

Peripheral, But Not Central, Administration of Adiponectin Reduces Visceral Adiposity and Upregulates the Expression of Uncoupling Protein in Agouti Yellow (A^y/a) Obese Mice

Takayuki Masaki,¹ Seiichi Chiba,¹ Tohru Yasuda,¹ Tetsuo Tsubone,¹ Tetsuya Kakuma,¹ Iichiro Shimomura,² Tohru Funahashi,³ Yuji Matsuzawa,³ and Hironobu Yoshimatsu¹

To examine the peripheral and central roles of adiponectin in energy intake and expenditure, we investigated the effects of adiponectin on food intake, adiposity, sympathetic nerve activity (SNA), and mRNA expressions of uncoupling protein (UCP) in the brown adipose tissue (BAT), white adipose tissue (WAT) and skeletal muscle in agouti yellow (A^y/a) obese mice. Intraperitoneal administration of adiponectin (1.5 mg/kg for 7 days) attenuated body weight gain and reduced visceral adiposity in A^y/a obese mice compared with PBS-treated controls. In addition, adiponectin treatment increased the expression of UCP1 mRNA in BAT, UCP2 mRNA in WAT, and UCP3 mRNA in skeletal muscle compared with PBS-treated A^y/a controls. Acute peripheral administration of adiponectin (1.5 mg/kg, one injection) also increased SNA in the BAT accompanied by an increase in rectal temperature. Finally, these above responses as well as expression of c-Fos-like immunohistochemistry in the hypothalamus were not induced by central application of adiponectin (0–15 μ g/kg). Taken together, adiponectin effectively regulated visceral adiposity, SNA, and UCP mRNA expression peripherally, suggesting that this substance can be used as a therapeutic tool, administered peripherally, in the treatment of visceral obesity and related metabolic disorders. *Diabetes* 52:2266–2273, 2003

Adiponectin, an adipocytokine, was identified in adipose tissue by screening adipose-specific genes in humans (1). The mouse homologue of adiponectin has been cloned as ACRP30 and AdipoQ (2–4), which contain a signal sequence and a collagen-repeat domain at the NH₂-terminus and a globular

domain at the C-terminus. Adiponectin mRNA is exclusively expressed in adipose tissue, and the protein is abundant in the plasma of humans and rodents (1–4). A negative correlation between plasma adiponectin levels and BMI has been demonstrated (5). The levels are lower in obese human than in normal-weight subjects (5–7). In addition, patients with type 2 diabetes also showed lower levels of plasma adiponectin, indicating the involvement of this adipocytokine in glucose metabolism (8–10). Hyperinsulinemic and euglycemic clamp studies in humans and monkeys showed that plasma adiponectin levels negatively correlate with insulin resistance (8). However, it is not clear whether the reduction of adiponectin is the cause or result of the development of obesity or insulin resistance. Recently, adiponectin treatment has been shown to improve insulin resistance with attenuation of body weight gain in mice (11–13). Furthermore, adiponectin treatment has been shown to increase free fatty acid β -oxidation via AMP kinase-dependent pathway (14), and adiponectin-deficient mice developed diet-induced obesity and/or have abnormal fatty acid oxidation (15–17). These findings suggest that adiponectin may regulate body weight and/or insulin sensitivity and also that reduced adiponectin production may be related to the pathogenesis of obesity and its complications.

With the discovery of leptin, an *ob* gene product, a variety of bioactive substances that regulate body weight and energy metabolism have been identified in the brain as well as peripheral tissue (18,19). Most of these regulatory substances, including leptin, have targets in the brain, and regulate body weight through affecting not only food intake but also peripheral energy expenditure (18,19). The peripheral administration of adiponectin attenuates body weight gain but does not affect food intake (11). It is highly probable that adiponectin may regulate body weight by affecting the energy expenditure in peripheral tissue. However, the mechanism of adiponectin in body weight regulation remains unclear. In particular, the central nervous system-mediated actions of adiponectin, such as hypothalamic regulation of feeding behavior and energy expenditure, have not been investigated.

Uncoupling protein (UCP)-1 in the brown adipose tissue (BAT) plays a major role in energy expenditure and nonshivering thermogenesis in rodents and neonates of larger mammalian species, including humans (20,21).

From the ¹Department of Internal Medicine, School of Medicine, Oita Medical University, Oita, Japan; the ²Department of Medicine and Pathophysiology, Graduate School of Medicine, Osaka University, Osaka, Japan; and the ³Department of Internal Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan.

Address correspondence and reprint requests to Dr. Takayuki Masaki, Department of Internal Medicine, School of Medicine, Oita Medical University, Hasama, Oita, 879-5593, Japan. E-mail: masaki@oita-med.ac.jp.

Received for publication 18 March 2003 and accepted in revised form 18 June 2003.

ARC, arcuate nucleus; BAT, brown adipose tissue; ICV, intracerebroventricular; PVN, paraventricular nucleus; SNA, sympathetic nerve activity; UCP, uncoupling protein; VMH, ventromedial hypothalamus; WAT, white adipose tissue.

© 2003 by the American Diabetes Association.

UCP2, another member of the UCP family, is expressed ubiquitously in peripheral tissue (22), and UCP3 is expressed mainly in the skeletal muscle and adipose tissues (23–25). Gene expression of these proteins is regulated by neuronal and humoral factors (26–28). In particular, the sympathetic nervous system and the hypothalamus have been well documented to regulate UCP1 levels (29–31). The central administration of leptin and other feeding-related neuropeptides, such as corticotropine releasing hormone and neuropeptide Y, has been shown to affect the activity of sympathetic nerves innervating BAT and/or UCP expression (26,32,33). Taken together, sympathetic activity and the function of UCP1 can be considered indicators of energy expenditure and seem to be targets for anti-obesity action of adiponectin. However, little is known about functional correlation between adiponectin and UCP1 in obese models.

