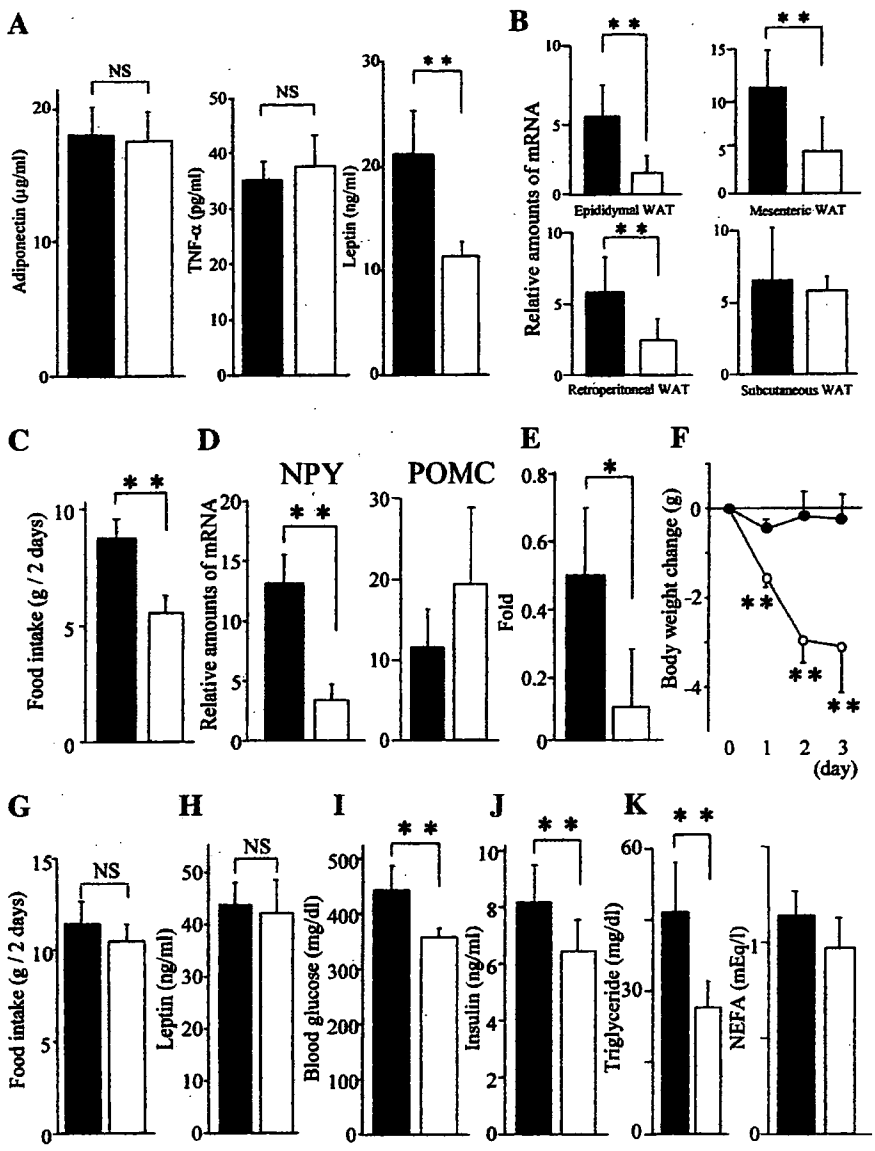

Figure 2. UCP1 expression in Epi improved leptin sensitivity

A–F) LacZ (black bars) or UCP1 (white bars) adenovirus was injected into Epi of mice with dietary obesity.

A) Serum adipocytokine levels (left: adiponectin, middle: TNFα, right: leptin) in LacZ mice and UCP1 mice after a 10 hr fast on day 3 after adenoviral administration.

B) Relative amounts of leptin mRNA in adipose tissues.

C) Total food intakes on days 2 and 3 after adenoviral administration.

D) Relative amounts of neuropeptide Y (left) and proopiomelanocortin (right) mRNA were measured by quantitative RT-PCR using total RNA obtained from the hypothalamus on day 2 after adenoviral administration. Data were corrected with β-actin as the standard (**B** and **D**).

E and F) Leptin-tolerance tests were performed on day 3 after adenoviral administration. Data were expressed as ratios to the food intakes of vehicle-treated mice (**E**). Mice were weighed at 12 hr after each daily injection of leptin or vehicle (**F**).

G–K) LacZ (black bars) or UCP1 (white bars) adenovirus was injected into Epi of db/db mice.

G) Total food intakes on days 2 and 3 after adenoviral administration are presented.

H–K) Blood leptin (**H**), glucose (**I**), and insulin (**J**) levels and serum lipid parameters ([**K**]; left: triglyceride, right: free fatty acids) of db/db mice were measured after a 10 hr fast. Data are presented as means ± SD (n = 8 per group). *$p < 0.05$; **$p < 0.01$ by unpaired t test.

leptin mRNA expression was markedly decreased in intra-abdominal fat tissues (Figure 2B). Thus, the effects of UCP1 expression in Epi are also exerted in fat tissues other than those injected with the adenovirus. Food intake was significantly suppressed (Figure 2C), indicating that hypothalamic leptin sensitivity was markedly improved despite the lack of significant changes in body weights. Decreased leptin expression in several adipose tissues suggests efferent sympathetic nerve activation, which also supports leptin signal enhancement.

Administration of green fluorescent protein-adenovirus exerted minimal metabolic effects (Figures S1F–S1J). On day 7, when adenoviral UCP1 expression was markedly decreased (Figure S1B), blood glucose, insulin, and leptin levels did not differ between the UCP1 and LacZ mice (Figure S2). In addition, we confirmed the metabolic effects of UCP1 expression in Epi using three other obese models: AKR mice on high-fat chow and KK mice and KK-Ay mice on normal chow. In these three models, similar metabolic impacts were observed with UCP1 adenovirus administration into Epi (Figure S3). Thus, UCP1 expression in Epi exerts acute, beneficial metabolic effects in both diet-induced and genetically obese models.

Increased leptin signals in the hypothalamus induced by UCP1 expression in Epi were further confirmed by changed levels of hypothalamic neuropeptide expression in UCP1 mice on day 3 after adenoviral administration. Real-time RT-PCR revealed adipose UCP1 expression to significantly decrease expression of neuropeptide Y, an orexigenic neuropeptide, while tending to increase that of proopiomelanocortin, a precursor of an anorexigenic neuropeptide, in the hypothalamus (Figure 2D).

To directly test whether leptin sensitivity was improved, we performed leptin-tolerance tests. When leptin was injected intraperitoneally into fasting mice on day 3, leptin-induced food-intake inhibition was far more profound in UCP1 mice than in LacZ mice (Figure 2E). In addition, when leptin was given daily, body weights were significantly decreased (Figure 2F). Thus,

even very limited UCP1 expression in Epi exerts a remote therapeutic effect on hypothalamic leptin resistance, which had already developed in response to preloading with high-fat chow. Transgenic overexpression of UCP1 (Kopecky et al., 1995) and rather minor induction of UCP1 in white adipose tissue (Cederberg et al., 2001; Leonardsson et al., 2004; Tsukiyama-Kohara et al., 2001; Um et al., 2004) result in resistance to high-fat-diet-induced obesity but do not reportedly cause hyperphagia. In this study, however, we expressed UCP1 after the development of obesity and leptin resistance and were thus able to observe acute, beneficial effects, i.e., improved leptin sensitivity, which would be difficult to detect using congenitally UCP1-overexpressing mice.

Increased leptin sensitivity is likely to be involved in the phenotype of UCP1 mice. If this is the case, at least some of the phenotypic features of UCP1 mice would presumably be absent in mice lacking the hypothalamic leptin signal. To test this, UCP1 or LacZ adenovirus was injected into Epi of db/db mice, leptin-receptor Ob-Rb mutants. Food intake (Figure 2G) and serum leptin (Figure 2H) did not differ between LacZ-expressing and UCP1-expressing db/db mice. These findings confirm that the effect of UCP1 expression in Epi on food intake is leptin-signal dependent. On the other hand, UCP1 expression in Epi of db/db mice caused small but significant decreases in blood glucose (Figure 2I), insulin (Figure 2J), and triglyceride (Figure 2K) levels, as well as tending to decrease serum free-fatty-acid levels (Figure 2K). These findings demonstrate that UCP1 expression in Epi improves insulin sensitivity, in part, independently of leptin signaling.

To eliminate the secondary effects of reduced food intake, pair-feeding experiments were performed using C57BL/6 wild-type mice (Figure S4). Pair feeding did not significantly alter the body weights of LacZ mice. Fasting blood glucose did not differ between UCP1 mice and pair-fed LacZ mice, but after glucose loading, blood glucose levels were significantly lower in UCP1 mice. In addition, serum insulin and leptin levels were significantly lower in UCP1 mice than in pair-fed LacZ mice. Taken together with the results obtained using db/db mice, the improved insulin sensitivity induced by UCP1 expression in Epi appears not to be mediated solely by decreased food intake.

The same amounts of recombinant adenovirus encoding UCP1 were directly injected into subcutaneous fat tissues in the flank of C57BL/6 mice with dietary obesity and diabetes. UCP1 expression levels were similar to those obtained by injection into Epi (data not shown). Food intake was significantly decreased by UCP1 expression, as compared with LacZ expression, in subcutaneous fat (Figure 3A), but the effects were much smaller than those produced by UCP1 expression in Epi (Figure 2C). Furthermore, there were no statistically significant decreases in blood glucose (Figure 3B), insulin (Figure 3C), or leptin (Figure 3D) levels. Thus, exogenous UCP1 expression in subcutaneous fat was far less effective in improving insulin and leptin resistance than that in intra-abdominal fat tissue. These findings suggest the anatomical location of the manipulated adipose tissue to be involved in the observed therapeutic effects, which would appear to be important for understanding the metabolic differences between visceral fat-dominant and subcutaneous fat-dominant obesity.

How does the signal (or signals) from intra-abdominal fat tissue exert these remote effects? The importance of anatomical fat-tissue location suggests the involvement of neuronal signaling. The afferent activity from Epi is reportedly transmitted through the nerve bundle, which runs alongside blood vessels supplying Epi, in rats (Niijima, 1998). To study the possible involvement of neuronal signals from Epi, we dissected this nerve bundle in mice with dietary obesity and diabetes. Ten days after bilateral nerve-bundle dissection, adenoviruses were injected into Epi. No significant differences in body weights or Epi weights were observed between sham-operated and nerve-dissected mice (data not shown). While UCP1 adenoviral administration significantly decreased food intake in sham-operated mice, nerve dissection blunted this decrease in food intake such that it was no longer statistically significant (Figure 3E). Similarly, nerve dissection blunted a decrease in hypothalamic NPY mRNA expression, rendering it statistically insignificant (NPY; LacZ versus UCP1: 12.06 ± 6.16 versus 6.39 ± 3.10; $p = 0.15$). These findings suggest that neuronal signals from intra-abdominal fat tissue are involved in food-intake regulation. In contrast, in nerve-dissected mice, blood glucose (Figure 3F) as well as serum insulin (Figure 3G) and leptin (Figure 3H) levels were significantly suppressed in a fashion similar to in sham-operated mice. Thus, improved insulin resistance is largely independent of this neuronal pathway.

To confirm that afferent-nerve signals are involved in UCP1-expression-mediated suppression of food intake, we next examined the effects of functional deafferentation by administering capsaicin (Fu et al., 2003), a selective neurotoxin for unmyelinated C fibers. In LacZ mice, food intake was not altered by capsaicin treatment 10 days prior to adenoviral administration. In contrast, capsaicin pretreatment significantly reversed the food-intake suppression induced by UCP1 expression in Epi (Figure 3I). The inhibitory effect of capsaicin pretreatment was very similar to that of local-nerve dissection (Figure 3E). Taken together, these observations suggest that afferent-nerve signals from Epi are involved in food-intake regulation. To elucidate the molecular mechanism whereby UCP1 expression in Epi modulates neuronal activity, we searched for genes upregulated by adipose UCP1 expression. Using the DNA microarray technique, gene expressions were examined in LacZ- and UCP1-adenovirus-treated Epi (Table S1) and in 3T3-L1 adipocytes (Table S2). With the exception of UCP1, however, there was no overlap in genes showing significantly increased expression. Although further expression profiling including proteomic approaches might elucidate the underlying mechanisms, the apparent lack of genes showing increased expression raises the possibility that the activation of afferent nerves does not involve gene-expression alterations. For instance, UCP1 generates heat, and a capsaicin receptor, TRPV1, is activated by a slightly above normal body temperature (Caterina et al., 1997). Capsaicin treatment affected UCP1-induced food-intake suppression (Figure 3I), raising the possibility that UCP1 expression activates capsaicin-sensitive nerves via TRPV1 activation. Another possibility is involvement of reactive oxygen species, which are affected by mitochondrial uncoupling (Bernal-Mizrachi et al., 2005; Jezek et al., 2004) and reportedly regulate capsaicin-sensitive afferent fibers (Ruan et al., 2005). Further studies are required to examine these hypotheses.

