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# Taurine (2-Aminoethanesulfonic Acid) Deficiency Creates a Vicious Circle Promoting Obesity

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**The relation between blood taurine (2-aminoethanesulfonic acid) concentrations and obesity was investigated. Taurine is supplied to the body by dietary ingestion as well as by *de novo* synthesis; it is anabolized by cysteine dioxygenase (CDO), which is abundantly expressed in liver and white adipose tissue. Overexpression of CDO in 3T3-L1 preadipocytes caused a decrease in the level of cysteine (precursor of taurine) and an increase in the level of taurine in the culture medium, suggesting that CDO is involved in biosynthesis and secretion of taurine in white adipose tissue. In high-fat diet-induced and/or genetically obese mice, a decrease in the blood taurine concentration was observed along with a decrease in CDO expression in adipose tissue but not in liver. Dietary taurine supplementation prevented high-fat diet-induced obesity with increased resting energy expenditure. Thus, taurine deficiency observed in association with obesity may create a vicious circle promoting obesity. Dietary taurine supplementation interrupts this vicious circle and may prevent obesity. (*Endocrinology* 147: 3276–3284, 2006)**

OBESITY AND DIABETES are serious health problems in Western countries. An increase in the amounts of ingested fat due to increased consumption of meat is thought to be one cause of obesity and type II diabetes. It is speculated that a fish- rather than meat-based diet reduces the risk of obesity and diabetes (1). 2-Aminoethanesulfonic acid (taurine) is a sulfur amino acid that is abundant in seafood but not in meat (2). Dietary taurine might be beneficial in preventing obesity and diabetes.

In animals, dietary taurine improved high blood pressure, liver damage, and hypercholesterolemia (3, 4). However, its effect on obesity is not clear. In mice, taurine administered in drinking water reduced high-fat (HF) diet-induced arterial lipid accumulation but did not reduce HF diet-induced obesity (5). Taurine reduced the body weight (BW) and abdominal fat pads in genetically obese KK mice, but these effects were not observed in BALB/C mice (6). In OLETF diabetic rats, dietary taurine supplementation improved insulin sensitivity but did not significantly reduce BW (7, 8).

In mammals, taurine is obtained via two pathways. The first is dietary ingestion, and the second is *de novo* synthesis. The key enzyme in the taurine biosynthetic pathway is cysteine dioxygenase (CDO), which catalyzes oxygenation of L-cysteine to yield L-cysteine sulfinate (9). Because CDO activity and CDO expression are highest in the liver (9–12), liver is considered the most important tissue for taurine synthesis. It was recently reported that CDO mRNA is also expressed in white adipose tissue (WAT) (13, 14). However, the role of CDO in WAT remains unclear.

Thus, we examined the role of blood taurine concentration in obesity. Results of this study suggest that taurine, obtained via dietary ingestion or *de novo* synthesis, plays a role in preventing obesity. Supplementation with taurine or activation of taurine synthesis might be a novel strategy for treating individuals with obesity.

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Abbreviations: BAT, Brown adipose tissue; BMI, body mass index; BW, body weight; CDO, cysteine dioxygenase; C/EBP, CCAAT/enhancer-binding protein; CoA, coenzyme A; DEXA, dual energy x-ray absorptiometry; en%, % of total energy; HC, high-carbohydrate; HF, high fat; KRHA, Krebs-Henseleit HEPES albumin; PGC, PPARγ coactivator; PPAR, peroxisome proliferator-activated receptor; PSL, phosphostimulated luminescence; taurine, 2-aminoethanesulfonic acid; UCP, uncoupling protein; WAT, white adipose tissue.

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## Materials and Methods

### *Animals*

Female C57BL/6J mice (7 wk old) were obtained from Tokyo Laboratory Animals Science Co. (Tokyo, Japan), and female KKA$^{y}$ mice and db/db mice were purchased from Clea Japan (Shizuoka, Japan). Mice were housed in cages at six per cage. Mice were exposed to a 12-h light, 12-h dark cycle and maintained at a constant temperature of 22 C. All procedures were in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals in Japan.

### *Northern blot analysis*

Liver, WAT (parametrial, retroperitoneal, sc), brown adipose tissue (BAT), muscle, spleen, kidney, heart, lung, and brain isolated from female C57BL/6J mice immediately after being killed were homogenized in guanidine-thiocyanate, and RNA was prepared (15). Total RNA (10 μg/lane) was denatured with glyoxal and dimethyl sulfoxide, fractionated by electrophoresis on 1% agarose gels, transferred to nylon membranes, and probed with $^{32}$P-labeled cDNA. cDNA fragments for probes were prepared as described previously (10). Amounts of mRNA were quantitated with an image analyzer (BAS 1800-II, FujiFilm, Tokyo,

Japan) and expressed as the intensity of phosphostimulated luminescence (PSL).

### *Isolation of adipocytes and nonadipocytes from adipose tissue*

Adipocytes and nonadipocytes were isolated by collagenase digestion from the parametrial adipose tissue of 12–18 mice. The tissue homogenates were fractionated by brief centrifugation (350 g for 20 sec) in Krebs-Henseleit HEPES albumin (KRHA) buffer [119 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 32.3 mM HEPES (pH 7.4), 20 mg/ml BSA (fraction V), and 2 mM glucose]. Floating cells were adipocytes, whereas the pelleted cells were nonadipocytes (16). The isolated adipocytes and nonadipocytes were homogenized immediately in guanidine-thiocyanate, and RNA was prepared (15). The adipocytes fractions were cultured in KRHA buffer at 37 C. Taurine concentrations were measured in culture media at 0, 30, 60, and 90 min after incubation.

### *3T3-L1 cell culture*

Murine 3T3-L1 preadipocytes were purchased from American Type Culture Collection (Rockville, MD). 3T3-L1 cells were cultured in DMEM (4500 mg glucose/liter) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin in a 5% $CO_2$ atmosphere at 37 C. Differentiation of 3T3-L1 cells into adipocytes was initiated by addition of 1 $\mu$M dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 $\mu$g/ml insulin to confluent cells for 2 d. Cells were refed every 2 d. Total RNA was isolated from 3T3-L1 cells at 0, 1, 2, 3, 5, 8, and 10 d after differentiation.

