Respiratory Uncoupling Lowers Blood Pressure Through a Leptin-Dependent Mechanism in Genetically Obese Mice

Carlos Bernal-Mizrachi, Sherry Weng, Bing Li, Lorraine A. Nolte, Chu Feng, Trey Coleman, John O. Holloszy, Clay F. Semenkovich

Abstract—Insulin resistance is commonly associated with hypertension, a condition that causes vascular disease in people with obesity and type 2 diabetes. The mechanisms linking hypertension and insulin resistance are poorly understood. To determine whether respiratory uncoupling can prevent insulin resistance–related hypertension, we crossed transgenic mice expressing uncoupling protein 1 (UCP1) in skeletal muscle with lethal yellow (A/y) mice, genetically obese animals known to have elevated blood pressure. Despite increased food intake, UCP-A/y mice weighed less than their A/y littermates. The metabolic rate was higher in UCP-A/y mice than in A/y mice and did not impair their ability to alter oxygen consumption in response to temperature changes, an adaptation involving sympathetic nervous system activity. Compared with their nontransgenic littermates, UCP-A/y mice had lower fasting insulin, glucose, triglyceride, and cholesterol levels and were more insulin sensitive. Blood pressure, serum leptin, and urinary catecholamine levels were also lower in uncoupled mice. Independent of sympathetic nervous system activity, low-dose peripheral leptin infusion increased blood pressure in UCP-A/y mice but not in their A/y littermates. These data indicate that skeletal muscle respiratory uncoupling reverses insulin resistance and lowers blood pressure in genetic obesity without affecting thermoregulation. The data also suggest that uncoupling could decrease the risk of atherosclerosis in type 2 diabetes.

Key Words: insulin resistance | hypertension | energy metabolism | uncoupling proteins | type 2 diabetes

Obesity is much more than a cosmetic problem. It is a potentially lethal disorder that increases the risk of cardiovascular events and death.1 Hypertension promotes vascular disease and is common in the obese. There is a graded relationship between obesity and blood pressure in humans.2 Obese individuals are severalfold more likely than lean people to develop hypertension. Obesity and hypertension may be related through insulin resistance, a complicated condition also associated with type 2 diabetes, dyslipidemia, and atherosclerosis.3 The mechanisms underlying hypertension in the setting of insulin resistance remain obscure.

Blood pressure increases with weight gain and decreases with weight loss,4,5 suggesting that adipose tissue itself affects vascular tone. Fat is the source of several molecules potentially involved in blood pressure and insulin sensitivity, including fatty acids, leptin, tumor necrosis factor-α, adiponectin, resistin, and others.6 Fatty acids and leptin have been implicated in hypertension.

Plasma free fatty acids are elevated in obesity.7 Insulin normally suppresses the activity of hormone-sensitive lipase in adipocytes. In obese people with insulin resistance, this suppression is lost, and unabated lipolysis in fat tissue increases circulating free fatty acids.8 Fatty acids could increase blood pressure through NO, protein kinase C, or peroxisome proliferator–activated receptor pathways. Short-term marked elevation of free fatty acids raises blood pressure in humans,9 but it is unclear whether specific therapies directed at insulin resistance affect blood pressure by altering the levels of fatty acids.

Leptin levels are elevated in obesity.10 Like blood pressure, leptin is directly related to fat mass. Blood pressure and leptin levels are correlated in people with hypertension and insulin resistance,11 but leptin physiology is complex. The hormone decreases appetite and promotes inefficient metabolism by acting on neurons in the arcuate nucleus of the hypothalamus.12 Elevated levels of leptin seen in those with extreme adiposity obviously do not prevent obese people from being obese, but they may contribute to the hypertension of obesity. Leptin increases sympathetic nervous activity, which is increased in obesity and implicated in some forms of hypertension.13 Leptin-deficient mice with severe obesity have low blood pressures, consistent with a role for leptin in blood pressure elevation.14 However, the exact effects of leptin on blood pressure are unresolved. Leptin has also been reported to lower blood pressure through NO-dependent vasodilation15 and to promote natriuresis.16 It is unknown whether specific
therapies that enhance insulin sensitivity affect blood pressure through the effects on leptin. An attractive potential therapy for enhancing insulin sensitivity is the promotion of inefficient metabolism. Uncoupling proteins promote inefficient metabolism by disrupting the electrochemical gradient across the inner mitochondrial membrane, causing energy to be released as heat instead of being used for ATP synthesis.17 We recently showed that transgenic mice expressing low levels of uncoupling protein 1 (UCP1, the protein responsible for brown fat–mediated adaptive thermogenesis in some mammals) in skeletal muscle are protected from diet-induced obesity.18

