.1 ± 7.7	1.4 ± 0.2
C57Bl/KsJ mice			
Histamine	103.8 ± 9.1	50.4 ± 5.3	0.4 ± 0.3
PBS pair-fed	108.6 ± 9.3	51.6 ± 3.4	0.5 ± 0.1
PBS	112.4 ± 8.5	54.1 ± 4.7	0.6 ± 0.2
db/db mice			
Histamine	303.6 ± 15.6†‡	82.7 ± 6.1*	2.1 ± 0.4*
PBS pair-fed	356.6 ± 26.2†	88.5 ± 8.3*	2.4 ± 0.2*
PBS	408.6 ± 20.1	92.1 ± 9.0	2.8 ± 0.3

Data are means ± SE. Histamine treatment was infused into the lateral cerebroventricle at a dose of 0.05 μmol/g body wt daily for 7 successive days. *$P < 0.05$ vs. PBS; †$P < 0.01$ vs. PBS; ‡$P < 0.05$ vs. PBS pair-fed.

weight decrease in *db/db* and DIO mice was greater than in the pair-fed controls [$F(1,11) = 2.53$, $P < 0.01$; $F(1,11) = 6.15$, $P < 0.01$] (Fig. 1*C* and *D*).

Effects on serum glucose, insulin, and FFA. Concentrations of serum glucose and FFAs were not changed in C57Bl6 and C57Ksj mice after histamine treatment (0.05 μmol · g body wt^{-1} · day^{-1} for 7 days), but serum insulin in C57Bl6 mice was significantly different ($P < 0.05$ vs. ad libitum vehicle controls) (Table 1). However, the histamine treatment attenuated or abolished hyperglycemia, hyperinsulinemia, and hyper–free fatty acidemia detectable in both DIO and *db/db* mice ($P < 0.05$ and $P < 0.01$ vs. vehicle controls). In addition, these moderating effects of histamine on serum glucose in *db/db* mice and insulin in DIO mice were more potent than those in pair-fed controls ($P < 0.05$ for each) (Table 1).

Effects on visceral adiposity in DIO and *db/db* mice. To examine net effects of histamine treatment (0.05 μmol · g body wt^{-1} · day^{-1} for 7 days) on fat distribution in DIO and *db/db* mice, corresponding pair-fed controls were used. As shown in Fig. 2, both pair-fed DIO and *db/db* mice reduced their visceral fat ($P < 0.05$ and $P < 0.01$ vs. the corresponding ad libitum controls). Although each showed similar food reduction, histamine treatment caused a greater decrease in visceral fat in the obese models than in the controls ($P < 0.05$ for each vs. the corresponding pair-fed controls). Decreases in Mes, Ret, and Epi fat in DIO mice were 21.8, 22.6, and 10.8%,

