Cardiovascular Abnormalities in Transgenic Mice With Reduced Brown Fat
An Animal Model of Human Obesity

Antonio Cittadini, MD; Christos S. Mantzoros, MD; Thomas G. Hampton, PhD; Kerry E. Travers, BS; Sarah E. Katz, BS; James P. Morgan, MD, PhD; Jeffrey S. Flier, MD; Pamela S. Douglas, MD

Background—A new model of murine obesity has recently been developed through transgenic ablation of brown adipose tissue that manifests typical metabolic complications of obesity, including insulin resistance and non–insulin-dependent diabetes mellitus. The cardiovascular phenotype has not been defined.

Methods and Results—Transthoracic echocardiography, aortic catheterization, isolated whole-heart studies, and morphometric histology defined cardiac structure and function in 30 transgenic mice with reduced brown fat and 30 matched wild-type controls. Obesity was indicated by a 77% increase in body weight and was accompanied by elevated systemic pressures (mean aortic blood pressure 85±1 versus 66±2 mm Hg; P<0.01), left ventricular dilation and hypertrophy (mass/body weight 4.0±0.2 versus 2.7±0.3 mg/g; P<0.01), and high cardiac output (cardiac index 3.2±0.4 versus 2.4±0.1 mL·kg⁻¹·min⁻¹; P<0.01). Baseline functional parameters assessed in vitro were not different, but after imposition of zero-flow ischemia, significant relaxation impairment developed in obese mice. Although morphometrically determined myocyte diameters were similar, the percentage of interstitial fibrosis was significantly increased in transgenic mice compared with wild-type controls (7.5±2% versus 4.2±0.2%; P<0.01).

Conclusions—Transgenic ablation of brown adipose tissue is associated not only with obesity but also with systemic hypertension, left ventricular hypertrophy with eccentric remodeling and fibrosis, and high cardiac output, a unique constellation of findings strikingly similar to that seen in human obesity. Mice with reduced brown fat may serve as a new model for the cardiovascular morbidity complications associated with obesity in humans. (Circulation. 1999;100:2177-2183.)

Key Words: brown fat ▪ hypertrophy ▪ echocardiography

Obesity is a common disease associated with increased morbidity and mortality, responsible for 7.8% of all healthcare costs.¹ A large portion of the increased risk is related to cardiovascular abnormalities, in particular hypertension and coronary artery disease. Cardiac structural and functional abnormalities, including left ventricular (LV) dilation, hypertrophy, and impaired function, have been reported in numerous studies of human obesity.²⁻⁴ Because ethical considerations limit the use of invasive techniques in humans, experiments with obese animals may offer greater insights into the cardiovascular changes related to obesity and its morbid complications. Nevertheless, such studies are limited.⁵⁻⁸ Furthermore, most rodent models of obesity display features that are atypical of human disease, such as reduced fertility, altered linear growth, elevated levels of corticosteroids, and reduced lean body mass. These peculiarities limit their relevance for studies of cardiovascular physiology. The recent introduction of transthoracic echocardiography in small rodents allows a more accurate assessment of in vivo cardiac physiology, which, combined with classic and innovative in vitro techniques (eg, Langendorff mouse heart), offers powerful tools to examine animal models of human disease.⁹

We recently developed a murine model of obesity based on the ablation of brown adipose tissue (BAT) using a transgenic toxigene approach.¹⁰ Mice with reduced BAT (UCP-DTA) develop decreased energy expenditure and hyperphagia leading to obesity.¹¹ More specifically, UCP-DTA mice have decreased body temperature and weight-specific metabolic rate but no differences in locomotor activity compared with normal mice.¹² Moreover, adjustment of food intake in relation to changes in ambient temperature is defective in the UCP-DTA mice.¹³ Importantly, when these mice are raised at thermoneutrality, obesity and hyperphagia are prevented.

Received January 25, 1999; revision received June 24, 1999; accepted July 2, 1999.

From the Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory, Cardiovascular and Endocrinology (C.S.M., J.S.F.) Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.


Correspondence to Antonio Cittadini, MD, Department of Internal Medicine, Federico II Medical School, Via Sergio Pansini, 5 (Edificio 18), 80131 Naples, Italy. E-mail cittadin@unina.it

© 1999 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org
indicating that BAT deficiency is responsible for the observed hyperphagia and obesity. At 16 days, UCP-DTA mice have a 68% reduction in uncoupling protein content of the interscapular brown fat depot, accompanied by moderate obesity. At 22 to 26 weeks of age, marked obesity develops in association with increased levels of glucose, insulin, and triglycerides and is markedly worsened by a high-fat diet.