In this study, very limited UCP1 expression in Epi markedly improved insulin and leptin resistance, thereby improving glucose tolerance and decreasing food intake. UCP1 mice were more insulin sensitive than pair-fed LacZ mice. In addition, in db/db mice, despite no food-intake suppression, blood glucose

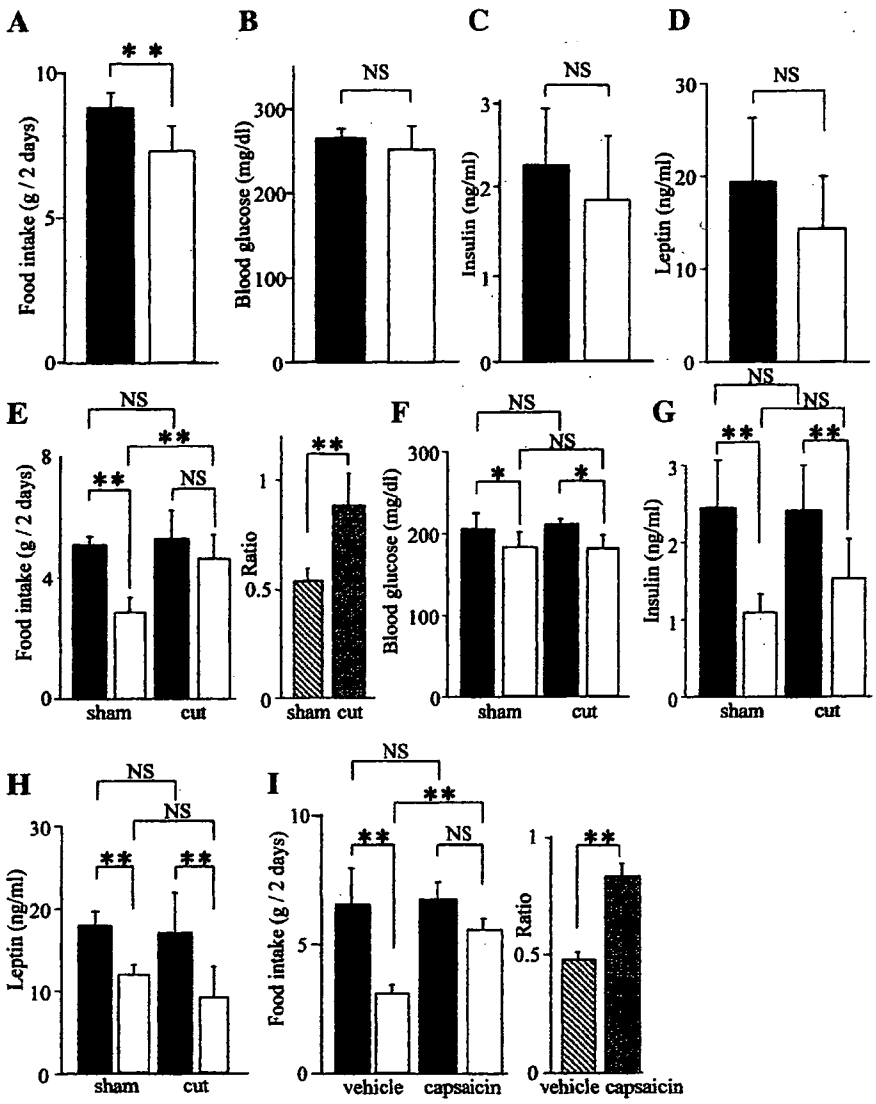

Figure 3. Neuronal signals are likely to be involved in food-intake regulation

A–D) LacZ (black bars) or UCP1 (white bars) adenovirus was injected into subcutaneous fat, and metabolic markers were measured. Total food intakes on days 2 and 3 after adenoviral administration are presented. Blood glucose (**B**), insulin (**C**), and leptin (**D**) levels were determined after a 10 hr fast on day 3 after adenoviral administration. $^{**}p < 0.01$ by unpaired t test.

E–H) Mice were subjected to local-nerve dissection 10 days prior to adenoviral injection into Epi. Total food intakes of sham-operated (sham) and nerve-dissected (cut) mice (**E**) on days 2 and 3 are presented graphically. Blood glucose (**F**), serum insulin (**G**), and leptin (**H**) levels were determined on day 3.

I) Mice were treated with capsaicin or vehicle 10 days prior to adenoviral injection into Epi. Total food intakes on days 2 and 3 after administration of LacZ (black bars) or UCP1 (white bars) adenovirus are presented. In (**E**) and (**I**), the food intakes of UCP1 mice are expressed in the right graph as ratios to those of LacZ mice. $^{**}p < 0.01$ assessed by one-factor ANOVA. Data are presented as means ± SD.

and insulin levels were modestly but significantly decreased by UCP1 expression in Epi. Thus, the mechanism underlying improved insulin sensitivity with UCP1 expression in Epi is, in part, independent of leptin signaling and food-intake suppression (Figure 4). Dissection of the nerve bundle from Epi did not alter the decreases in blood glucose and insulin levels. Taken together with the findings that UCP1 expression in subcutaneous fat did not significantly decrease blood glucose or insulin levels, our observations indicate that nonneuronal signals including humoral factors from intra-abdominal adipose tissue possibly participate in systemic improvement of insulin resistance. Since UCP1 expression was observed in a very limited population of adipocytes in Epi, suppression of insulin-resistant adipocytokine secretion is unlikely to explain the beneficial effects. Serum adiponectin levels were not altered, suggesting involvement of other unknown insulin-sensitizing factor (or factors).

On the other hand, decreased food intake is likely to be, at least partially, mediated by afferent-nerve signals from Epi (Figure 4). Afferent-nerve signals from Epi to the central nervous system reportedly result in a reflex from epididymal fat to white adipose tissues via efferent sympathetic-nerve activation (Niijima, 1998; Tanida et al., 2000). In addition, vagal afferent

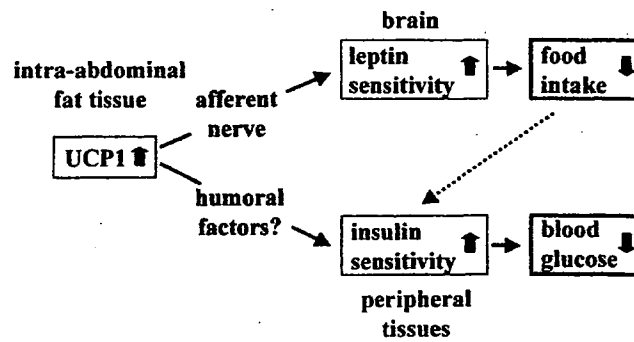

Figure 4. The proposed mechanism whereby UCP1 expression in Epi decreases food intake and improves glucose tolerance

neuronal signals from intra-abdominal tissues, including the gut (Fu et al., 2003; Smith et al., 1981) and the liver (Friedman, 1998; Scharrer, 1999), are known to play a part in regulating food intake. We also reported that UCP1 gene administration into the liver modulates food intake (Ishigaki et al., 2005). Herein we report that intra-abdominal fat tissue is likely to convey metabolic signals to the brain via a neuronal pathway, in addition to via the circulation, resulting in modulation of food intake. Although the precise molecular mechanism remains to be elucidated, this neuronal pathway might play a role in development of the metabolic syndrome, making it a potentially novel therapeutic target.

Experimental procedures

Preparation of recombinant adenovirus

Recombinant adenovirus containing murine UCP1 cDNA (Ishigaki et al., 2005) was constructed as described previously (Katagiri et al., 1996). Recombinant adenoviruses bearing the bacterial β-galactosidase gene (*Adex1CAlacZ*) and green fluorescent protein (AdCMV-GFP) were used as controls.

Animals and in vivo adenovirus injection into fat pad

Animal studies were conducted in accordance with the institutional guidelines for animal experiments at Tohoku University. Male C57BL/6N and AKR/N mice were housed individually, and high-fat-chow feeding (32% safflower oil, 33.1% casein, 17.6% sucrose, and 5.6% cellulose) (Ishigaki et al., 2005) was initiated at 5 weeks of age. After 4 weeks of high-fat-chow loading, body-weight-matched mice were anesthetized prior to dissection of the skin and body wall. The adenoviral preparation (1×10^8 plaque-forming units in a volume of 20 μl) was injected at two points each on each side of the epididymal fat pad or subcutaneous fat tissues in the flank, i.e., a total of four points. KK mice and KK-Ay mice maintained on a standard diet (65% carbohydrate, 4% fat, 24% protein) were similarly administered adenoviruses at 9 weeks and 5 weeks of age, respectively.

Immunoblotting

Tissue protein extracts (250 μg total protein) were boiled in Laemmli buffer containing 10 mM dithiothreitol, subjected to SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose filters. The filters were incubated with anti-UCP1 antibody (Santa Cruz Biotechnology, Santa Cruz, California) and then with anti-goat immunoglobulin G coupled to horseradish peroxidase. The immunoblots were visualized with an enhanced chemiluminescence detection kit (Amersham, Buckinghamshire, UK). The intensities of bands were quantified with the NIH Image 1.62 program.

Histological analysis

Mouse epididymal fat and BAT were immunostained as previously reported (Ishigaki et al., 2005). Mature white adipocytes were identified by their characteristic unilocular appearance. Diameters of 100 or more white adipocytes per mouse in each group were traced manually and analyzed.

Oxygen consumption

Oxygen consumption was measured as previously reported (Ishigaki et al., 2005).

Pair-feeding experiments

Pair-feeding experiments were performed as previously described (Ishigaki et al., 2005).

Blood analysis

Blood glucose and serum insulin, leptin, adiponectin, TNFα, total cholesterol, triglyceride, and free-fatty-acid levels were determined as previously described (Ishigaki et al., 2005).

Measurement of quantitative RT-PCR-based gene expression

The skull was reflected from the brain and the hypothalamus was isolated by snap freezing in liquid nitrogen as previously reported (Bjorbaek et al., 1998). Total RNA was isolated from mouse hypothalamus, fat tissues, or 3T3-L1 adipocytes with ISOGEN (Wako Pure Chemical Co., Osaka, Japan), and cDNA synthesized from total RNA was evaluated with a real-time PCR quantitative system (Light Cycler Quick System 350S; Roche Diagnostics GmbH, Mannheim, Germany).The relative amount of mRNA was calculated with β-actin mRNA as the invariant control. The primers used are shown in Table S3.

Glucose-, insulin-, and leptin-tolerance tests

Glucose-tolerance tests were performed on fasted (10 hr, daytime) mice. Mice were given glucose (2 g/kg of body weight) intraperitoneally, followed by measurement of blood glucose levels. Insulin-tolerance tests were performed on ad libitum-fed mice. Mice were intraperitoneally injected with human regular insulin (0.75 U/kg of body weight; Eli Lilly Co., Kobe, Japan).

Leptin-tolerance tests were carried out as described in a previous report (Igel et al., 1997), with slight modification. Fasted (12 hr) mice were injected with mouse leptin (7.2 mg/kg of body weight; R&D Systems, Inc.) intraperitoneally, and food intakes were monitored for 12 hr after the injection. To examine effects on body-weight change, these two groups of mice were given leptin daily starting on the day of adenoviral administration. Each mouse was then weighed.

Capsaicin treatments

Capsaicin treatment was performed as described in a previous report (Fu et al., 2003), with minor modification. Mice were anesthetized prior to subcutaneous injection of capsaicin solution (50 mg/kg, 12.5 mg/ml dissolved in vehicle). The control group received vehicle treatment (10% Tween 80, 10% ethanol, and 80% saline) under identical administration conditions. Adenoviral administration into Epi was carried out 10 days later.

Local-nerve dissection

The small nerve bundle which runs along side blood vessels supplying Epi was dissected as previously reported (Niijima, 1998). Ten days after bilateral dissection of this nerve bundle, adenoviruses were injected into epididymal fat pad.

Measurement of ATP

Fully differentiated 3T3-L1 adipocytes were infected with recombinant adenoviruses as previously described (Katagiri et al., 1996). Intracellular ATP levels were measured using an ATP determination kit (TOYO B-Net, Tokyo, Japan).

Microarray experiments

Total RNA from epididymal fat or 3T3-L1 adipocytes was used to synthesize cRNA, which was then hybridized to an HG-U133A oligonucleotide array (Affymetrix, Santa Clara, California) according to standard protocols, as described previously (Hippo et al., 2002).

Statistical analysis

All data were expressed as means ± SD. The statistical significance of differences was assessed by the unpaired t test and one-factor ANOVA.

Supplemental data

Supplemental Data include four figures and three tables and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/3/3/223/DC1/.

Acknowledgments

We appreciate Drs. L.P. Kozak (Pennington Biomedical Research Center) and H. Mizuguchi (National Institute of Biomedical Innovation) for the generous gifts of UCP1 cDNA and GFP-adenovirus, respectively. We thank Ms. H. Meguro (Tokyo University) for technical support. This work was supported by a Grant-in-Aid for Scientific Research (B2, 15390282) and a Grant-in-Aid for Exploratory Research (15659214) to H.K. from the Ministry of Education, Science, Sports and Culture of Japan and a Grant-in-Aid for Scientific Research (H16-genome-003) to Y.O. from the Ministry of Health, Labor and Welfare of Japan. This work was also supported by the 21st Century COE Programs "CRESCENDO" (H.K.) and "the Center for Innovative Therapeutic Development for Common Diseases" (Y.O.) of the Ministry of Education, Science, Sports and Culture.

Received: June 22, 2005
Revised: October 12, 2005
Accepted: February 1, 2006
Published: March 7, 2006

References

Bernal-Mizrachi, C., Gates, A.C., Weng, S., Imamura, T., Knutsen, R.H., DeSantis, P., Coleman, T., Townsend, R.R., Muglia, L.J., and Semenkovich, C.F. (2005). Vascular respiratory uncoupling increases blood pressure and atherosclerosis. Nature *435*, 502–506.

Bjorbaek, C., Elmquist, J.K., Frantz, J.D., Shoelson, S.E., and Flier, J.S. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. Mol. Cell *1*, 619–625.

Bjorntorp, P. (1992). Abdominal fat distribution and disease: an overview of epidemiological data. Ann. Med. *24*, 15–18.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature *389*, 816–824.

Cederberg, A., Gronning, L.M., Ahren, B., Tasken, K., Carlsson, P., and Enerback, S. (2001). FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell *106*, 563–573.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., Bauer, T.L., et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. *334*, 292–295.

Flier, J.S. (2004). Obesity wars: molecular progress confronts an expanding epidemic. Cell *116*, 337–350.

Friedman, J.M. (2003). A war on obesity, not the obese. Science *299*, 856–858.

Friedman, J.M., and Halaas, J.L. (1998). Leptin and the regulation of body weight in mammals. Nature *395*, 763–770.

Friedman, M.I. (1998). Fuel partitioning and food intake. Am. J. Clin. Nutr. *67*, 513S–518S.

Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., and Piomelli, D. (2003). Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature *425*, 90–93.

Heymsfield, S.B., Greenberg, A.S., Fujioka, K., Dixon, R.M., Kushner, R., Hunt, T., Lubina, J.A., Patane, J., Self, B., Hunt, P., and McCamish, M. (1999). Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA *282*, 1568–1575.

Hippo, Y., Taniguchi, H., Tsutsumi, S., Machida, N., Chong, J.M., Fukayama, M., Kodama, T., and Aburatani, H. (2002). Global gene expression analysis of gastric cancer by oligonucleotide microarrays. Cancer Res. *62*, 233–240.

Igel, M., Becker, W., Herberg, L., and Joost, H.G. (1997). Hyperleptinemia, leptin resistance, and polymorphic leptin receptor in the New Zealand obese mouse. Endocrinology *138*, 4234–4239.

Ishigaki, Y., Katagiri, H., Yamada, T., Ogihara, T., Imai, J., Uno, K., Hasegawa, Y., Gao, J., Ishihara, H., Shimosegawa, T., et al. (2005). Dissipating excess energy stored in the liver is a potential treatment strategy for diabetes associated with obesity. Diabetes *54*, 322–332.

Jezek, P., Zackova, M., Ruzicka, M., Skobisova, E., and Jaburek, M. (2004). Mitochondrial uncoupling proteins—facts and fantasies. Physiol. Res. *53* Suppl. 1, S199–S211.

