

# Targeted Disruption of Histamine H<sub>1</sub>-Receptor Attenuates Regulatory Effects of Leptin on Feeding, Adiposity, and UCP Family in Mice

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**Histamine neurons are widely distributed in the brain and suppress food intake through the histamine H<sub>1</sub> receptor (H<sub>1</sub>-R) in the hypothalamus. To examine the role of neuronal histamine in leptin signaling pathways, we investigated the effects of H<sub>1</sub>-R knockout (H1KO) mice on both food intake and mRNA expressions of uncoupling proteins (UCPs) as regulated by leptin, and concomitantly on basal changes in both expression of hypothalamic neuropeptides and diet-induced fat deposition in adipose tissues. H1KO mice showed no change in daily food intake, growth curve, body weight, or adiposity. Reflecting no specificity in these parameters, H1KO mice induced no basal changes in mRNA expression of hypothalamic neuropeptides, *ob* gene, or peripheral UCPs. Loading H1KO mice with a high-fat diet accelerated fat deposition and *ob* gene expression compared with the controls. Leptin-induced feeding suppression was partially attenuated in H1KO mice, indicating involvement of histamine neurons in feeding regulation as a downstream signal of leptin. Upregulation of fat UCP mRNA and reduction of body fat induced by central infusion of leptin were attenuated in the H1KO mice. These results show that H1KO mice are a novel leptin-resistant model and that H<sub>1</sub>-R is a key receptor for downstream signaling of leptin in the brain that contributes to regulation of feeding, fat deposition, and UCP mRNA expression. *Diabetes* 50:385–391, 2001**

**H**istamine neurons originating from the tuberomammillary nucleus of the posterior hypothalamus project diffusely in the brain to regulate energy homeostasis (1,2). Neuronal histamine has been shown to suppress food intake through histamine H<sub>1</sub>-receptors (H<sub>1</sub>-Rs) in the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN) (3,4). It also alters thermoregulation (5). Energy deficiency in the brain, i.e.,

neural glucoprivation, activates histamine neurons in the hypothalamus (6) and augments glycogenolysis in the brain (7). Histamine neurons stimulate the sympathetic nervous system to increase lipolysis in the adipose tissue (8,9).

Leptin, an *ob* gene product (10), has been recently demonstrated to promote histamine turnover by affecting the post-transcriptional process of histidine decarboxylase formation or histamine release per se (11). In addition, concentration or turnover rate of hypothalamic histamine was lowered in leptin-deficient *ob/ob* and leptin receptor-mutated *db/db* mice, but it was increased in diet-induced obese animals (11). Leptin regulates metabolic efficiency and exerts anorectic action (12–14) through its hypothalamic long-form receptors, in the VMH, the dorsomedial hypothalamus, the arcuate nucleus, and the ventral premammillary nucleus (15–17). The VMH, the PVN, and the arcuate nucleus are known as controlling centers of appetite and receive projections from histamine neurons (3,4,18,19).

From the viewpoint of energy metabolism, the uncoupling protein (UCP) family plays an essential role in energy homeostasis (20–22). Gene expression of these proteins is regulated by humoral and neuronal factors (23–28). Central administration of leptin upregulated gene expression of the UCP family (28). These findings suggest that signal transduction between leptin and histamine neurons may be involved in central regulation of the UCP family.

A growing body of rapidly advancing information on functional roles of histamine neurons together with their central signaling pathways is consistent with a concept that hypothalamic histamine neurons are very likely to contribute to central regulation of energy balance governed by leptin. To address this issue, we hypothesized that knockout of H<sub>1</sub>-R (H1KO) might disrupt leptin signaling messages ranging from expression of hypothalamic neuropeptides to that of the UCP family and *ob* gene. A goal of the present study was to examine the essential roles of H<sub>1</sub>-R in regulation of food intake and UCP expression.

## RESEARCH DESIGN AND METHODS

**Animals.** Mature male C57Bl/6J mice (Seac Yoshitomi, Fukuoka, Japan) and H1KO mice (Kyushu University, Fukuoka, Japan), at 0–30 weeks of age, were housed in a room illuminated daily from 0700 to 1900 (a 12:12 h light-dark cycle) at a temperature at 21 ± 1°C and humidity at 55 ± 5%. The mice were allowed access to standard powdered mouse food (CLEA Japan, Tokyo, Japan) and tap water ad libitum. Daily food consumption and body weight of the mice were measured at 0800. The measurement was monitored at least 7 days before each experiment. 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 maintained for backcrossing at Medical Institute of Bioregulation (Kyushu Uni-

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BAT, brown adipose tissue; H<sub>1</sub>-R, H<sub>1</sub>-receptor; HFD, high-fat diet; LFD, low-fat diet; NPY, neuropeptide Y; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; POMC, proopiomelanocortin; PVN, paraventricular nucleus; UCP, uncoupling protein; VMH, ventromedial hypothalamus; WAT, white adipose tissue, WT, wild-type.