Although the metabolic phenotype of these mice resembles human syndrome X, an important cardiovascular risk factor, a systematic investigation of cardiac structure and function has not yet been performed. Such studies would provide insight into the cardiovascular complications of obesity and insulin resistance and simultaneously establish the usefulness of UCP-DTA mice as a new model for studying obesity and insulin resistance and their cardiovascular complications.

The aim of the present studies was to characterize the cardiovascular phenotype of UCP-DTA mice. An integrated approach using transthoracic echocardiography, isolated whole-heart studies, and morphometric histology was used to define cardiac structure and function.

Methods

The development of UCP-DTA transgenic mice is described elsewhere. Transgenic FVB mice carrying the toxigene construct were bred as heterozygotes. All the methods are consistent with the Panel on Euthanasia of the American Veterinary Medical Association, conform to the requirements of the American Heart Association, and were approved by the Animal Care Committee of the Beth Israel Deaconess Medical Center. Mice were weaned at the age of 19 days, housed individually, and maintained on a 12:12-hour light-dark cycle under controlled temperature and humidity. Water and food (Purina Chow No. 5008) were available ad libitum. Experiments were performed on male 12-week-old mice.

Echocardiography

Previous reports from our laboratory have demonstrated the accuracy and reproducibility of transthoracic echocardiography in mice. Briefly, mice were anesthetized with ketamine HCl 100 mg/kg IP (Parke Davis) and xylazine 5 mg/kg IP (Lloyd Laboratories). Echocardiograms were performed with a Hewlett-Packard Sonos 2500 sector scanner equipped with a 7.5-MHz phased-array transducer. Two-dimensionally guided M-mode tracings were recorded with a strip-chart recorder at a paper speed of 100 mm/s. Anterior and posterior wall thickness and LV dimensions were measured in standard fashion, offline (Cardiac Workstation, Freeland Systems), by 1 observer blinded to prior results and were based on the average of 3 consecutive cardiac cycles. LV mass was determined by the cube formula, as well as LV volumes. Relative wall thickness, stroke volume, and cardiac output were calculated according to standard formulas. When appropriate, structural and functional indexes were normalized to body weight and to fat-free body weight, calculated by multiplying body weight by 0.72 for transgenic and 0.81 for wild-type (total body fat content is 28% and 19% in wild-type; total body fat content is 28% and 19% in UCP-DTA and wild-type mice of the same age and sex and on the same diet, respectively). However, because an ideal frame of reference for expressing cardiac structural and functional data has not been defined in obesity because of the relative underperfusion of fat tissue, we also report absolute values and percent changes from reference for expressing cardiac structural and functional data has not yet been performed. Such studies would provide insight into the cardiovascular complications of obesity and insulin resistance and simultaneously establish the usefulness of UCP-DTA mice as a new model for studying obesity and insulin resistance and their cardiovascular complications.

Histology

Specimens for histological examination were obtained from the 5 hearts used for the hemodynamic studies. Each heart was cut into cross sections at 4 levels from apex to base. The tissues were immersion-fixed in 10% buffered formalin. The samples were embedded in paraffin and stained with hematoxylin and eosin for muscle fiber diameter and with Masson’s trichrome for interstitial fibrosis. Quantitative evaluation was carried out by morphometry, according to previously described methods.

Blood Work

Blood was collected from the retro-orbital sinus of animals fasted overnight. Plasma insulin was assayed by radioimmunoassay with rat insulin standards (Linco). Leptin was assayed as reported previously.

Statistical Analysis

All values are mean ± SEM. Statistical analysis was performed with Statview. After tests for normal distribution, comparisons between the 2 study groups were performed with the unpaired 2-tailed Student's t-test.
Student’s t test. Linear regression analysis was used as appropriate. A value of $P<0.05$ was considered significant.

**Results**

Body weight was increased by 77% in UCP-DTA mice. Insulin and serum leptin levels were increased by ~18 and 16 times, respectively (Table 1). A detailed description of the metabolic consequences of BAT ablation is reported elsewhere.10–16 Maximal, mean, and minimal values of aortic blood pressure were all significantly higher in transgenic mice (Table 1). The average increase of mean blood pressure was ~29%.