Katagiri, H., Asano, T., Ishihara, H., Inukai, K., Shibasaki, Y., Kikuchi, M., Yazaki, Y., and Oka, Y. (1996). Overexpression of catalytic subunit p110alpha of phosphatidylinositol 3-kinase increases glucose transport activity with translocation of glucose transporters in 3T3-L1 adipocytes. J. Biol. Chem. *271*, 16987–16990.

Klingenberg, M., and Huang, S.G. (1999). Structure and function of the uncoupling protein from brown adipose tissue. Biochim. Biophys. Acta *1415*, 271–296.

Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., and Kozak, L.P. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. J. Clin. Invest. *96*, 2914–2923.

Leonardsson, G., Steel, J.H., Christian, M., Pocock, V., Milligan, S., Bell, J., So, P.W., Medina-Gomez, G., Vidal-Puig, A., White, R., and Parker, M.G. (2004). Nuclear receptor corepressor RIP140 regulates fat accumulation. Proc. Natl. Acad. Sci. USA *101*, 8437–8442.

Matsuzawa, Y., Shimomura, I., Nakamura, T., Keno, Y., and Tokunaga, K. (1995). Pathophysiology and pathogenesis of visceral fat obesity. Ann. N Y Acad. Sci. *748*, 399–406.

Niijima, A. (1998). Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. J. Auton. Nerv. Syst. *73*, 19–25.

Ruan, T., Lin, Y.S., Lin, K.S., and Kou, Y.R. (2005). Sensory transduction of pulmonary reactive oxygen species by capsaicin-sensitive vagal lung afferent fibres in rats. J. Physiol. *565*, 563–578.

Scharrer, E. (1999). Control of food intake by fatty acid oxidation and ketogenesis. Nutrition *15*, 704–714.

Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., and Simansky, K.J. (1981). Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science *213*, 1036–1037.

Tanida, M., Iwashita, S., Ootsuka, Y., Terui, N., and Suzuki, M. (2000). Leptin injection into white adipose tissue elevates renal sympathetic nerve activity dose-dependently through the afferent nerves pathway in rats. Neurosci. Lett. *293*, 107–110.

Tsukiyama-Kohara, K., Poulin, F., Kohara, M., DeMaria, C.T., Cheng, A., Wu, Z., Gingras, A.C., Katsume, A., Elchebly, M., Spiegelman, B.M., et al. (2001). Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat. Med. *7*, 1128–1132.

Um, S.H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P.R., Kozma, S.C., Auwerx, J., and Thomas, G. (2004). Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature *431*, 200–205.

Neuronal Pathway from the Liver Modulates Energy Expenditure and Systemic Insulin Sensitivity

Kenji Uno,[1,2*] Hideki Katagiri,[2*†] Tetsuya Yamada,[1*] Yasushi Ishigaki,[1] Takehide Ogihara,[2] Junta Imai,[1,2] Yutaka Hasegawa,[1,2] Junhong Gao,[1,2] Keizo Kaneko,[1,2] Hiroko Iwasaki,[2] Hisamitsu Ishihara,[1] Hironobu Sasano,[3] Kouichi Inukai,[4] Hiroyuki Mizuguchi,[5] Tomoichiro Asano,[6] Masakazu Shiota,[7] Masamitsu Nakazato,[8] Yoshitomo Oka[1]

Coordinated control of energy metabolism and glucose homeostasis requires communication between organs and tissues. We identified a neuronal pathway that participates in the cross talk between the liver and adipose tissue. By studying a mouse model, we showed that adenovirus-mediated expression of peroxisome proliferator–activated receptor (PPAR)–γ2 in the liver induces acute hepatic steatosis while markedly decreasing peripheral adiposity. These changes were accompanied by increased energy expenditure and improved systemic insulin sensitivity. Hepatic vagotomy and selective afferent blockage of the hepatic vagus revealed that the effects on peripheral tissues involve the afferent vagal nerve. Furthermore, an antidiabetic thiazolidinedione, a PPARγ agonist, enhanced this pathway. This neuronal pathway from the liver may function to protect against metabolic perturbation induced by excessive energy storage.

The incidence of obesity, insulin resistance, hyperlipidemia, and hypertension, collectively referred to as the metabolic syndrome, is increasing at an alarming rate in Western cultures (*1*). Secreted humoral factors, including leptin (*2*), convey information about energy storage from adipose tissue to the central nervous system (CNS). As in adipose tissues, fat storage in the liver is dynamically changed by overall energy balance, but our understanding of how the liver transmits metabolic signals to other tissues remains incomplete. Studies of mouse models created by tissue-specific genetic engineering (*3*, *4*) or adenoviral gene transfer (*5*, *6*) have shown the importance of cross talk between tissues in the regulation of energy metabolism. Mice with tissue-specific knockout of peroxisome proliferator–activated receptor γ (PPARγ) provide an example of such intertissue communication (*7*). PPARγ activates genes involved in lipid storage and metabolism (*8*). Although PPARγ expression in the liver is low compared with that in adipose tissues (*9*), hepatic expression of PPARγ (*10*, *11*), especially that of PPARγ2 (*12*), is functionally enhanced in a number of obesity models. In addition, liver-specific disruption of PPARγ in obese (ob/ob) mice prevents hepatic steatosis but increases peripheral adiposity and decreases insulin sensitivity in muscle and fat (*13*). Thus, hepatic PPARγ2 plays important roles not only in the development of liver steatosis but also in the regulation of peripheral lipid storage and insulin sensitivity.

To investigate the mechanism by which hepatic PPARγ2 expression affects metabolism in peripheral tissues, we overexpressed PPARγ2 in the livers of C57BL/6 mice using adenoviral gene transfer. After being fed a high-fat diet for 4 weeks, the mice developed obesity-associated diabetes (*14*). The PPARγ2 adenovirus vector was then administered intravenously to mice (PPARγ2 mice). Control mice given the LacZ adenovirus (LacZ mice) showed no alterations in blood glucose levels, food intake, or plasma lipid parameters after virus administration (*14*). Systemic infusion of the PPARγ2 adenovirus into mice resulted in expression of the transgene primarily in the liver (Fig. 1A), without increased expression in peripheral tissues, including white adipose tissue (WAT) (Fig. 1B).

The livers of PPARγ2 mice were pale and enlarged as compared with those of control mice (fig. S1A). Liver weights were significantly increased (Fig. 1C) because of increased triglyceride content (Fig. 1D). Histological analysis of PPARγ2 mice revealed an abundance of large lipid droplets in the livers, without apparent inflammation or structural change (fig. S1B). Thus, hepatic PPARγ2 expression induced severe hepatic steatosis. Hepatic PPARγ2 expression enhanced the expression of lipogenesis-related genes (fig. S2), suggesting that increased uptake and synthesis of fatty acids induce severe steatosis.

In contrast, WAT in PPARγ2 mice was notably diminished in size (fig. S1A); for example, epididymal fat weight was decreased by 46.6% in PPARγ2 mice versus controls (Fig. 1E). Cell diameters in WAT and brown adipose tissue (BAT) were also markedly decreased in PPARγ2 mice (fig. S1C). The increases in body weights induced by a high-fat diet were suppressed in PPARγ2 mice (Fig. 1F). Resting oxygen consumption was increased by 29.4% in PPARγ2 mice (Fig. 1G), whereas food intake did not differ from that of LacZ mice (Fig. 1H).

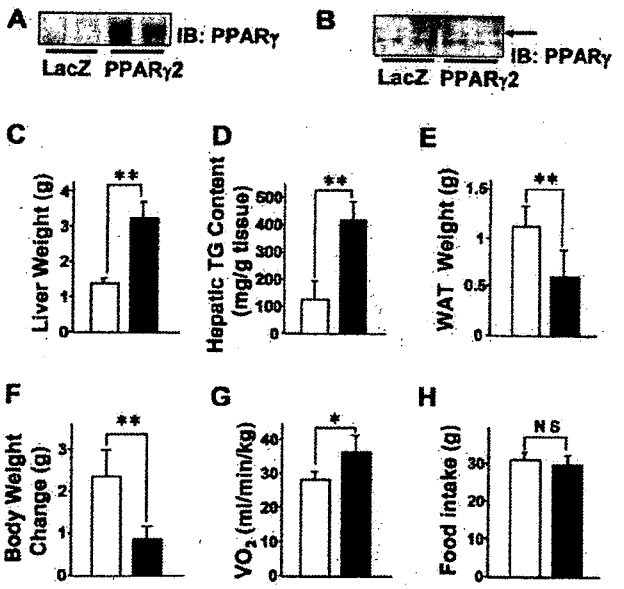

Fig. 1. Hepatic PPARγ2 expression aggravates hepatic steatosis but diminishes peripheral adiposity. (**A** and **B**) Immunoblotting (IB) with an antibody to PPARγ of liver (A) and epididymal fat (B) extracts. Liver weight (**C**), triglyceride (TG) content (**D**), and epididymal fat tissue (WAT) weights (**E**) are shown. Experiments in (A) to (E) were performed on day 7 after adenoviral administration. (**F**) Body weight changes during the 7 days after adenoviral administration. (**G**) Resting oxygen consumption (VO_2) was measured on day 3 after adenoviral injection. (**H**) Total food intake was measured for 7 days after adenoviral administration. In (C) to (H), white and black bars indicate results from LacZ mice and PPARγ2 mice, respectively. Significance as compared to LacZ mice is indicated (**$P < 0.01$ and *$P < 0.05$) by an unpaired t test. NS, not significant.

[1]Division of Molecular Metabolism and Diabetes, [2]Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research, [3]Department of Pathology, Tohoku University Graduate School of Medicine, Sendai 980–8575, Japan. [4]The Fourth Department of Internal Medicine, Saitama Medical School, Moroyama, Iruma-gun, Saitama 350–0495, Japan. [5]Laboratory of Gene Transfer and Regulation, National Institute of Biomedical Innovation, Osaka 567–0085, Japan. [6]Department of Physiological Chemistry and Metabolism, University of Tokyo, Tokyo 113–8655, Japan. [7]Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN 37232, USA. [8]Third Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki 889–1692, Japan.

*These authors contributed equally to this work.
†To whom correspondence should be addressed E-mail: katagiri@mail.tains.tohoku.ac.jp

Thus, hepatic PPARγ2 expression increased systemic energy expenditure, thereby suppressing high-fat diet–induced weight gain.

Control mice were hyperglycemic, hyperinsulinemic, and hyperleptinemic in response to a 5-week-long high-fat diet. Hepatic PPARγ2 expression decreased fasting blood glucose and insulin levels (Fig. 2A), indicating markedly improved systemic insulin sensitivity. As shown in Fig. 2B, PPARγ2 mice also showed a 79% reduction in serum leptin levels. Although serum adiponectin levels were similar to those in control mice, tumor necrosis factor–α (TNF-α) levels were significantly decreased in PPARγ2 mice. These findings are consistent with a reduction in peripheral adiposity.

Glucose tolerance (Fig. 2C) and insulin tolerance (Fig. 2D) tests showed that hepatic expression of PPARγ2 markedly improved insulin sensitivity and glucose tolerance. Furthermore, improved insulin sensitivity in muscle (fig. S3A) and epididymal fat tissue (fig. S3B) was confirmed by enhanced tyrosine phosphorylation of the insulin receptor and insulin receptor substrate–1 in response to insulin administration. Thus, hepatic PPARγ2 expression clearly exerts remote beneficial effects on insulin sensitivity in muscle and WAT. Although insulin sensitivity in the liver was impaired (fig. S3C), hepatic PPARγ coactivator (PGC)–1α and hepatic phosphoenolpyruvate carboxykinase (PEPCK) expression was decreased (Fig. 2E), suggesting decreased hepatic glucose output.

To further examine insulin sensitivity and endogenous glucose production in PPARγ2 mice, we performed hyperinsulinemic euglycemic clamp experiments. Basal glucose production in PPARγ2 mice was decreased by 22% as compared with that in LacZ mice, whereas insulin's ability to suppress endogenous glucose production was severely blunted in PPARγ2 mice (Fig. 2F). In addition, glucose infusion rates in PPARγ2 mice were markedly increased (Fig. 2G). Thus, hepatic PPARγ2 expression improved insulin sensitivity in the periphery and decreased glucose output from the liver despite hepatic insulin resistance.

Serum free–fatty-acid (FFA) levels were markedly increased in PPARγ2 mice (fig. S4A), suggesting that hepatic PPARγ2 expression promotes hydrolysis of triglycerides stored in adipose tissues. Increased expression levels of the uncoupling protein (UCP)–1 (*15*), PGC1α (*16*), and hormone-sensitive lipase (*17*) in BAT (fig. S4B) and WAT (fig. S4C) indicate high tonus of the sympathetic nerves innervating these adipose tissues. In addition, the administration of bupranorol, a pan–β-adrenergic blocker (*18*), decreased serum FFA in PPARγ2 mice but had no effect in LacZ mice (fig. S4D), confirming that the β-adrenergic nerve function enhances lipolysis in adipose tissues of PPARγ2 mice.

To examine whether afferent nerves originating in the liver mediate the remote effects, we dissected the hepatic branch of the vagus nerve. Seven days after selective hepatic vagotomy (HV), we administered recombinant adenovirus encoding LacZ or PPARγ2 to mice. Hepatic PPARγ2 expression similarly altered liver weights, hepatic triglyceride content, and PEPCK expression in mice subjected to HV and sham operation (SO) (Table 1). In contrast, selective HV completely blocked the decreases in WAT weights and brown adipocyte size as well as the increases in serum FFA, resting oxygen consumption, and WAT UCP1 expression in PPARγ2 mice (Table 1), indicating that the hepatic vagus mediates the remote effects of hepatic PPARγ2 expression.