### *Ectopic overexpression of CDO in 3T3-L1 cells*

cDNA for rat CDO was subcloned into the *Eco*RI site of the pLXSN retroviral expression vector (BD Bioscience CLONTECH, Palo Alto, CA). Retroviral constructs were purified with an endotoxin-free plasmid preparation kit (QIAGEN K.K., Tokyo, Japan). For the preparation of recombinant retroviruses, expression constructs were transfected transiently into Phoenix 293 packaging cells (17) with Lipofect Amine Plus (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Viral supernatants were harvested 48 h after transfection. Viral supernatants were applied to 3T3-L1 cells in DMEM containing 10% fetal bovine serum and 5 $\mu$g/$\mu$l polybrene. Cells infected with retrovirus were selected for by the addition of 1.5 mg/ml neomycin (G418) to the culture medium. Taurine and cysteine concentrations in media after 3 d of culture were measured with an amino acid analyzer (SRL, Tokyo, Japan). Levels of taurine and cysteine in media before culture were subtracted from the experimental levels determined after 3 d of culture, and the resulting values were expressed as taurine secretion and cysteine use.

### *Mice models of diet-induced and genetically based obesity*

C57BL/6J mice were fed a high-carbohydrate (HC) diet [10% fat of total energy (10 en% fat)] or a safflower oil-based HF diet (60 en% fat) for 23 wk. Compositions of the diets and the protocol were as described previously (18). $KKA^y$ mice were fed a HC diet (10 en% fat) or a safflower oil-based HF diet (60 en% fat) for 25 wk. db/db mice were fed laboratory chow for 1 wk. Insulin tolerance tests were conducted at 6 wk after the HF diet was begun, as previously described (19). The HC diet, HF diet, and laboratory chow diet did not contain any taurine.

### *Histologic analysis and morphometry*

Parametrial adipose tissue from mice was fixed in 4% paraformaldehyde in PBS and then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. To determine the size of adipocytes, sectional areas of WAT in hematoxylin and eosin-stained preparations were analyzed. For analysis of CDO protein, deparaffinized sections were reacted with anti-CDO antisera in 0.05 M Tris buffer (pH 7.6) at 4 C for 14 h. After being washed with 0.05 M Tris buffer (pH 7.6), sections were reacted with biotinylated antirabbit Ig in 0.05 M Tris buffer (pH 7.6) for 30 min. Sections were then visualized with streptavidin-peroxidase conjugate. For negative control, sections were treated by the same procedure but without anti-CDO antisera.

### *Dietary taurine supplementation*

C57BL/6J mice were fed a HC diet (10 en% fat), safflower oil-based HF diet (60 en% fat), or a HF plus taurine diet (5% wt/wt) for 18 wk. Compositions of the HC and HF diets and the protocol were as described previously (18). Energy intake was measured every day for 18 wk. To estimate insulin resistance, insulin tolerance tests were conducted under feeding conditions 14 wk after the diets were started, as described previously (19). Sigma glucose levels were determined 0, 30, 90, and 120 min after insulin injection. Body fat levels were estimated by dual-energy x-ray absorptiometry (DEXA) at 18 wk after the diets were started (Lunar PIXI Mus2 Densitometer, Lunar Corp., Madison, WI). Oxygen consumption was measured with the use of a metabolic chamber at 9–15 wk after the diets were started, as described previously (19). Each group of mice (1 mouse per cage) was placed in a metabolic chamber of the open-circuit oxygen consumption measuring system for 24 h. Oxygen consumption was measured by a computerized system with a 1-liter chamber maintained at 25 C with air flow of 0.2 ml/min and an oxygen consumption monitor (Osaka Microsystems, Osaka, Japan). Mice were unstrained and given free access to the experimental food and water. The oxygen consumption rate was monitored in the resting (0801–1600 h) and active (2001–0600 h) states and normalized to $BW^{0.75}$. Physical activity was measured as running wheel activity. C57BL/6J mice were housed individually in cages ($9 \times 22 \times 9$ cm) equipped with a running wheel (20 cm in diameter, Shinano Co., Tokyo, Japan) for 4 d. Experimental diet feeding was then started. Each wheel revolution was registered by a magnetic switch, which was connected to a counter. The number of revolutions was recorded daily for 9 d.

### *Statistical methods*

Differences between two groups were analyzed by Student's unpaired *t* test. Differences between more than two groups were analyzed by one-way ANOVA. When differences were significant, values in each group were compared with values in the other groups by means of Fisher's protected least significant difference test. All statistical analyzes were performed with StatView 5.0 (Abacus Concepts, Inc., Berkeley, CA). Statistical significance was defined as $P < 0.05$. Values are presented as mean ± SEM.

## Results

### *CDO is expressed in murine adipocytes and differentiated 3T3-L1 adipocytes*

Initially, levels of CDO mRNA were measured in various tissues of mice. In C57BL/6J mice, CDO mRNA levels were highest in liver, parametrial WAT, and BAT, and significant levels were detected in kidney and lung (Fig. 1A, *left*). When WAT subtypes were compared, CDO mRNA levels were found to be higher in visceral fat (parametrial and retroperitoneal WAT) than in sc WAT (Fig. 1A, *right*). To examine whether CDO is expressed in adipocytes and nonadipocytes (including preadipocytes, blood cells, and macrophages) from adipose tissues, WAT was digested by collagenase, each fraction was separated by brief centrifugation, and CDO mRNA levels were measured in each fraction (Fig. 1B). CDO was expressed mostly in adipocytes and was hardly expressed in nonadipocytes. In 3T3-L1 cells, the expression of CDO mRNA gradually increased during differentiation from preadipocytes to mature adipocytes. Adipocyte differentiation over 10 d resulted in a 6-fold increase in the CDO mRNA