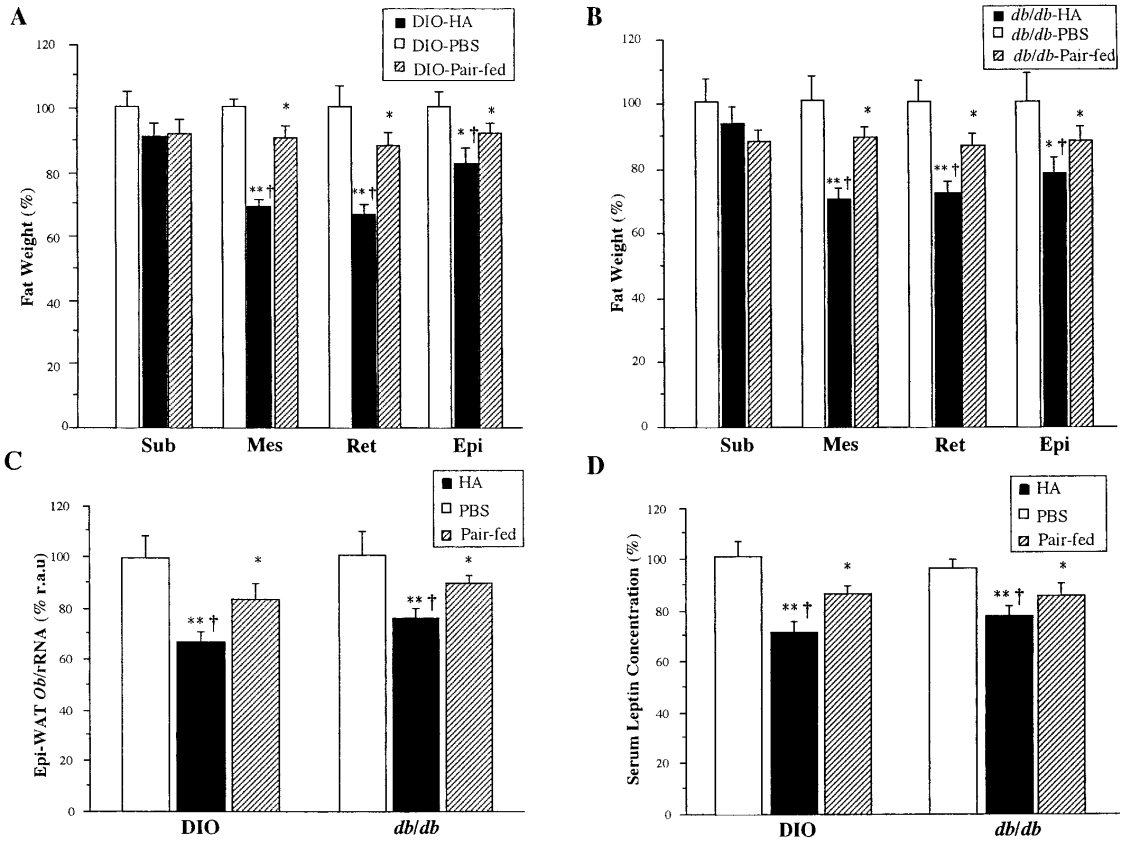

FIG. 2. Central effects of chronic histamine (HA) infusion on fat weight (*A*, *B*), *ob* gene expression (*C*), and serum leptin concentration (*D*) in DIO and *db/db* obese mice. Each pair-fed group was pair-fed with the corresponding HA-treated mice. HA treatment and other procedures were the same as those in Fig. 1, as applicable. Values are means ± SE ($n = 6$ for each). Each value is expressed as percentage of PBS controls. *db/db*-HA, *db/db* mice with HA; *db/db*-PBS, *db/db* mice with PBS; DIO-HA, DIO mice treated with HA; DIO-PBS, DIO mice with PBS; Sub, subcutaneous; r.a.u., relative arbitrary unit.*$P < 0.05$ and **$P < 0.01$ vs. the corresponding PBS controls; †$P < 0.05$ vs. the corresponding pair-fed PBS controls.