versity). The methods used to produce these mice are reported in detail elsewhere (29). Backcrossing H<sub>1</sub>R<sup>-/-</sup> homozygous mice to the C57Bl/6J strain for five generations resulted in the inbred congenial N4 mice of three genotypes (H<sub>1</sub>R<sup>-/-</sup>, H<sub>1</sub>R<sup>+/-</sup>, H<sub>1</sub>R<sup>+/+</sup>) used here. All genotypes were confirmed using Southern blotting. In total, 178 offspring of heterozygous males and females were analyzed for the genotype at the age of 28 days. The following distribution was observed: +/+, *n* = 42; +/-, *n* = 90; -/-, *n* = 46. These results closely appropriate the expected ratio of 1:2:1, indicating that H<sub>1</sub>-R deficiency does not adversely affect pre- or postnatal viability.

**Measurement of food consumption and growth curve.** The growth rate of the male H1KO and wild-type (WT) mice (6 mice/group) was monitored from their weaning at 1 week of age up to a 30-week period. Their daily food intake over 24 h was measured at 12 and 30 weeks of age. These mice were housed alone throughout the 30-week monitoring period under the acclimatized ambient condition described above.

**Measurement of body composition and blood sampling procedures.** Body weight, total fat weight, and percent fat were measured to detect the difference in body fat accumulation between male H1KO and WT mice at 12 and 30 weeks of age using 6 subjects for each. Total body weight, fat weight, and percent fat were obtained using an analytical balance (Mettler, Toledo, Osaka, Japan) and dual energy X-ray absorptiometry for rodents (Muromachi, Tokyo, Japan). Epididymal white adipose tissue (WAT) and brown adipose tissue (BAT) were dissected, immediately frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Each mouse was chronically implanted with a silastic catheter (No. 00; Shinetsu, Tokyo, Japan) for serum sample collection. A catheter was inserted through the right jugular vein with the inner end placed just outside the right atrium. The sampling tube was attached to a 30-gauge Multi Sampling Needle to prevent air from being sucked into the system. Details on the sampling procedures were described elsewhere (30). Samples taken through the catheter were separated into serum and immediately frozen at -20°C until their use for assay. Serum glucose, insulin, and triglyceride were taken after overnight fasting to assay with commercially available kits (Eiken Chemical, Tokyo, Japan).

**Load of mice with high- or low-fat diet.** Matched for body weight at 8 weeks of age, H1KO and WT mice were divided into high-fat diet (HFD) and low-fat diet (LFD) groups (*n* = 6 for each subgroup). The HFD consisted of 45% fat, 35% carbohydrate, and 20% protein, with an energy density of 4.73 kcal/g. The LFD consisted of 10% fat, 70% carbohydrate, and 20% protein, with an energy density of 3.85 kcal/g. Body weight in each subgroup was measured weekly from 8 to 16 weeks of age. Total fat weight, percent fat, and *ob* gene expression in WAT were measured at 16 weeks of age.

**Chronic implantation with a cannula into the lateral ventricle.** Male adult mice at 12–14 weeks of age were anaesthetized with intraperitoneal injection of nembutal (1 mg/kg). Mice were placed in a stereotaxic device to implant a 29-gauge stainless steel cannula chronically into the left lateral cerebroventricle (0.5 mm posterior, 1.0 mm lateral, and 2.0 mm ventral to the bregma). After surgery, a 30-gauge wire plug was inserted into each cannula to prevent blood coagulation. All the mice were allowed 1 week of postoperative recovery before they were handled daily to equilibrate their arousal levels. After cessation of all experiments, cannula placement was verified in each mouse infused with the dye India green.

**Procedures of leptin treatment.** Murine recombinant leptin (Amgen, Thousand Oaks, CA) was dissolved in phosphate-buffered saline (PBS). To acquire a dose-response relationship, leptin was infused into the left lateral cerebroventricle at doses of 0.1, 0.25, and 0.5 µg/mouse daily for 3 successive days. The procedures of PBS infusion in the control group were the same as those in the leptin group, where applicable. The intracerebroventricular infusion volume of leptin and PBS was 0.1 µl. Matched according to basal body weight at 12–14 weeks of age, H1KO and WT mice were divided into the leptin and control groups (*n* = 6 for each). On the day before and for 3 days after treatment, food intake was measured daily in each subgroup (*n* = 6 for each subgroup). To prevent a difference in food consumption between the leptin and control groups, the control mice in each leptin infusion study were pair-fed daily with the appropriate leptin-treated mice. After the feeding evaluation, adipose tissues were surgically removed according to the procedures mentioned above and analyzed for fat accumulation and *UCP* expression.

