Perspectives in Diabetes

Dissipating Excess Energy Stored in the Liver Is a Potential Treatment Strategy for Diabetes Associated With Obesity

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For examining whether dissipating excess energy in the liver is a potential therapeutic approach to high-fat diet–induced metabolic disorders, uncoupling protein-1 (UCP1) was expressed in murine liver using adenoviral vectors in mice with high-fat diet–induced diabetes and obesity, and in standard diet–fed lean mice. Once diabetes with obesity developed, hepatic UCP1 expression increased energy expenditure, decreased body weight, and reduced fat in the liver and adipose tissues, resulting in markedly improved insulin resistance and, thus, diabetes and dyslipidemia. Decreased expressions of enzymes for lipid synthesis and glucose production and activation of AMP-activated kinase in the liver seem to contribute to these improvements. Hepatic UCP1 expression also reversed high-fat diet–induced hyperphagia and hypothalamic leptin resistance, as well as insulin resistance in muscle. In contrast, intriguingly, in standard diet–fed lean mice, hepatic UCP1 expression did not significantly affect energy expenditure or hepatic ATP contents. Furthermore, no alterations in blood glucose levels, body weight, or adiposity were observed. These findings suggest that ectopic UCP1 in the liver dissipates surplus energy without affecting required energy and exerts minimal metabolic effects in lean mice. Thus, enhanced UCP expression in the liver is a new potential therapeutic target for the metabolic syndrome. Diabetes 54:322–332, 2005

A n explosive increase in the number of diabetic patients, which has become a major public health concern in most industrialized countries in recent decades (1), is mainly the result of excess energy intake and physical inactivity. When food intake chronically exceeds metabolic needs, efficient metabolism causes excess energy storage and results in obesity, a common condition associated with diabetes, hyperlipidemia, and premature heart disease. Excess energy in cells lowers the response to insulin, namely insulin resistance. However, the major treatment modalities for diabetes, including insulin injection and oral sulfonylureas, aim at lowering blood glucose levels by driving glucose into cells in peripheral tissues such as muscle and fat. This further exacerbates insulin resistance when energy intake is in excess, resulting in a vicious cycle. Therefore, novel therapies that promote increased energy expenditure are needed.

Inefficient metabolism, such as the generation of heat instead of ATP, is a potential treatment strategy for type 2 diabetes associated with obesity. Uncoupling proteins (UCPs) were discovered members of the mitochondrial inner membrane carrier family. These proteins leak protons into the mitochondrial matrix, dissipating energy as heat rather than allowing it to be captured in ATP (2). UCP1 (thermogenin) was originally identified in brown adipose tissue and demonstrated to mediate nonshivering thermogenesis. UCP1 plays an important role in mediating cold exposure–induced thermogenesis (3) and is also a likely regulator of diet-induced thermogenesis (4).

Several laboratories have reported overexpression of UCPs, using the transgenic approach, in mice (5–8). These reports indicate that overexpression of UCPs in white adipose tissue and skeletal muscle has preventive effects on development of genetic and dietary obesity and the resultant insulin resistance. However, it is still unclear whether ectopic UCP1 expression exerts therapeutic effects after the development of diabetes associated with obesity.

The liver is one of the major metabolic organs involved in glucose and lipid metabolism and insulin action. In addition, the liver can store and release abundant fat...
dynamically, in response to the energy balance. We reported that hepatic AKT activation resulted in marked alterations in glucose and lipid metabolism (9), suggesting that the liver is a potential site of ectopic expression. We herein expressed UCP1 protein in the liver, before or after diabetes associated with obesity had developed. We found that hepatic UCP1 expression improved diabetes and obesity under high-fat diet conditions through local effects in the liver as well as remote effects in adipose tissues, muscle, and the hypothalamus. However, in standard diet−fed lean mice, effects on glucose and lipid metabolism were minimal. Using gene transduction after disease development, as in this study, provides useful information allowing analysis of therapeutic, rather than preventive, effects that would be difficult to examine using congenitally gene-engineered animal models.