Thus, the functional roles of adiponectin in body weight regulation together with their signaling pathways are still not clear, although its contribution to energy metabolism is certain. To address this issue, we investigated the effects of adiponectin on 1) food intake and body weight change, 2) adiposity in peripheral tissue, 3) sympathetic nerve activity (SNA), and 4) UCP1 mRNA expression as indicators of energy expenditure, and its central effects on these parameters in agouti yellow (*A^{y/a}*) obese mice. The goal of the present study was to examine the possibility of adiponectin as an anti-obesity substance.

RESEARCH DESIGN AND METHODS

Animals. Mature male *A^{y/a}* obese mice (Seac Yoshitomi, Fukuoka, Japan) at 12 weeks of age were housed in a room illuminated daily from 0700 to 1900 (a 12-h light-dark cycle) with temperature at $21 \pm 1^\circ\text{C}$ and humidity at $55 \pm 5\%$. 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. The animals used were treated in accordance with the Oita Medical University Guidelines for the Care and Use of Laboratory Animals based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Reagent. Recombinant full-length adiponectin (Osaka University, Osaka, Japan) was dissolved in PBS to concentrations of $0.5 \mu\text{g}/\mu\text{l}$. Each solution was freshly prepared on the day of administration. The pH of each solution was adjusted to 6.8–7.4. The adiponectin preparation was free of any contaminants such as endotoxin.

Implantation of a cannula in the cerebroventricle. The mice were anesthetized with an intraperitoneal injection of nembutal (1 mg/kg) and placed in a stereotaxic device (Narishige, Tokyo, Japan) to implant a 29-gauge stainless steel cannula chronically into the left lateral intracerebroventricular (ICV) (0.5 mm posterior, 1.0 mm lateral, and 2.0 mm ventral to the bregma) (31,34). After the procedure, a stainless wire stylet was inserted into the cannula to prevent coagulation. The mice had 1 week of postoperative recovery, after which they were handled daily to equilibrate their arousal levels. After the completion of the experiments, the animals were decapitated and the cannula location was verified histologically.

Adiponectin treatment procedures. Matched on the basis of body weight and food intake during the adaptation period, mice were divided into adiponectin and PBS groups. Mice were infused with 0, 150 ng/kg, 1.5 $\mu\text{g}/\text{kg}$, and 15 $\mu\text{g}/\text{kg}$ adiponectin ICV and 0, 15 $\mu\text{g}/\text{kg}$, 150 $\mu\text{g}/\text{kg}$, and 1.5 mg/kg i.p. per mice for 10 min at an infusion rate of 0.1 $\mu\text{l}/\text{min}$ (ICV) and 10 $\mu\text{l}/\text{min}$ (intraperitoneal), respectively ($n = 4$ –6 subjects for each adiponectin ICV, intraperitoneal adiponectin, PBS ICV, or intraperitoneal PBS experimental groups in the below experiments). These doses and the infusion speed were selected on the basis of earlier results (11,12) and our preliminary study.

Experiment 1. For the analysis of food intake, body weight, histological changes, and UCP mRNA expression, adiponectin ICV, intraperitoneal adiponectin, PBS ICV, or intraperitoneal PBS were administered at 1700 once daily for 7 days. Daily food intake of these mice was measured once daily for 7 days. Body weight change, body composition, serum parameters, histological changes of tissues, BAT UCP1, WAT UCP2, and skeletal muscle UCP3

mRNA expression were examined in each group after 7 days of ICV and intraperitoneal treatment (15–16 h after the last injection).

Experiment 2. For the analysis of SNA, a bolus adiponectin ICV, intraperitoneal adiponectin, PBS ICV, or intraperitoneal PBS infusion was performed, and SNA in BAT was recorded using an analogue-digital converter (Neurodata ER98; Cygnus Technology, Tokyo, Japan) from 20 min before until 80 min after the infusion.

Experiment 3. For the analysis of rectal temperature, a bolus adiponectin ICV, intraperitoneal adiponectin, PBS ICV, or intraperitoneal PBS infusion was performed, and rectal temperature was measured 60 min after the infusion.

Experiment 4. For the analysis of *c-fos*-like immunoreactivity, a bolus adiponectin ICV or PBS ICV infusion was performed, and *c-fos*-like immunoreactivity in hypothalamus was measured 90 min after the infusion.

Measurement of body composition and blood sampling procedures. Body fat weights were measured to detect the difference in body fat accumulation in *A^{y/a}* obese mice (4–6 subjects for each). Fat weights were obtained using an analytical balance (Mettler, Toledo, Osaka, Japan), and epididymal, mesenteric, retroperitoneal WAT, BAT, heart, and kidney were dissected, weighed, immediately frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Blood samples taken through the jugular vein were separated into serum and immediately frozen at -20°C until their use for assay. Serum glucose, insulin, and triglycerides were taken after an overnight fast and assayed with commercially available kits (Eiken Chemical, Tokyo, Japan).

Histological analysis. Small pieces of BAT and retroperitoneal WAT and liver were removed and rinsed with saline. The tissues were fixed with 10% formalin and embedded in paraffin. Tissue sections were cut at a thickness of 20 μm and stained with hematoxylin and eosin.

Triglycerides in adipose tissues. Adipose tissue (100 mg) was homogenized in 2 ml of solution containing 150 mmol/l sodium chloride, 0.1% Triton X-100, and 10 mmol/l Tris, pH 8.0, at 50°C using a polytron homogenizer (NS-310E; Micro Tech Nichion, Chiba, Japan) for 1 min. The triglyceride content of this 100- μl homogenized solution was determined by a determiner triglyceride kit (Eiken Med, Tokyo, Japan).

Preparation of cDNA probe. Primers for PCR were designed for the coding region of the UCP1 gene (GenBank accession no. U63419; upstream primer, 5'-TACACGGGGACCTACAATGCT-3'; reverse primer, 5'-TCGCACAGCTTGG T-ACGCTT-3'). Reverse-transcription of 10 μg total RNA from C57BL/6 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- μmol primers. 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 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). The UCP2 and UCP3 probes were generated in an analogous fashion according to a previous report (35).