HV involves dissection of both afferent and efferent vagal branches innervating the liver. To determine whether the remote effects of hepatic PPARγ2 expression are mediated by the afferent vagus, we applied a specific afferent neurotoxin, capsaicin, to the hepatic branch of the vagus of diet-induced obese male Sprague-Dawley (SD) rats. Seven days after perivagal application of capsaicin or vehicle, we administered recombinant adenovirus encoding LacZ or PPARγ2. Expression of calcitonin gene–related peptide, a sensory neuropeptide, was markedly decreased in the capsaicin-treated vagal nerve, whereas immunoreactivity for S100 proteins was similar in vehicle- and capsaicin-treated nerves (fig. S5A). Furthermore, transmission electron microscopic analyses (fig. S5B) revealed selective degradation of unmyelinated fibers in the vagal hepatic branch. In addition, application of capsaicin to this branch did not affect the esophageal branch of the posterior vagal trunk (fig. S5). These observations indicate selective deafferentation of the hepatic branch of the vagus. Under these conditions, perivagal capsaicin treatment completely blocked the hepatic PPARγ2 expression–induced decrease in WAT weight (Table 1). When taken together, these findings strongly suggest that afferent vagal nerve ac-

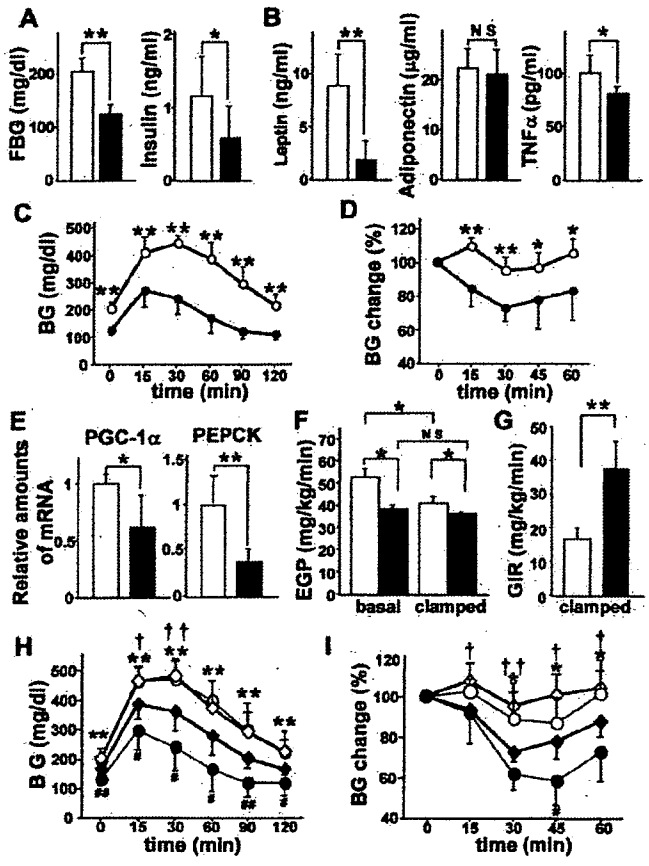

Fig. 2. Hepatic PPARγ2 expression improves peripheral insulin resistance. Fasting blood glucose (FBG) and serum insulin (**A**) and adipocytokines (**B**) were measured in LacZ mice (white bars) and PPARγ2 mice (black bars) on day 7 after adenoviral administration. These serum parameters were measured after a 10-hour fast. (**C** and **D**) LacZ mice (open circles) and PPARγ2 mice (solid circles) were subjected to glucose tolerance (C) and insulin tolerance (D) tests. BG, blood glucose. (**E**) Relative amounts of PGC-1α and PEPCK mRNA in the liver were measured by quantitative reverse transcriptase polymerase chain reaction. (**F** and **G**) Metabolic variables during hyperinsulinemic euglycemic clamp. Endogenous glucose production (EGP) in basal and clamped states (F) and rates of glucose infusion (GIR) were required to maintain euglycemia during the clamp study (G). Experiments in (A) to (G) were performed on day 7 after adenoviral administration. In (A), (B), and (E) to (G), white and black bars indicate results from LacZ mice and PPARγ2 mice, respectively. Significance as compared to LacZ mice is indicated (**$P < 0.01$ and *$P < 0.05$) by an unpaired t test. NS, not significant. (**H** and **I**) HV or SO was performed 7 days before the administration of LacZ or PPARγ2 adenovirus. Mice were subjected to glucose tolerance (H) and insulin tolerance (I) tests on day 7 after adenoviral administration. Open and solid circles indicate SO LacZ mice and SO PPARγ2 mice, respectively. Open and solid diamonds indicate HV LacZ mice and HV PPARγ2 mice, respectively. Data are presented as mean ± SD. **($P < 0.01$) and *($P < 0.05$) indicate significance in SO LacZ mice versus SO PPARγ2 mice, ††($P < 0.01$) and †($P < 0.05$) indicate significance in HV LacZ mice versus HV PPARγ2 mice, and ##($P < 0.01$) and #($P < 0.05$) indicate significance in HV PPARγ2 mice versus SO PPARγ2 mice, by unpaired t tests.

tivation originating in the liver mediates the remote effects of hepatic PPARγ2 expression on peripheral lipolysis.

We next examined the effects of HV on glucose (Fig. 2H) and insulin (Fig. 2I) tolerance test results in PPARγ2 mice. In SO mice, glucose tolerance and insulin sensitivity were improved by hepatic PPARγ2 expression, but these improvements were partially suppressed by hepatic branch vagotomy. These findings suggest that hepatic PPARγ2 expression improved glucose tolerance and systemic insulin sensitivity via both improved peripheral insulin sensitivity and decreased hepatic glucose output; the former requires afferent vagal and efferent sympathetic nerves, whereas the latter does not.

Next, to determine whether the neuronal system, consisting of afferent vagal and efferent sympathetic nerves, functions in the physiological setting of enhanced endogenous PPARγ2 expression in the liver, we examined the effects of an antidiabetic thiazolidinedione (TZD, a PPARγ agonist) using db/db mice, which are a murine model of genetic obesity and diabetes. In db/db mice, endogenous expression of PPARγ2, at both the mRNA (Fig. 3A) and the protein (fig. S6A) levels, is markedly enhanced in the liver. To eliminate the secondary effects of body weight changes, troglitazone, a TZD derivative, was given to db/db mice for 2 days, followed by an evaluation of acute effects. The TZD administration did not alter body weights (Fig. 3B) but did increase resting oxygen consumption (Fig. 3C) and UCP1 expression in BAT (Fig. 3D) and WAT (Fig. 3E), suggesting activation of sympathetic nerves to BAT and WAT. Dissection of the hepatic branch of the vagus 7 days before TZD administration reversed the increases in resting oxygen consumption (Fig. 3C) as well as UCP1 expression in BAT (Fig. 3D) and WAT (Fig. 3E). These findings indicate that the neuronal pathway originating in the liver is also involved in the acute systemic effects of TZDs, under conditions in which hepatic PPARγ expression is upregulated, such as in obese subjects.

To further examine whether endogenous PPARγ in the liver affects energy metabolism, we knocked down hepatic PPARγ in db/db mice. Administration of recombinant adenovirus expressing short hairpin RNA for PPARγ (*19*) 7 days before TZD treatment substantially decreased endogenous PPARγ expression in the liver (fig. S6B) as well as hepatic triglyceride content (fig. S6C) and sterol regulatory element binding protein–1c expression (fig. S6D), indicating functional knockdown of hepatic PPARγ (*20*). Under these conditions, TZD-enhanced energy expenditure was partially but significantly suppressed (fig. S6E). Thus, endogenous PPARγ in the liver regulates acute energy metabolism in vivo. TZD treatment reportedly alleviates insulin resistance in adipose-tissue–ablated mice (*10*) and adipose-specific-PPARγ–deficient mice (*21*), which may involve the aforementioned hepatic-PPARγ–induced neuronal activation in addition to a muscle PPARγ contribution (*22*).

We have shown that a neuronal pathway, consisting of the afferent vagus from the liver and efferent sympathetic nerves to adipose tissues, is involved in the regulation of energy expenditure, systemic insulin sensitivity, glucose metabolism, and fat distribution between the liver and the periphery. Because hepatic PPARγ expression is physiologically associated with obesity, the liver may convey information regarding excess energy balance to the CNS via the afferent vagus. This neuronal system may underlie chronic adaptive thermogenesis, resulting in protection against metabolic perturbation induced by excessive energy storage. There are two avenues of communication between the

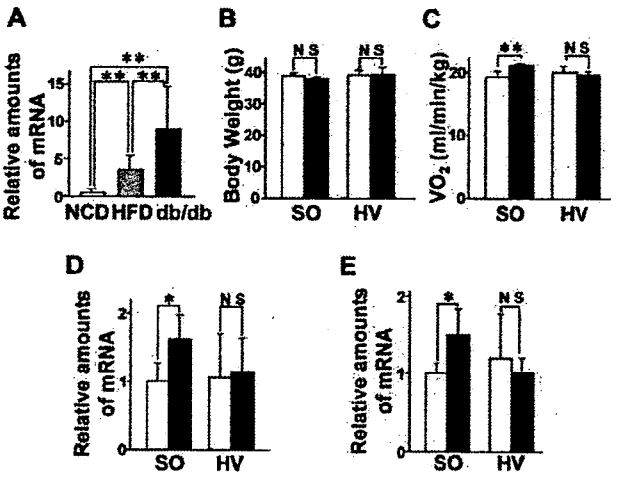

Fig. 3. HV inhibits TZD-enhanced energy expenditure in obese mice. (**A**) Relative amounts of PPARγ2 mRNA in the livers of normal chow diet–fed (NCD) C57BL/6 mice, high-fat diet–fed (HFD) C57BL/6 mice, and normal chow diet–fed db/db (db/db) mice. (**B** to **E**) db/db mice were subjected to HV or SO 7 days before the 2-day administration of TZD (black bars) or vehicle (white bars), after which body weights (B) and resting oxygen consumptions (C) were measured. Relative amounts of UCP1 mRNA in BAT (D) and epididymal fat tissue (E) from mice fed ad libitum. Data are presented as mean ± SD. Significance as compared to control mice is indicated (**$P < 0.01$ and *$P < 0.05$) by an unpaired t test. NS, not significant.

Table 1. Afferent vagal activation from the liver is involved in remote effects of hepatic PPARγ2 expression. (Upper section) Mice were subjected to HV or SO 7 days before administration of LacZ or PPARγ2 adenovirus. Resting oxygen consumption (VO_2) was measured on day 3 after adenoviral injection. Mice were killed after a 10-hour fast on day 7 after adenoviral injection. (Lower section) Male SD rats with high-fat diet–induced obesity were subjected to application of capsaicin or vehicle to the vagal hepatic branch 7 days before administration of LacZ or PPARγ2 adenovirus. Seven days after adenoviral administration, epididymal fat weights were determined. Significance as compared to LacZ mice is indicated (P values) by an unpaired t test. LW, liver weight; HTG, hepatic TG content; P, PEPCK; CD, cell diameter; NS, not significant.

	SO			HV		
	LacZ	PPARγ2	P	LacZ	PPARγ2	P
LW (g)	1.11 ± 0.13	2.30 ± 0.39	<0.001	1.12 ± 0.07	2.07 ± 0.32	<0.001
HTG (mg/g tissue)	78.71 ± 46.50	171.26 ± 43.90	0.008	62.02 ± 24.92	215.09 ± 75.78	<0.001
P mRNA (liver)	1.00 ± 0.21	0.50 ± 0.17	0.003	1.356 ± 0.460	0.54 ± 0.22	0.002
WAT weight (g)	1.13 ± 0.13	0.85 ± 0.14	<0.001	1.04 ± 0.26	1.06 ± 0.19	NS
BAT CD (μm)	11.55 ± 4.45	7.69 ± 2.09	<0.001	10.63 ± 3.38	10.55 ± 3.93	NS
FFA (μEq/l)	556.14 ± 87.33	860.47 ± 206.04	0.005	533.14 ± 59.50	558.38 ± 151.58	NS
VO_2 (ml/min/kg)	30.25 ± 2.38	34.38 ± 3.03	0.015	32.73 ± 4.54	31.98 ± 4.05	NS
UCP1 mRNA (WAT)	1.00 ± 0.24	2.36 ± 0.77	0.019	2.05 ± 0.64	1.82 ± 1.15	NS
	Vehicle			Capsaicin		
WAT weight (g)	8.95 ± 0.99	7.06 ± 1.32	0.024	8.70 ± 1.14	8.85 ± 1.71	NS

brain and other tissues: humoral factors and neuronal pathways. Leptin, a humoral factor from adipocytes, is a mediator of metabolic information from adipose tissue to the hypothalamus (*2*). In addition, circulating nutrients reportedly affect food intake and alter hepatic glucose production via the efferent vagal pathway (*23*, *24*). An afferent vagal signal originating in the liver is likely to be another metabolic information pathway. In this way, the brain may integrate information obtained from several tissues and organs via both humoral and neuronal pathways. When the brain receives information regarding excess energy storage, the sympathetic nervous system is activated to enhance energy expenditure and lipolysis, thereby maintaining energy homeostasis. Disturbance of the control system is implicated in the development of the metabolic syndrome (*25*). Targeting of this neuronal pathway is a potential therapeutic strategy for treating the metabolic syndrome.

References and Notes

1. J. S. Flier, *Cell* **116**, 337 (2004).
2. J. M. Friedman, J. L. Halaas, *Nature* **395**, 763 (1998).
3. Y. Minokoshi, C. R. Kahn, B. B. Kahn, *J. Biol. Chem.* **278**, 33609 (2003).
4. T. Kitamura, C. R. Kahn, D. Accili, *Annu. Rev. Physiol.* **65**, 313 (2003).
5. J. An *et al.*, *Nat. Med.* **10**, 268 (2004).
6. T. Yamada *et al.*, *Cell Metab.* **3**, 223 (2006).
7. O. Gavrilova *et al.*, *J. Biol. Chem.* **278**, 34268 (2003).
8. V. Bocher, I. Pineda-Torra, J. C. Fruchart, B. Staels, *Ann. N.Y. Acad. Sci.* **967**, 7 (2002).
9. L. Fajas *et al.*, *J. Biol. Chem.* **272**, 18779 (1997).
10. C. F. Burant *et al.*, *J. Clin. Invest.* **100**, 2900 (1997).
11. L. Chao *et al.*, *J. Clin. Invest.* **106**, 1221 (2000).
12. R. Rahimian *et al.*, *Mol. Cell. Biochem.* **224**, 29 (2001).
13. K. Matsusue *et al.*, *J. Clin. Invest.* **111**, 737 (2003).
14. Y. Ishigaki *et al.*, *Diabetes* **54**, 322 (2005).
15. I. Nagase *et al.*, *J. Clin. Invest.* **97**, 2898 (1996).
16. J. Gomez-Ambrosi, G. Fruhbeck, J. A. Martinez, *Mol. Cell. Endocrinol.* **176**, 85 (2001).
17. Y. Hatakeyama, Y. Sakata, S. Takakura, T. Manda, S. Mutoh, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R336 (2004).
18. A. Weilstein, D. Palm, G. G. Belz, *J. Cardiovasc. Pharmacol.* **8** (suppl. 11), S36 (1986).
19. T. Hosono *et al.*, *Gene* **348**, 157 (2005).
20. S. Herzig *et al.*, *Nature* **426**, 190 (2003).
21. W. He *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15712 (2003).
22. A. L. Hevener *et al.*, *Nat. Med.* **9**, 1491 (2003).
23. A. Pocai, S. Obici, G. J. Schwartz, L. Rossetti, *Cell Metab.* **1**, 53 (2005).
24. T. K. Lam *et al.*, *Nat. Med.* **11**, 320 (2005).
25. M. W. Schwartz, D. Porte Jr., *Science* **307**, 375 (2005).