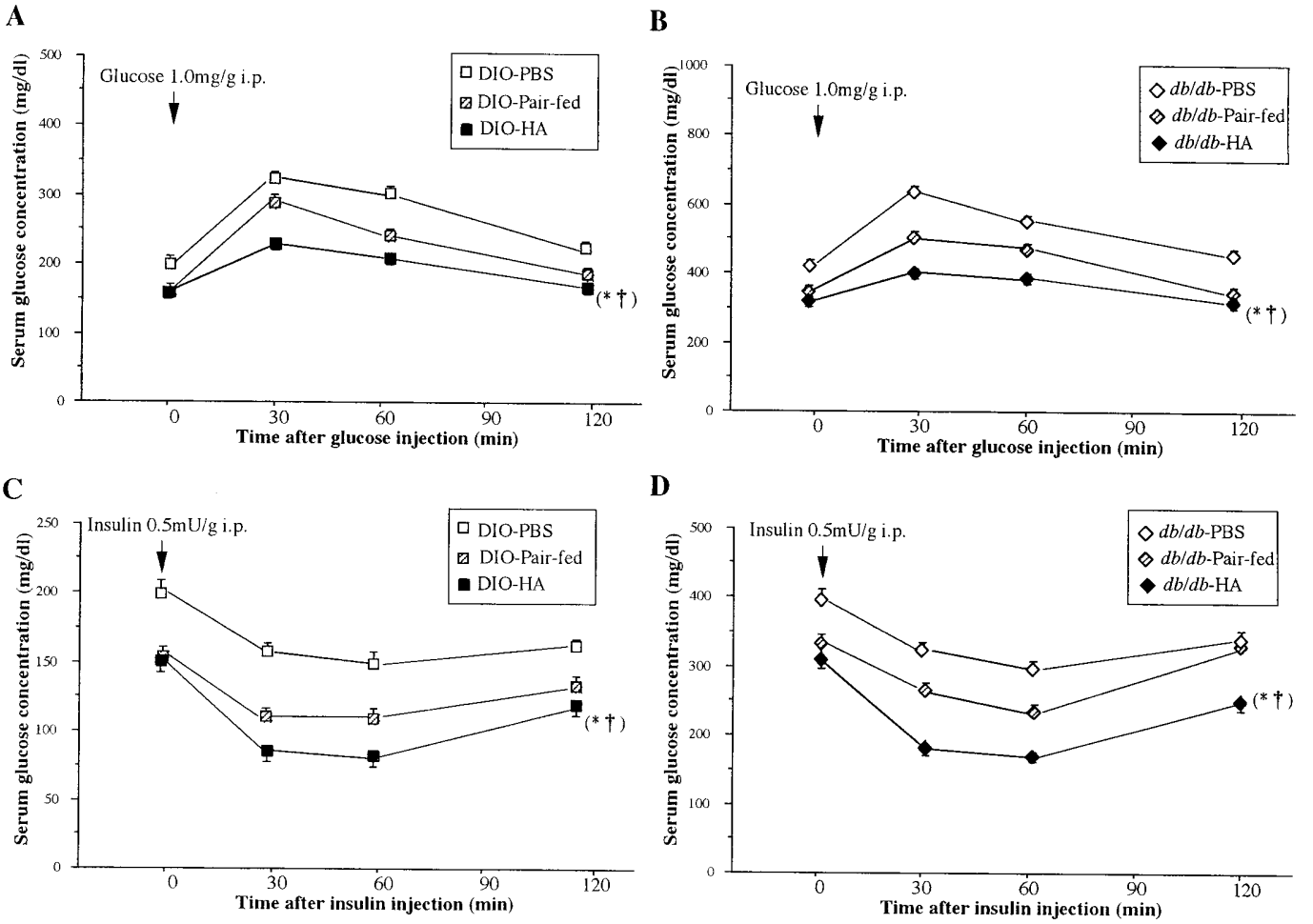

FIG. 3. Effects of histamine (HA) on intraperitoneal glucose tolerance (*A*, *B*) and insulin tolerance (*C*, *D*) in DIO and *db/db* obese mice. Mice in *A* and *B* were injected i.p. with 1.0 mg/g body wt glucose. Mice in *C* and *D* were injected i.p. with 0.5 mU/g body wt insulin. Each pair-fed group was pair-fed with the corresponding HA-treated mice. Values and vertical bars are means ± SE (*n* = 6 for each). *db/db*-HA, *db/db* mice after 7-day HA treatment; *db/db*-PBS, *db/db* mice after 7-day PBS treatment; DIO-HA, DIO mice after 7-day HA treatment; DIO-PBS, DIO mice after 7-day PBS treatment. **P* < 0.05 vs. the corresponding PBS controls; †*P* < 0.05 vs. the corresponding pair-fed control.

respectively ($P < 0.05$ for each vs. the corresponding pair-fed DIO controls) (Fig. 2*A*). Similar inhibitory effects of histamine were manifest in *db/db* mice (22.6%, 16.2%, and 11.1% decrease in Mes, Ret, and Epi fat, respectively; $P < 0.05$ for each vs. the corresponding pair-fed *db/db* controls) (Fig. 2*B*). Of note, subcutaneous fat in either DIO or *db/db* mice was not affected by histamine treatment (Fig. 2).

Effects on *ob* gene expression and serum leptin concentration. Histamine treatment i.c.v. (0.05 µmol · g body wt^{-1} · day^{-1} for 7 days) reduced Epi-fat *ob* gene mRNA expression in both DIO and *db/db* mice compared with pair-fed controls or ad libitum controls (Fig. 2*C*), consistent with the results in Fig. 2. The percent decrease in *ob* gene expression in DIO and *db/db* mice was 34.4 and 22.3%, respectively, compared with ad libitum controls ($P < 0.01$ for each), and 18.4 and 11.3%, respectively, compared with pair-fed controls ($P < 0.05$ for each) (Fig. 2*C*). Reflecting the reduced mRNA, serum leptin concentration after histamine infusion was 15.4 and 11.2% less in DIO and *db/db* mice, respectively, than in pair-fed controls ($P < 0.05$ for each), and 30.4 and 21.1% less, respectively, than in ad libitum controls ($P < 0.01$ for each) (Fig. 2*D*).

Glucose and insulin tolerance tests. We examined the effects of histamine on glucose and insulin tolerance after administration of standard intraperitoneal glucose or insulin after 7 days of histamine treatment. Serum glucose concentrations during glucose tolerance tests were lowered in both histamine-treated DIO and *db/db* mice compared with PBS-treated DIO and pair-fed PBS-treated *db/db* mice [DIO: $F(1,3) = 3.94$, $P < 0.05$ and $F(1,3) = 3.76$, $P < 0.05$; *db/db*: $F(1,3) = 4.03$, $P < 0.05$ and $F(1,3) = 3.23$, $P < 0.05$] (Fig. 3). As with glucose loading, the insulin tolerance test showed that hypoglycemic responses were exaggerated more in histamine-treated DIO and *db/db* mice than in PBS-treated DIO and pair-fed PBS-treated *db/db* mice [DIO: $F(1,3) = 3.20$, $P < 0.05$ and $F(1,3) = 4.06$, $P < 0.05$; *db/db*: $F(1,3) = 3.35$, $P < 0.05$ and $F(1,3) = 3.44$, $P < 0.05$] (Fig. 3). There were no significant differences between PBS and pair-fed PBS groups (Fig. 3).