To test the hypothesis that skeletal muscle respiratory uncoupling can reverse insulin resistance–related hypertension, we crossed UCP1 skeletal muscle transgenic mice with lethal yellow (A/y) mice, a genetic model of obesity/insulin resistance known to have increased blood pressure.14 Our results suggest that skeletal muscle respiratory uncoupling lowers blood pressure through a leptin-dependent mechanism and prevents insulin resistance in genetic obesity.

**Methods**

**Animals and Tissue Characterization**

Mice expressing UCP1 in skeletal muscle have been described previously.14 Two independent founders (UCP-L, with low expression, and UCP-H, with high expression) had different physiological effects depending on the level of transgene expression. UCP-L mice, shown to have normal skeletal muscle mass, normal high-energy phosphate content, and normal exercise capacity, were used for the present experiments. Female A/y mice (Jackson Laboratories, Bar Harbor, Me) were crossed with male UCP-L mice to generate the F1 animals used in the present study. UCP-A/y mice and their nontransgenic A/y littermates were identified by their distinctive coat color (see Figure 2). The presence of the transgene was determined by polymerase chain reaction (PCR) as described.18 Mice had free access to water and mouse chow (with a fat content of 4.5%). For measurements of food intake, mice were individually housed, and food measurements of food intake, mice were individually housed, and food intake was determined daily by weighing food troughs and accounted for spilling food. The Washington University Animal Studies Committee approved the protocols.

Tissues from euthanized mice were immediately removed and separated into aliquots for preparation of total RNA and a protein extract. Total RNA was subjected to reverse transcription–PCR for UCP1 and GAPDH as described.18 Amplified bands were sequenced to verify their identity. Protein extracts were subjected to Western blotting by using rabbit anti-mouse UCP1 as described.18

**Analytical Procedures**

Body composition was performed on anesthetized living mice by dual-energy x-ray absorptiometry with the use of a small animal densitometer (Lunar). Serum samples from mice fasted for 4 hours were assayed for glucose, cholesterol, triglycerides, and nonesterified fatty acids as described.19 Tissue triglycerides were determined after the extraction of lipids with chloroform/methanol.20 Insulin levels were measured by ELISA with the use of commercial reagents (Crystal Chem Inc.). Leptin concentrations were determined by radioimmunoassay with the use of an antibody directed against murine leptin (product ML-82K, Linco). For glucose and insulin tolerance tests, whole blood glucose measurements were made by using a blood glucose meter (Hemocue).

**Indirect Calorimetry**

Animals were studied in the fed state in a single-chamber indirect calorimetry system (Columbus Instruments). The same UCP-A/y and A/y littermates were studied at 3 different ambient temperatures in sessions separated by >1 week. Mice were characterized at room temperature (25°C), at thermoneutrality (34°C), and in the cold (6°C to 9°C). Warming was achieved by using heat lamps to an ambient temperature of 34°C for several hours. Chilling was achieved by placing animals in a cold room (6°C to 9°C) for 12 hours, followed by transfer to a metabolic chamber that was encased in ice to maintain the same temperature as the cold room.

**Glucose/Insulin Tolerance Tests and Glucose Transport**

Mice were accustomed to handling for several days before each study.20 Glucose tolerance testing preceded insulin tolerance testing by at least 1 week, and both tests were performed after an overnight fast. Mice received an intraperitoneal injection of 10% D-glucose (1 g/kg body wt) for glucose tolerance testing and human regular insulin (0.75 U/kg, Lilly) for insulin tolerance testing. For both, 5 to 10 μL of blood was drawn from the tail at 0, 30, 60, and 120 minutes and assayed for blood glucose.