RNA extraction and Northern blot analysis. Total cellular RNA was prepared from various mouse tissues with the use of TRIzol (Lifetech, Tokyo, 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, ON, Canada) in $20\times$ saline sodium citrate by capillary blotting and immobilized by exposure to ultraviolet light (0.80 J). Prehybridization and hybridization were carried out according to the method described by the manufacturer's protocol. After washing the membranes, the hybridization signals were analyzed with a BIO-image analyzer BAS 2000 (Fuji Film Institution, Tokyo, Japan). The membranes were stripped by exposure to boiling at 0.1% SDS and rehybridized with a ribosomal RNA that was used to quantify the amounts of RNA species on the blots.

Measurement of SNA. Experiments were carried out using a mixture of urethane (0.8 g/kg) and α -chloralose (80 mg/kg) as anesthesia. After dissection of the fine branches of the intercostal nerves supplying BAT, the nerves were cut in the region where they entered the BAT. Nerve activity was detected by a pair of silver wire electrodes that were immersed in a mixture of liquid paraffin and white petroleum jelly to prevent dehydration. The action potential was amplified and filtered at low- and high-frequency cutoffs. The nerve signal was distinguished from background noise using a window discriminator that enabled selection of action potentials right above a background threshold voltage level. All of the nerve activities were analyzed based on the values obtained after conversion of raw data to standard pulses

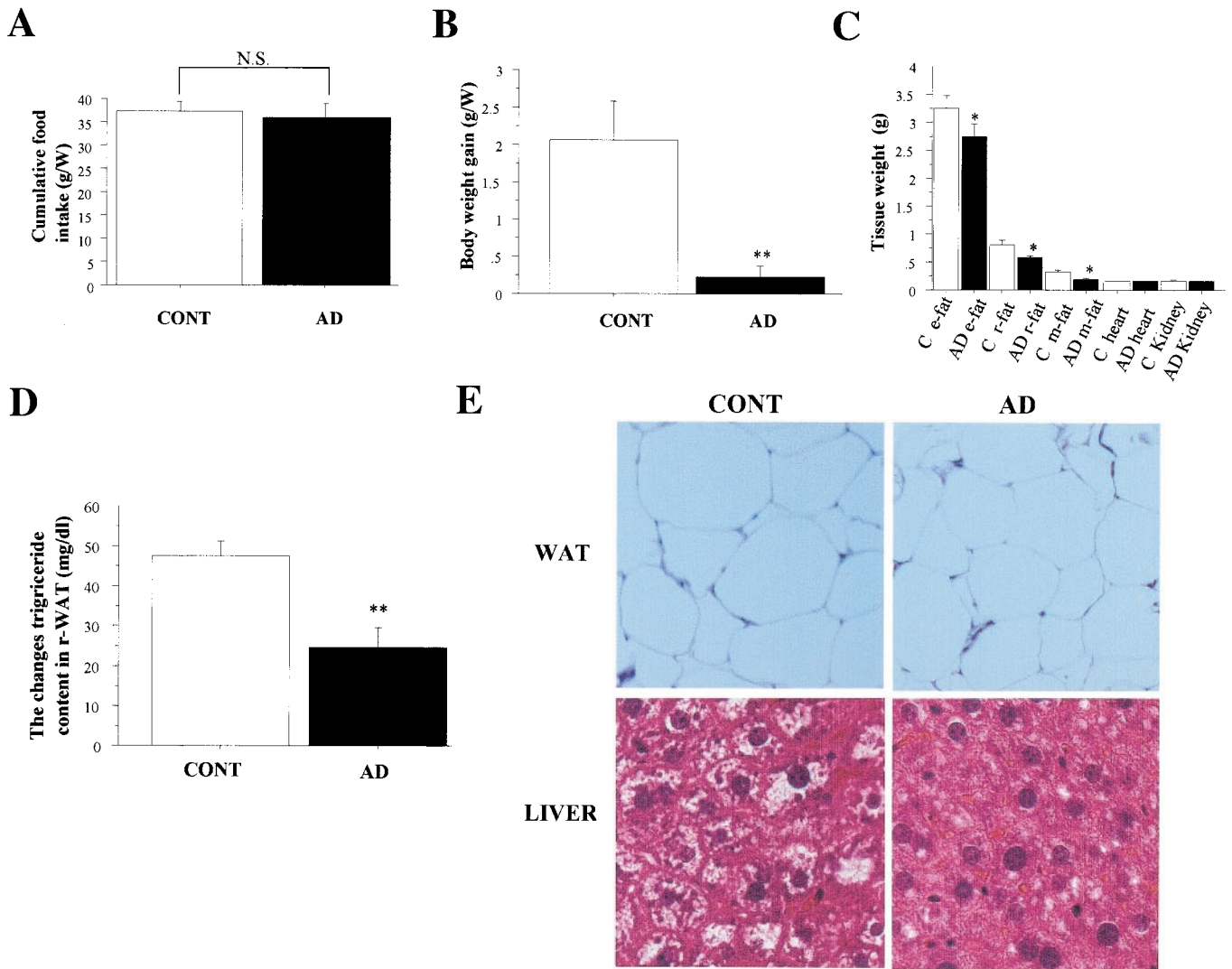


FIG. 1. Effects of intraperitoneal adiponectin infusion on cumulative food intake (A), body weight change (B), tissue weight (C), triglyceride content (D), and histology of WAT and/or liver (E) in *A^{y/a}* obese mice treated with either adiponectin (AD), PBS (control [CONT]). M, mesenteric; r, retroperitoneal fat; e, epididymal fat. Each value and vertical bar represents the mean \pm SE. * $P < 0.05$, ** $P < 0.01$ vs. the corresponding PBS controls. N.S., not significant vs. the corresponding PBS controls. Scale bar denotes 100 μ m.

using an analog-digital converter. Impulses were integrated by a rate meter with a reset time of 5 s, and they were recorded by a pen recorder. To determine the background firing rate of sympathetic nerves, changes in the nerve activity following a bolus ICV infusion of adiponectin (15 μ g/kg PBS, over 10 min) and intraperitoneal infusion of adiponectin (1.5 mg/kg PBS, over 10 min) or PBS were measured up to a maximum of 80 min. Calculation of the nerve activity was carried out at 20 min before, immediately before, and 15, 30, 45, and 60 min after adiponectin infusion. Each value after the infusion was expressed as the percentage of difference from the initial value. Details of the recording of nerve activity have been described elsewhere (36).