Effects of histamine treatment on food intake and body weight in H1KO mice. Figure 4 shows time-course changes in food intake and body weight of DIO and *db/db* controls and mice combined with H1KO after i.c.v. infusion of histamine (0.05 µmol · g body wt^{-1} · day^{-1}) for 7 days. Histamine infusion into DIO and *db/db* mice induced decreases in cumu-

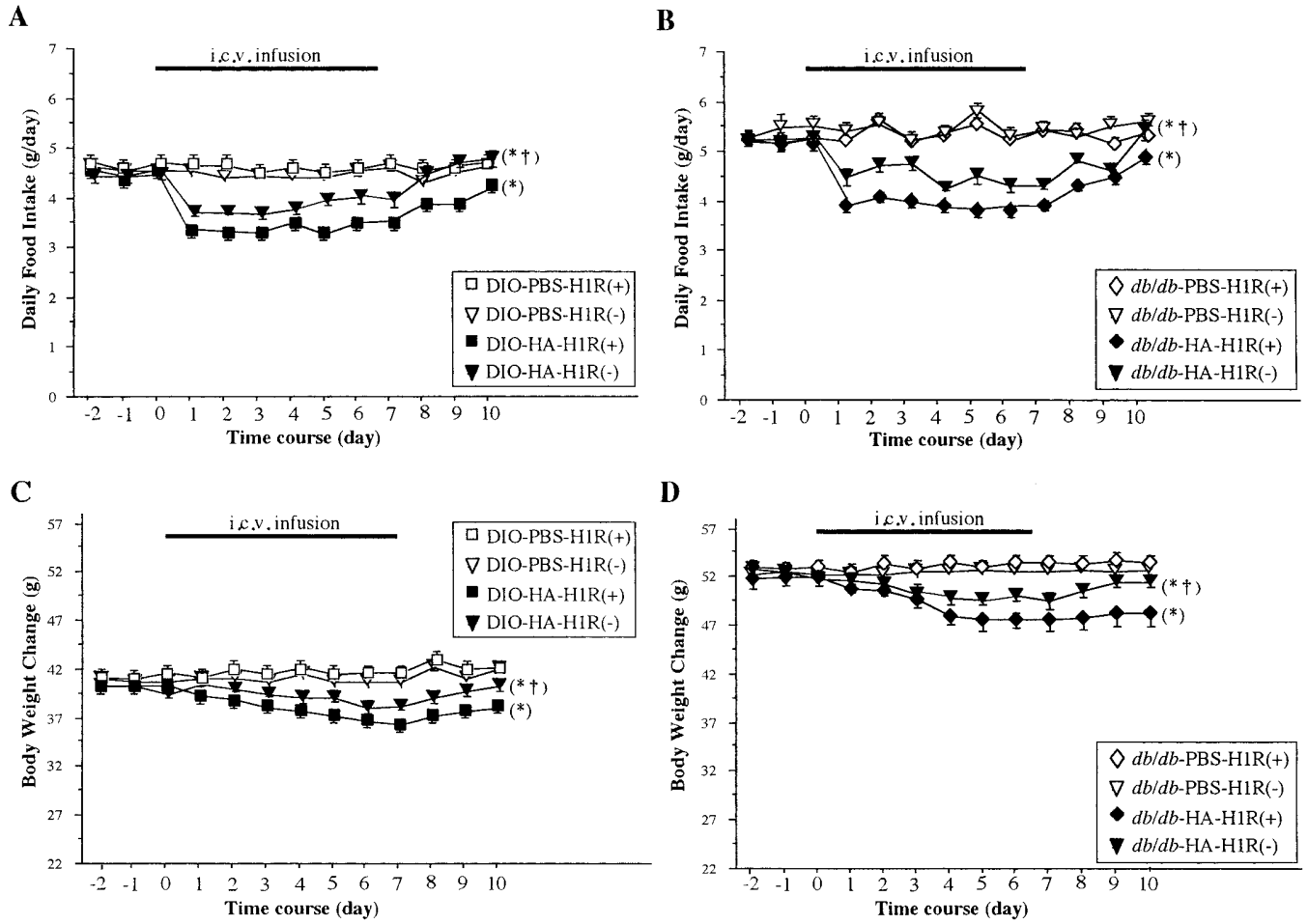

FIG. 4. Central effects of chronic histamine (HA) infusion on food intake (*A*, *B*) and body weight (*C*, *D*) in DIO (*A*, *C*) and *db/db* obese (*B*, *D*) mice with and without H1KO. Values and vertical bars are means ± SE (n = 6 for each). Horizontal bars show the i.c.v. infusion period of test solution. *db/db*-HA-H_1R(−), H1KO-*db/db* mice treated with HA; *db/db*-HA-H_1R(+), wild *db/db* mice with HA; *db/db*-PBS-H_1R(−), H1KO-*db/db* mice with PBS; *db/db*-PBS-H_1R(+), wild *db/db* mice with PBS; DIO-HA-H_1R(−), H1KO-DIO mice with HA; DIO-HA-H_1R(+), wild-type DIO mice with HA; DIO-PBS-H_1R(−), H1KO-DIO mice with PBS; DIO-PBS-H_1R(+), wild DIO mice with PBS. *P < 0.01 vs. the corresponding PBS control; †P < 0.01 vs. the corresponding wild-type control.