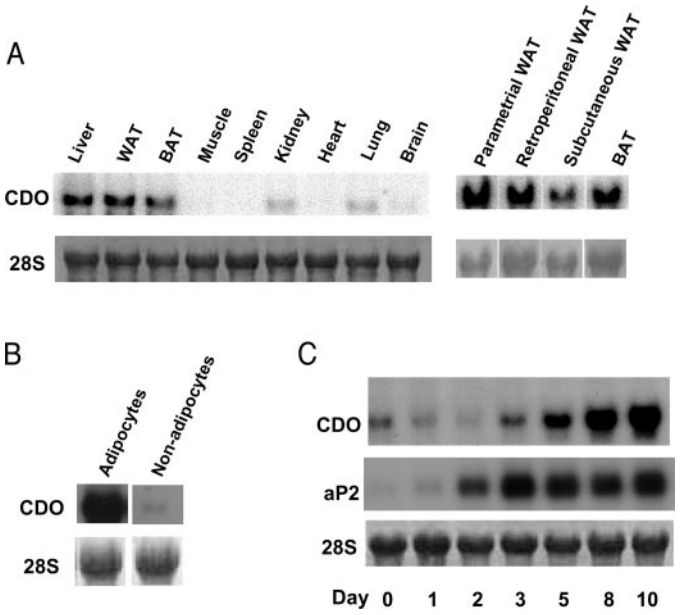


FIG. 1. CDO is expressed in murine adipocytes and differentiated 3T3-L1 adipocytes. A, Distribution of CDO mRNA in mice. Total RNA for liver, parametrial WAT, BAT, muscle, spleen, kidney, heart, lung, brain (left), parametrial WAT, retroperitoneal WAT, sc WAT, and interscapular BAT (right) were isolated from female C57BL/6J mice. The Northern blot membrane was hybridized with $^{32}$P-labeled cDNA probe for mouse CDO. Equal sample loading was confirmed by ethidium bromide staining of *28S* ribosomal RNA. B, Expression of CDO mRNA by adipocytes and nonadipocytes isolated from parametrial WAT. Adipose tissue pooled from 12 mice was digested by collagenase and then separated into adipocytes and nonadipocytes by brief centrifugation. The isolated adipocytes and nonadipocytes were homogenized immediately in guanidine-thiocyanate, and RNA was prepared (12). Equal sample loading was confirmed by ethidium bromide staining of *28S* ribosomal RNA. C, Effect of adipocyte differentiation on expression of CDO mRNA by 3T3-L1 cells. 3T3-L1 cells were induced to differentiate with 1 $\mu$M dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 $\mu$g/ml insulin for 2 d. Cells were refed every 2 d. Equal sample loading was confirmed by ethidium bromide staining of *28S* ribosomal RNA. aP2, Adipocyte fatty acid binding protein 2.

level in comparison with the level in 3T3-L1 preadipose cells (Fig. 1C). These results indicate that CDO is expressed in mature adipocytes.

### *CDO may increase taurine secretion in adipocytes*

When primary adipocytes isolated from parametrial WAT were incubated in culture media, the taurine concentration increased gradually (Fig. 2A). This suggests that adipocytes secrete taurine. To examine whether CDO is involved in taurine secretion in adipocytes, CDO was overexpressed in 3T3-L1 cells by means of a retroviral vector, and taurine and cysteine concentrations were measured in culture media (Fig. 2B). The level of taurine secreted into culture media for 3 d was increased significantly by CDO overexpression in comparison with the control level. In contrast, the level of used cysteine, which is the substrate of CDO for synthesis of taurine, was decreased significantly by CDO overexpression. These results suggest that CDO increases the synthesis of taurine from cysteine and that the taurine is secreted from adipocytes.

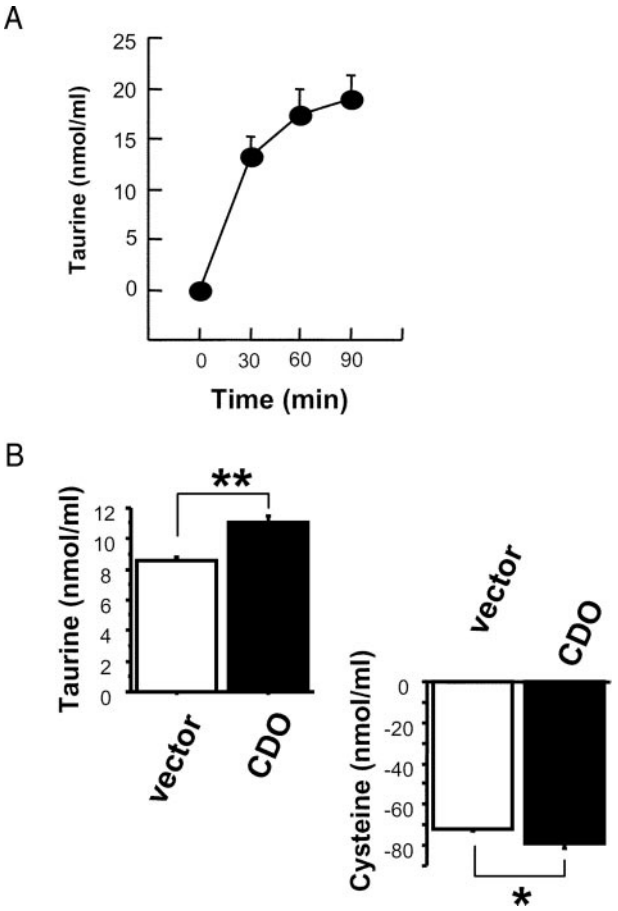


FIG. 2. CDO mediates taurine secretion in adipocytes. A, Taurine concentration in media of cultured primary adipocyte isolated from parametrial WAT of C57BL/6J mice. Primary adipocyte fractions isolated by collagenase digestion were incubated in KRHA buffer at 37 C. Taurine concentrations were measured in culture media at 0, 30, 60, and 90 min after incubation. B, Taurine and cysteine concentrations in culture media in response to CDO overexpression in 3T3-L1 cells achieved by retrovirus-mediated gene transfer. Stable cells expressing CDO were induced to differentiate with 1 $\mu$M dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 $\mu$g/ml insulin for 2 d. Taurine and cysteine concentrations in media after 3 d of culture were measured with an amino acid analyzer. Levels of taurine and cysteine in media before culture were subtracted from the experimental levels determined after 3 d of culture and expressed as taurine secretion and cysteine use. Values are expressed as mean ± SEM (n = 5). **, $P < 0.01$; *, $P < 0.05$.