26. We thank M. Kaji, T. Takai, Y. Sato, H. Yawo, T. Hashikawa, M. Kanzaki, Y. Minokoshi, and M. Tominaga for advice and discussions. Supported by grants-in-aid from the Ministry of Education, Science, Sports, and Culture of Japan (H.K.), a grant-in-aid from the Ministry of Health, Labor, and Welfare of Japan (Y.O.), and the 21st Century Center of Excellence Programs (H.K. and Y.O.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5780/1656/DC1
Materials and Methods
SOM Text
Figs. S1 to S6
Table S1
References

9 February 2006; accepted 8 May 2006
10.1126/science.1126010

Synaptic Amplifier of Inflammatory Pain in the Spinal Dorsal Horn

Hiroshi Ikeda,* Johanna Stark, Harald Fischer, Matthias Wagner, Ruth Drdla, Tino Jäger, Jürgen Sandkühler†

Inflammation and trauma lead to enhanced pain sensitivity (hyperalgesia), which is in part due to altered sensory processing in the spinal cord. The synaptic hypothesis of hyperalgesia, which postulates that hyperalgesia is induced by the activity-dependent long-term potentiation (LTP) in the spinal cord, has been challenged, because in previous studies of pain pathways, LTP was experimentally induced by nerve stimulation at high frequencies (~100 hertz). This does not, however, resemble the real low-frequency afferent barrage that occurs during inflammation. We identified a synaptic amplifier at the origin of an ascending pain pathway that is switched-on by low-level activity in nociceptive nerve fibers. This model integrates known signal transduction pathways of hyperalgesia without contradiction.

Inflammation of peripheral tissues causes spontaneous pain and hyperalgesia. Amplification of pain-related information in the spinal dorsal horn lamina I contributes to inflammatory pain (*1*–*6*). Inflammation causes release of neuromodulators, including substance P and glutamate in spinal dorsal horn (*7*, *8*), potentially leading to Ca^{2+}-dependent LTP. In all previous studies, spinal LTP was induced by brief (1 s), high-frequency (100 Hz) burstlike stimulation (HFS) of afferent nerve fibers. High-frequency bursts do not, however, resemble the continuous low-frequency afferent barrage that occurs during inflammation. Low-frequency presynaptic activity normally fails to induce LTP but rather induces synaptic long-term depression (LTD) (*9*). The LTP model of inflammatory hyperalgesia thus may be questioned. Here, we evaluated the effect of low-frequency afferent barrage on synaptic transmission in ascending pain pathways and asked if synaptic plasticity is differentially induced in distinct ascending pain tracts. We labeled lamina I projection neurons by retrograde fluorescent marker DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate), injected into either of two major projection areas of spinal lamina I neurons: the parabrachial (PB) area or the periaqueductal gray (PAG) (*10*, *11*) (Fig. 1, A and B). To circumvent confounding developmental factors, we used only juvenile or adult rats in this study. Transverse spinal cord slices with long dorsal roots attached were prepared 3 to 4 days after DiI injections to allow whole-cell recordings from identified projection neurons in 21- to 28-day-old rats (*10*). In the presence of tetrodotoxin, bath application of substance P (2 μM) induced transient inward currents in 21 out of 27 spino-PB and in 9 out of 12 spino-PAG neurons (Fig. 1C), confirming the expression of functional neurokinin 1 receptors (NK1Rs). Spinal release of substance P following electrical stimulation of primary afferents at C-fiber strength was assessed by the internalization of NK1R in lamina I neurons. HFS parameters (100-Hz bursts) similar to all previously used conditioning stimulation protocols to induce classical LTP in pain pathways, or low-frequency stimulation (LFS, 2 Hz), was used. Both types of stimulation elicited substantial NK1R internalization in 89 ± 1% and in 78 ± 4% of 150 neurons evaluated in three rats per group (Fig. 1D). We then used these stimulation protocols for conditioning.

Conditioning HFS induces LTP at synapses between C-fibers and lamina I neurons that project to the PB (*12*). We confirmed these results by showing LTP of monosynaptically evoked excitatory postsynaptic currents (EPSCs) to 172 ± 15% of the control value at 30 min after conditioning (n = 8) (Fig. 2A). However, conditioning electrical stimulation within the typical frequency band of C-fibers during inflammation (2 Hz) (*13*) did not change synaptic strength in any of the spino-PB neurons tested (108 ± 19% of control, n = 7) (Fig. 2C). LFS, however, did modify synaptic strength in spinal lamina I neurons with a projection to the PAG. In all spino-PAG neurons tested, LFS induced a robust LTP of monosynaptic C-fiber–evoked EPSCs [to 262 ± 30% of the control value at 30 min after stimulation (n = 18) and to 346 ± 33% at 60 min (n = 8)] (Fig. 2D). In all seven lamina I neurons with a projection to the PAG, conditioning stimulation at high frequency was ineffective (98 ± 10%, n = 7) (Fig. 2B). Monosynaptic, A-fiber–evoked

Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, Vienna, Austria.

*Present address: Department of Human and Artificial Intelligence Systems, University of Fukui, 3-9-1 Bunkyo, Fukui 910-8507, Japan.

†To whom correspondence should be addressed. E-mail: juergen.sandkuehler@meduniwien.ac.at

This Review is part of a thematic series on **Adipocyte Signaling in the Cardiovascular System**, which includes the following articles:

Adipose Tissue, Inflammation, and Cardiovascular Disease
Adipocyte Signaling and Lipid Homeostasis: Sequelae of Insulin Resistant Adipose Tissue
Diabetic Cardiomyopathy: The Search for a Unifying Hypothesis

Adiposity and Cardiovascular Disorders: Disturbance of the Regulatory System Consisting of Humoral and Neuronal Signals

PPARγ Activation and the Effects on the Vasculature

Philipp Scherer, Guest Editor

Adiposity and Cardiovascular Disorders

Disturbance of the Regulatory System Consisting of Humoral and Neuronal Signals

Hideki Katagiri, Tetsuya Yamada, Yoshitomo Oka

Abstract—Obesity, a major healthcare issue, is associated with significant cardiovascular morbidities, including hypertension and atherosclerosis. Numerous intensive studies conducted this decade have revealed that adipose tissue is a major endocrine organ that secretes a variety of bioactive substances, termed adipocytokines. Adipocytokine secretion profiles are altered as obesity develops, which may increase the risk of obesity-related cardiovascular disorders. For instance, leptin is upregulated in obese subjects and plays important roles in the pathophysiology of obesity-related atherogenesis through multiple mechanisms, such as its proliferative, proinflammatory, prothrombotic, and prooxidant actions. In contrast, adiponectin, which is downregulated in obese subjects, has protective effects against cardiovascular disorders at various atherogenic stages. In addition to these factors secreted by adipose tissue, neuronal circuits involving autonomic nerves are now being recognized as an important metabolic regulatory system and have thus attracted considerable attentions. Alterations in fat accumulation in intraabdominal organs, such as visceral adipose tissue and the liver, send afferent neuronal signals to the brain, leading to modulation of sympathetic tonus and thereby affecting the vasculature. Moreover, these humoral and neuronal signaling pathways communicate with each other, resulting in cooperative metabolic regulation among tissues/organs throughout the body. Further elucidation of these regulatory systems is anticipated to lead to new approaches to devising therapeutic strategies for the metabolic syndrome. (***Circ Res.*** **2007;101:27-39.**)

Key Words: adipocytokines ■ autonomic nervous system ■ metabolic syndrome ■ atherosclerosis ■ hypertension

Excess food intake and physical inactivity underlie the growing worldwide epidemic of obesity, not only in the industrialized nations but also in developing countries. A variety of common disorders, eg, hyperglycemia, hyperlipidemia, and hypertension, are common in obese individuals.[1,2] Such disorders are not clustered coincidently, and intraabdominal visceral adiposity has been suggested to play a fundamental role in the simultaneous development of these

Original received March 5, 2007; revision received May 4, 2007; accepted May 16, 2007.

From the Division of Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research (H.K.); and Division of Molecular Metabolism and Diabetes (T.Y., Y.O.), Tohoku University Graduate School of Medicine, Sendai, Japan.

Correspondence to Hideki Katagiri, MD, PhD, Division of Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. E-mail katagiri@mail.tains.tohoku.ac.jp

Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/CIRCRESAHA.107.151621**

disorders,[3] collectively termed the metabolic syndrome.[4] In addition, one of the major challenges of this syndrome is the high prevalence of cardiovascular diseases arising from atherosclerosis.

Visceral fat accumulation may be directly associated with the development of cardiovascular disease. Epidemiological studies have suggested that visceral adiposity, as evaluated by the waist-to-hip ratio[5] or computed tomography scanning,[6] is related to coronary artery disease independently of body mass index. Recent intensive studies have revealed that humoral factors secreted by adipose tissue contribute to the development of the metabolic syndrome and vascular diseases.

Adipose tissues were long regarded as nothing more than passive fuel storage sites. However, recent studies have revealed that adipocytes, as well as other cells within fat tissues, release numerous biologically active substances, termed adipocytokines, leading to the concept of adipose tissue as a versatile endocrine gland. Obesity, especially visceral fat accumulation, alters adipocytokine secretion profiles, and obesity-related disorders are now recognized as a state of adipose tissue dysfunction. Cardiovascular morbidity in obese individuals might be explained by adipocytokine secretion profile alterations, which result mainly from enlargement of adipocytes and proinflammatory changes in adipose tissue. In addition, recent studies, including ours, have revealed that adiposity in intraabdominal tissues, such as the liver and visceral adipose tissues, directly influences the autonomic nervous system, and thereby modulates sympathetic tonus.

The present review focuses on the effects of different adipocytokines on vascular functions. In addition, we further discuss intertissue communication of metabolic information via the autonomic nervous system in obesity-related disorders.

Humoral Factors Involved in Metabolic Regulation

Humoral Factors Derived From Adipose Tissue

Adipocytes produce and secrete a number of bioactive substances, including polypeptides and nonprotein factors that are known to exert a wide variety of effects on glucose and lipid metabolism, energy homeostasis, and cardiovascular function, among others. These substances, collectively called adipocytokines, include leptin, adiponectin, resistin, angiotensinogen, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor (PAI)-1, visfatin, retinol-binding protein (RBP)4, fatty acids, sex steroids, and various growth factors. Insulin resistance is an important factor in the development of coronary heart disease, as evidenced by studies in both animal models and humans. Adipocytokines act synergistically or competitively with insulin. Therefore, these factors directly or indirectly affect vascular function and have the potential to provide useful insights into the pathogenesis of vascular disease.

Here we present the current understanding of the complex roles of adipocyte-derived hormones, in particular leptin and adiponectin, in endothelial cell function and the pathogenesis of atherosclerotic vascular disease (Figure 1).

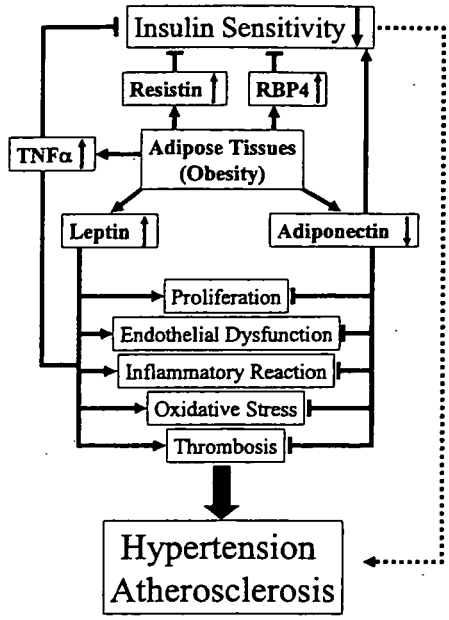

Figure 1. Adipocytokines interact in a complex way to regulate vascular function and ultimately the development of cardiovascular diseases.

Leptin

Leptin was identified by positional cloning in the *ob/ob* mouse model[7] as a key molecule in the regulation of body weight and energy balance. Leptin is a 167-aa secreted protein encoded by the *ob* gene. Leptin is mainly produced and secreted by adipocytes. Leptin acts on the hypothalamus, altering energy intake by decreasing appetite and increasing energy expenditure via sympathetic stimulation of several tissues.[8] Adipocyte leptin expression is transcriptionally regulated, as determined mainly by the status of the energy stores in white adipose tissue and the size of adipocyte sizes. Thus, leptin plays versatile role in maintaining energy homeostasis by communicating information regarding the energy-storage status of adipose tissue to the brain. For instance, with increasing energy storage, the energy balance is negatively regulated by decreased food intake and increased energy expenditure.[9]

Leptin receptors were first isolated from the mouse choroid plexus by expression cloning[10] but are also present in several other tissues, including the hypothalamus. Positional cloning of the *db* locus encoding leptin receptors revealed at least 6 alternatively spliced forms, leptin receptor (Ob-R)a through Ob-Rf. Among these receptor isoforms, Ob-Rb, also termed the long isoform, is highly expressed in the hypothalamus and mediates the anorectic effect of leptin. Ob-Rb contains the longest intracellular domain, which, on ligand binding, activates protein tyrosine kinases of the Janus kinase family–signal transducers and activators of transcription (JAK-STAT) pathway. Other short isoforms, including Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf, do not activate the JAK-STAT pathway.[9] Subsequent research demonstrated that the effects of leptin are not restricted to the energy balance. The long form Ob-Rb is expressed throughout the body and has also been detected in endothelial cells.[11] Leptin is a pleiotropic molecule with a wide range of biological actions, including

reproductive functions, regulating the hypothalamic–pituitary–adrenal axis, glucose and insulin metabolism, lipolysis, immune responses, hematopoiesis, and angiogenesis.

Leptin and the Vasculature

Several reports have suggested either a vasodilatory or vasoconstrictive action of leptin, which would be direct on the vascular wall. First, the vasodilatory action of leptin is supported by experimental results showing that endothelial-dependent vasorelaxant responses to acetylcholine are markedly impaired in microvessels from leptin-deficient *ob/ob* mice and that leptin restoration reverses the endothelial dysfunction observed in these mice.[12] Leptin has been shown to promote nitric oxide (NO) release from the vascular endothelium, thereby potentially decreasing blood pressure.[13,14] However, in these reports, decreased blood pressure in response to leptin treatment was observed in only sympathectomized rats. In addition, systemic leptin administration does not attenuate the renal and hindlimb vasoconstriction resulting from sympathetic nerve stimulation.[15] These findings suggest that the NO-dependent vasodilatory effects of leptin are insufficient to counter sympathetically mediated vasoconstriction. Furthermore, in vitro treatment of human umbilical vein endothelial cells (HUVECs) with leptin induced endothelin-1, known to be a potent vasoconstrictor.[16] Thus, although high concentrations of leptin may exert vasodilatory effects, the exact vasodilatory actions of leptin remain uncertain.