**Preparation of cDNA probe.** Polymerase chain reaction (PCR) primers of 5'-CATCTTCTGGGA-GGTAGC-3' and 5'-AAGACAGGGCAGGAATGG-3' were designed to the coding region of the *UCP2* gene. Primers of 5'-GTTACCTTCCACTGGACAC-3' and 5'-CCGTTTCAGCTGCTCATAGG-3' were designed to the *UCP3* gene. Reverse transcription of 10 µg total RNA from C57Bl/6J 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 primers. The reaction profile was 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 dideoxynucleotide 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 *UCP-1*, *ob*, neuropeptide Y (NPY), and proopiomelanocortin (POMC) (GenBank accession no. U63419, U18812, M15880, and AH005319) probes were generated in an analogous fashion.

**RNA extraction and Northern blot analysis.** Total cellular RNA was prepared from various mouse 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, and the separated RNA was transferred onto a Biotrans 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, Japan). The membranes were stripped by exposure to boiling 0.1% SDS and rehybridized with a ribosomal RNA that was used to quantify the amounts of RNA species on the blots.

**Statistical analysis.** All the data were expressed as the mean ± SE. The statistical analysis of difference was assessed by Sheffe's or repeated two-way analysis of variance (Figs. 1–4), and the unpaired *t* test for multiple comparisons was used where appropriate (Table 1, Fig. 1). To evaluate the dose-response curve as to the effects of intracerebroventricular leptin infusion on food intake, the Spearman's correlation coefficient by rank was carried out.

## RESULTS

### Effects of H<sub>1</sub>-R deficiency on bodily growth, body fat accumulation, and serum glucose, insulin, and triglyceride.

As shown in Fig. 1A, no difference was found in growth rate and body weight between H1KO and WT mice from the weaning period of 1 week of age up to 30 weeks of age. Food intake (Fig. 1B), body weight, fat deposition, and fasting serum concentration of glucose, insulin, and triglyceride measured at 12 and 30 weeks of age did not differ between H1KO and WT mice (Table 1).

**Effects of H<sub>1</sub>-R deficiency on brain neuropeptides, UCP, and *ob* genes.** Figure 1C and D show changes in gene expression involved in regulation of energy intake and expenditure in H1KO and WT mice at 12 (Fig. 1C) and 30 (Fig. 1D) weeks of age. Compared with the WT controls, no significant change was found in H1KO mice at either parameter, including hypothalamic mRNA of NPY or POMC and expression of BAT *UCP-1*, WAT *UCP-2*, WAT *UCP-3*, or WAT *ob* gene.

**Effect of high-fat loading on H1KO mice.** Loading of both male H1KO and WT mice with an HFD for 8 weeks starting at 8 weeks of age increased body weight more than that of mice with LFD (*P* < 0.01 for each), whereas no significant difference was found in body weight between H1KO and WT mice loaded with the same diet (either HFD or LFD) (Fig. 2A). Mean daily food intake in H1KO mice did not differ from that in WT mice throughout loading of these diets (data not shown). The notable result was that H1KO mice loaded with HFD increased total fat weight and percent fat more than WT mice with HFD (*P* < 0.01 for each), although there was no difference between the two types of mice loaded with LFD in either parameter (Fig. 2B and C). In agreement with these results, expression of *ob* gene in epididymal WAT was upregulated in H1KO mice loaded with HFD compared with that in the corresponding WT controls (*P* < 0.01) (Fig. 2D and E). There was no difference in serum glucose between H1KO and WT mice loaded with HFD, but serum insulin was increased in H1KO mice loaded with HFD