Ex vivo basal and insulin-stimulated glucose transport was performed as described.18 Epitrochlearis muscles were allowed to recover from the dissection procedure by shaking for 1 hour in oxygenated Krebs-Henseleit buffer supplemented with 8 mmol/L glucose, 32 mmol/L mannitol, and 0.1% BSA. Muscles were then transferred to fresh media in the absence or presence of a maximally effective concentration of porcine insulin (2 mU/mL). They were incubated for 30 minutes by using 2-deoxy-o-[1,2-3H]glucose (American Radiolabeled Chemicals) and [U-14C]mannitol (DuPont-NEN) as described.18

**Blood Pressure and Leptin Infusions**

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in conscious A/y and UCP-A/y mice by using a tail-cuff system (Kent Scientific).21 Animals were accustomed to handling and placement in the apparatus daily for 1 week before the measurement of blood pressure. Multiple measurements were made at each of 3 daily sessions for each mouse. Baseline blood pressure was determined in 20-week-old A/y and UCP-A/y mice (16 total animals, 8 per genotype, equal numbers of males and females). Baseline 24-hour urine collections (obtained by using metabolic cages) were assayed for norepinephrine and epinephrine by radioimmunoassay. Mice were then anesthetized with pentobarbital (50 mg/kg), and 200 μL osmotic minipumps (Alza Corp) delivering 1 μL/h for 7 days were implanted intraperitoneally. Mice from each genotype received either recombinant mouse leptin (Sigma Chemical Co) at a dose of 1.2 μg/g per day or vehicle (PBS). Serum leptin was measured 2 days after minipump placement. After leptin was measured, 3 consecutive 24-hour urine collections were obtained for each mouse and assayed for catecholamines. Blood pressures were recorded over 3 days by an observer who was blinded to the treatment status of the animals.

**Results**

**Skeletal Muscle Expression of UCP1 in UCP-A/y Mice**

UCP1 mRNA was expressed in skeletal muscle in UCP-A/y mice (Figure 1A, lane 1) and was absent in A/y skeletal muscle (not shown). The ectopic expression of UCP1 in skeletal muscle did not affect the levels of native UCP1 protein in brown fat (Figure 1B, lanes 1 and 2). UCP1 protein was absent from control muscle (Figure 1B, lane 3) but present in skeletal muscle of UCP-A/y animals (lane 4) at ~1% of the level found in brown fat (lanes 3 and 4 were loaded with 20-fold more protein than lanes 1 and 2).

**Body Weight, Food Intake, and Metabolic Rate**

UCP-A/y mice and their nontransgenic A/y littermates were weaned to a chow diet at the age of 21 days and weighed
frequently. Differences in body weight were detected early and persisted over time (Figure 2). The inset presents a side-by-side comparison of a representative Ay/a mouse (top) and a UCP-Ay/a littermate (bottom). The data in Figure 2 were derived from males; the same results were seen in females (see below). Food intake expressed per mouse was not different for UCP-Ay/a and Ay/a littermates (4.7±0.1 g/d per mouse for UCP-Ay/a [n=7] versus 5.2±0.3 g/d per mouse for Ay/a [n=6], P=NS) but was 22% greater in the uncoupled mice when intake was normalized to lean body mass (0.200±0.010 g/d per gram body weight for UCP-Ay/a mice versus 0.164±0.011 g/d per gram body weight for Ay/a mice, P=0.034). There was no difference in body temperature.

The resting whole-body metabolic rate was 27% greater in UCP-Ay/a mice than in Ay/a mice (Table 1). The constitutive expression of UCP1 in skeletal muscle did not compromise the ability of animals to adapt to changes in ambient temperature. Regardless of genotype, the mice significantly decreased oxygen consumption in response to heat and increased consumption in response to cold (Table 1).

Body Composition and Serum Determinations
Adiposity and lean body mass were determined in 24 mice of both sexes and genotypes. For Ay/a mice, females tended to have more adiposity than males (P=0.809), and males tended to have greater body mass than females (P=0.693), but these
differences were not significant. For littermate UCP-Ay/a mice, females tended to have less adiposity than males (P=0.293), and males tended to have greater body mass than females (P=0.556), but again, these differences were not significant. Body composition and size are shown in Table 2. UCP-Ay/a mice weighed 41% less and had less fat mass, less adiposity, and greater lean mass compared with their nontransgenic littermates. There was no effect of genotype on nasal-anal length.