lative food intake [F(1,11) = 17.65, P < 0.01; F(1,11) = 40.87, P < 0.01]. In the DIO- and *db/db*-H1KO mice, the suppressive effects of histamine on food intake were decreased compared with histamine-treated wild type (+/+) controls [F(1,11) = 4.51, P < 0.01; F(1,11) = 4.64, P < 0.01] (Fig. 4*A* and *D*). Histamine infusion at the same dose and for the same period as described in DIO and *db/db* mice decreased body weight [F(1,11) = 7.69, P < 0.01; F(1,11) = 12.08, P < 0.01]. In the DIO- and *db/db*-H1KO mice, however, the suppressive effects on body weight were decreased compared with histamine-treated wild type (+/+) controls [F(1,11) = 2.53, P < 0.01; F(1,11) = 4.37, P < 0.01] (Fig. 4*A*, and *D*).

Effects of histamine to regulate adiposity and BAT and WAT UCP gene expression are partially mediated by H_1-R. Intracerebroventricular infusion of histamine (0.05 µmol · g body wt^{-1} · day^{-1} for 7 days) decreased visceral fat in DIO and *db/db* mice compared with pair-fed controls (P < 0.01 for each). In contrast to these mice, induced changes in such adiposity were attenuated in H_1-R knockout DIO and *db/db* mice compared with pair-fed controls (P < 0.01 for each). Intralateralventricular treatment with histamine (0.05 µmol · g body wt^{-1} · day^{-1} for 7 days) remarkably increased BAT UCP-1 mRNA expression by 172.4 and 154.5% in DIO and *db/db* mice, respectively, compared with pair-fed controls. These effects of histamine were attenuated in the DIO and *db/db* mice with targeted disruption of the histamine H_1-R gene (Fig. 5*A*). Central treatment with histamine (0.05 µmol · g body wt^{-1} · day^{-1} for 7 days) remarkably increased WAT UCP-3 expression by 173.2 and 165.9% in DIO and *db/db* mice, respectively, compared with pair-fed controls. However, these effects of histamine were also attenuated in the DIO and *db/db* mice with targeted disruption of the histamine H_1-R gene (Fig. 5*B*).

DISCUSSION

The present study shows that chronically central treatment with histamine contributes to improvement of the abnormality in energy metabolism of DIO and *db/db* mice. DIO mice are known to be an acquired leptin-resistant model, whereas *db/db* mice are an inherited model with a leptin-receptor mutation (28). In the present study, i.c.v. treatment with histamine reduced food intake in DIO mice similar to that observed in *db/db* mice. However, the difference in leptin resistance between DIO and *db/db* mice leaves the possibility