RESEARCH DESIGN AND METHODS
Preparation of recombinant adenovirus. Murine UCP1 cDNA (10) was provided by Professor Leslie P. Kozak (Pennington Biomedical Research Center). Murine liver carnitine palmitoyltransferase 1 (CPT1a) cDNA was obtained by RT-PCR with liver total RNA and primers designed from the reported sequence (GenBank accession no. NM_013495). Recombinant adenovirus, containing murine UCP1 (11) or CPT1a cDNA under the CAG promoter, was prepared as described previously (12). A recombinant adenovirus bearing the bacterial β-galactosidase gene (Ad5/1C1αlac-Z) (13) was used as a control.

Animals. Animal studies were conducted under protocols in accordance with the institutional guidelines for animal experiments at Tohoku University. Male C57BL/6N mice were housed individually and divided into high-fat diet (32% fat)-fed and normal diet-fed groups. Two groups of mice of age, when body weights were 21.2 ± 0.5 g, were separated, body weight−matched mice for each group received an injection of adenovirus via the tail vein. Hindlimb muscles were removed and fixed with 10% formalin and subjected to SDS-PAGE. Four weeks after infection, body weight−matched mice for each group received an injection of adenovirus via the tail vein. Viruses were administered intravenously at a dose of 1×1010vp/kg body wt; the latter LacZ mice were given their daily food allotments on the basis of the previous day’s consumption by UC1P1 mice.

Antibodies. UC1P1, acetyl-CoA carboxylase 1 (ACC 1), and insulin receptor antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The α-subunit of AMP-activated protein kinase (AMPK), phospho-AMPK (Thr172), and phospho-ACC (Ser79) antibodies were obtained from Cell Signaling Technology (Beverly, MA). Affinity-purified antibody against insulin receptor substrate 1 (IRS1) was prepared as described previously (15).

Immunoblotting. Tissue samples were prepared as previously described (9), and tissue protein extracts (250 μg of total protein) were boiled in Laemmli buffer that contained 10 mmol/l dithiothreitol and subjected to SDS-PAGE. The immunoblots were visualized with an enhanced chemiluminescence detection kit (Amersham, Buckinghamshire, U.K.).

Oxygen consumption. Oxygen consumption was measured with an O2/CO2 metabolism measuring system (model MK-5000RQ; Muromachi Kikai, Tokyo, Japan). Each mouse was kept unrestrained in a sealed chamber with an air flow of 0.5 l/min for 5 h at 25°C without food or water during the light cycle. Air was sampled every 3 min, and the consumed oxygen concentration (Vo2) was calculated.

Histological analysis. Livers as well as epididymal fat (white adipose tissue) and brown adipose tissues were removed and fixed with 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin. Total adipocyte areas were traced manually and analyzed. Brown and white adipocyte areas were measured in 100 or more cells per mouse in each group.

Measurement of body temperature. Rectal temperature was measured with a ThermaLink TH-5 (Physitemp, Clifton, NJ).

Measurement of AMPK activity. AMPK activity in the immunoprecipitates was assessed as a function of SAMS peptide phosphorylation, as previously described (18).

Tyrosine phosphorylation of insulin receptor and IRS1. Mice that were fasted for 16 h received an injection of 100 μl of normal saline (0.9% NaCl), and blood glucose was assayed immediately before and at 15, 30, 60, and 120 min after administration. Insulin tolerance tests were performed on fed mice. Mice received an injection of human regular insulin (0.75 units/kg body wt), and blood glucose was assayed immediately before and at 15, 30, 60, and 120 min after injection. Leptin tolerance tests were performed as reported previously (19) with slight modification. Fasted (12 h) mice received an injection of mouse leptin (7.2 ng/kg body wt; B&D Systems) into the intraperitoneal space, and food intake amounts for 12 h thereafter were determined. Ratios of food intake amounts to those of vehicle-injected mice were calculated.