### *Blood taurine concentration and CDO expression in WAT are reduced in obese mice fed a HF diet*

To examine whether the blood taurine concentration and CDO expression in WAT are related to obesity, levels were measured in obese C57BL/6J mice fed a HF diet. The blood taurine concentration and parametrial WAT CDO mRNA level in the obese mice fed a HF diet were reduced by 33 and 52%, respectively, in comparison with levels in mice fed a lean HC diet (Fig. 3, A and B). The protein level of CDO was also decreased by the HF diet in parametrial WAT slices (Fig. 3C). As expected, the HF diet *vs.* the HC diet increased BW (Fig. 3D), visceral WAT mass (Fig. 3E), and the diameter of adipocytes (Fig. 3F), and it induced insulin resistance (Fig. 3G). The level of CDO mRNA in liver and BAT was not

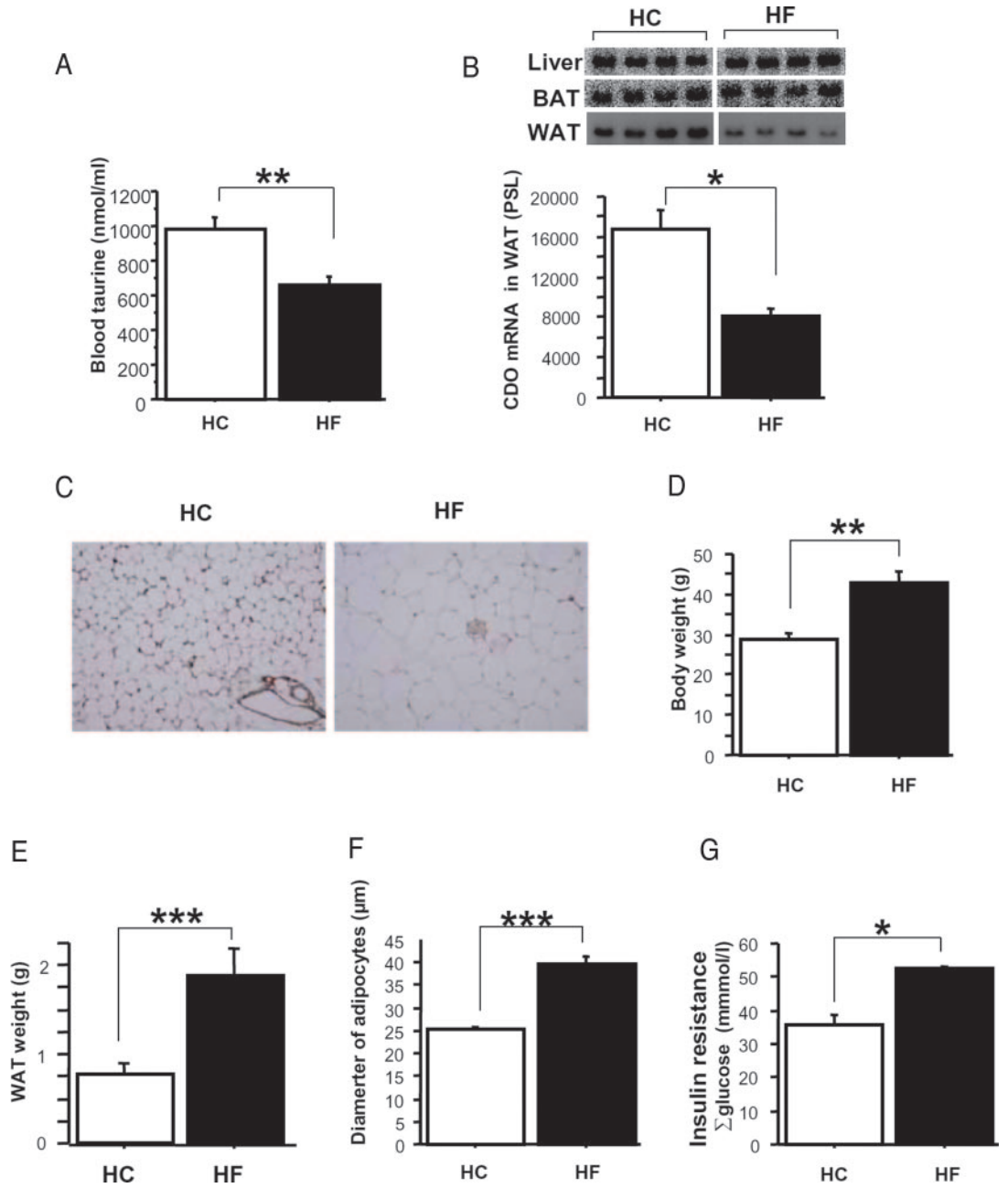


FIG. 3. Blood taurine concentration and WAT CDO expression are reduced in HF diet-fed obese mice. A, Plasma taurine concentration in mice fed a HC or HF diet. Plasma taurine was measured at 23 wk under feeding conditions. Each data point represents the mean ± SE of six mice. **, $P < 0.01$. B, Expression of CDO mRNA levels in parametrial WAT, BAT, and liver. Parametrial WAT, BAT, and liver in mice fed HC or HF diet were used for preparation of total RNA. A typical autoradiogram of parametrial WAT, BAT, and liver and PSL levels of parametrial WAT are shown. In the autoradiogram, each lane represents a sample from an individual mouse. Each data point represents the mean ± SE of six mice. *, $P < 0.05$. C, Immunohistology of parametrial WAT with anti-CDO antisera. Aliquots of parametrial WAT from mice fed HC or HF diet were fixed with paraformaldehyde, and histological sections were prepared. Anti-CDO antisera were used in the WAT sections. A typical photomicrograph with results similar to those obtained from four independent mice is shown. D, Final BW. BW was measured at 23 wk after mice were fed HC or HF diet. Each data point represents the mean ± SE of six mice. **, $P < 0.01$. E, Parametrial WAT weight. Parametrial WAT weight was measured at 23 wk after mice were fed HC or HF diet. Each data point represents the mean ± SE of six mice. ***, $P < 0.001$. F, Adipocyte diameter. The size of adipocytes in a fixed area were determined at 23 wk after mice were fed HC or HF diet (n = 237–809). The sections were stained with hematoxylin and eosin. Each data point represents the mean ± SE of four independent mice. ***, $P < 0.001$. G, Sigma glucose levels during insulin tolerance tests in C57BL/6J mice fed HC or HF diet. Sigma glucose levels were determined 0, 30, 90, and 120 min after insulin injection. Three independent HF diet experiments provided similar data. Values are means ± SE of six mice. *, $P < 0.05$.

decreased by the HF diet (Fig. 3B). Thus, the decrease in the CDO mRNA level in WAT, but not in liver and BAT, was positively associated with the blood taurine concentration, supporting the hypothesis that CDO in WAT regulates the blood taurine concentration. The down-regulation of CDO mRNA expression caused by the HF diet was detected at 14 d

after the HF diet was started, but not at 1, 2, 3, or 7 d, and body fat was also increased significantly at 14 d, but not at 1–7 d, after the start of the HF diet ($P < 0.05$) (data not shown). These results suggest that dietary obesity decreases CDO expression in WAT and then decreases the blood taurine concentration.