Measurement of *c-fos*-like immunoreactivity. On the test day, mice were given an ICV infusion of 0, 150 ng/kg, 1.5 μ g/kg, and 15 μ g/kg adiponectin or PBS ($n = 4-6$ for each). Then, 1.5 h after the injection, mice were anesthetized with Nembutal (3.3 ml/kg i.p.) and perfused transcardially with isotonic PBS, followed by 4% paraformaldehyde in 0.1 mol/l phosphate buffer. Brains were removed and postfixed for 24 h before processing for *c-fos*-like immunoreactivity. Slices (40 μ m) were cut from the brain with a vibratome. Slices were then transferred without rinsing to the primary antibody solution, consisting of 0.005 g/ml polyclonal rabbit antiserum (Santa Cruz Biotechnology, Santa Cruz, CA), with specificity to residues 3-16 of the *c-fos* protein. After 24 h of incubation on ice, the slices were washed three times in PBS and processed with the ABC method (Vector Laboratories, Burlingame, CA). Slices were transferred to biotinylated anti-rabbit antibody for 1 h, washed, transferred to avidin-biotinylated peroxidase for 1 h, washed, and developed with diaminobenzidine substrate for 6 min. Slices were then washed, mounted

on slides, and coverslipped with Permount. Sections were examined for *c-fos*-like immunoreactivity (Olympus Optical, Tokyo, Japan) including the paraventricular nucleus (PVN), dorsomedial hypothalamus, lateral hypothalamic area, arcuate nucleus (ARC) and ventromedial hypothalamus (VMH) area based on Paxinos and Flanklins mice atlas (34).

Statistical analysis. All the data were expressed as the mean \pm SEM. The statistical analysis of difference was assessed by the unpaired *t* test, and two-way ANOVA for multiple comparisons was used where appropriate.

RESULTS

Effects of adiponectin on cumulative food intake and body weight. As shown in Fig. 1A, cumulative food intake was not affected by adiponectin treatment (1.5 mg/kg i.p. for 7 days) compared with PBS-treated *A^{y/a}* controls ($P > 0.1$). On the contrary, the physiological increase in body weight was attenuated by 1.5 mg/kg i.p. adiponectin treatment compared with PBS-treated *A^{y/a}* mice ($P < 0.05$) (Fig. 1B). The body weight change was dependent on the peripheral dose of adiponectin when given intraperitoneally for 7 days (0, 2.2 ± 0.4 g; 15 μ g/kg, 1.7 ± 0.2 g; 150