Quantitative RT-PCR-based gene expression. Total RNA was isolated from 0.1 g of mouse hepatic tissue with ISOGEN (Wako Pure Chemical, Osaka, Japan) and treated with DNase I and RNase. Real-time quantitative PCR analysis was performed using Power SYBR Green PCR Master Mix (Applied Biosystems). The expression of the target genes was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

TABLE 1  Sequences of quantitative RT-PCR primers

<table>
<thead>
<tr>
<th>Probe</th>
<th>Primer 1</th>
<th>Primer 2</th>
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<tbody>
<tr>
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<td>5′-gccccgttagcttggtggttgta-3′</td>
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<tr>
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<td>5′-gctggtcctggaagg-3′</td>
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<tr>
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<td>5′-gctggtcctggaagg-3′</td>
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FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; FAT, fatty acid transporter; MCAD, medium-chain acyl-CoA dehydrogenase; PEPCk, phosphoenolpyruvate carboxylase; G6Pase, glucose-6-phosphatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
cDNA synthesis was performed with a Cloned AMV First Strand Synthesis Kit (Invitrogen, Rockville, MD) using 5 μg of total RNA. cDNA synthesized from total RNA was evaluated in a real-time PCR quantitative system (Light Cycler Quick System 350S; Roche Diagnostics, Mannheim, Germany). The relative amount of mRNA was calculated with glyceraldehyde-3-dehydrogenase mRNA as the invariant control. The primers used are described in Table 1.

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

RESULTS

Hepatic UCP1 expression increased energy expenditure and reduced body weight and blood glucose levels in mice that had high-fat diet–induced obesity and diabetes. C57BL/6 mice were on a high-fat diet for 4 weeks, resulting in diabetes associated with obesity. The UCP1 adenovirus vector (11) was then administered intravenously (UCP1 mice). Mice that were given the LacZ adenovirus were used as a control (LacZ mice). No significant alterations were observed in body weights (Fig. 1B), blood glucose levels (Fig. 1C), food intake amounts, body temperature, or plasma lipid parameters (data not shown) before versus after LacZ adenovirus administration. Systemic infusion of recombinant adenoviruses into mice through the tail vein primarily resulted in expression of transgenes in the liver, with no detectable expression in peripheral tissues such as muscle, fat, kidney, or brain (data not shown), as reported previously (20). As shown in Fig. 1A, immunoblotting revealed that ectopic UCP1 expression in the liver peaked on day 3. Maximal expression was maintained through day 8. After day 9, hepatic expression of UCP1 decreased, and very small amounts of UCP1 protein were detected on day 14 (Fig. 1A).

In UCP1 mice, body weight and blood glucose levels were markedly decreased (Fig. 1B and C) concomitantly with hepatic expression levels of UCP1. On day 7, body weights of UCP1 mice were significantly lower, by 13%, than those of control mice. After day 9, body weight and

FIG. 1. Hepatic UCP1 expression reduced body weight and blood glucose levels. A: Ectopic UCP1 expression in the liver in high-fat–fed mice was detected by immunoblotting of hepatic extracts (250 μg total protein/lane). Liver samples were collected at different times after adenovirus injection. B and C: Body weights (B) and blood glucose levels (C) in the ad libitum–fed state after adenoviral administration in control (LacZ) mice (●) and UCP1 mice (○) (n = 4 per group). D: Resting VO₂ was measured on day 3 after adenoviral injection with open-circuit indirect calorimetry. All mice were kept in a cage for ~5 h in the daytime without food or water (n = 5 per group). E: Rectal temperature was measured in the ad libitum–fed state on day 7 after adenoviral injection (n = 6 per group). F: Average daily food intake amounts over the first and the second weeks after adenoviral administration are presented. Regarding all panels, similar results were obtained from 10 or more experiments, and representative results are presented as means ± SE. *P < 0.05, **P < 0.01 assessed by unpaired t test.
blood glucose levels began to increase as the expression of hepatic UCP1 declined. These findings indicate that hepatic UCP1 expression exerted therapeutic effects on diabetes associated with diet-induced obesity.