Fasting glucose and insulin were lower in UCP-Ay/a mice (Table 2) than Ay/a mice. Fasting cholesterol and triglyceride levels were also lower, but free fatty acid levels were higher in UCP-Ay/a mice (Table 2). Serum leptin concentrations were higher in Ay/a mice (Table 2), consistent with their increased fat mass. Leptin normalized to fat mass remained elevated in Ay/a mice (1.79 ± 0.293), and males tended to have greater body mass than females (P=0.02 1 for 5 males of each genotype. Within each genotype, each temperature change also resulted in significant changes in oxygen consumption: *P<0.002 vs room temperature; †P<0.001 vs room temperature; ‡P<0.001 vs room temperature.

TABLE 1. Whole-Animal Oxygen Consumption in UCP-Ay/a and Control Ay/a Mice

<table>
<thead>
<tr>
<th>Ambient Temperature (°C)</th>
<th>A/a</th>
<th>UCP-Ay/a</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24°</td>
<td>10.28±0.40</td>
<td>13.10±0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6° to 9°</td>
<td>12.77±0.40*</td>
<td>15.74±0.54‡</td>
<td>0.002</td>
</tr>
<tr>
<td>34°</td>
<td>5.54±0.36†</td>
<td>6.76±0.22§</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Whole-body metabolic rate was determined by indirect calorimetry with a single chamber Oxymax system. The same male mice were studied in the fed state under three conditions separated by one week: room temperature (24°C), overnight exposure to cold (6–9°C), and a several-hour exposure to thermoneutrality (34°C). Data are mean ± SE in mL oxygen · g⁻¹ · h⁻¹ for 5 males of each genotype. Within each genotype, each temperature change also resulted in significant changes in oxygen consumption: *P<0.002 vs room temperature; †P<0.001 vs room temperature; ‡P<0.001 vs room temperature.

Figure 3. Enhanced glucose tolerance and insulin responsiveness in UCP-Ay/a mice. A, Fasted A/a (open circles) and UCP-Ay/a (solid squares) mice were given intraperitoneal D-glucose (1 g/kg), followed by determination of tail vein glucose at 30, 60, and 120 minutes. B, One week after the glucose tolerance test, the same mice were fasted and then injected with insulin (0.75 U/kg). For both panels, data are expressed as mean ± SE. *P<0.05 and **P<0.01 vs UCP-Ay/a at the same time point (n=11 for each genotype). For panel B, the data are presented as the percentage of the blood glucose level at time 0. P=0.012), suggesting the presence of leptin resistance in the obese mice.

Glucose Metabolism
UCP-Ay/a mice had lower glycemic excursions during glucose tolerance testing (Figure 3A) and lower glucose levels during insulin tolerance testing (Figure 3B). This enhanced sensitivity to insulin was due to accelerated glucose transport in skeletal muscle. Ex vivo insulin-stimulated glucose transport was significantly greater in muscle from the UCP-Ay/a mice (Figure 4A) despite the fact that triglyceride content was 52% greater in these muscles than in muscles isolated from nontransgenic littermates (Figure 4B).

Blood Pressure and Response to Leptin Infusion
SBP measured noninvasively at the tail was 10% lower in UCP-Ay/a mice than in nontransgenic littermate Ay/a mice (75±1.3 versus 83±2.4 mm Hg, respectively, P<0.01; Figure 5A and 5D). DBP was 8% lower in mice with the UCP1 transgene (62±1.1 versus 67±2.0 mm Hg, respectively, P<0.02; Figure 5A and 5D). As expected, nonobese wild-type (a/a) littermates from this F1 intercross had lower blood pressure than obese Ay/a mice (SBP 76±1.5 mm Hg in a/a nontransgenic mice, P=0.027 versus Ay/a mice). F1 intercross a/a littermates that were transgenic for UCP1 had the lowest body mass (as expected) and the lowest blood

TABLE 2. Body Composition and Serum Measurements in UCP-Ay/a and Control Ay/a Mice