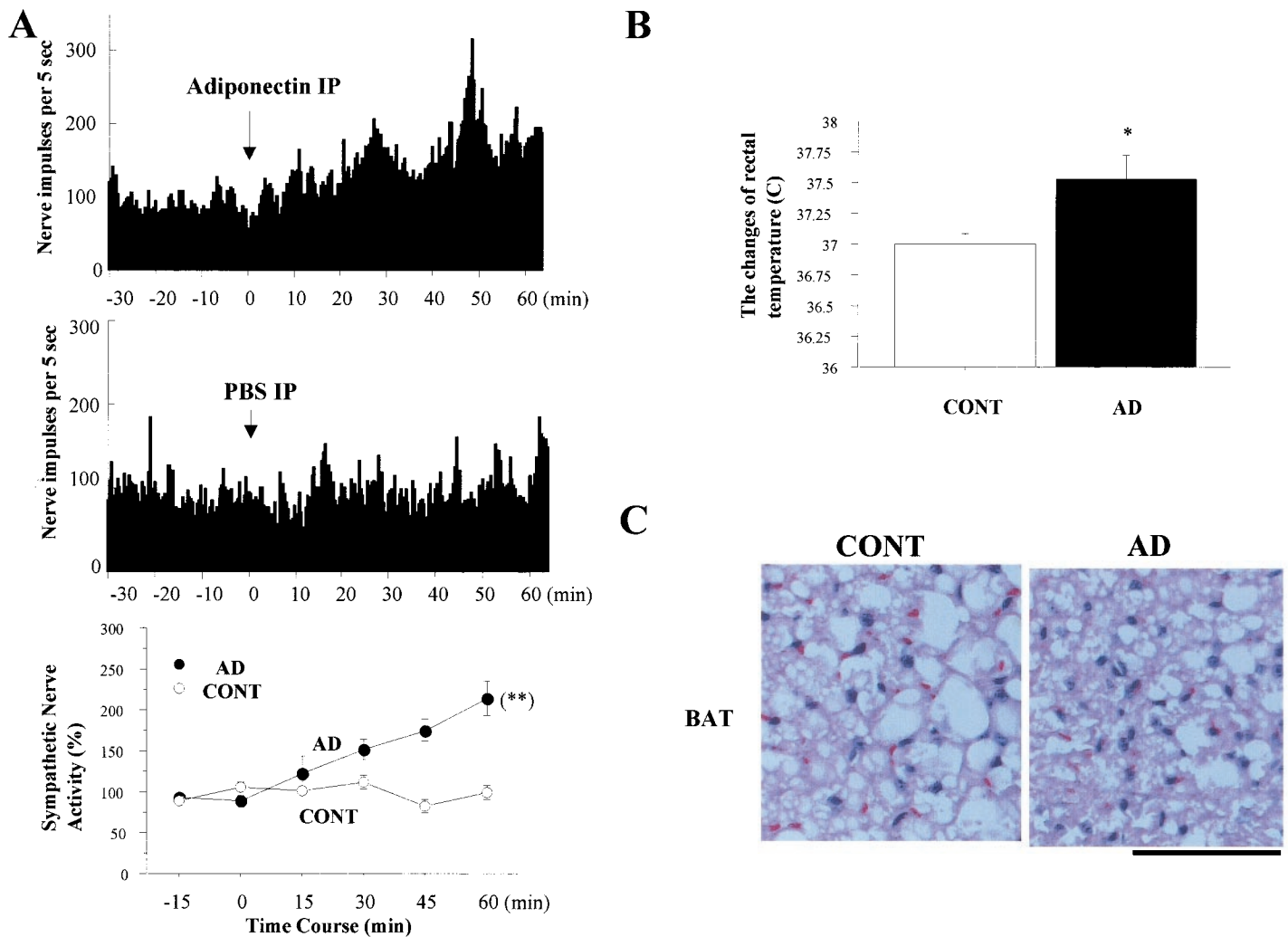


FIG. 2. Effects of adiponectin infusion on SNA (A) in BAT, rectal temperature (B), and histology of BAT (C) treated with either adiponectin (AD) or PBS (control [CONT]). Changes in discharge of sympathetic nerve after intraperitoneal infusion of adiponectin. Vertical axis, nerve impulses per 5 s. Horizontal bars, scale for 10 min. Each value is expressed as percentage of PBS controls with statistical significance marked in parentheses. * $P < 0.05$, ** $P < 0.01$ vs. the corresponding PBS controls. Scale bar denotes 100 μm .

$\mu\text{g}/\text{kg}$, 1.5 ± 0.2 g; and 1.5 mg/kg, 0.3 ± 0.1 g; i.p.: correlation = 0.83, $P < 0.01$).

Effects of adiponectin on serum glucose, insulin, and triglyceride. Adiponectin treatment (1.5 mg/kg i.p. for 7 days) decreased serum glucose (137.8 ± 29.9 vs. 231.5 ± 31.8 mg/dl), insulin (2.2 ± 0.8 vs. 7.3 ± 1.5 ng/dl), and triglyceride (119.3 ± 11.2 vs. 194.5 ± 26.4 mg/dl) levels compared with PBS-treated A^y/a mice (all $P < 0.05$).

Effects of adiponectin on body fat accumulation and triglyceride content in BAT, WAT, and liver. Adiponectin treatment showed a decrease in the weight of visceral WAT including epididymal, retroperitoneal, and mesenteric fat tissue compared with the control mice ($P < 0.01$) but no change in the weight of kidney or heart (Fig. 1C). Adiponectin treatment also decreased triglyceride content of the retroperitoneal WAT (23.7 ± 4.8 vs. 47.6 ± 4.4 mg/dl) (Fig. 1D) and liver (21.6 ± 0.9 vs. 34.2 ± 2.5 mg/dl) as compared with the A^y/a control mice, respectively ($P < 0.01$). Histological analysis showed that adiponectin treatment decreased fat deposition in BAT, retroperitoneal WAT, and liver compared with A^y/a controls (Figs. 1E and 2C).

Effects of adiponectin on SNA in BAT and body temperature. Figure 2A shows the typical response of BAT SNA to the acute administration of adiponectin (1.5 mg/kg i.p., one injection). Adiponectin treatment increased BAT SNA immediately after administration. The changes in activity of intraperitoneal sympathetic efferent nerves innervating BAT following intraperitoneal infusion of adiponectin are shown in Fig. 2. Figure 2A illustrates a typical recording of the efferent nerve activity. After intraperitoneal infusion of adiponectin, sympathetic efferent nerve activity showed a sustained increase throughout the infusion. The percentage change from the baseline value of the nerve activity after the infusion is shown in Fig. 2A. The mean discharge rate was 90.1 ± 7.7 , 80.5 ± 8.2 , 125.1 ± 15.4 , 152.3 ± 7.5 , 170.1 ± 7.3 , and 217.5 ± 12.3 impulses/5 s at 15 min before; 0 immediately before; and 15, 30, 45, and 60 min after the infusion, respectively ($P < 0.01$) (Fig. 2A). PBS infusion did not show any remarkable change in sympathetic nerve activity ($P > 0.1$) (Fig. 2A). As shown in Fig. 2B, adiponectin treatment (1.5 mg/kg i.p., one injection) also increased rectal temperature by 0.3–0.5°C com-

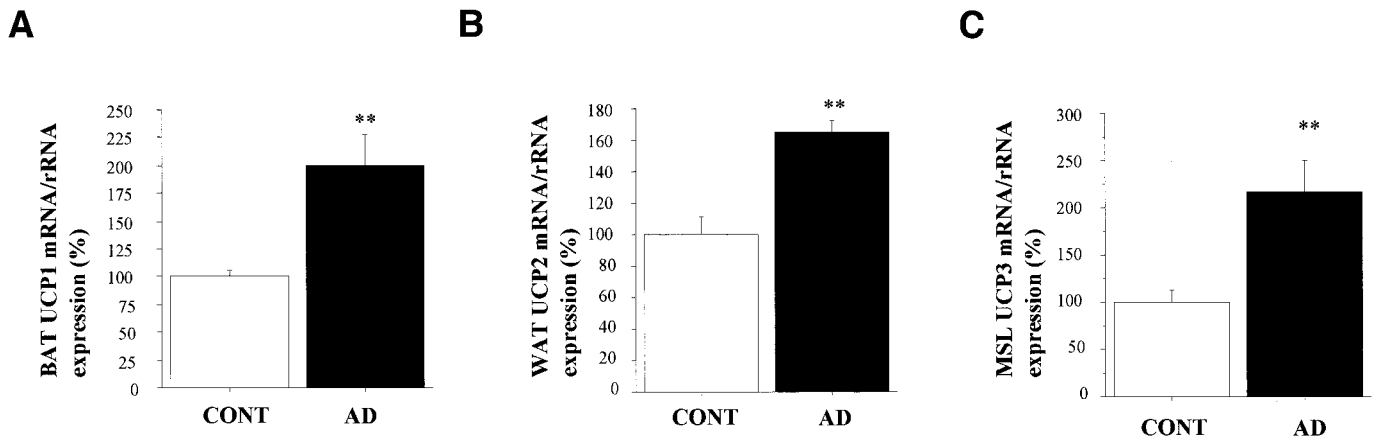


FIG. 3. Effects of intraperitoneal adiponectin infusion on UCP1 mRNA expression in BAT (A), UCP2 mRNA expression in WAT (B), UCP3 mRNA expression in skeletal muscle (MSL) (C) in $A^{y/a}$ obese mice treated with either adiponectin (AD) or PBS (control [CONT]). The columns represent the mean \pm SE with each value expressed as percentage of PBS controls. ** $P < 0.01$ vs. the corresponding PBS controls.

pared with $A^{y/a}$ controls 60 min after the infusion ($P < 0.05$).