Resting oxygen consumption on day 3 was markedly increased, by 12%, in UCP1 mice compared with controls (Fig. 1D), whereas rectal temperature did not differ between the two (Fig. 1E). Thus, ectopic UCP1 in the liver, like endogenous UCP1 in brown adipocytes, promoted inefficient metabolism, thereby enhancing energy expenditure and leading to weight reduction. This effect, however, was not sufficient to raise whole-body temperature. In addition, hepatic UCP1 expression changed food intake. Whereas without hepatic UCP1 expression, food intake amounts in high-fat–fed mice were markedly increased compared with those in standard diet–fed lean mice (compare Figs. 1F and 5D), hepatic UCP1 expression reversed hyperphagia in mice with high-fat diet–induced obesity and diabetes (Fig. 1F). After day 8, concomitantly with the drop in hepatic UCP1 expression, hyperphagia was restored (Fig. 1F). In contrast, mice received an intravenous injection of adenovirus encoding CPT1a, another mitochondrial protein, did not show significantly altered food consumption (data not shown), suggesting that food intake suppression induced by hepatic UCP1 expression is not a nonspecific effect of expression of any of the hepatic mitochondrial proteins.

To eliminate any secondary effects of reduced food intake induced by hepatic UCP1 expression, we performed pair-feeding experiments. In contrast to UCP1 mice, pair-fed LacZ mice exhibited only slight decreases in body weights and blood glucose levels (of 3.1 and 6.9%, respectively, on day 7 after adenoviral administration). These results suggest that increased energy expenditure is an important mechanism underlying marked improvements of obesity and diabetes in UCP1 mice.

**Hepatic UCP1 expression decreased fat contents in the liver and adipose tissues.** Hepatic and adipose fat accumulations were examined on day 7 after adenoviral gene delivery. In the high-fat–fed control mice, liver weight and triglyceride content were markedly increased compared with the standard chow–fed lean mice (compare Fig. 2A and B with Fig. 5E and F, respectively). Hepatic UCP1 expression significantly decreased liver weight (Fig. 2A) and triglyceride content (Fig. 2B) compared with LacZ mice, with high-fat feeding. It is interesting that hepatic UCP1 expression also decreased fat content in their adipose tissues. For example, epididymal fat weight was significantly decreased in UCP1 mice compared with that in controls (Fig. 2C). Thus, hepatic expression of UCP1 exerts not only local effects in the liver but also remote effects on metabolism in other tissues.

These results were confirmed by the histological findings. No apparent infiltration or structural change was observed in the livers of either LacZ mice or UCP1 mice, indicating the absence of adenovirus-induced liver damage (Fig. 2D). Whereas abundant lipid droplets were present in the livers of control mice, these lipid droplets were markedly diminished in UCP1 mouse livers, indicating marked improvement of fatty liver findings in response to UCP1 expression (Fig. 2D). Furthermore, the cell diameters in epididymal fat (Fig. 2E) and brown adipose (Fig. 2F) tissues were significantly decreased in UCP1 mice. Expression levels of endogenous UCP1 protein in brown adipocytes were similar in the two groups (Fig. 2G), suggesting that energy expenditure in brown adipocytes was not increased in UCP1 mice. These findings suggest that hepatic UCP1 expression promotes hydrolysis of triglycerides already stored in adipose tissues, leading to smaller adipocytes with the resultant fatty acids being mobilized and metabolized as a substrate for oxidation in the liver.