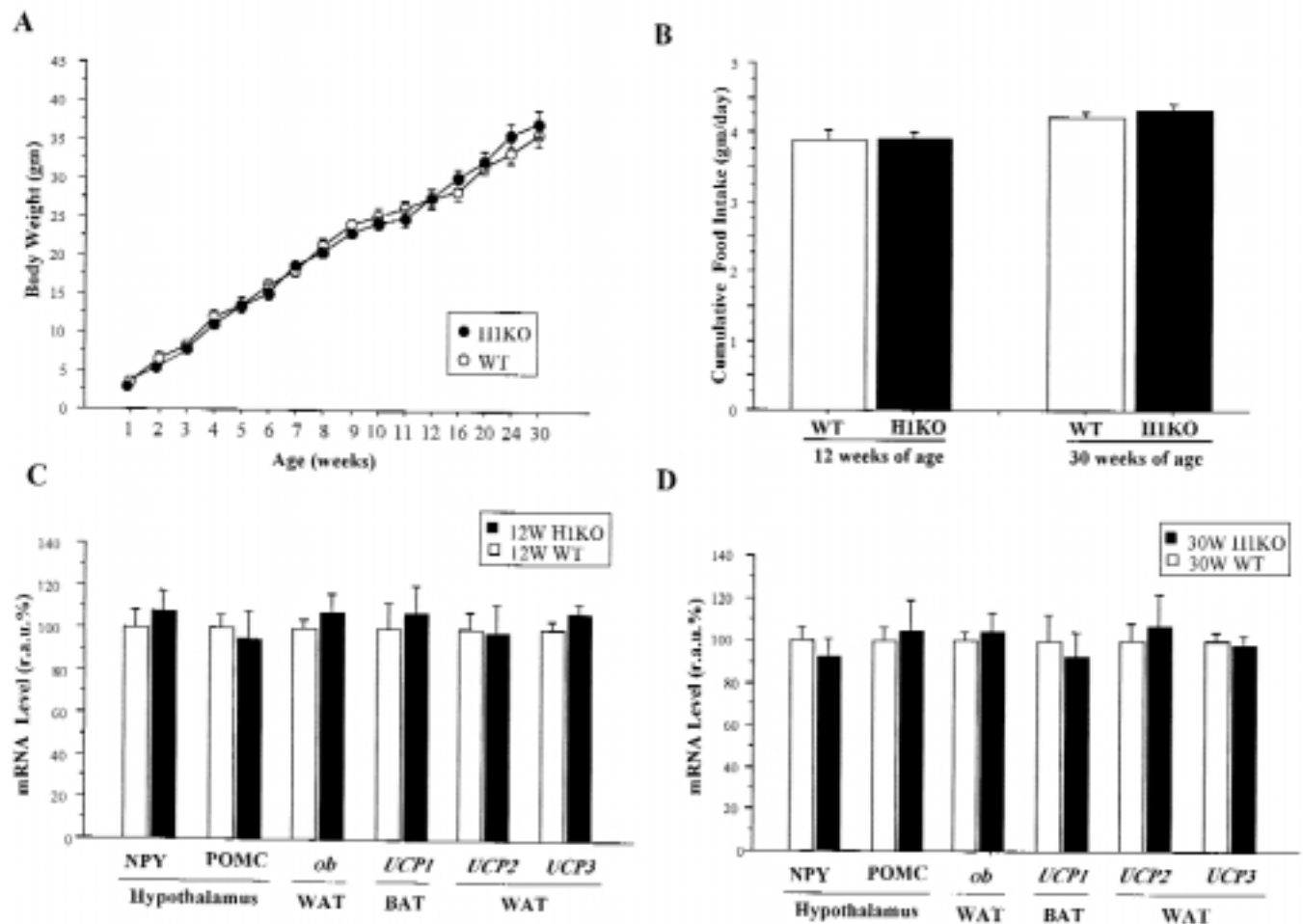


FIG. 1. Growth curves and 24-h food intake of H1KO and WT control mice (A). Note that no difference was found between H1KO and WT mice in the growth curve measured from weaning at 1 week of age up to 30 weeks of age or in daily food intake at 12 and 30 weeks of age (B). Values and vertical bars are means  $\pm$  SE ( $n = 6$  for each). C and D: Gene expression of NPY and POMC mRNAs in the hypothalamus, *UCP-1* mRNA in BAT, *UCP-2* and *UCP-3* mRNAs in epididymal WAT, and *ob* mRNA in WAT in H1KO and WT mice at 12 (C) and 30 (D) weeks of age. Northern blot analysis was performed in total RNA (20  $\mu$ g/lane) of the samples. No difference was found between the groups in either parameter. Values and vertical bars are means  $\pm$  SE ( $n = 6$  for each). r.a.u.%, Percent of relative arbitrary unit.

(202.1  $\pm$  10.4  $\mu$ U/ml) compared with corresponding WT controls (151.2  $\pm$  8.3  $\mu$ U/ml) ( $P < 0.05$ ).

**Effects of leptin infusion on food intake.** The effect of central administration of leptin on 24-h food intake was investigated in H1KO and WT mice at 12–14 weeks of age. Intracerebroventricular infusion of 0.1–0.5  $\mu$ g leptin/mouse daily produced dose-dependent feeding suppression (0.1, 0.25, and

0.5  $\mu$ g leptin produced –9%, –16% and –34% food intake, respectively, compared with PBS controls;  $r = 0.79$ ,  $P < 0.01$ ). Leptin infusion into the left lateral cerebroventricle at the highest dose of 0.5  $\mu$ g/mouse daily for 3 days decreased food intake in both H1KO and WT mice ( $P < 0.01$  for each) (Fig. 3). Leptin-induced feeding suppression was attenuated in H1KO mice compared with corresponding WT controls ( $P < 0.05$ ) (Fig. 3).