*CDO expression in WAT is reduced in genetically obese mice*

To examine whether decreases in the blood taurine concentration and CDO mRNA in WAT are also found in genetically based obesity/diabetes, levels were measured in KKA$^y$ mice. The plasma taurine concentration was slightly decreased in KKA$^y$ mice, but not significantly, and the CDO mRNA level in WAT was decreased by 50% (Fig. 4, A and B). In BAT, the CDO mRNA level was not decreased in KKA$^y$ mice. In db/db mice, another type of genetically obese mice, the level of CDO mRNA in WAT was also decreased (data not shown). When KKA$^y$ mice were fed a HF diet, they showed further marked obesity (Fig. 4C) and insulin resistance (Fig. 4D) with a marked decrease in the CDO mRNA levels in WAT (Fig. 4B) and the blood taurine concentration (Fig. 4A). Thus, decreases in CDO mRNA levels in WAT were also observed in genetically obese mice, and the decrease corresponded well with the degree of obesity.

*Taurine supplementation reverses HF diet-induced obesity*

To examine whether the decreased blood taurine concentration affects development of obesity, taurine (5% wt/wt) was supplemented orally to C57BL/6J mice fed a HF diet, and phenotypic changes were monitored. Surprisingly, the addition of taurine prevented the HF diet-induced increase in BW (Fig. 5A), parametrial WAT weight (Fig. 5B), percentage of body fat estimated by DEXA scan (Fig. 5C), and adipocyte size (Fig. 5D). Mice fed dietary taurine had a 58% higher blood taurine concentration than that of mice fed the HF diet alone (data not shown). The average energy intake did not differ significantly. The average energy intake of mice fed the HC diet was 8.6 ± 0.2 kcal/mouse·d, that of mice fed the HF diet was 8.5 ± 0.2 kcal/mouse·d, and that of mice fed the HF diet plus taurine was 8.4 ± 0.2 kcal/mouse·d. The effects of taurine are not likely due to nonspecific aversive effects of the taurine supplements because food intake was apparently not changed.

*Taurine supplementation increases energy expenditure*

To examine the mechanism underlying the antiobesity effects of taurine supplementation, we measured the energy expenditure and physical activity levels of each group of

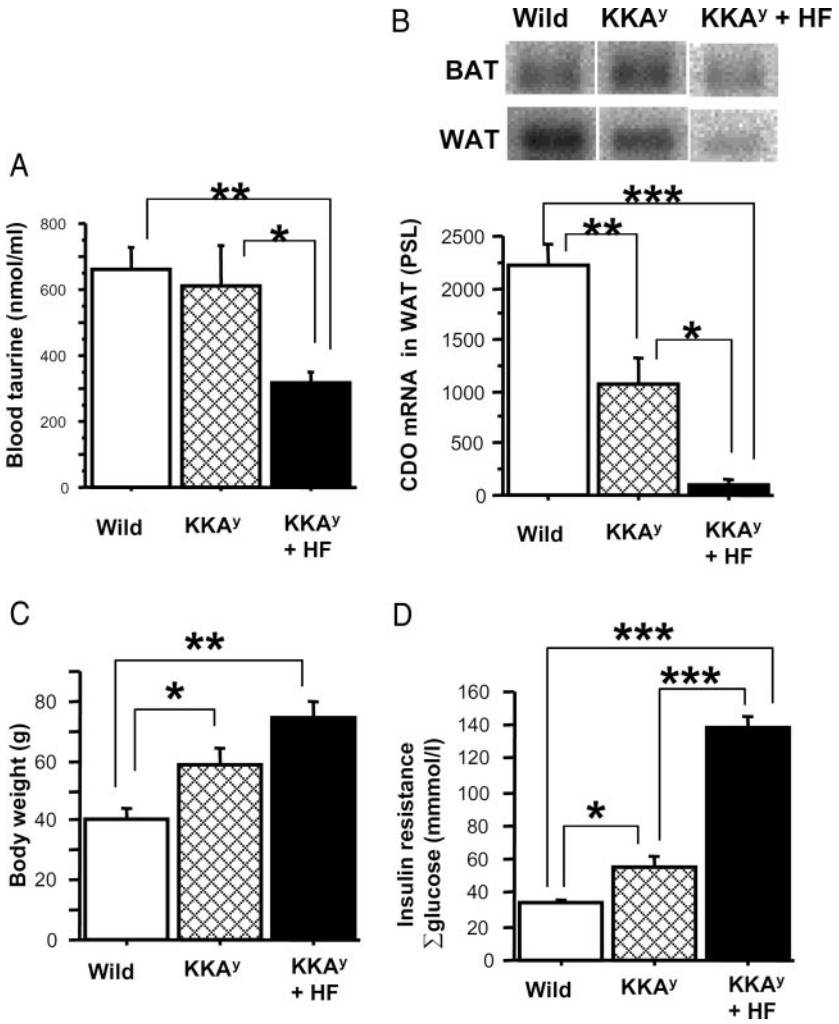


FIG. 4. CDO expression in WAT is reduced in KKA$^y$ and obese KKA$^y$ mice given a HF diet. A, Plasma taurine concentration in wild-type and KKA$^y$ mice fed a HC or HF diet. Plasma taurine was measured at 25 wk under feeding conditions. Each data point represents the mean ± SE of three to nine mice. *, $P < 0.05$; **, $P < 0.01$. B, Level of CDO mRNA expression in BAT and parametrial WAT. BAT and parametrial WAT in wild-type and KKA$^y$ mice fed HC or HF diet was used for preparation of total RNA. A typical autoradiogram of BAT and parametrial WAT and its PSL levels are shown. Each data point represents the mean ± SE of three to five mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. C, Final BW. BW in wild-type and KKA$^y$ mice was measured at 25 wk after mice were fed HC or HF diet. Each data point represents the mean ± SE of three to nine mice. *, $P < 0.05$; **, $P < 0.01$. D, Sigma glucose levels during insulin tolerance tests in wild-type and KKA$^y$ mice ingesting HC or HF diet. Sigma glucose levels were determined 0, 30, 90, and 120 min after insulin injection. Values are means ± SE of three to six mice. *, $P < 0.05$; ***, $P < 0.001$.