**Hepatic expressions of enzymes involved in lipid metabolism and glucose production.** To elucidate the underlying mechanism whereby stored fat was decreased in the liver by hepatic UCP1 expression, we examined the expressions of proteins involved in lipid metabolism by quantitative RT-PCR. Significant reductions in the expressions of the lipogenic enzymes, including stearoyl-CoA desaturase-1 and fatty acid synthase, were observed in UCP1 mice (Fig. 3A). Sterol regulatory element binding protein 1c (SREBP1c) expression in the liver tended to be diminished. In contrast, hepatic expressions of enzymes involved in fatty acid oxidation tended to be increased. In particular, expressions of fatty acid transporter and UCP2 were significantly increased (Fig. 3B).

We further examined expression levels of key enzymes for hepatic glucose production. Hepatic phosphoenolpyruvate carboxykinase and glucose-6-phosphatase expressions were significantly decreased in UCP1 mice (Fig. 3C), suggesting a decrease to contribute to improvement of diabetes.

UCP1 expression may activate AMPK as a result of decreased generation of ATP. AMPK activation reportedly decreases malonyl-CoA generation via inhibition of ACC (21), resulting in enhancement of fatty acid oxidation. Therefore, ATP levels and AMPK phosphorylation in the liver were examined in LacZ and UCP1 mice under ad libitum feeding conditions. Hepatic ATP concentrations in UCP1 mice were approximately half those in control mice (Fig. 3D) but still ~2.3-fold those in standard diet–fed control mice. Hepatic AMPK activity was increased 1.6-fold in UCP1 mice compared with LacZ mice (Fig. 3E). The phosphorylation state of the α-subunit of AMPK in the liver was enhanced in UCP1 mice (Fig. 3F). Furthermore, resultant enhancement of hepatic ACC phosphorylation was observed (Fig. 3G). These findings suggest that AMPK activation induced by UCP1 expression plays an important role in the observed marked improvement of fatty liver findings via enhanced fatty acid oxidation.

**Glucose and lipid metabolism in UCP1 mice.** The results of oral glucose tolerance (Fig. 4A) and insulin tolerance (Fig. 4B) tests on day 7 after adenoviral administration clearly showed that hepatic expression of UCP1 markedly improved glucose tolerance and insulin sensitivity in obese and diabetic mice. Improved insulin sensitivity in muscle was confirmed by enhanced insulin receptor and IRS1 phosphorylation (Fig. 4C) in response to insulin administration. Thus, hepatic UCP1 expression exerts a remote beneficial effect on insulin sensitivity in muscle.

In addition, plasma lipid parameters were decreased in UCP1 mice. Total plasma cholesterol levels tended to be decreased in UCP1 mice compared with controls, although the changes were not statistically significant (Fig. 4D). Plasma triglyceride and free fatty acid levels were signifi-
cantly decreased in UCP1 mice (Fig. 4D). Thus, hepatic UCP1 expression also improved diet-induced dyslipidemia.

Serum insulin levels were markedly decreased, by 57% (Fig. 4E), in UCP1 mice, despite lower blood glucose levels (Fig. 1C), indicating marked improvement of systemic insulin sensitivity. Serum adiponectin and TNF-α levels were similar in these groups (Fig. 4F), suggesting that these adipocytokines are not involved in the improvement of insulin resistance in UCP1 mice. In contrast, serum leptin levels were significantly decreased, by 56%, in
UCP1 mice compared with those in control mice (Fig. 4F) concomitantly with decreased food intake (Fig. 1F). In control mice that were fed a high-fat diet, marked hyperleptinemia was observed (serum leptin concentrations, standard diet–fed mice versus high-fat diet–fed mice: 0.48 ± 0.08 vs. 32.0 ± 4.6 ng/ml) despite increased food intake (compare Fig. 1F with Fig. 5D), indicating leptin resistance. The present results suggest that hepatic UCP1 expression improves hypothalamic leptin resistance in obese and diabetic mice. To directly test whether leptin sensitivity was improved, we performed leptin tolerance tests (Fig. 4G). Leptin was injected intraperitoneally into fasted mice, followed by measurement of 12-h food intakes. The food intake inhibition by leptin administration was far more profound in UCP1 mice than in LacZ mice. Thus, UCP1 mice responded strongly to leptin administration, clearly showing that hepatic UCP1 expression exerts a therapeutic effect on hypothalamic leptin resistance.