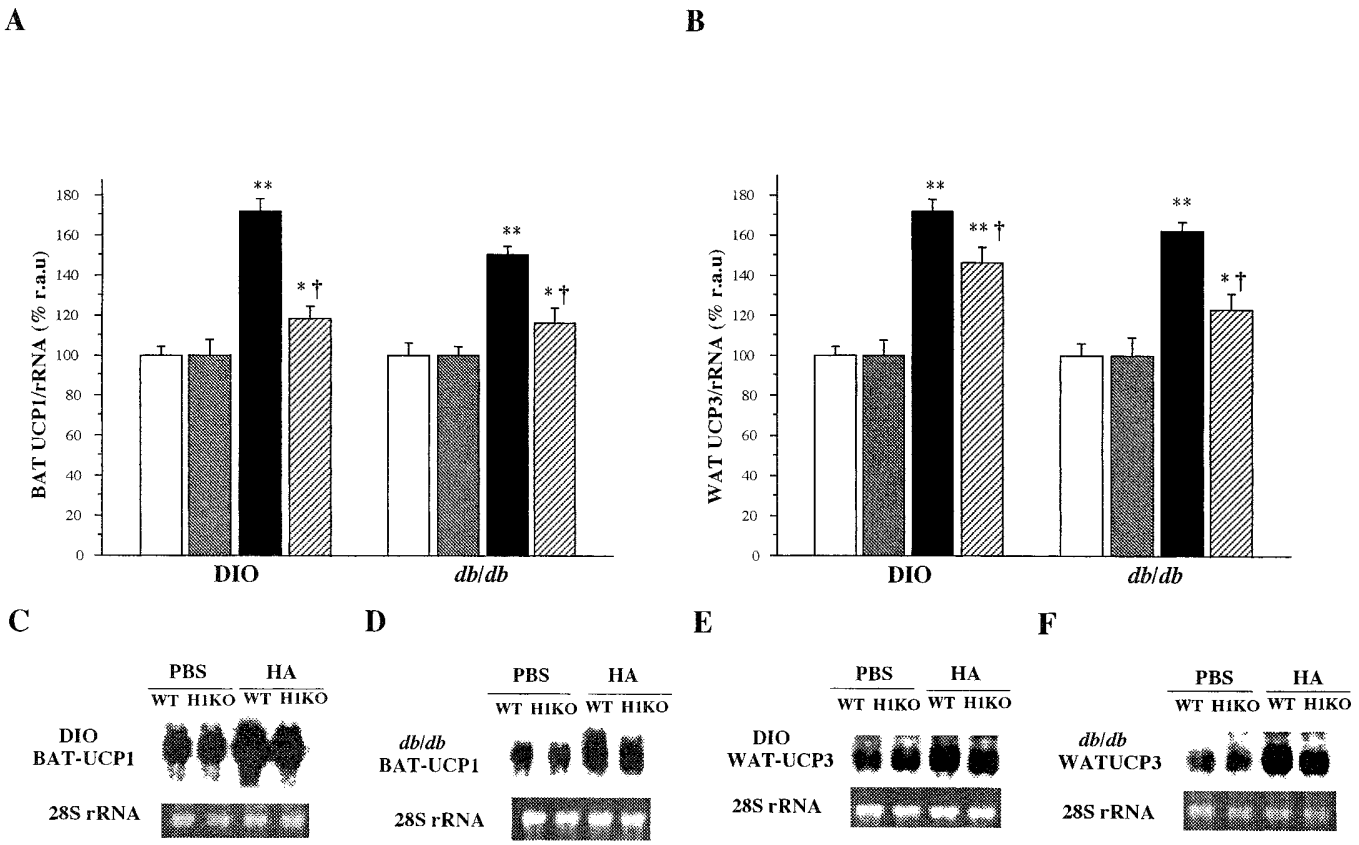

FIG. 5. Chronic central effects of histamine (HA) on gene expression of UCP-1 in BAT (*A*) and UCP-3 in WAT (*B*) in PBS-treated wild-type (WT) (□), PBS-treated H1KO (▩), HA-treated WT (■), and HA-treated H1KO (▨) (n = 6) DIO and *db/db* obese mice. Values are means ± SE (n = 6 for each). Each value is expressed as percentage of PBS controls. *P < 0.05, **P < 0.01 vs. PBS control; †P < 0.05 vs. HA-treated control. *C* and *D:* Representative blots of BAT UCP-1 gene expression in PBS-treated WT mice (PBS-WT), PBS-treated H1KO mice (PBS-H1KO), HA-treated WT mice (HA-WT), and HA-treated H1KO mice (HA-H1KO) in DIO (*C*) and *db/db* (*D*) mice. *E* and *F*: Representative blots of WAT UCP-3 gene expression in PBS-WT, PBS-H1KO, HA-WT, and HA-H1KO in DIO (*E*) and *db/db* (*F*) mice. r.a.u., relative arbitrary unit.

that if leptin were actually infused i.c.v. in DIO mice, it would cause responses similar to those observed after histamine treatment in DIO mice. Histamine-treated DIO and *db/db* mice lost more body weight than pair-fed PBS controls. The results suggest that the weight loss after histamine treatment may be attributable not only to the decrease in food intake but also, at least in part, to the histamine-derived increase in lipolysis. In fact, the present data showed a greater decrease in fat pads in histamine-treated mice than in the pair-fed PBS controls. In particular, the histamine treatment was predominantly effective to reduce visceral fat, leaving subcutaneous fat unaffected. Histamine has been shown to activate peripheral lipolysis through H_1- and/or H_2-Rs (40,41). Previous studies showed that activation of histamine signaling promoted lipolysis through sympathetic nerves (38,41). Selective agonists of the b_3 adrenoceptor were found to accelerate lipolysis of visceral fat more than that of subcutaneous fat (42). Ultimately, the suppressive and selective effect of histamine on visceral fat deposition depends most likely on activation of the sympathetic nervous system. In this context, it is understandable why the present histamine treatment reduced *ob* gene expression in WAT and serum leptin concentration. In other words, the downregulation of *ob* mRNA expression and the resultant decrease in leptin production reflect reduction in body fat content by histamine treatment because WAT *ob* mRNA level and serum concentration of leptin are positively and tightly correlated with body fat mass (25,26).