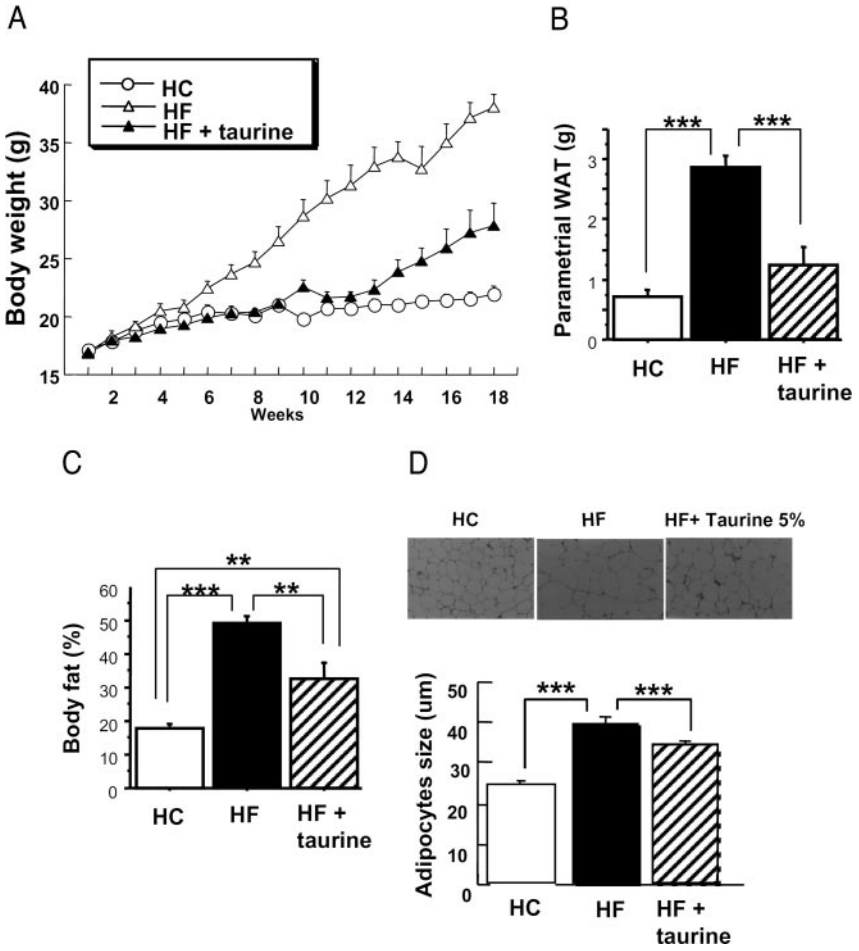


FIG. 5. Dietary taurine reverses obesity induced by a HF diet. A, BW change. BW was measured every week in C57BL/6J mice fed a HC, HF, or HF plus taurine (5% wt/wt) diet. Each data point represents the mean ± SE of five to six mice. B, Parametrial WAT weight. Parametrial WAT weight was measured at 18 wk after mice were fed HC, HF, or HF plus taurine diet. Each data point represents the mean ± SE of five to six mice. ***, $P < 0.001$. C, Percent body fat. Body fat levels were estimated by DEXA at 18 wk after feeding. Each data point represents the mean ± SE of five to six mice. **, $P < 0.01$; ***, $P < 0.001$. D, Diameter of adipocytes. The sizes of adipocytes in a fixed area were determined at 18 wk after mice were fed HC, HF, or HF plus taurine diet. The sections were stained with hematoxylin and eosin. Each data point represents the mean ± SE of four independent mice. ***, $P < 0.001$.

mice. Oxygen consumption was monitored in the light phase as a resting state (0801–1600 h) and in the dark phase as an active state (2001–0600 h). In comparison with the HC diet, the HF diet decreased resting oxygen consumption in the light phase (Fig. 6A). However, taurine reversed this decrease. The taurine supplementation diet significantly increased resting oxygen consumption in comparison with the HF diet without taurine (Fig. 6A). The average resting oxygen consumption of mice fed the HC diet was 695.2 ± 12.3 $\mu$mol/min·kg $BW^{0.75}$, that of mice fed the HF diet was 606.4 ± 13.3 $\mu$mol/min·kg $BW^{0.75}$, and that of mice fed the HF diet plus taurine was 683.9 ± 27.9 $\mu$mol/min·kg $BW^{0.75}$. In the activity-related oxygen consumption in the dark phase that reflected the physical activity levels, there was no difference between the groups of mice (Fig. 6B). Furthermore, running wheel activity (spontaneous locomotive activity) was measured as physical activity in mice fed the three different diets. Mice were transferred to cages with a running wheel, and the number of wheel revolutions made daily was monitored for 9 d. HF diet-fed mice showed slightly fewer average wheel revolutions in comparison with mice fed the other diets (Fig. 6C). However, the difference was not statistically significant. Because the data differed considerably from day to day, a constant tendency was not observed (Fig. 6D).

Consistent with the increased resting oxygen consumption, significant increases in the expression level of energy expenditure-related genes in WAT of taurine-supplemented mice were observed (Fig. 6E). Dietary taurine supplementation in comparison with the HF diet alone increased mRNA levels of transcription factors and cofactor involved in energy expenditure such as peroxisome proliferator-activated receptor (PPAR) $\gamma$ coactivator (PGC-1$\alpha$), PPAR$\alpha$, PPAR$\gamma$, and nuclear respiratory factor 2$\alpha$ in WAT. mRNA levels of their target genes, lipoprotein lipase, acyl-coenzyme A (CoA) oxidase, acyl-CoA synthetase, and medium-chain acyl-CoA dehydrogenase in WAT, enzymes for fatty acid $\beta$-oxidation, were higher than in mice fed the HF diet without taurine. In addition, expression of the $\beta$-subunit of ATP synthetase and uncoupling protein (UCP) 2, which is important for mitochondrial respiration, was also increased. In other tissues (BAT, liver, gastrocnemius), the mRNA levels of PGC-1$\alpha$, PPAR$\alpha$, PPAR$\gamma$, and nuclear respiratory factor 2$\alpha$ were not increased by dietary taurine (Fig. 6F). Most target genes were not increased in BAT, liver, and gastrocnemius from mice fed the taurine-supplemented diet. Interestingly, the UCP1 mRNA level in BAT, which greatly influences the energy expenditure of rodents, did not change with the addition of taurine.