<table>
<thead>
<tr>
<th></th>
<th>A/a</th>
<th>UCP-Ay/a</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>51±3.6</td>
<td>30±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass, g</td>
<td>16±2.5</td>
<td>7±0.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Adiposity, %</td>
<td>31±3</td>
<td>23±3</td>
<td>0.008</td>
</tr>
<tr>
<td>Lean mass, g</td>
<td>33±0.7</td>
<td>24±1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Leanness, %</td>
<td>66±3</td>
<td>80±3</td>
<td>0.002</td>
</tr>
<tr>
<td>Nasa-anal length, cm</td>
<td>10.55±0.02</td>
<td>10.52±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>255±25</td>
<td>148±23</td>
<td>0.009</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>1.89±0.31</td>
<td>0.59±0.09</td>
<td>0.006</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>73±4</td>
<td>50±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>128±10</td>
<td>81±12</td>
<td>0.033</td>
</tr>
<tr>
<td>NEFA, mmol/L</td>
<td>0.32±0.05</td>
<td>0.44±0.02</td>
<td>0.040</td>
</tr>
<tr>
<td>Leptin, pg/mL</td>
<td>23.1±2.4</td>
<td>6.9±1.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Body composition was determined by dual-energy x-ray absorptiometry in 12 lethal yellow (Ay/a) mice and 12 lethal yellow littermates carrying the skeletal muscle UCP1 transgene (UCP-Ay/a). Data (mean ± SE) from equal numbers of age-matched males and females are presented because there was no sex-specific effect on body composition. Chemistries were performed on samples from 10 Ay/a and 9 UCP-Ay/a mice after a 4-hour fast. NEFA indicates nonesterified fatty acids. P values were calculated by unpaired, two-tailed t test.
pressure (SBP 70±1.3 mm Hg in UCP-A/a mice, P=0.009 versus a/a nontransgenic mice).

To determine whether leptin contributes to blood pressure in this model, mice were implanted with osmotic pumps delivering either leptin or PBS for 1 week. The leptin infusion rate (1.2 μg/g per day) achieved leptin concentrations in UCP-A/a mice similar to those in nontransgenic A/a mice with higher blood pressure. The mean baseline leptin concentration in UCP-A/a mice was 11.1±1.7 ng/mL. During the control (PBS) infusion, serum leptin was unchanged compared with baseline at 13.7±4.8 ng/mL. With leptin infusion, leptin concentration increased to 36.3±3.8 ng/mL (P<0.05 versus both baseline and control infusion by the Tukey test). The mean baseline leptin concentration in the obese A/a mice was 22.8±6.4 ng/mL. During the control (PBS) infusion, serum leptin was unchanged compared with baseline at 23.3±3.0 ng/mL. With leptin infusion, leptin concentration increased to 55.1±11.4 ng/mL (P<0.05 versus both baseline and control infusion by the Tukey test).

Control infusion (Figure 5B and 5E) had no effect on blood pressure in either genotype. Leptin infusion elevated blood pressure in UCP-A/a animals (Figure 5F). With leptin, SBP was 17% higher (90±2.1 mm Hg for leptin versus 77±1.2 mm Hg for PBS, P=0.002), and DBP was 20% higher (71±2.2 mm Hg for leptin versus 59±1.4 mm Hg for PBS, P=0.004) than control perfusion. Leptin infusion had no effect on blood pressure in obese A/a mice (Figure 5C).

This rate of leptin infusion did not affect whole-body oxygen consumption in UCP-A/a mice (12.1±0.10 mL/g0.75 per hour with leptin versus 11.8±0.24 mL/g0.75 per hour with PBS, P=0.292) or A/a mice (9.7±0.12 mL/g0.75 per hour with leptin versus 9.6±0.08 mL/g0.75 per hour with PBS, P=0.514). Infusions had no genotype-specific effect on food intake.