Effects of adiponectin on BAT UCP1, WAT UCP2, and skeletal muscle UCP3 mRNA expression. Figure 3A shows the change in BAT UCP1 mRNA levels after treatment with adiponectin (1.5 mg/kg i.p. for 7 days). BAT UCP1 mRNA expression was increased by 98% after treatment with adiponectin compared with the $A^{y/a}$ controls ($P < 0.05$) (Fig. 3A). WAT UCP2 mRNA expression and skeletal muscle UCP3 mRNA expression were also increased by 68 and 125%, respectively, after treatment with adiponectin compared with PBS-treated $A^{y/a}$ mice ($P < 0.01$ for each) (Fig. 3).

Effect of central administration of adiponectin on food intake, body weight, UCP mRNA expression, and BAT SNA hypothalamic *c-fos*-like immunoreactivity. Compared with the controls, food intake, body weight, and UCP mRNA expression were not significantly changed by the ICV application of adiponectin (0, 150 ng/kg, 1.5 μ g/kg, and 15 μ g/kg ICV for 7 days). Figure 4A and B shows the changes in food intake and body weight in response to 7 days central administration of adiponectin (15 μ g/kg ICV for 7 days). Figure 4C shows changes in BAT SNA in response to the acute central administration of adiponectin (15 μ g/kg ICV, one injection). BAT SNA showed no remarkable changes after administration of adiponectin ($P > 0.1$) (Fig. 4C). The central administration of adiponectin did not induce expression of *c-fos*-like immunoreactivity in discrete hypothalamic regions (cell counts; PVN: 25 ± 8 vs. 30 ± 12 vs. ICV group 25 ± 8 , VMH: 10 ± 4 vs. 8 ± 3 vs. ICV group 10 ± 4 , ARC: 8 ± 2 vs. 7 ± 3 vs. ICV group, and LH: 6 ± 2 vs. 5 ± 2 for all treated versus controls, $P > 0.1$). Figure 4D shows an example of a *c-fos*-like immunoreactivity study indicating no remarkable change in *c-fos*-like immunoreactivity expression in the PVN after adiponectin treatment (15 μ g/kg ICV, one injection) (Fig. 4E).

DISCUSSION

In agreement with previous studies (14,15), the present study demonstrated that the peripheral administration of adiponectin attenuated body weight gain and reduced body adiposity. The present study indicates that the adi-

ponectin-induced attenuation of body weight gain may be the result of a reduction in visceral adiposity, since the weight of other organs, such as heart and kidney, have shown no remarkable change after treatment. The histological analysis also showed a reduction in fat deposition in visceral WAT and liver after adiponectin treatment. It is well established that visceral fat plays a key role in the pathogenesis of obesity-related metabolic disorders (37). In fact, reflecting the reduction in visceral adiposity, hyperglycemia and hyperinsulinemia in $A^{y/a}$ obese mice were partially improved by adiponectin treatment. Taken together, previous studies and the present results indicate that the reduction of visceral fat by adiponectin may contribute to the normalization of these metabolic disorders.

Another interesting point of the present study is that adiponectin treatment affects body weight and adiposity without influencing food intake. From the viewpoint of energy balance, we must focus on the effect of adiponectin on energy expenditure. It is well known that BAT plays an important role in thermogenesis and energy expenditure through the function of UCP1 (38–41). The physiological functions of BAT and gene expression of UCP1 are under the control of the sympathetic nervous system and hypothalamus (29–31). In the $A^{y/a}$ obese mice, brown adipocytes showed hypertrophy reflecting increased fat deposition. These morphologic changes of brown adipocytes in obese animals may induce disturbance of BAT function to regulate energy expenditure and thermogenesis. In fact, body temperature and/or mRNA expressions of BAT UCP1 in obese mice were lower than that in their lean littermates (40,41). A previous study demonstrated that transgenic mice with a BAT deficiency due to UCP1 promoter-driven diphtheria toxin A expression develop obesity that is initially due to decreased energy expenditure (42). In the present study, peripheral adiponectin treatment induced an increase in BAT SNA and BAT UCP1 mRNA expression accompanied by morphologic changes of brown adipocytes, such as reduced fat deposition. In addition, our unpublished observations (T.Y. and H.Y.) demonstrate that similar changes of BAT SNA by adiponectin are observed in both lean and obese models. Rectal temperature was also elevated by adiponectin