Hepatic UCP1 expression exerted minimal effects in standard diet–fed lean mice. Hepatic UCP1 expression reduced body weight and blood glucose and lipid levels in obese and diabetic mice. These are very promising results suggesting that ectopic UCP1 expression may be useful in treating diabetic individuals who are obese. However, if

FIG. 3. Hepatic expressions of enzymes involved in lipid metabolism and glucose production and phosphorylations of AMPK and ACC. A–C: Relative amounts of mRNA were measured by quantitative RT-PCR and corrected with glyceraldehyde-3-dehydrogenase as the standard. Hepatic total RNA of mice, on day 3 after adenoviral administration in the 10-h–fasted state, was isolated. Expressions of lipogenic enzymes and SREBP1c (A), enzymes for fatty acid oxidation and PPAR-α (B), and enzymes for hepatic glucose production (C) in the liver were assayed (n = 6 per group). D and E: ATP concentrations (D) and AMPK activity (E) in the liver were measured. Data are presented as the relative amounts compared with those in standard diet–fed control mice (n = 6 per group). F and G: Immunoblots using anti–phospho-AMPK (F) or anti–phospho-ACC (G) antibody (top), as well as anti-AMPK (F) or anti-ACC1 (G) antibody (bottom) revealed the phosphorylation state of the AMPK α-subunit in the liver on day 3 after adenoviral injection (n = 2 per group). Data are presented as means ± SE. *P < 0.05 assessed by unpaired t test.
this were also the case in lean individuals, then these individuals would become leaner, possibly even developing malnutrition and hypoglycemia. We therefore performed experiments with a similar design but used 9-week-old standard diet–fed lean mice, i.e., the same age as the high-fat–fed mice.

It is intriguing that although ectopic UCP1 expression levels in the liver were similar under high-fat and standard diet conditions (Fig. 5A), the resultant phenotypes were completely different. In standard diet–fed lean mice, hepatic UCP1 expression did not alter body weight (Fig. 5B), fasting blood glucose levels (Fig. 5C), or food intake.
amounts (Fig. 5D). In addition, hepatic weight (Fig. 5E), triglyceride content (Fig. 5F), and epididymal fat weight (Fig. 5G) were not changed. Thus, hepatic UCP1 expression did not exert significant effects on glucose metabolism or adiposity in lean mice.

To determine why hepatic UCP1 expression in lean mice did not significantly alter metabolic conditions, we measured basal energy expenditure and hepatic ATP contents. Hepatic UCP1 expression did not significantly change basal energy expenditure (Fig. 5H) or hepatic ATP levels (Fig. 5I), suggesting that UCP1 ectopically expressed in the liver is minimally involved in mitochondrial uncoupling, when surplus energy is not stored in the liver. Thus, hepatic UCP1 is likely to dissipate excess energy while having no effect on required energy. These characteristics are favorable in terms of therapeutic strategies for the metabolic syndrome.

**DISCUSSION**

In this study, after mice had developed obesity-associated diabetes, ectopically expressing UCP1 in the liver resulted in marked improvements in both disease conditions. UCP1 expression would be expected to decrease ATP generation in the liver and thus to activate hepatic AMPK. Indeed, ATP contents were decreased, and AMPK and ACC phosphorylations were increased. AMPK reportedly phosphorylates and inactivates ACC, resulting in a decrease in malonyl-CoA generation (21). Because malonyl-CoA is a negative regulator via suppression of CPT1, a rate-limiting enzyme for fatty acid oxidation (22), a decrease in malonyl-CoA generation is likely to enhance fatty acid oxidation to meet respiratory demands. Furthermore, hepatic expressions of lipogenic enzymes were decreased by UCP1 expression in the liver, which may be explained by...
AMPK activation and possible SREBP1 reduction in the liver; metformin reportedly activates AMPK and inhibits hepatic SREBP1 expression (23). Taken together, the results suggest that fatty acid synthesis was suppressed with concomitant enhancement of fatty acid oxidation, resulting in the marked decrease in hepatic triglyceride contents.