There were genotype-specific responses to catecholamine metabolism (Figure 6), but these were unrelated to blood pressure. Baseline norepinephrine excretion (Figure 6, left panels) was substantially higher in the obese A/a mice (216±28.3 ng/24 h for A/a mice versus 28.1±10.4 ng/24 h for UCP-A/a mice, P=0.001). Baseline epinephrine excretion (Figure 6, right panels) was also increased in the obese animals (37.2±3.5 ng/24 h for A/a mice versus 9.0±2.4 ng/24 h for UCP-A/a mice, P<0.001). In the UCP-A/a mice, both infuses (PBS and leptin) significantly increased the excretion of norepinephrine (Figure 6, bottom left panel) and epinephrine (Figure 6, bottom right panel) compared with baseline. However, compared with PBS infusion, leptin infusion sufficient to elevate blood pressure (compare Figure 5E with Figure 5F) did not increase norepinephrine or epinephrine excretion. Compared with PBS infusion, leptin infusion increased norepinephrine excretion in obese A/a mice (Figure 6, top left panel), but this increase in sympathetic activity did not affect blood pressure (compare Figure 5B with Figure 5C).

![Figure 4. Basal and insulin-stimulated glucose transport in skeletal muscle. A, Ex vivo 2-deoxyglucose uptake determined in epitrochlearis muscle from A/a mice (open bars) and UCP-A/a mice (solid bars). *P<0.05 vs insulin-stimulated glucose transport in A/a mice by pairwise multiple comparison testing after ANOVA. B, Skeletal muscle triglyceride content. Triglycerides were assayed in epitrochlearis muscle from A/a mice (open bars) and UCP-A/a mice (solid bars). *P=0.019 vs A/a by t test. For both panels, data are presented as mean±SE for tissues from 4 mice per genotype.](http://atvb.ahajournals.org/content/5/5/965/F5.large.jpg)

![Figure 5. SBP and DBP in A/a mice (open bars) and UCP-A/a mice (solid bars). Each animal was accustomed to the tail-cuff blood pressure apparatus, and then at least 12 measurements were made over several days for baseline determinations (A and D) and after control infusion (PBS, B and E) and low-dose leptin infusion (C and F). Baseline SBP and DBP were measured in 16 animals, 8 obese A/a mice (A) and 8 lean UCP-A/a mice (D). For each genotype, half of the mice were implanted with pumps infusing PBS (B and E), and half of the mice were implanted with pumps infusing low-dose leptin (C and F). Blood pressure determinations began 2 days after pump placement. Data are expressed as mean±SE. †P<0.01 and ††P<0.02 for baseline and PBS-treated UCP-A/a mice compared with A/a mice treated the same way. *P=0.002 and **P=0.004 compared with UCP-A/a mice receiving PBS.](http://atvb.ahajournals.org/content/5/5/965/F6.large.jpg)
Defective signaling at melanocortin 4 receptor represents the most common monogenic form of obesity in humans. The mechanisms underlying hypertension in insulin resistance are unknown. Fatty acids have been implicated, but in the present study, respiratory uncoupling lowered blood pressure in the setting of mildly increased fatty acids. One possible interpretation of these data is that fatty acids are necessary but not sufficient for the development of hypertension.

UCP-A/y/a mice with lower blood pressures were lean and insulin sensitive, and they had lower levels of leptin. Low-dose peripheral leptin infusion in lean UCP-A/y/a mice increased blood pressure to the same level as obese A/y/a mice. These results suggest that leptin-dependent processes contribute to the hypertension of obesity.

In UCP-A/y/a mice, leptin infusion achieved levels of \( \approx 36 \) ng/mL and increased blood pressure but did not increase catecholamine excretion beyond that associated with a control infusion. Sympathetic responses were intact in these mice, inasmuch as they were able to alter oxygen consumption in response to temperature changes. In obese A/y/a mice, leptin infusion achieved levels of \( \approx 55 \) ng/mL and increased catecholamine excretion but had no effect on blood pressure. These data suggest that the effect of leptin on blood pressure is saturable, with levels beyond a certain threshold having little capacity to alter vascular tone. Leptin transport across the vessels of the blood-brain barrier is also thought to be saturable.