TABLE 1  
Body composition and serum concentration of mice at 12 and 30 weeks

Parameters	12 Weeks		30 Weeks	
	+/+	-/-	+/+	-/-
Body weight (g)	30.4 $\pm$ 1.1	30.3 $\pm$ 1.3	35.4 $\pm$ 1.2	36.1 $\pm$ 1.9
Body fat (g)	2.68 $\pm$ 0.28	2.85 $\pm$ 0.22	3.41 $\pm$ 0.30	3.75 $\pm$ 0.42
Body fat (%)	8.81 $\pm$ 0.92	9.40 $\pm$ 0.85	9.63 $\pm$ 0.84	10.39 $\pm$ 0.80
Glucose (mg/dl)	174.4 $\pm$ 15.3	167.5 $\pm$ 12.5	192.4 $\pm$ 15.1	190.0 $\pm$ 14.9
Insulin ( $\mu$ U/ml)	56.6 $\pm$ 7.6	59.3 $\pm$ 11.0	60.1 $\pm$ 8.7	69.8 $\pm$ 13.7
Triglyceride (mg/dl)	97.5 $\pm$ 9.4	96.8 $\pm$ 7.9	128.7 $\pm$ 11.4	138.9 $\pm$ 12.5

Data are means  $\pm$  SE.