How might a change in hepatic lipid metabolism affect the energy balance of the entire body? It is noteworthy that the weight and/or cell sizes of epididymal fat and brown adipose tissues were markedly decreased by hepatic UCPI expression in the present study. Inhibition of fat accumulation in adipose tissues was also observed in UCPI and in UCPI transgenic mice under the control of muscle-specific promoters (7,8). Mice lacking ACC2, which is predominantly expressed in the heart and muscle of wild-type mice, also markedly inhibited fat accumulation in their adipose tissues (24). In reports using transgenic models, muscle is a site of increasing energy expenditure, through mitochondrial uncoupling, which prevents obesity. In the present study, hepatic expression of UCPI reduced fat contents, rather than inhibiting fat accumulation, not only in the liver but also in adipose tissues, indicating promotion of hydrolysis of triglycerides already stored in the adipose tissues. Thus, hepatic uncoupling is likely to convey signals to peripheral adipose tissues. These signals might involve an autonomic nervous network, because the hydrolysis of triglycerides stored in adipose tissues is controlled mainly by the cAMP-mediated pathway, including sympathetic nerve activation (25). Alternatively, a decline in serum fatty acid concentrations, observed in UCPI mice, or some unknown factors secreted by the liver might trigger lipolysis in adipose tissues. Although more work is required to elucidate the mechanism underlying this remote effect, enhancement of hepatic uncoupling is likely to exert therapeutic, rather than preventive, effects on insulin resistance associated with obesity. Thus, the liver is a potential therapeutic target for diabetes with obesity. Furthermore, unraveling the underlying mechanism may lead to development of antiobesity pharmacological agents that promote lipolysis in adipose tissues.

The present results are also interesting with respect to appetite regulation. Transgenic mice overexpressing UCP3 in skeletal muscle are reportedly hyperphagic (8), whereas UCPI transgenic mice show no changes in food intake (7). In these transgenic mice, UCPIs are continuously overexpressed throughout life, including in the fetal stage. In contrast, the UCP was expressed after development of adipose tissues, indicating promotion of hydrolysis of triglycerides already stored in the adipose tissues. Thus, hepatic uncoupling is likely to convey signals to peripheral adipose tissues. These signals might involve an autonomic nervous network, because the hydrolysis of triglycerides stored in adipose tissues is controlled mainly by the cAMP-mediated pathway, including sympathetic nerve activation (25). Alternatively, a decline in serum fatty acid concentrations, observed in UCPI mice, or some unknown factors secreted by the liver might trigger lipolysis in adipose tissues. Although more work is required to elucidate the mechanism underlying this remote effect, enhancement of hepatic uncoupling is likely to exert therapeutic, rather than preventive, effects on insulin resistance associated with obesity. Thus, the liver is a potential therapeutic target for diabetes with obesity. Furthermore, unraveling the underlying mechanism may lead to development of antiobesity pharmacological agents that promote lipolysis in adipose tissues.

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increases. A high mitochondrial electrochemical gradient is associated with the production of reactive oxygen species that may damage tissues, a possible cause of diabetes complications and atherosclerosis (30). Thus, the respiratory uncoupling increment in the liver may protect tissues from oxidative stress. Taken together with the results of the present study, enhancement of UCPs in the liver may protect against diabetes complications and atherosclerosis (39). Thus, the possible reactive oxygen species in obese and diabetic individuals.

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