Our findings represent an initial demonstration that leptin can increase blood pressure independent of the sympathetic nervous system. Leptin signals the central nervous system through neurons with multiple downstream effects, including autonomic stimulation. Central nervous system or peripheral infusion of high-dose leptin increases sympathetic activity and blood pressure in rodents. Transgenic mice that express leptin from a liver-specific promoter have plasma leptin levels of \( \approx 60 \) ng/mL, elevated blood pressure, and increased urinary catecholamine excretion. However, short-term peripheral infusion of leptin at doses higher than those used in the present study increases sympathetic activity but not blood pressure, perhaps because of the concomitant stimulation of vascular NO.

Our observations are consistent with a deleterious effect of leptin on the vasculature. Leptin increases fatty acid oxidation and the generation of reactive oxygen species in endothelial cells, events that are likely to promote increased blood pressure and vascular injury. Consistent with this concept, mice with defective fatty acid oxidation have lower blood pressure and are protected from atherosclerosis. Diet-induced diabetes promotes vascular calcification in mice by activating osteoblast differentiation. Leptin appears to participate in this process. Leptin may also have prothrombotic effects in the vasculature. These mechanisms may explain why leptin is an independent risk factor for coronary heart disease in humans.

Uncoupling lowered blood pressure in part by lowering adiposity, leading to lower leptin levels. However, uncoupling itself may affect blood pressure. UCP1 prevents the activation of protein kinase C and might alter signaling.

Discussion

Uncoupling proteins uncouple respiration and oxidative phosphorylation and may protect some humans from obesity and insulin resistance. Human UCP2 promoter polymorphisms may predict obesity. UCP1 or UCP3 expression in skeletal muscle prevents insulin resistance and diet-induced obesity in mice.

More than half of the adults in developed countries are overweight or obese, placing them at risk for insulin resistance, diabetes, hypertension, and atherosclerosis. The ominous increase in insulin-resistant diabetes among children portends a new epidemic of vascular disease. Hypertension promotes vascular dysfunction in type 2 diabetes, but the relationship between insulin resistance and blood pressure is poorly understood.

In the present study, we show that respiratory uncoupling in skeletal muscle lowers blood pressure and prevents obesity in lethal yellow (A/y/a) mice without compromising their ability to respond to changes in ambient temperature. Adipose UCP1 expression also prevents obesity in A/y/a mice. We specifically studied lethal yellow mice for 2 reasons. First, their blood pressure is increased compared with that in control mice. Second, susceptibility to obesity is genetically determined. Lethal yellow mice are obese because of hypothalamic expression of the coat color protein agouti, which impairs normal signaling by the melanocortin 4 receptor.
molecules affecting vascular tone in muscle, a major vascular bed.

Our observations also support the notion that respiratory uncoupling in skeletal muscle mimics chronic vigorous exercise. Exercise decreases adiposity, serum glucose and lipids, blood pressure, catecholamine excretion, and insulin resistance and appears to increase skeletal muscle triglyceride content in athletes. All of these effects were also seen in UCP-A/y mice. Curiously, elevated skeletal muscle fat is observed in insulin-resistant obese people and insulin-sensitive athletes, suggesting that neutral lipids alone are not responsible for insulin resistance.

Accelerating metabolism in skeletal muscle, at least in mice, reverses insulin resistance and lowers blood pressure in genetic obesity. Modest blood pressure lowering is more effective than blood glucose lowering at decreasing cardiovascular event rates in people with type 2 diabetes. Thus, promoting respiratory uncoupling in skeletal muscle represents a potential approach for decreasing vascular disease associated with insulin resistance.

Acknowledgments
This study was supported by National Institutes of Health grants AG-20091, HL-58427, and DK-18986, by Clinical Nutrition Research Unit grant DK-56341, by Diabetes Research and Training Center grant DK-20579, and by an American Diabetes Association/Johnson & Johnson Mentor-Based Postdoctoral Fellowship.

References


Respiratory Uncoupling Lowers Blood Pressure Through a Leptin-Dependent Mechanism in Genetically Obese Mice
Carlos Bernal-Mizrachi, Sherry Weng, Bing Li, Lorraine A. Nolte, Chu Feng, Trey Coleman, John O. Holloszy and Clay F. Semenkovich

Arterioscler Thromb Vasc Biol. 2002;22:961-968; originally published online April 25, 2002; doi: 10.1161/01.ATV.0000019404.65403.71
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/22/6/961

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/