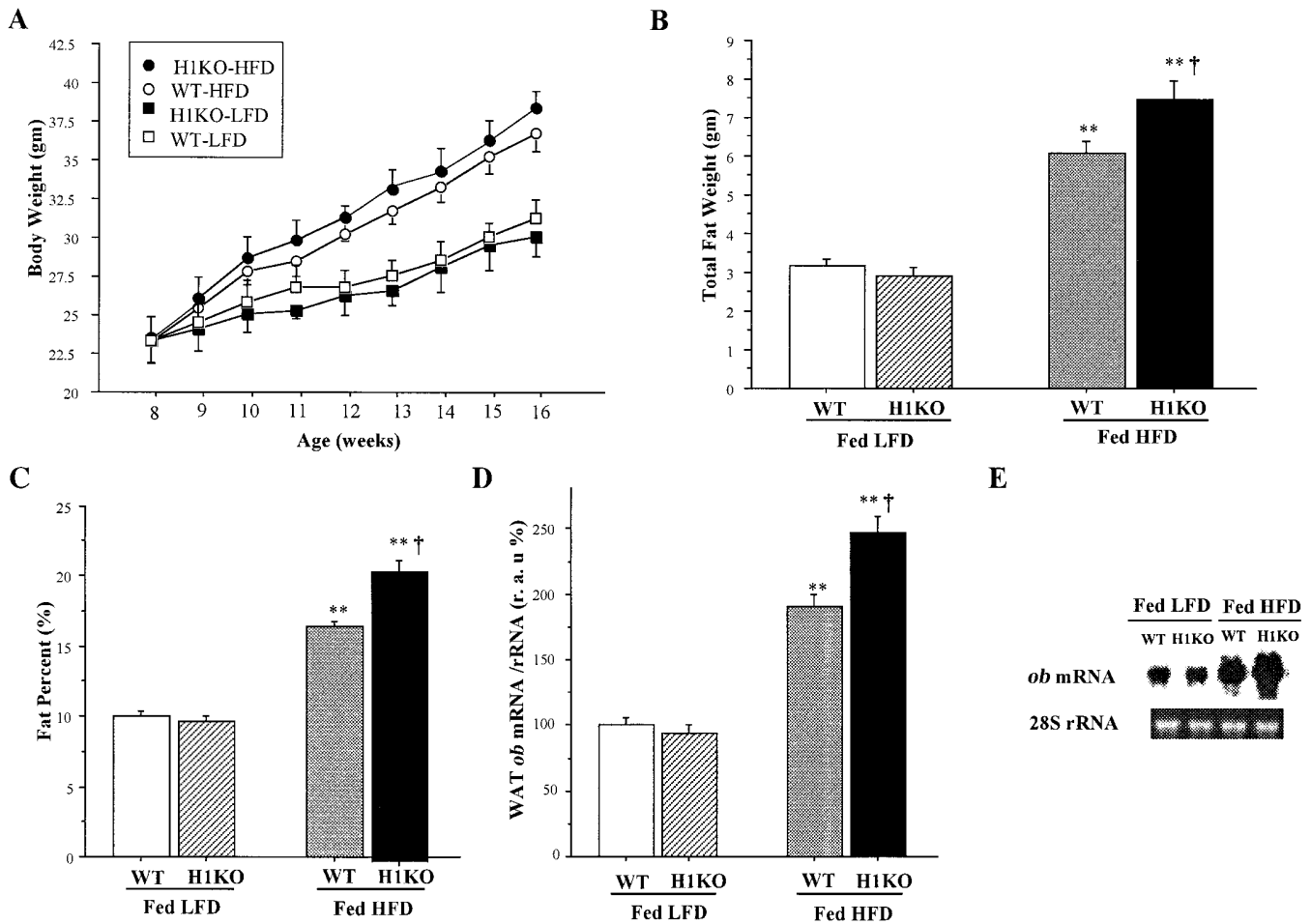


FIG. 2. Effects of HFD and LFD on body weight growth (A), fat weight (B), fat percent (C), and epididymal WAT *ob* gene expression (D, E) in H1KO and WT mice. Total fat weight, fat percent, and *ob* gene expression in H1KO mice fed with HFD were increased more than those in the corresponding WT mice. Values and vertical bars are means  $\pm$  SE ( $n = 6$  for each). r.a.u.%, Percent of relative arbitrary unit. \*\* $P < 0.01$  vs. corresponding LFD controls; † $P < 0.05$  vs. corresponding WT controls.

**Effect of intracerebroventricular leptin infusion on adiposity and *UCP* expression in BAT and WAT.** Effects of chronic intracerebroventricular leptin infusion on adiposity and *UCP* expression in fat tissues were examined in pair-fed H1KO and WT mice at 12–14 weeks of age. Infusion of murine leptin into the left lateral cerebroventricle at a dose of 0.5  $\mu$ g/mouse daily for 3 successive days lowered percent body fat in the pair-fed WT group compared with that in the corresponding controls with PBS ( $P < 0.05$ ) (Fig. 4A). Leptin caused a more remarkable decrease of visceral fat weight than of subcutaneous fat (data not shown). The suppressive effect of leptin was attenuated in H1KO mice compared with that in the corresponding pair-fed WT controls ( $P < 0.05$ ) (Fig. 4A). Gene expression of both *UCP-1* and *UCP-3* in BAT and *UCP-3* in WAT was upregulated more in both pair-fed H1KO ( $P < 0.05$  for each) and WT mice ( $P < 0.01$  for each) after intracerebroventricular leptin infusion than those in the corresponding PBS controls (Fig. 4B and G). Note that the accelerated effects of leptin on adiposity and *UCP* expression were attenuated more predominantly in H1KO mice than in the pair-fed corresponding WT controls ( $P < 0.05$  for each) (Fig. 4B and G).

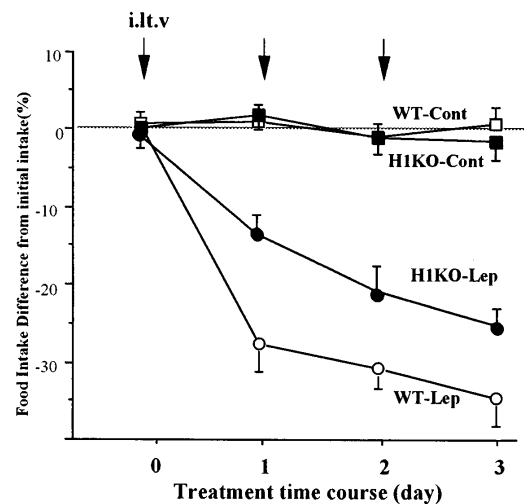


FIG. 3. Effect of leptin (Lep) infusion on food intake in H1KO and WT mice at 12–14 weeks of age. Suppressive effect of Lep on feeding was attenuated in H1KO mice with Lep. Values are the percent difference from corresponding initial baseline value. Values and vertical bars are means  $\pm$  SE ( $n = 6$  for each). The arrows indicate infusion of Lep or PBS (Cont) into the lateral cerebroventricle (i.l.t.v.). See the text for details about significant differences between the subgroups.