Elevation of circulating FFAs in obese animals has been regarded as a major determinant of decreased insulin sensitivity because it increases hepatic glucose output and decreases glucose disposal in muscle (43). Treatment of DIO and *db/db* obese mice with histamine lowered serum concentrations of glucose and insulin in the present study. In addition, both intraperitoneal glucose and insulin tolerance tests showed that histamine treatment improved glucose tolerance and insulin sensitivity. The lowered serum FFA concentration produced simply by virtue of histamine-induced reduction of visceral fat may be a major factor that improved insulin sensitivity.

To clarify the possibility of reduction in body weight and adiposity induced by increased energy expenditure, we investigated the effects of histamine on UCP expression in peripheral tissues. Intracerebroventricular infusion of histamine in DIO and *db/db* obese mice upregulated mRNA expression of BAT UCP-1 and WAT UCP-3 in the present study. It is well known that BAT is richly innervated by sympathetic nerves (6). Expression of UCP-1 and UCP-3 in BAT and WAT is known to be modulated, in part, by β_3 agonists, indicating sympathetic influence on UCP expression (44). According to previous studies, hypothalamic neuronal histamine affects peripheral lipid metabolism and autonomic function (38,41). Such obser-

vations suggest that pharmacological potentials of enhanced histamine signaling in the hypothalamus may regulate UCP expression through the sympathetic nervous system.

Further experiments as to whether the H_1-R per se may be involved in energy intake and expenditure were carried out to examine the effects of H1KO on both food intake and expression of BAT and WAT UCPs in DIO and *db/db* mice. The present data from DIO and *db/db* mice revealed that the effects of histamine treatment on food intake and gene expression of BAT UCP-1 and WAT UCP-3 were attenuated in H1KO mice compared with those in the histamine-treated wild controls, although H1KO mice were not restored to the levels of the PBS controls. Intracerebroventricular infusion of histamine thus suppresses energy intake and accelerates energy expenditure through the H_1-R in the hypothalamus. It is intriguing that the partial but not complete abolishment of the effects on food intake and UCP expression in DIO and *db/db* mice is confirmed by targeted disruption of H_1-R. In this regard, histamine receptors other than H_1-R may be involved in the control of feeding and UCP expression.

In conclusion, activation of hypothalamic histamine signaling induced by histamine treatment contributes to maintenance of energy balance even in leptin-resistant DIO and *db/db* mice through reduction of food intake, visceral adiposity, *ob* gene expression, and circulating leptin concentration together with upregulation of BAT and WAT UCPs gene expression. The improvement of adiposity results in recovery of insulin sensitivity in DIO and *db/db* mice. Evidence that regulatory actions of histamine are mediated at least in part through H_1-R has been shown by targeted disruption of H_1-R.

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REFERENCES

1. Kaplan NM: The deadly quartet: upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med* 149:1514–1520, 1989
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
3. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
4. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* 269:540–543, 1995
5. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P: Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546–549, 1995
6. Nicholls DG, Locke RM: Thermogenic mechanism in brown fat. *Physiol Rev* 64:1–64, 1984
7. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D, Warden CH: Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* 15:269–272, 1997
8. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP: Uncoupling protein-3: a new member of the mitochondrial Carrier family with tissue-specific expression. *FEBS Lett* 408:39–42, 1997
9. Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB: UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophys Res Commun* 235:79–82, 1997
10. Hidaka S, Kakuma T, Yoshimatsu H, Sakino H, Fukuchi S, Sakata T: Streptozotoxin treatment upregulates uncoupling protein 3 gene in the rat heart. *Diabetes* 48:430–435, 1999
11. Zhou Y-T, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, Newgard CB, Unger RH: Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc Natl Acad Sci U S A* 94:6386–6390, 1997
12. Masaki T, Yoshimatsu H, Kakuma T, Hidaka S, Kurokawa M, Sakata T: Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone. *FEBS Lett* 418:323–326, 1997
13. Masaki T, Yoshimatsu H, Chiba S, Hidaka S, Tajima D, Kakuma T, Kurokawa M, Sakata T: Tumor necrosis factor-α regulates in vivo expression of the rat UCP family differentially. *Biochim Biophys Acta* 1436:585–592, 1999
14. Cusin I, Zakrzewska KE, Boss O, Muzzin P, Giacobino J-P, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F: Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling protein expression and energy expenditure. *Diabetes* 47:1014–1019, 1998
15. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS: Unraveling the central nervous system pathways underlying responses to leptin. *Nat Neurosci* 1:445–450, 1998
16. Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG: Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* 46:2119–2123, 1997
17. Ebihara K, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Satoh N, Tamaki M, Yoshioka T, Hayase M, Matsuoka N, Aizawa-Abe M, Yoshimasa Y, Nakao K: Involvement of agouti-related protein, an endogenous antagonist of hypothalamic melanocortin receptor, in leptin action. *Diabetes* 48:2028–2033, 1999
18. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr, Woods SC, Seeley RJ, Weigle DS: Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. *Diabetes* 45:531–535, 1996
19. Li C, Chen P, Smith MS: Corticotropin releasing hormone neurons in the paraventricular nucleus are direct targets for neuropeptide Y neurons in the arcuate nucleus: an anterograde tracing study. *Brain Res* 854:122–129, 2000
20. Huang Q, Rivest R, Richard D: Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (*ob/ob*) mice. *Endocrinology* 139:1524–1532, 1998
21. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M: Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:573–585, 1998
22. Tritos NA, Vicent D, Gillette J, Ludwig DS, Flier ES, Maratos-Flier E: Functional interactions between melanin-concentrating hormone, neuropeptide Y, and anorectic neuropeptides in the rat hypothalamus. *Diabetes* 47:1687–1692, 1998
23. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang P, Sinha MK, Considine RV: Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348:159–161, 1996
24. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr: Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2:589–593, 1996
25. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS: Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1:1311–1314, 1995
26. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM: Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med* 1:1155–1161, 1995
27. Gura T: Leptin not impressive in clinical trial. *Science* 286:881–882, 1999
28. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP: Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* 84:491–495, 1996
29. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B: A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392:398–401, 1998
30. Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP,