On the other hand, considerable evidence obtained from animal studies indicates that leptin may modulate arterial pressure through sympathetic mechanisms. In rats, acute intravenous[8] and intracerebroventricular[17] administration of leptin has been shown to increase sympathetic nerve signals to brown adipose tissue, kidneys, adrenal glands, and hindlimbs. Chronic intracarotid[18] and intracerebroventricular[19] administration of leptin also raises blood pressure in rats. Transgenic mice overexpressing leptin in the liver develop hypertension, which is reversed by α_1-adrenergic, β-adrenergic, or ganglionic blockers.[20] Furthermore, despite severe obesity, leptin-deficient *ob/ob* mice have lower blood pressure than lean controls,[21] whereas administering exogenous leptin to *ob/ob* mice raises blood pressure to the levels of lean controls.[20] Thus, leptin has unequivocal sympathoexcitatory actions in rodents. In humans as well, there is a positive relationship between mean blood pressure and serum leptin levels in lean subjects with essential hypertension.[22] In human subjects with widely differing degrees of adiposity, renal norepinephrine spillover correlates with plasma leptin concentrations after adjusting for adiposity,[23] whereas giving leptin to lean subjects for 6 days had no impact on norepinephrine, dopamine, or epinephrine levels in 24-hour urine samples.[24] Further studies are needed to obtain conclusive evidence of the sympathoexcitatory effects of leptin on blood pressure in humans.

Leptin Resistance and Hypertension

Obese subjects remain hyperphagic despite their high circulating leptin levels, indicating hypothalamic insensitivity to leptin, a state termed leptin resistance. This was confirmed by clinical trials in which leptin given to obese patients produced only modest effects on body weight.[25] However, despite severe leptin resistance, the sympathoexcitatory effect of leptin, as evaluated by neurography of renal sympathetic nerves, is reportedly preserved after either systemic or central neural administration of leptin.[26]

In mice with dietary obesity, food intake suppression and body weight gain induced by intraperitoneal or intracerebroventricular leptin were significantly attenuated, whereas the renal sympathoexcitatory response to leptin was preserved, leading to substantially elevated arterial pressure. The leptin-dependent increases in arterial pressure were of similar magnitude in mice fed either a high-fat diet or normal chow.[27] These findings led to the notion of selective leptin resistance in which, despite resistance to the anorexigenic effect of leptin, sympathetic nerves are normally activated in response to leptin. In human subjects, there is a strong correlation between leptin plasma concentrations and renal sympathetic activation, as shown in men with widely differing degrees of adiposity.[23] Thus, selective leptin resistance and the resultant sympathetic activation in response to hyperleptinemia may contribute to development of hypertension in patients afflicted with the metabolic syndrome.

Leptin and Atherosclerosis

A number of observations indicate a correlation between serum leptin and the pathogenesis of atherosclerotic vascular disease. Human plasma leptin concentrations are independently associated with intima–media thickness in the common carotid artery, an early marker of atherosclerosis.[28] Elevated leptin concentrations in healthy adolescents are associated with decreased arterial distensibility within a broad range of body mass indices.[29] In a major prospective cohort investigation, the West of Scotland Coronary Prevention Study, serum leptin levels were moderately associated with coronary heart disease, independently of other risk factors.[30] In addition, leptin levels independently predict future cardiovascular events in subjects with established coronary atherosclerosis.[31]

In mouse studies as well, there is growing evidence of the contribution of leptin to the development of atherosclerosis. Wild-type mice on an atherogenic diet show leptin elevation and greater neointimal wall thickening after carotid artery injury with high leptin receptor expression in the lesion. In contrast, *ob/ob* mice are markedly resistant to diet-induced formation of atherosclerosis, despite the presence of atherosclerosis risk factors such as diabetes, obesity, and hyperlipidemia. Exogenously administered leptin induces wall thickening in *ob/ob* mice but not in *db/db* mice.[32] Thus, there might be a direct link between hyperleptinemia and an increased risk for cardiovascular disease development in obese subjects. Possible mechanisms underlying the atherogenic actions of leptin will be discussed below.

Proliferative Actions of Leptin

The vascular proliferative actions of leptin are exerted mainly via activations of mitogenic factors. For instance, leptin in culture media dose-dependently increases both the migration and the proliferation of rat vascular smooth muscle cells through activation of phosphatidylinositol-3-kinase and mitogen-activated protein kinases.[33] Neointimal formation

after endovascular arterial injury is markedly attenuated in *db/db* mice,[34] suggesting a role for leptin in endothelial intimal layer regeneration after vascular injury. Thus, leptin may contribute to vascular remodeling and perhaps arterial restenosis after angioplasty.

Proinflammatory Actions of Leptin

Stimulation of low-grade vascular inflammation is another mechanism whereby leptin may promote both endothelial dysfunction and atherogenesis.[35] In *ob/ob* and *db/db* mice, phagocytosis and the expressions of proinflammatory cytokines, such as TNF-α, interleukin (IL)- 6, and IL-12, in macrophages are impaired both in vivo and in vitro. Administering exogenous leptin upregulates both phagocytosis and proinflammatory cytokine production in macrophages collected from *ob/ob*, but not from *db/db*, mice.[36] These observations strongly suggest a physiological role of leptin in modulating inflammatory process.

In a cross-sectional investigation involving healthy young males, leptin was independently associated with C-reactive protein,[37] a widely recognized marker of atherosclerotic vascular risk, although whether this is a causal association is unknown. At present, information regarding the interactions between leptin and various inflammatory reactions in humans is limited, but the proinflammatory actions of leptin are speculated to be involved in vascular remodeling.

Prothrombotic Actions of Leptin

Obese subjects appear to be predisposed to thrombosis formation, raising the risk of deep venous thrombosis and pulmonary embolism. Experimental evidence obtained with animal models suggests that leptin might be an important procoagulant factor. Thrombi originating from arterial lesions in *ob/ob* mice are unstable as compared with those in littermate controls. Platelet aggregation is blunted in *ob/ob* and *db/db* mice. Exogenous leptin normalizes thrombus formation and platelet aggregation in *ob/ob*, but not in *db/db*, mice.[38] Bone marrow transplantation from *db/db* to normal mice delays thrombus formation in recipients, suggesting the importance of leptin signaling in platelets in thrombosis formation. Leptin accelerates thrombogenesis by acting on platelets of *ob/ob* mice after vascular injury in vivo.[39] In addition, leptin modestly decreases the expression of thrombomodulin, an antithrombotic protein, in cultured HUVECs.[40] These prothrombotic actions of leptin together might contribute to the elevated risk of developing acute coronary events, venous thrombosis, pulmonary thromboembolism, and thrombotic events after plaque rupture, in obese subjects.

Prooxidant Actions of Leptin

Increased oxidative stress has been recognized in experimental animal and human obesity and may contribute pathogenically to the metabolic syndrome.[41]

Numerous reports have shown that leptin increases oxidative stress via multiple mechanisms. In bovine aortic endothelial cells, leptin induces mitochondrial superoxide production by increasing fatty acid oxidation via activation of protein kinase A.[42] In rats, leptin administration for 7 days decreased the activity of paraoxonase-1, an antioxidant enzyme contained in plasma lipoproteins, followed by increased plasma and urinary concentrations of isoprostanes, reflecting increased oxidative stress.[43] By increasing oxidative stress and activating protein kinase C, leptin promotes secretion of atherogenic lipoprotein lipase from macrophages in vitro.[44] Thus, leptin-induced oxidative stress is likely not only to directly damage endothelial and vascular smooth muscle cells but also to increase serum atherogenic factors, contributing to development of atherosclerosis.

Collectively, data from animal and human studies suggest that leptin plays major roles in the pathophysiology of obesity-related atherogenesis by impacting multiple steps, including vascular inflammation, proliferation, calcification, and elevated oxidative stress.

Adiponectin

Adiponectin, also termed Acrp30,[45] apM1,[46] AdipoQ,[47] or GBP28,[48] was identified independently by 4 research groups using different approaches, as a protein that is specifically and most abundantly[46] produced in adipose tissue. It has a 20-residue signal sequence, collagen-like motif and globular domain and shows significant homology with collagens X and VIII and complement factor C1q.[49] Adiponectin molecules combine via its collagen domain, producing a wide range of multimer complexes in plasma: a low-molecular-weight trimer, a middle-molecular-weight hexamer, and a high-molecular-weight 12- to 18-mer adiponectin.[50,51]

Plasma adiponectin levels in humans are quite high, normally ranging from 3 to 30 μg/mL. In contrast to leptin, adiponectin plasma levels correlate negatively with body mass index.[52,53] The negative correlation is stronger between plasma adiponectin levels and visceral adiposity than between this protein levels and subcutaneous adiposity.[54,55] The expression of adiponectin in adipose tissue is reportedly regulated by several mechanisms via humoral and neuronal pathways. As an example, insulin and insulin-like growth factor-1 both upregulate adiponectin expression,[56] whereas TNF-α and activation of the peroxisome proliferators-activated receptor (PPAR)α have the opposite effect.[57] Angiotensin II also reportedly reduces adiponectin production, as described below.[58] In addition, sympathetic activation suppresses adiponectin expression via adrenergic β function.[59,60] The mechanism underlying the adiponectin reduction in obese subjects remains unclear, but a plausible explanation is that inflammatory cytokines, eg, TNF-α, cause transcriptional suppression and secretory inhibition of adiponectin.[57]

Different types of putative adiponectin receptors have been described. T-cadherin was identified as a receptor for the hexameric and high-molecular-weight species of adiponectin but for neither the trimeric nor the globular species.[61] On the other hand, novel family proteins, designated AdipoR1 and AdipoR2, were found to be receptors for globular and full-length adiponectin.[62] This family of adiponectin receptors is predicted to contain 7-transmembrane domains, despite being structurally and functionally distinct from G protein–coupled receptors. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is expressed mainly in the liver. Very recently, simultaneous disruption of both AdipoR1 and -R2 was reported to abolish adiponectin binding as

well as its actions.[63] The molecular pathways by which adiponectin mediates its effects apparently involve activation of AMP-activated protein kinase (AMPK), PPARα, and p38 mitogen-activated protein kinase signaling pathways,[64] although further investigation is needed in this field.

Adiponectin and Hypertension

Lower concentrations of plasma adiponectin have been associated with essential hypertension. Patients with hypertension appear to have significantly lower plasma adiponectin levels than normotensive patients.[65,66] The mechanism underlying this observation may involve the effects of angiotensin II. Infusion of angiotensin II in rats decreased plasma adiponectin levels via signaling through the angiotensin II type 1 receptor.[58] Human subjects with essential hypertension, treated with angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors, had increased adiponectin concentrations without affecting body mass indices.[67] However, the molecular mechanisms whereby angiotensin II signaling reduces adiponectin production have yet to be clarified.

Adiponectin and Atherosclerosis

Lines of evidence obtained from experimental animal models, such as adiponectin overexpression and knockout mice, have indicated protective effects of adiponectin against the development of obesity-related vascular diseases including atherosclerosis.

Adenovirus-mediated overexpression of adiponectin in apolipoprotein E (apoE)-deficient mice attenuates atherosclerotic lesion formation in the aortic sinus as compared with control apoE-deficient mice.[68] Transgenic overexpression of globular adiponectin also ameliorates atherosclerotic lesion formation and diminishes the expression of the class A scavenger receptor in apoE-knockout mice, despite the absence of changes in blood glucose and lipid levels.[69] These effects of adiponectin were confirmed by studies using adiponectin-knockout mice. Adiponectin-knockout mice show increased neointimal hyperplasia and proliferation of smooth muscle cells following acute vascular injury.[70,71] Conversely, adenovirus-mediated reexpression of adiponectin blunts the increase in neointimal thickening observed in adiponectin-knockout mice.[71] These in vivo experiments have demonstrated that adiponectin plays a role in preventing atherosclerotic progression. This conclusion appears to be supported by reports showing that, in humans, mutations and polymorphisms within the adiponectin gene, which are associated with lower adiponectin levels, are associated with coronary artery disease.[72,73]

Adiponectin expression in adipocytes and its plasma levels are upregulated by treatment with thiazolidinediones, agonists for PPARγ.[74] There is mounting evidence that PPARγ agonists reduce the incidence of cardiovascular diseases, including myocardial infarction and stroke, in patients with type 2 diabetes who are at a high risk for macrovascular events.[75] Adiponectin deficiency diminishes the ability of thiazolidinediones to improve glucose tolerance,[76] suggesting involvement of adiponectin in the protective effects of thiazolidinediones against the development of cardiovascular diseases.

Protective Role of Adiponectin Against Endothelial Dysfunction

A series of in vitro and in vivo studies has suggested that adiponectin exerts protective actions on endothelial cells, thereby preventing the pathogenic effects of obesity on vascular function.

Adiponectin may exert antiinflammatory properties in part by altering NO levels in the endothelium. In human aortic endothelial cells, adiponectin promotes endothelial NO synthase mRNA and its protein expression, resulting in enhanced NO production via AMPK pathway activation[77,78] Globular adiponectin also reverses oxidized LDL-induced suppression of endothelial NO synthase activity.[78,79] Adiponectin-knockout mice show impaired endothelial-dependent vasodilation when given an atherogenic diet.[66] In addition, adiponectin has antiapoptotic effects on endothelial cells.[80,81] Taken together, these observations indicate that adiponectin protects against endothelial dysfunction through multiple mechanisms.

Adiponectin also inhibits nuclear factor-κB (NF-κB) activation in both endothelial cells and macrophages. Inhibition of endothelial NF-κB signaling by adiponectin treatment suppresses TNF-α–stimulated expression of the proinflammatory cytokine IL-8 as well as adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, such that the attachment of monocytes to endothelial cells is attenuated.[82,83] Adiponectin-induced suppression of these adhesion molecules was also demonstrated in vivo with adenovirus-mediated overexpression of adiponectin in apoE-deficient mice.[68] In addition, in macrophages as well, adiponectin suppresses NF-κB signaling[84,85] and the expression of class A scavenger receptors, resulting in reduced foam cell formation and the secretion of proinflammatory cytokines.[86] Foam-cell formation is further reduced by adiponectin-induced downregulation of acyl-coenzyme A:cholesterol acyltranferase-1, the enzyme that catalyzes the formation of cholesteryl esters,[87] in macrophages. Adiponectin also enhances expression of the antiinflammatory cytokine IL-10 and the tissue inhibitor of metalloproteinase-1 in macrophages.[88] Through this variety of mechanisms, adiponectin limits the initiation of atherosclerotic plaque formation.