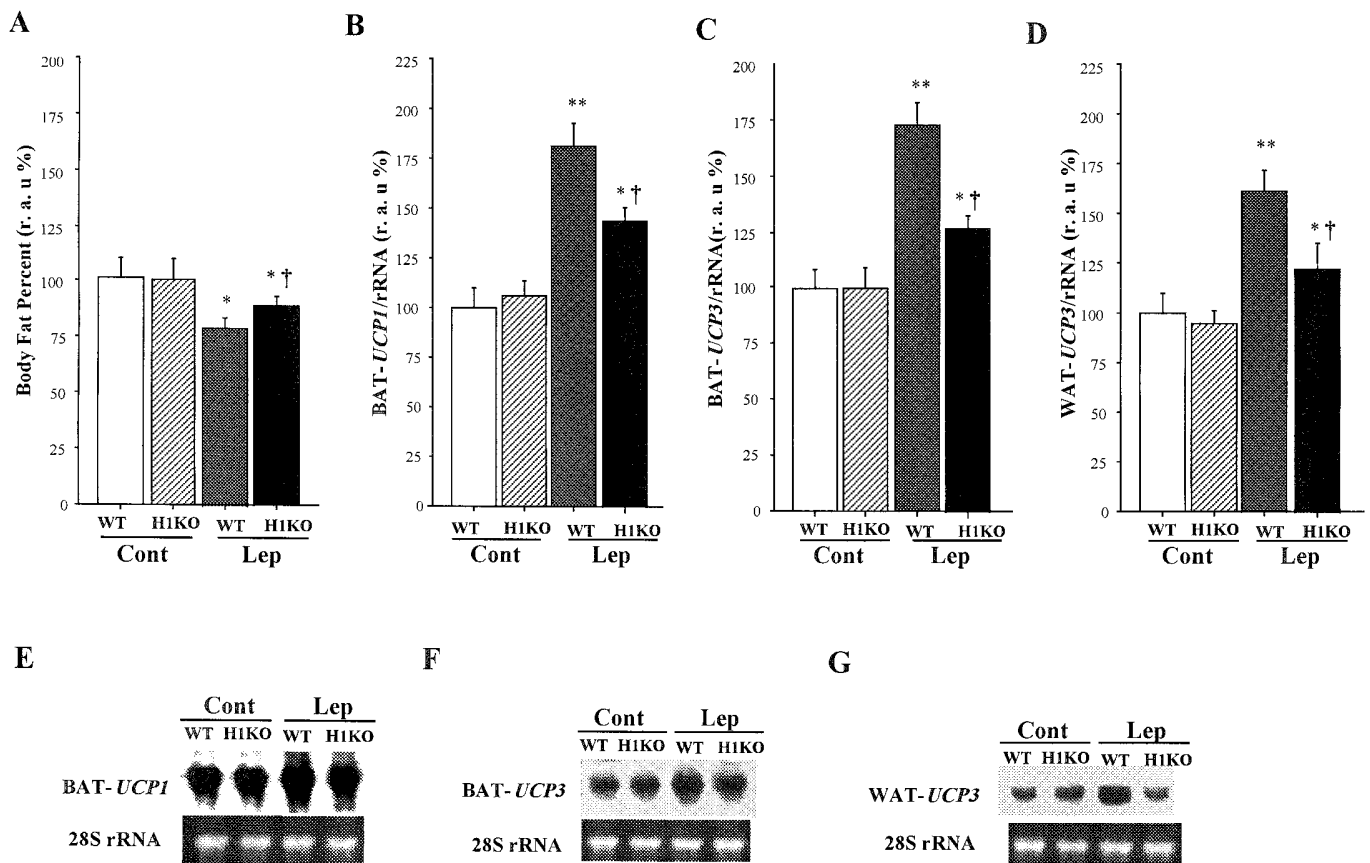


FIG. 4. Effect of leptin (Lep) infusion on fat percent (A) and *UCP* gene expression in H1KO and WT mice at 12–14 weeks of age. Each parameter was attenuated or enhanced in H1KO mice after Lep treatment compared with PBS controls (Cont). Values are the percent differences from the corresponding initial baseline value. Values and vertical bars are means  $\pm$  SE ( $n = 6$  for each). r.a.u.%, Percent of relative arbitrary unit. \* $P < 0.05$ , \*\* $P < 0.01$  vs. corresponding untreated controls; † $P < 0.05$  vs. corresponding WT controls. Gene expression of BAT *UCP-1* (E), BAT *UCP-3* (F), and WAT *UCP-3* (G) in H1KO and WT mice after Lep or PBS (Cont) treatment.

## DISCUSSION

The effects of histamine neurons on brain functions are mediated by histamine  $H_1$ -R,  $H_2$ -R, and  $H_3$ -R, which belong to a family of guanine nucleotide-binding regulatory G protein-coupled receptors (2). These histamine receptors are involved in a variety of physiological functions: mediation of immune response (31), circadian rhythm (29), and food intake through  $H_1$ -R (3,4); endocrine system and gastric secretion through  $H_2$ -R (32); and auto-inhibition for negative feedback regulation through  $H_3$ -R that affects histamine contents and turnover (33). The present study focused on the role of  $H_1$ -R in the leptin signaling pathway, which regulates feeding behavior and *UCP* expression.