Judging by these data, an increase of the physical activity level and energy expenditure in BAT, liver, and muscle might not greatly contribute to weight loss related to taurine intake. In WAT from mice given the taurine-supplemented

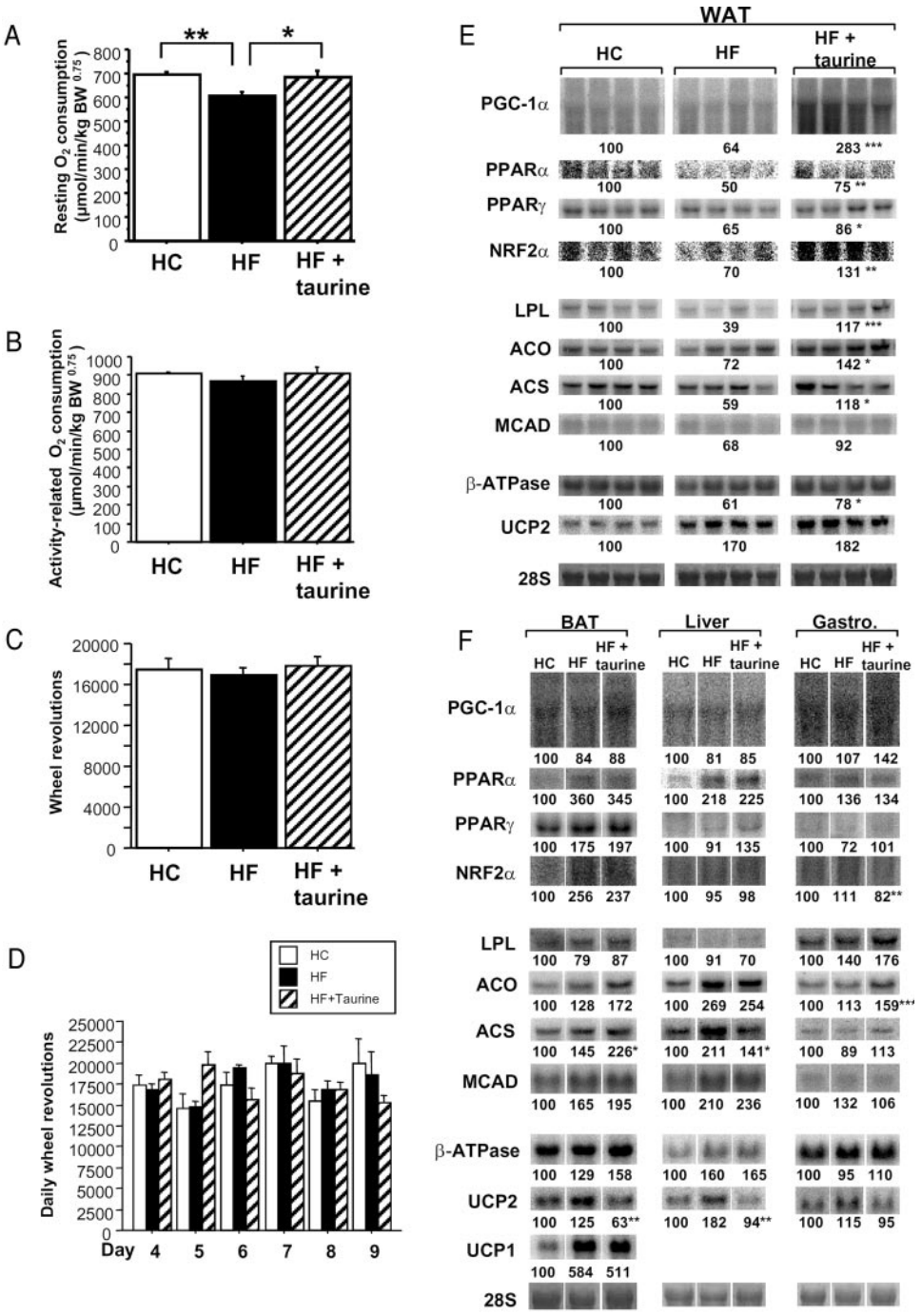


FIG. 6. Taurine supplementation increases energy expenditure. A and B, Oxygen consumption. Oxygen consumption of C57BL/6J mice fed a HC, HF, or HF plus taurine (5% wt/wt) diet was measured in a metabolic chamber. Resting oxygen consumption (A) was calculated as the light phase (0801–1600 h), and activity-related oxygen consumption (B) was calculated as the dark phase (2001–0600 h). Each data point represents the mean ± SE of average oxygen consumption per five individual mice. *, $P < 0.05$; **, $P < 0.01$. C and D, Running wheel activity of C57BL/6J mice fed HC, HF, or HF plus taurine (5% wt/wt) diet. Mice were housed individually in cages equipped with a running wheel (20 cm in diameter). The number of revolutions made was recorded daily for 9 d, and the average values (C) and daily values (D) are shown. E, mRNA expression in parametrial WAT from C57BL/6J mice fed HC, HF, or HF plus taurine (5% wt/wt) diet. Each lane represents a sample from an individual mouse. Values are the means (n = 4–6). The data are shown as values relative to mRNA levels of HC diet-fed mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* HF diet. F, mRNA expression in BAT, liver, and gastrocnemius (Gastro.) muscle from C57BL/6J mice fed HC, HF, or HF plus taurine (5% wt/wt) diet. A typical autoradiogram, representative of four to six independent mice with similar results, is shown. Values are the means (n = 4–6). The data are shown as values relative to mRNA levels of HC diet-fed mice. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ *vs.* HF diet. NRF2α, Nuclear respiratory factor 2α; LPL, lipoprotein lipase; ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; MCAD, medium-chain acyl-CoA dehydrogenase; β-ATPase, β-subunit of ATP synthetase.

diet, up-regulation of PGC-1α, PPARα, and PPARγ and their target genes may lead to increased energy expenditure, including fatty acid β-oxidation, which prevented obesity.

## Discussion

To examine the role of taurine in obesity, we focused on CDO, the key enzyme for taurine synthesis, in adipose tissue. The increased taurine concentration in culture media from CDO-overexpressing 3T3-L1 cells indicates that CDO in adipocytes increases taurine secretion into the blood. A decrease in the blood taurine concentration concomitant with a decrease in CDO expression in adipose tissue, but not in liver, was observed in mice with diet-induced and/or genetically based obesity and insulin resistance. Dietary taurine supplementation increased the blood taurine concentration and prevented obesity with induction of resting energy expenditure and of gene expression involved in energy metabolism in WAT, but it did not affect food intake and physical activity. Obesity causes depletion of the blood taurine concentration, which then promotes further obesity, creating a vicious cir-

cle. Dietary taurine supplementation interrupts this vicious circle and might prevent obesity (Fig. 7).