Protective Role of Adiponectin Against Vascular Remodeling

The evolution of a fatty streak into a complex lesion is characterized by the proliferation of smooth muscle cells, their migration toward the intima, and their synthesis of collagen. Adiponectin may modulate smooth muscle cell proliferation during the development and progression of vascular lesions. Physiological concentrations of adiponectin significantly suppress both proliferation and migration of human aortic smooth muscle cells in vitro, induced by platelet-derived growth factor-BB, via direct binding with platelet-derived growth factor-BB.[89] Adiponectin was also shown to generally inhibit growth factor-stimulated extracellular signal-regulated kinase signaling. Similarly, adiponectin was found to inhibit smooth muscle cell proliferation through its ability to bind to various growth factors and to interfere

with receptor-mediated cellular responses.[90] As described above, these effects of adiponectin were confirmed by in vivo studies with adiponectin-knockout mice.[70,71] Thus, adiponectin may act as a modulator of vascular remodeling and may favor plaque stabilization via these various mechanisms.

Protective Role of Adiponectin Against Thrombosis Formation

Investigations using adiponectin-knockout mice further revealed adiponectin to potentially be an endogenous antithrombotic factor. Compared with wild-type control mice, adiponectin-knockout mice showed enhanced thrombus formation and platelet aggregation at sites of vascular injury, with no differences in either platelet counts or coagulation parameters. Adenovirus-mediated supplementation of adiponectin blunted this enhanced thrombus formation.[91] The antithrombotic actions of adiponectin might well play a protective role against developing acute coronary events and some thrombotic diseases.

Role of Adiponectin in Protection From Ischemic Heart Disease

Obesity-related disorders have a major impact on both the incidence and the severity of ischemic heart disease,[92,93] and adiponectin may have a protective function in this setting. Adiponectin treatment inhibits apoptosis of cardiac myocytes and fibroblasts exposed to hypoxia-reoxygenation stress. Blockade of the AMPK pathway by dominant-negative AMPK expression inhibits this adiponectin effect of protecting against apoptosis. In addition, cyclooxygenase-2 is upregulated by adiponectin, leading to increased prostaglandin E_2 synthesis. Adiponectin thus appears to protect against myocardial ischemia/reperfusion injury through AMPK-dependent and cyclooxygenase-2–dependent pathways.[94] In adiponectin-knockout mice, larger infarcts are observed after ischemia/reperfusion, which is associated with greater myocardial cell apoptosis and TNF-α expression. Adiponectin replenishment attenuates these damaging effects.[94] Thus, adiponectin may protect myocardial cells from hypoxic stress via both antiapoptotic and antiinflammatory mechanisms. Therefore, adiponectin administration might have a practical clinical application in the treatment of acute myocardial infarction.

Other Adipocytokines

Tumor Necrosis Factor α

The first clear links among obesity, insulin resistance, and chronic inflammation were provided by a report showing enhanced expression of TNF-α, a proinflammatory cytokine, in adipose tissue of obese mice.[95] Lack of TNF-α function improves insulin resistance in obese mice,[96] suggesting an important role for TNF-α in the development of insulin resistance. TNF-α is suggested to be involved in vascular remodeling via proinflammatory and insulin resistant effects. Interestingly, obesity is associated with macrophage accumulation in adipose tissue[97] and TNF-α is apparently derived from infiltrating macrophages,[98] suggesting macrophage infiltration of adipose tissue to play a role in development of obesity-related morbidities.

Plasminogen Activator Inhibitor-1

PAI-1 is another adipocytokine, which is highly expressed in adipose tissue and has thrombotic effects.[99] During progressive fat accumulation, PAI-1 expression is markedly enhanced in visceral adipose tissue. Plasma PAI-1 levels correlated significantly with visceral adiposity, as evaluated by computed tomography scanning, in humans.[100] Therefore, PAI-1 secreted from accumulated visceral adipose tissue might play an important role in the development of thrombotic disorders, ie, the ultimate consequences of atherosclerosis.

Retinol-Binding Protein 4

In subjects with obesity and type 2 diabetes, GLUT4 glucose transporter expression is selectively decreased in adipocytes.[101] Conversely, adipose-specific GLUT4 disruption secondarily induces insulin resistance in muscle and liver.[102] In this mouse model, RBP4 was identified as an upregulated protein in adipose tissue.[103] Transgenic expression or injections of RBP4 caused insulin resistance in mice, whereas experimentally decreasing RBP4 levels ameliorated insulin resistance in diet-induced obesity. RBP4 enhances hepatic gluconeogenesis and attenuates insulin signaling in skeletal muscle.[103] Serum RBP4 is elevated in insulin-resistant mice and humans with obesity and type 2 diabetes.[104] Thus, RBP4 might play a major role in the development of insulin resistance, although the impact of RBP4 on obesity-related hypertension and vascular diseases remains uncertain.

Resistin

Resistin is a member of the newly recognized family of cysteine-rich secretory proteins called resistin-like molecules (RELMs) or FIZZ (found in the inflammatory zone). Resistin is expressed almost exclusively in white adipose tissue and leads to insulin resistance in mice.[105] A few studies focusing on the link between resistin and endothelial functions have recently been published. Resistin promotes endothelin-1 release and also upregulates the expressions of adhesion molecules, monocyte chemoattractant chemokine-1, and pentraxin 3, a marker of NF-κB–dependent inflammation, while downregulating the expression of TNF-receptor–associated factor-3, an inhibitor of CD40 ligand signaling in endothelial cells.[106,107] These results suggest that resistin contributes to initiation or perpetuation of the atherosclerotic state. However, unlike murine resistin, human resistin expression is very low in adipocytes while being readily detectable in mononuclear blood cells.[108–110] Therefore, the role of resistin in the development of obesity-related vascular diseases in humans is still uncertain.

Humoral Factors Derived From the Liver

In addition to adipocytokines, circulating factors secreted by the liver are also involved in systemic metabolic regulation. Members of the angiopoietin-like (Angptl) family of proteins are structurally related to angiopoietins, although their receptors are currently unknown. Angptl3 and Angptl6 (angiopoietin-related growth factor) expressions are restricted mainly to the liver, whereas Angptl4 expression is most abundant in the liver and adipose tissue. Angptl3, -4, and -6

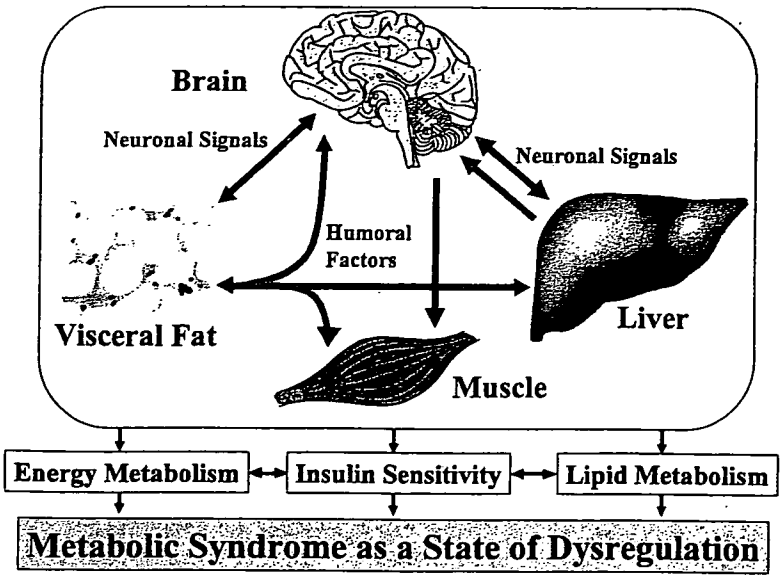

Figure 2. Communications among organs/tissues via humoral and neuronal pathways.

are detected in the systemic circulation, suggesting an endocrine function.

Like the angiopoietins, these Angptl proteins play important roles in angiogenesis, but there are also several reports showing their involvement in triglyceride and energy metabolism as well as insulin sensitivity. Angptl3, a downstream target of the oxysterol receptor liver X receptor,[111] is involved in development of the hypertriglyceridemia.[112] The underlying mechanism appears to be reductions in very-low-density lipoprotein clearance secondary to lipoprotein lipase inhibition[113] and direct activation of lipolysis in adipocytes.[114] In contrast, Angptl6 is suggested to function in counteracting obesity and related insulin resistance through increased energy expenditure.[115]

Angptl4 is also expressed mainly in the liver and adipose tissue, and its expression changes with nutrition status[116] and also according to the activation state of PPARs.[117] Adenovirus-mediated expression of Angptl4 potently decreased blood glucose and improved glucose tolerance, whereas it induced hyperlipidemia, fatty liver, and hepatomegaly. In addition, in patients with type 2 diabetes, serum Angptl4 were lower than in healthy subjects.[118]

Thus, the function, or even dysfunction, of pathways mediated by these humoral factors derived from the liver may contribute to the development of hyperlipidemia and insulin resistance, both major elements of the metabolic syndrome. However, further intensive studies are needed to elucidate the contributions of these factors to cardiovascular disease.

Neuronal Signals From Intraabdominal Tissues in Response to Metabolic Alterations

In addition to humoral pathways, autonomic nervous system is likely to play an important role in both metabolic and cardiovascular regulation. The central nervous system (CNS) integrates signals from peripheral sites, thereby modulating glucose and energy metabolism as well as blood pressure. At least 2 avenues for these signals, humoral and neuronal, are involved in the underlying mechanisms. Whereas humoral signals including adipocytokines have been intensively investigated in recent years, neuronal signals from adipose tissue and the liver remain largely a mystery. Several recent reports, including ours, have indicated the importance of afferent neuronal signals in response to metabolic alterations, such as adiposity, in intraabdominal organs/tissues. In this regard, afferent signals from intraabdominal organs transmitted by autonomic neurons have attracted considerable attention. Organs/tissues communicate metabolic information each other via humoral and neuronal pathways (Figure 2).

Neuronal Signals From Adipose Tissues

Fat pads have rich sympathetic fiber innervation. Numerous studies have revealed a role for efferent sympathetic nerves in lipolysis. Various signals from the brain modulate the rate of lipolysis in adipose tissue via sympathetic β-adrenergic action.[119] In contrast, only a few studies have examined afferent nerve signals from adipose tissue. According to these reports, activation of afferent nerves from intraabdominal (epididymal) adipose tissue results in reflex signals being sent to white adipose tissues via efferent sympathetic nerve activation.[120,121] The functional significance of these afferent signals, however, was not clarified. Research performed by our group has suggested that neural afferent signals from intraabdominal adipose tissue to the brain affect hypothalamic leptin sensitivity, thereby modulating food intake and sympathetic outflow.[122]

Our goal was to determine whether a local reduction in the adiposity of intraabdominal adipose tissue would reverse obesity-related metabolic disorders, in particular, insensitivity to leptin and insulin. Therefore, adenoviral-mediated expression of uncoupling protein (UCP)1, which functions to dissipate energy as heat, was attempted in epididymal adipose tissue of diet-induced obese and diabetic mice in which insulin and leptin resistance had already developed. Despite UCP1 being expressed in epididymal adipose tissue at only very low levels, food intake clearly declined in association with decreased serum leptin levels as well as downregulation of orexigenic neuropeptide Y and upregulation of the anorexigenic precursor neuropeptide proopiomelanocortin in the

hypothalamus. The response to exogenous leptin was enhanced in these mice. In addition, hypophagia could not be duplicated in db/db mice with mutant leptin receptors. Collectively, these findings convincingly demonstrate that very limited UCP1 expression in the intraabdominal fat pad dramatically ameliorates the hypothalamic leptin resistance induced by high-fat-diet feeding. Local dissection of nerves from the epididymal fat pad as well as pharmacological deafferentation abrogated the anorectic effects of adipose UCP1 expression. Taken together, our results suggest afferent nerve signals originating in epididymal fat pads to modulate hypothalamic leptin sensitivity.

Hypothalamic leptin resistance is an important mechanism that maintains the obese state. Therefore, the perturbation of the afferent signals from adipose tissue might contribute to the development of obesity-related disorders, including hypertension and atherosclerosis. Adipose UCP1 expression increases sympathetic outflow, also suggesting the effects of adipose tissue-derived afferent signals on vascular systems. Adipose tissues were long recognized as passive energy storage sites. The discovery of various adipocytokines has raised adipose tissue to the status of a versatile endocrine organ. The aforementioned recent studies may provide additional evidence of the key role of adipose tissue as an important base from which neuronal signals originate. Further elucidation of this new pathway could open a new paradigm enhancing our understanding of adipose functions and dysfunctions, and thereby the pathophysiology of vascular diseases.

Neuronal Signals From the Liver

Nutrients absorbed from the gut enter the portal vein, a major route to the liver, thereby reaching the liver directly. Thus, given its anatomical location, it seems reasonable for the liver to function as a nutrient sensor and to send signals that regulate systemic metabolism. Signals regarding serum glucose levels from the so-called hepatoportal glucose sensor to the brain have been demonstrated to be carried along afferent vagal nerve pathways.[123] Raising portal vein glucose levels decreases vagal afferent discharges reaching the nuclei of solitary tract neurons, which in turn activates sympathetic efferents to the adrenal glands, liver, splanchnic bed, and pancreas. Because these reflex efferent outputs are all blocked by hepatic vagotomy, it appears that signals triggered by high levels of portal glucose are transmitted through vagal afferents.[123,124] Similarly, hepatic portal infusions of linoleic acid raised hepatic vagal afferent activity, suggesting hepatic vagal afferent involvement in the transmission of signals regarding lipid metabolism to the CNS.[125] In addition, infusion of long-chain fatty acids into the portal vein activates the sympathetic nervous system, thereby elevating blood pressure.[126–128] Therefore, portal nutrient signals may influence systemic blood pressure through afferent vagal and efferent sympathetic nerves. Our recent study provided further evidence of the link between hepatic metabolism and peripheral adiposity[129] through an autonomic nerve circuit consisting of afferent vagal and efferent sympathetic nerve activity.[130]

Hepatic expression of PPARγ, especially PPARγ2, has been shown to be functionally enhanced in a number of obesity models.[131,132] Therefore, to identify the mechanism underlying the interorgan/-tissue communications between the liver and peripheral tissues, including muscle and fat, we overexpressed PPARγ2 in the livers of mice and produced hepatic steatosis using adenoviral gene transfer. Contrary to the increased adiposity in the liver, hepatic PPARγ2 expression markedly reduced adiposity in the periphery with enhanced lipolysis. Systemic metabolic rates were increased, and peripheral insulin sensitivity and glucose tolerance were thus markedly improved. These remote effects were attributed to increased sympathetic outflow into muscle and adipose tissues. Selective hepatic branch vagotomy and pharmacological deafferentation of the vagus completely reversed these remote effects. Thus, hepatic PPARγ2 expression and/or hepatic lipid accumulation stimulates afferent vagal nerve fibers, communicating metabolic information to the brain and producing antiobesity and antiinsulin-resistant effects in muscle and adipose tissue via efferent sympathetic pathways.[130] Fat storage in the liver changes dynamically in accordance with the systemic energy balance and is associated with several features of the metabolic syndrome. Because hepatic PPARγ expression is physiologically associated with obesity, these findings indicate that the liver transmits information regarding excess energy to the CNS via the afferent vagus. When the brain receives this information regarding excess energy storage mediated by leptin from adipose tissues and via the afferent vagus from the liver, the sympathetic nervous system is activated, which in turn enhances energy expenditure and lipolysis, thereby maintaining energy homeostasis. Notably, liver-specific disruption of PPARγ in *ob/ob* mice prevented hepatic steatosis but increased peripheral adiposity, resulting in aggravation of the diabetic phenotype attributable to decreased insulin sensitivity in muscle and fat.[133] Thus, this system consisting of an autonomic nervous circuit appears to function as a protective mechanism against excess calorie intake in physiological settings.