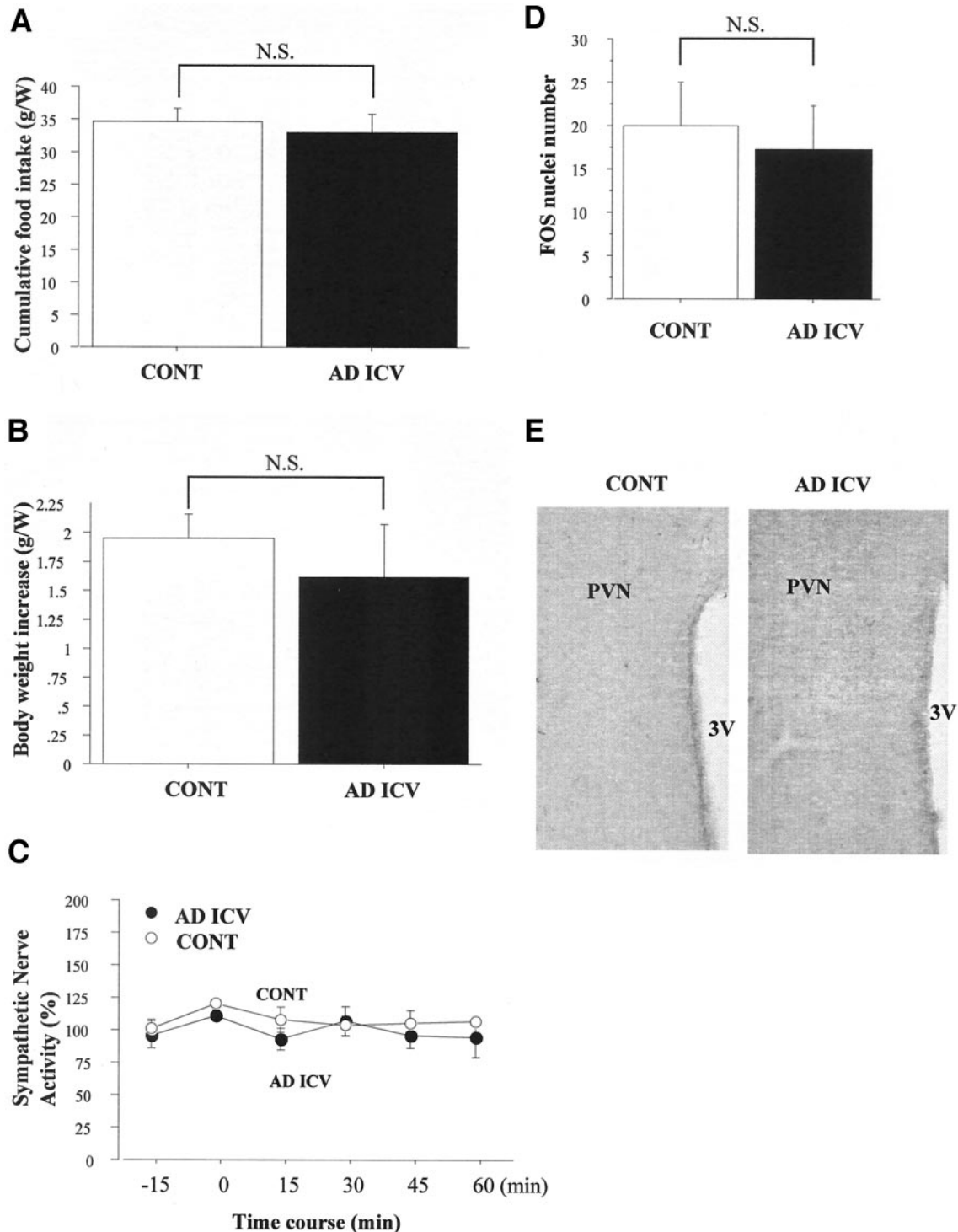


FIG. 4. Central effects of adiponectin infusion on cumulative food intake (A), body weight change (B), SNA in BAT (C), and hypothalamic *c-fos*-like immunoreactivity (D) in *A^{y/a}* obese mice treated with either ICV adiponectin (AD ICV) or PBS (control [CONT]). E: Representative photomicrographs of *c-fos*-like immunoreactivity in PVN. N.S., not significant vs. the corresponding PBS controls.

treatment, indicating acceleration of BAT thermogenesis. These findings indicate that adiponectin may enhance energy expenditure by an increase in BAT SNA and consequent upregulation of BAT UCP1. Thus, it is likely that the combined effects of these morphologic and functional changes of brown adipocytes induced by adiponectin treatment may have a protective effect against the development of obesity in *A^{y/a}* mice.

In the present study, adiponectin treatment increased UCP2 mRNA expression in WAT, in agreement with a previous report (14), and increased UCP3 mRNA expression in skeletal muscle. Adiponectin treatment increased BAT SNA in the present study. Since both WAT UCP2 and skeletal muscle UCP3 mRNA expression are influenced by sympathetic nerve activity (43), it is probable that adiponectin accelerates WAT UCP2 and skeletal muscle

UCP3 expression through activation of sympathetic nerve, innervating both tissues. However, the functional roles of adiponectin-induced WAT UCP2 and skeletal muscle UCP3 are still unknown, since there is little evidence supporting energy expenditure functions for UCP2 and UCP3.

The present study showed that peripheral administration of adiponectin failed to affect food intake, although it clearly changed adiposity in peripheral tissue. Since adiponectin has a high molecular weight (1–4,11), it is expected that it cannot cross the blood-brain barrier under physiological conditions. This may be one of the reasons why peripheral treatment with adiponectin cannot induce central action, such as the regulation of food intake. Leptin, another fat tissue-derived regulator for energy metabolism, regulates both food intake and energy expenditure through action in the hypothalamus (18,19). Therefore, the difference in the actions of adiponectin and leptin, especially the effect on food intake, led us to hypothesize that the target of adiponectin differs from that of leptin. To examine whether adiponectin regulates adiposity as well as energy expenditure by peripheral or central mechanisms, we analyzed the effect of ICV administration of adiponectin on food intake, body weight, BAT SNA, and expressions of BAT UCP1 mRNA and hypothalamic *c-fos*-like immunoreactivity. ICV administration of adiponectin induced no remarkable change in these parameters compared with the controls. These data indicate that adiponectin may regulate body weight, SNA, and UCP mRNA expression predominantly by acting directly on the peripheral mechanism. However, the possibilities that a smaller form, such as globular adiponectin, centrally affects energy metabolism or that the dose of adiponectin given was too low for central adiponectin effects cannot be excluded.