Deficit in  $H_1$ -R per se has been found to produce no manifest change in gene expression of peripheral *UCPs* or hypothalamic neuropeptides such as NPY and POMC. Consistently, food consumption, growth rate, and body weight did not change in H1KO mice. These results are compatible with our previous finding that histamine depletion by chronic treatment with  $\alpha$ -fluoromethylhistidine did not affect daily food intake or body weight growth, although acute depletion of neuronal histamine induced a transient increase in feeding (34). Together with the resulting and foregoing findings, it would be reasonable to argue that the effectiveness of histamine depletion or defect in  $H_1$ -R may not be detectable

under normal conditions, because histamine neurons in the hypothalamus appear to play homeostatic rather than direct roles in energy balance (1). In this context, histamine neurons in parallel with other regulatory systems of energy metabolism may act indirectly to influence major physiologic determinants of energy balance. The assumption is supported by the present study on feeding mice an HFD. More rapid increase in body fat deposition was induced in H1KO mice after access to HFD. Here, an essential question can be raised as to why body weight did not differ between the WT and H1KO groups, although the adipose parameters of total fat weight, percent fat, and *ob* gene expression did differ between those groups. There is no definite answer to the query to date. One possible and reasonable explanation is that the increase in body fat may be too small to affect body weight. Another possibility is that the difference in mean body weight gain between those groups is relatively small compared with the deviation of body weight in both groups.

The resulting information indicates that a genetic defect in histamine signaling through  $H_1$ -R is capable of enhancing the development of adiposity leading to obesity. In normal conditions, histamine neurons function through  $H_1$ -R to prevent the living body from excessive fat accumulation induced by high-energy intake. There are four possible explanations for the action of neuronal histamine against obesity develop-

ment. First, neuronal histamine has been demonstrated to suppress food intake through H<sub>1</sub>-R in the VMH and the PVN, both of which are known as satiety centers (3,4). However, the mechanism is not likely to have played a main role in the present study, because no significant difference was found in daily consumption of HFD between H1KO and WT mice. Second, histaminergic activation may lead to increased sympathetic outflow and lipolysis. Indeed, previous reports demonstrated increases in lipolytic responses together with elevation of serum free fatty acids through the sympathetic pathway (8,9). Third, the present study revealed that H1KO mice increased insulin levels in response to an HFD. The result indicates that besides sympathetic outflow to fat pads, HFD-induced insulin secretion in H1KO mice may promote lipogenesis in adipocytes because insulin increases leptin synthesis (35), as the present result showed WAT *ob* mRNA was upregulated. Finally, it seems quite reasonable that histamine neurons may be involved in central regulation of *UCP* expression as indicators of energy expenditure. *UCP-1* and *UCP-3* in BAT and *UCP-3* in WAT are upregulated by a  $\beta_3$ -agonist, indicating effects of sympathetic activity on their gene expression (36). Administration of leptin has been shown to affect BAT and WAT *UCP* mRNAs through the activation of sympathetic nerve (28,37). BAT *UCP-1* is shown modulated not only by sympathetic nerve activity (20,38) but by upper brain function (28,39). Under this scenario, histamine neurons may guard against fat accumulation, depending on histamine-induced upregulation of *UCPs*.

The question arises as to what kind of information activates histamine neurons in response to HFD loading. Leptin is a most probable candidate for the activation. HFD-promoted fat accumulation leads to elevation of leptin secretion from expanded adipose tissues (40,41). Thereafter, leptin activates histamine release in the hypothalamus and limits food intake (11). Ultimately, the activation of histamine neurons drives a negative feedback loop that downregulates adiposity and feeding by returning them to those initial levels. Blockade of the histamine signaling pathway at an H<sub>1</sub>-R level may limit leptin's actions on food intake and regulation of *UCP* expression. It would be very much in line with our present results indicating that the signaling pathways of leptin interact with those of histamine neurons in the regulation of feeding, adiposity, and *UCP* expression. Notably, the suppressive effects of leptin on feeding behavior and fat accumulation and the accelerating effects of leptin on *UCP* expression were partially attenuated in H1KO mice. Taken together with the foregoing explanation of leptin-induced *UCP* upregulation (28,37) and the interaction of leptin signaling messages with those of histamine neurons (11), this attenuation may result from reduced sympathetic nerve activity. The present study demonstrates that histamine neurons play an important role in the leptin signaling pathway through H<sub>1</sub>-R. Coordinate interactions of histamine neuron systems with leptin signaling thus regulate suppression of feeding behavior, acceleration of lipolysis, and upregulation of the *UCP* family.

Evidence has rapidly emerged to indicate that development of obesity results from interaction between genetic and environmental factors. Developmental patterns of H1KO mice when fed an HFD resemble middle-aged human obesity. More recently, brain serotonin systems were reported to have a causal relation with fat deposition in middle-aged and diet-induced obesity (42). In this context, a genetic defect in hist-

amine signaling transduction and/or the intake of a high-fat, high-energy diet tend to develop obesity. It is not surprising for the foregoing reasons that the inhibition of brain histamine neurons will predispose animal models and humans to obesity.

In conclusion, H1KO mice are not only useful as a new leptin-resistant model but also provide a novel insight that H<sub>1</sub>-R contributes as a key receptor in the regulation of energy homeostasis when mice are fed an HFD and acts as a downstream signal of leptin's actions in the brain.

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