A similar autonomic nerve circuit appears to play an essential role in development of glucocorticoid-induced insulin resistance and hypertension. Glucocorticoid excess is well known to result in insulin resistance and hypertension. In particular, accelerated conversion of glucocorticoid from the inactive to the active form in adipose tissue has phenotypic similarities with the metabolic syndrome.[134] In mice, chronic glucocorticoid exposure leads to insulin resistance and hypertension associated with increased sympathetic tone, renin activity and urinary sodium retention. The underlying mechanism involves hepatic activation of PPARα.[135] Deafferentation, whether surgical or pharmacological, of the hepatic vagus reversed these phenotypic features following chronic glucocorticoid exposure.[136] Taken together, these observations indicate the importance of the vagal afferent pathway in regulating insulin sensitivity and blood pressure. The development of hypertension is attributable to sympathetic activation. Thus, autonomic nerve circuit consisting of hepatic vagal afferent and sympathetic efferent nerves may contribute to the development of obesity-related hypertension. Elucidation of the molecular mechanisms, including the mediators influencing vagal activity, could lead to new therapeutic

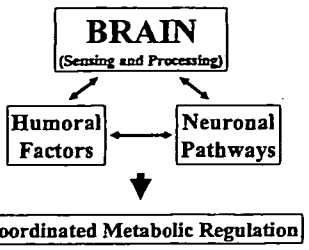

Figure 3. The CNS receives peripheral metabolic information and regulates systemic metabolism via humoral factors and neuronal pathways in a coordinated manner.

approaches to the metabolic syndrome and cardiovascular diseases.

Conclusion

There is a growing body of evidence for a link between obesity and cardiovascular diseases, such as hypertension and atherosclerosis. During this decade, the versatility of adipose tissue as an endocrine organ and as a contributor to disease development has been established. Adipocytokine-mediated crosstalk between adipose tissue and the vascular system is clearly important. In addition, a number of recent studies have shown that tissue-specific knockout mice exhibit unexpected phenotypes, suggesting the presence of currently unknown crosstalk among organs/tissues. Further unraveling the complexities of this interorgan communication would enhance our understanding of the development of obesity-related disorders.

Metabolism is not an independent process, segregated among different organs/tissues, but rather is coordinated and regulated throughout the body. Metabolic regulation coordinated among organs/tissues, which requires communication among these organs/tissues, is apparently essential for maintaining the homeostasis of systemic metabolism, particularly glucose and energy metabolism. Therefore, perturbation of this coordinated control system may lead to the development of metabolic disorders. Recent research advances in this field have revealed myriad complex and important roles of the CNS. The brain receives various forms of metabolic information from peripheral organs/tissues through humoral and neuronal avenues (Figure 3). For instance, leptin acts on the hypothalamus and other brain areas, mediating divergent effects on lipid metabolism and insulin signaling in the brain.[137] Adiponectin also appears to exert central effects on energy metabolism.[138] These inputs are probably integrated and processed in the brain, leading to the transmission of regulatory signals, which in turn induce appropriate systemic responses. In addition, humoral and neuronal signals affect each other, as exemplified by the findings that leptin and adiponectin expressions are regulated by sympathetic activity.[23,60] Further elucidation of these regulatory systems, in much greater detail, may facilitate unraveling the mechanisms underlying metabolic homeostasis and thereby reveal the mechanisms underlying the development of the metabolic syndrome as a state of dysregulation (Figure 2). Moreover, targeting of the coordinated regulatory system consisting of these humoral and neuronal pathways is a potential therapeutic strategy for obesity-related disorders, including cardiovascular diseases.

Sources of Funding

This work was supported by grant-in-aids for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (B2, 18390267 to H.K.; and B2, 17390258 to Y.O.), by a grant-in-aid for scientific research from the Ministry of Health, Labor and Welfare of Japan (H16 genome-003 to Y.O.), and by the 21st Century Center of Excellence Programs of the Ministry of Education Science, Sports and Culture of Japan (to H.K. and Y.O.).

Disclosures

None.

References

1. Kaplan NM. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med.* 1989; 149:1514–1520.
2. Reaven GM. Role of insulin resistance in human disease (syndrome X): an expanded definition. *Annu Rev Med.* 1993;44:121–131.
3. Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism.* 1987;36:54–59.
4. Hasegawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, Gao J, Kaneko K, Ishihara H, Sasano H, Nakauchi H, Oka Y, Katagiri H. Bone Marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. *Endocrinology.* 2007;148: 2006–2015.
5. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *Br Med J (Clin Res Ed).* 1984;288:1401–1404.
6. Nakamura T, Kobayashi H, Yanagi K, Nakagawa T, Nishida M, Kihara S, Hiraoka H, Nozaki S, Funahashi T, Yamashita S, Kameda-Takemura K, Matsuzawa Y. Importance of intra-abdominal visceral fat accumulation to coronary atherosclerosis in heterozygous familial hypercholesterolaemia. *Int J Obes Relat Metab Disord.* 1997;21:580–586.
7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372:425–432.
8. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest.* 1997;100:270–278.
9. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature.* 1998;395:763–770.
10. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell.* 1995;83:1263–1271.
11. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science.* 1998;281:1683–1686.
12. Winters B, Mo Z, Brooks-Asplund E, Kim S, Shoukas A, Li D, Nyhan D, Berkowitz DE. Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in obese (Lep(ob)) mice. *J Appl Physiol.* 2000; 89:2382–2390.
13. Fruhbeck G. Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes.* 1999;48:903–908.
14. Lembo G, Vecchione C, Fratta L, Marino G, Trimarco V, d'Amati G, Trimarco B. Leptin induces direct vasodilation through distinct endothelial mechanisms. *Diabetes.* 2000;49:293–297.
15. Jalali A, Morgan DA, Sivitz WI, Correia ML, Mark AL, Haynes WG. Does leptin cause functional peripheral sympatholysis? *Am J Hypertens.* 2001;14:615–618.
16. Quehenberger P, Exner M, Sunder-Plassmann R, Ruzicka K, Bieglmayer C, Endler G, Muellner C, Speiser W, Wagner O. Leptin induces endothelin-1 in endothelial cells in vitro. *Circ Res.* 2002;90: 711–718.

17. Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes*. 1997;46:2040–2043.
18. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension*. 1998;31:409–414.
19. Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension*. 2001;37:936–942.
20. Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K. Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest*. 2000;105:1243–1252.
21. Mark AL, Shaffer RA, Correia ML, Morgan DA, Sigmund CD, Haynes WG. Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens*. 1999;17:1949–1953.
22. Agata J, Masuda A, Takada M, Higashiura K, Murakami H, Miyazaki Y, Shimamoto K. High plasma immunoreactive leptin level in essential hypertension. *Am J Hypertens*. 1997;10:1171–1174.
23. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M. Interactions between leptin and the human sympathetic nervous system. *Hypertension*. 2003;41:1072–1079.
24. Mackintosh RM, Hirsch J. The effects of leptin administration in nonobese human subjects. *Obes Res*. 2001;9:462–469.
25. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*. 1999;282:1568–1575.
26. Correia ML, Rahmouni K. Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. *Diabetes Obes Metab*. 2006;8:603–610.
27. Rahmouni K, Morgan DA, Morgan GM, Mark AL, Haynes WG. Role of selective leptin resistance in diet-induced obesity hypertension. *Diabetes*. 2005;54:2012–2018.
28. Ciccone M, Vettor R, Pannacciulli N, Minenna A, Bellacicco M, Rizzon P, Giorgino R, De Pergola G. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. *Int J Obes Relat Metab Disord*. 2001;25:805–810.
29. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, Lucas A, Deanfield J. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation*. 2002;106:1919–1924.
30. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation*. 2001;104:3052–3056.
31. Wolk R, Berger P, Lennon RJ, Brilakis ES, Johnson BD, Somers VK. Plasma leptin and prognosis in patients with established coronary atherosclerosis. *J Am Coll Cardiol*. 2004;44:1819–1824.
32. Schafer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, Konstantinides S. Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol*. 2004;24:112–117.
33. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci*. 2001;47:141–150.
34. Stephenson K, Tunstead J, Tsai A, Gordon R, Henderson S, Dansky HM. Neointimal formation after endovascular arterial injury is markedly attenuated in db/db mice. *Arterioscler Thromb Vasc Biol*. 2003;23:2027–2033.
35. Cleland SJ, Sattar N, Petrie JR, Forouhi NG, Elliott HL, Connell JM. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. *Clin Sci (Lond)*. 2000;98:531–535.
36. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM. Leptin regulates proinflammatory immune responses. *FASEB J*. 1998;12:57–65.
37. Kazumi T, Kawaguchi A, Hirano T, Yoshino G. C-reactive protein in young, apparently healthy men: associations with serum leptin, QTc interval, and high-density lipoprotein-cholesterol. *Metabolism*. 2003;52:1113–1116.
38. Konstantinides S, Schafer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. *J Clin Invest*. 2001;108:1533–1540.
39. Bodary PF, Westrick RJ, Wickenheiser KJ, Shen Y, Eitzman DT. Effect of leptin on arterial thrombosis following vascular injury in mice. *JAMA*. 2002;287:1706–1709.
40. Maruyama I, Nakata M, Yamaji K. Effect of leptin in platelet and endothelial cells. Obesity and arterial thrombosis. *Ann N Y Acad Sci*. 2000;902:315–319.
41. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004;114:1752–1761.
42. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzmań M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem*. 2001;276:25096–25100.
43. Beltowski J, Wojcicka G, Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis*. 2003;170:21–29.
44. Maingrette F, Renier G. Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C. *Diabetes*. 2003;52:2121–2128.
45. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem*. 1995;270:26746–26749.
46. 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*. 1996;221:286–289.
47. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem*. 1996;271:10697–10703.
48. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo)*. 1996;120:803–812.
49. Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol*. 1998;8:335–338.
50. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem*. 2003;278:9073–9085.
51. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem*. 2003;278:40352–40363.
52. Takahashi M, Funahashi T, Shimomura I, Miyaoka K, Matsuzawa Y. Plasma leptin levels and body fat distribution. *Horm Metab Res*. 1996;28:751–752.
53. 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*. 1999;257:79–83.
54. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia*. 2003;46:459–469.
55. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. *Circ J*. 2004;68:975–981.
56. Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, Brichard SM. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun*. 2001;288:1102–1107.
57. 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. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*. 2001;50:2094–2099.
58. Ran J, Hirano T, Fukui T, Saito K, Kageyama H, Okada K, Adachi M. Angiotensin II infusion decreases plasma adiponectin level via its type

1 receptor in rats: an implication for hypertension-related insulin resistance. *Metabolism.* 2006;55:478–488.
59. Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Brichard SM. Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies. *Biochem J.* 2002;367:677–685.
60. Imai J, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Uno K, Hasegawa Y, Gao J, Ishihara H, Sasano H, Oka Y. Cold exposure suppresses serum adiponectin levels through sympathetic nerve activation in mice. *Obesity (Silver Spring).* 2006;14:1132–1141.
61. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. *Proc Natl Acad Sci U S A.* 2004;101:10308–10313.
62. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature.* 2003;423:762–769.
63. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med.* 2007;33:332–339.
64. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest.* 2006;116:1784–1792.
65. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens.* 2003;16:72–75.
66. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension.* 2003;42:231–234.
67. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, Yoshida D, Shimamoto K. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension.* 2003;42:76–81.
68. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation.* 2002;106:2767–2770.
69. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem.* 2003;278:2461–2468.
70. 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.* 2002;277:25863–25866.
71. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem.* 2002;277:37487–37491.
72. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, Kawamoto T, Matsumoto S, Nagaretani H, Kumada M, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Hiraoka H, Iwashima Y, Ishikawa K, Ohishi M, Katsuya T, Rakugi H, Ogihara T, Matsuzawa Y. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *J Am Coll Cardiol.* 2004;43:1195–1200.
73. Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, Verrienti A, Fanelli M, Fallarino M, Sorropago G, Baroni MG. The adiponectin gene SNP+276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age <or=50 years). *J Mol Med.* 2005;83:711–719.
74. Combs TP, Wagner JA, Berger J, Doebber T, Wang WJ, Zhang BB, Tanen M, Berg AH, O'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE, Moller DE. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology.* 2002;143:998–1007.
75. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Koranyi L, Laakso M, Mokan M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;366:1279–1289.
76. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, Rossetti L, Scherer PE. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem.* 2006;281:2654–2660.
77. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem.* 2003;278:45021–45026.
78. Hattori Y, Suzuki M, Hattori S, Kasai K. Globular adiponectin upregulates nitric oxide production in vascular endothelial cells. *Diabetologia.* 2003;46:1543–1549.
79. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. *Biochem Biophys Res Commun.* 2004;315:264–271.
80. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res.* 2004;94:e27–e31.
81. Lin LY, Lin CY, Su TC, Liau CS. Angiotensin II-induced apoptosis in human endothelial cells is inhibited by adiponectin through restoration of the association between endothelial nitric oxide synthase and heat shock protein 90. *FEBS Lett.* 2004;574:106–110.
82. Kobashi C, Urakaze M, Kishida M, Kibayashi E, Kobayashi H, Kihara S, Funahashi T, Takata M, Temaru R, Sato A, Yamazaki K, Nakamura N, Kobayashi M. Adiponectin inhibits endothelial synthesis of interleukin-8. *Circ Res.* 2005;97:1245–1252.
83. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation.* 2000;102:1296–1301.
84. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun.* 2004;316:924–929.
85. Yamaguchi N, Argueta JG, Masuhiro Y, Kagishita M, Nonaka K, Saito T, Hanazawa S, Yamashita Y. Adiponectin inhibits Toll-like receptor family-induced signaling. *FEBS Lett.* 2005;579:6821–6826.
86. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation.* 2001;103:1057–1063.
87. Furukawa K, Hori M, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Miyazaki A, Nakayama H, Horiuchi S. Adiponectin down-regulates acyl-coenzyme A:cholesterol acyltransferase-1 in cultured human monocyte-derived macrophages. *Biochem Biophys Res Commun.* 2004;317:831–836.
88. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation.* 2004;109:2046–2049.
89. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation.* 2002;105:2893–2898.