Sybertz EJ, Strader CD, Davis HR Jr: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest* 99:385–390, 1997
31. Yoshimatsu H, Itateyama E, Kondou S, Hidaka S, Tajima D, Himeno K, Kurokawa M, Sakata T: Hypothalamic neuronal histamine as a target of leptin action on feeding behavior in the central nervous system. *Diabetes* 48:1342–1346, 1999
32. Sakata T, Yoshimatsu H, Kurokawa M: Hypothalamic neuronal histamine: implication of its homeostatic control of energy metabolism. *Nutrition* 13:403–411, 1997
33. Sakata T, Ookuma K, Fukagawa K, Fujimoto K, Yoshimatsu H, Shiraishi H, Wada H: Blockade of the histamine H_1-receptor in the rat ventromedial hypothalamus and feeding elicitation. *Brain Res* 441:403–407, 1988
34. Ookuma K, Yoshimatsu H, Sakata T, Fujimoto K, Kurokawa M: Hypothalamic sites of neuronal histamine action on food intake by rats. *Brain Res* 490:299–301, 1989
35. Kang K, Yoshimatsu H, Ogawa R, Oohara A, Tamari Y, Sakata T: Hypothalamic neuronal histamine modulates physiological responses induced by interleukin-1 beta. *Am J Physiol* 269:R1308–R1313, 1995
36. Oohara A, Yoshimatsu H, Kurokawa M, Oishi R, Saeki K, Sakata T: Neuronal glucoprivation enhances hypothalamic histamine turnover in rats. *J Neurochem* 63:677–682, 1994
37. Sakata T, Kurokawa M, Oohara A, Yoshimatsu H: A physiological role of brain histamine during energy deficiency. *Brain Res Bull* 35:135–139, 1994
38. Bugajski J, Janusz Z: Lipolytic responses induced by intracerebroventricular administration of histamine in the rat. *Agents Actions* 11:147–150, 1981
39. Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Nimura K, Watanabe T, Watanabe T: Impaired locomotor activity and exploratory behavior in mice lacking histamine H_1 receptors. *Proc Natl Acad Sci U S A* 93:13316–13320, 1997
40. Akiyama Y, Mukai T, Kamei C, Tasaka K: Histamine lipolysis. I. Changes in the free fatty acid levels of dog plasma after intravenous infusion of histamine. *Methods Find Exp Clin Pharmacol* 12:315–324, 1990
41. Takahashi A, Shimazu T: Hypothalamic regulation of lipid metabolism in the rat: effect of hypothalamic stimulation on lipolysis. *J Auton Nerv Syst* 4:195–205, 1981
42. Himms-Hagen J, Cui J, Danforth E Jr, Taatjes DJ, Lang SS, Waters BL, Claus TH: Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol* 266:R1371–R1382, 1994
43. Jenkins AB, Storlien LH, Chisholm DJ, Kraegen EW: Effects of nonesterified fatty acid availability on tissue-specific glucose utilization in rats in vivo. *J Clin Invest* 82:293–299, 1988
44. Yoshitomi H, Yamazaki K, Abe S, Tanaka I: Differential regulation of mouse uncoupling proteins among brown adipose tissue, white adipose tissue, and skeletal muscle in chronic beta 3 adrenergic receptor agonist treatment. *Biochem Biophys Res Commun* 253:85–91, 1998