In Western countries, levels of dietary taurine may be decreased due to the greater consumption of meat than of seafood; serum taurine concentrations are correlated with daily dietary intake of taurine from fish and shellfish (20). In addition, our observations indicate that taurine synthesis in WAT is reduced in obesity. Thus, the blood taurine concentration is expected to be decreased in obese/diabetic patients. Indeed, plasma taurine levels were significantly lower in patients with diabetes [plasma taurine = 32.1 ± 1.9 μmol/liter, body mass index (BMI) = 28.4 ± 0.5] than in control subjects (plasma taurine = 48.6 ± 4.9 μmol/liter, BMI = 26.8 ± 0.9) (21). In healthy populations, obese subjects showed a decrease in plasma taurine levels (plasma taurine = 48 ± 4 μmol/liter, BMI = 36 ± 1) in comparison with age- and sex-matched nonobese subjects (plasma taurine = 85 ± 6 μmol/liter, BMI = 25 ± 1) (22).

Taurine given to female C57BL/6J mice in their drinking water (1.7 mg/g BW·d) for 6 months did not prevent HF diet-induced obesity (5). Our dosage was 3 mg/g BW·d, indicating that a large amount of taurine is required for antiobesity effects. Supplementation of 5% (wt/wt) taurine prevented an increase in BW in obese KK mice (estimated taurine intake, 11 mg/g BW·d) but not in lean BALB/C mice (estimated taurine intake, 8.7 mg/g BW·d) (6), suggesting that when a large amount of dietary taurine is given in the taurine-deficient state that accompanies extreme obesity, taurine supplementation may have an antiobesity effect.

In OLETF diabetic rats, 5% (wt/wt) taurine-supplemented diet (estimated taurine intake, 2.3 mg/g BW·d) or 3% (wt/vol) taurine added to water (estimated taurine intake, 2.1 mg/g BW·d) slightly decreased visceral fat mass (7, 8). The antiobesity effect of taurine supplementation was much weaker in obese rats than in obese mice; rats might require much more taurine to reduce BW than mice require. It is also conceivable that taurine sensitivity differs between species. Taurine decreased energy expenditure in OLETF diabetic rats more than in obese mice (8). This might explain the difference in antiobesity effects between rats and mice.

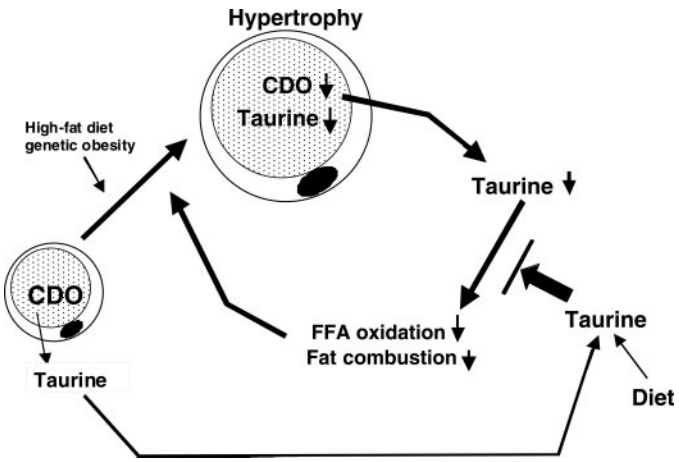


FIG. 7. Proposed model of taurine-mediated vicious circle in obesity. Obesity causes a depletion of blood taurine concentration, which then further promotes obesity (vicious circle). Dietary taurine supplementation interrupts this vicious circle and might prevent obesity.

The amount of taurine needed to correct human obesity is uncertain. Ingestion of 3 g taurine/d for 7 wk has been reported to prevent obesity in humans (23). However, because neither the physical activity nor dietary habits of the subjects were controlled, it is uncertain whether the effect was from the taurine alone.

CCAAT/enhancer-binding protein (C/EBP) α might be involved in obesity-mediated down-regulation of CDO. C/EBPα plays a critical role in the late stages of adipogenesis and is important for maintaining the functions of mature adipocytes (24). The mouse, rat, and human CDO genes contain a putative C/EBP-binding site in their 5′-flanking regions (10–12). It was shown in C/EBPα-null mice that CDO expression in WAT requires C/EBPα (13).

PGC-1α is known to be a powerful regulator of energy expenditure in adipose tissues (25). PGC-1α binds not only to PPARγ but also to PPARα and activates their target genes (26). However, dietary taurine did not increase PGC-1α, these transcription factors, or their target genes in BAT, which greatly influence energy expenditure in rodents. An increase in WAT PGC-1α with dietary taurine supplementation might lead to the up-regulation of genes involved in energy expenditure including fatty acid oxidation in WAT. In rat β-cells, taurine increases the amount of ATP in UCP2-overexpressing β-cells, probably by increasing mitochondrial $Ca^{2+}$ influx through the $Ca^{2+}$ uniporter, and activates mitochondrial metabolic function (27). Although the mechanisms might differ, increased energy consumption was observed both in WAT and β-cells. It is thought that dietary taurine burns fat directly or indirectly through an increase of PGC-1α in WAT.

Adipocytes secrete various molecules and hormones. It has been proposed that larger adipocytes secrete molecules that cause insulin resistance, such as free fatty acid, TNF-α, and resistin, and that smaller adipocytes secrete molecules that increase insulin sensitivity, such as adiponectin and leptin (28, 29). Thus, the size of adipocytes could be essential to the management of obesity-related diseases. The molecules secreted from adipocytes are called adipocytokines (29). CDO expression in WAT and the blood taurine concentration were decreased in our obese mice, suggesting that large adipocytes are less able to synthesize and secrete taurine. This is similar to what occurs with adiponectin, which can reverse insulin resistance and HF diet-induced obesity. Thus, although not a classical hormone, taurine could be a new adipocytokine with antiobesity effects in promoting basal metabolic rate.

Further analysis of the molecular mechanisms of taurine in WAT is important from the clinical as well as the nutritional perspective. More studies examining whether taurine supplementation in obese subjects can reduce body fat are needed.

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