Next, we must discuss how adiponectin upregulates BAT SNA and UCP1, if this regulatory function does not originate from the hypothalamic neural circuit. It is possible that adiponectin may regulate BAT SNA through neuronal reflex loop from peripheral adiponectin sensor to sympathetic nerve. For example, an infusion of glucose into the hepatic portal vein increases efferent sympathetic nerve activity to BAT (44). If such a reflex pathway is involved in adiponectin signaling to regulate BAT SNA, several factors including peripheral sensor, afferent vagal nerve, relay neurons in the brain stem, and efferent sympathetic nerve may constitute this reflex loop. In any case, the mechanism needs further clarification and merits further study.

In summary, we demonstrated that administration of adiponectin attenuates body weight gain and decreases adiposity in *A^{y/a}* obese mice by affecting BAT SNA and UCP mRNA expression. It is suggested that the reduction in visceral fat mass, prevention of adipocyte hypertrophy, and acceleration of energy expenditure under the influence of adiponectin may contribute to the amelioration of obesity and insulin resistance.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid 10470233 from the Japanese Ministry of Education, Science and Culture; Research Grants for Intractable Diseases from the Japa-

nese Ministry of Health and Welfare, 1998–2000; and research grants from the Japanese Fisheries Agency for Research into Efficient Exploitation of Marine Products for Promotion of Health, 1998–2000.

REFERENCES

- Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K: cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant gene transcript 1). *Biochem Biophys Res Commun* 221:286–289, 1996
- Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996
- Sato C, Yasukawa Z, Honda N, Matsuda T, Kitajima K: Identification and adipocyte differentiation-dependent expression of the unique disialic acid residue in an adipose tissue-specific glycoprotein, adipo Q. *J Biol Chem* 276:28849–28856, 2001
- Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
- Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
- Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, Vettor R: Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. *Obes Res* 10:1095–1103, 2002
- Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
- Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, Olefsky JM: The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 51:2968–2974, 2002
- Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPAR γ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094–2099, 2001
- Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nat Med* 7:941–946, 2001
- Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001
- Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
- Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–1295, 2002
- Ma K, Cabrero A, Saha PK, Kojima H, Li L, Chang BH, Paul A, Chan L: Increased beta-oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin. *J Biol Chem* 277:34658–34661, 2002
- Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
- Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S,

- Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002
18. Inui A: Feeding and body-weight regulation by hypothalamic neuropeptides: mediation of the actions of leptin. *Trends Neurosci* 22:62–67, 1999
 19. Spiegelman BM, Flier JS: Obesity and the regulation of energy balance. *Cell* 104:531–543, 2001
 20. Nicholls DG, Locke RM: Thermogenic mechanism in brown fat. *Physiol Rev* 64:1–64, 1984
 21. Goubern M, Chapey MF, Senault C, Laury MC, Yazbeck J, Miroux B, Ricquier D, Portet R: Effect of sympathetic de-activation on thermogenic function and membrane lipid composition in mitochondria of brown adipose tissue. *Biochim Biophys Acta* 1107:159–164, 1992
 22. 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
 23. 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
 24. 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
 25. Gong DW, He Y, Karas M, Reitman M: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J Biol Chem* 272:24129–24132, 1997
 26. 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 proteins. *Diabetes* 47:1014–1019, 1998
 27. 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
 28. 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
 29. Scarpace PJ, Matheny M: Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *Am J Physiol* 275:E259–E264, 1998
 30. Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T: Targeted disruption of histamine H₁-receptor attenuates regulatory effects of leptin on feeding, adiposity, and UCP family expression in mice. *Diabetes* 50:385–391, 2001
 31. Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T: Central infusion of histamine reduces fat accumulation and upregulates UCP family in leptin-resistant obese mice. *Diabetes* 50:376–384, 2001
 32. Egawa M, Yoshimatsu H, Bray GA: Effect of corticotropin releasing hormone and neuropeptide Y on electrophysiological activity of sympathetic nerves to interscapular brown adipose tissue. *Neuroscience* 34:771–775, 1990
 33. Egawa M, Yoshimatsu H, Bray GA: Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats. *Am J Physiol* 260:R328–R334, 1991
 34. Paxinos GT, Franklin KBJ: *The Mouse Brain in Stereotaxic Coordinates*. 2nd ed. San Diego, CA, Academic Press, 2001
 35. Watson PM, Commins SP, Beiler RJ, Hatcher HC, Gettys TW: Differential regulation of leptin expression and function in A/J vs. C57BL/6J mice during diet-induced obesity. *Am J Physiol* 279:E356–E365, 2000
 36. Tsuda K, Yoshimatsu H, Nijima A, Chiba S, Okeda T, Sakata T: Hypothalamic histamine neurons activate lipolysis in rat adipose tissue. *Exp Biol Med* 227:208–213, 2002
 37. Kaplan NM: The deadly quartet: upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med* 149:1514–1520, 1989
 38. Nedergaard J, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B: UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim Biophys Acta* 1504:82–106, 2001
 39. Ricquier D, Bouillaud F, Toumelin P, Mory G, Bazin R, Arch J, Penicaud L: Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue: evidence for a rapid beta-adrenoreceptor-mediated and transcriptionally regulated step during activation of thermogenesis. *J Biol Chem* 261:13905–13910, 1986
 40. Himms-Hagen J: Defective brown adipose tissue thermogenesis in obese mice. *Int J Obes* 9 (Suppl. 2):17–24, 1985
 41. Masaki T, Yoshimatsu H, Chiba S, Sakata T: Impaired response of UCP family to cold exposure in diabetic (*db/db*) mice. *Am J Physiol* 278:R494–R498, 2000
 42. Mantzoros CS, Frederich RC, Qu D, Lowell BB, Maratos-Flier E, Flier JS: Severe leptin resistance in brown fat-deficient uncoupling protein promoter-driven diphtheria toxin A mice despite suppression of hypothalamic neuropeptide Y and circulating corticosterone concentrations. *Diabetes* 47:230–238, 1998
 43. 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
 44. Sakaguchi T, Yamazaki M: Hepatic portal injection of glucose elevates efferent sympathetic discharges of interscapular brown adipose tissue. *Exp Neurol* 101:464–469, 1998