**Echocardiography**

Figure 1 depicts representative echocardiographic tracings from a UCP-DTA mouse and a wild-type control. LV mass was increased by 135% in UCP-DTA mice (Table 2) as a result of higher posterior and anterior diastolic wall thickness (+35% and +64%, respectively) and by a concomitant 34% increase of LV cavity diameter. The ratios of LV mass to body weight and to fat-free body weight were significantly increased, indicating LV hypertrophy. The pattern of hypertrophy was eccentric, with unchanged relative wall thickness. Ejection-phase indexes were similar, suggesting normal pump function in vivo in UCP-DTA mice. Cardiac output was increased by 130% in transgenic mice; even after normalization to body weight and fat-free body weight, cardiac output was still significantly higher than in nontransgenic littermates, indicating a high-output syndrome. Peripheral vascular resistance index was slightly but not significantly lower in UCP-DTA mice.

**In Vitro LV Function**

Systolic and developed pressures were significantly increased in UCP-DTA obese mice compared with control over a wide range of preload, ie, balloon volumes (Figure 2, Table 3), with a trend toward lower developed wall stress. Diastolic

---

**TABLE 1. Animal Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>UCP-DTA</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g (n=11)</td>
<td>30±8</td>
<td>53±1*</td>
<td>+77</td>
</tr>
<tr>
<td>FF body weight, g (n=11)</td>
<td>25±2</td>
<td>38±1*</td>
<td>+52</td>
</tr>
<tr>
<td>Insulin, ng/mL (n=11)</td>
<td>1.4±0.2</td>
<td>25±3*</td>
<td>+178</td>
</tr>
<tr>
<td>Leptin, ng/mL (n=11)</td>
<td>0.23±0.09</td>
<td>3.83±1.2*</td>
<td>+166</td>
</tr>
<tr>
<td>HW, g (n=11)</td>
<td>0.14±0.01</td>
<td>0.29±0.01*</td>
<td>+107</td>
</tr>
<tr>
<td>HW/body weight, mg/g (n=11)</td>
<td>4.2±0.8</td>
<td>5.6±0.4</td>
<td>+33</td>
</tr>
<tr>
<td>HW/FF body weight, mg/g (n=11)</td>
<td>5.9±0.3</td>
<td>7.6±0.6*</td>
<td>+29</td>
</tr>
<tr>
<td>Aortic pressure max, mm Hg (n=5)</td>
<td>69±2</td>
<td>91±1*</td>
<td>+32</td>
</tr>
<tr>
<td>Aortic pressure mean, mm Hg (n=5)</td>
<td>66±2</td>
<td>85±1*</td>
<td>+29</td>
</tr>
<tr>
<td>Aortic pressure min, mm Hg (n=5)</td>
<td>63±2</td>
<td>79±1*</td>
<td>+25</td>
</tr>
</tbody>
</table>

FF indicates fat-free; HW, heart weight; and n, number of animals studied for each variable in each group. Values are mean±SEM.

*P<0.01 vs wild-type.

---

Figure 1. Representative echocardiographic monodimensional tracing obtained in a UCP-DTA mouse (left) and a wild-type control (right). Note larger diameters and wall thicknesses of transgenic animals with normal fractional shortening.
pressure–volume curves normalized to Vol$_{max}$ were superimposable, indicating similar compliance. In both normal and obese mice, wall stress–volume relationships were linear, with high correlation coefficients ($r=0.99$). Despite similar baseline in vitro function, obese mice had an increased susceptibility to ischemia, with significant prolongation of diastolic relaxation on reperfusion (Table 3).

Morphometric histology showed no differences in myocyte diameter between the groups (6.8±0.4 μm in wild-type versus 7.0±0.9 μm in transgenic, $P=NS$), whereas percentage of interstitial tissue was significantly higher in obese mice than in controls (4.2%±2% versus 7.5±2%, $P<0.01$).

**Discussion**

The present study demonstrates that mice with BAT ablation display a distinct cardiovascular phenotype in addition to the development of obesity and insulin resistance, manifest by elevation of blood pressure, LV hypertrophy with an eccentric remodeling pattern and increased interstitial tissue, and reduced tolerance to ischemia. Such alterations very closely parallel those observed in human obesity.$^{2,4,18,23-27}$ The UCP-DTA mouse may therefore serve as a new model for studying the cardiac morbidity complications of obesity and insulin resistance.

**Obesity and the Heart in Humans**

LV enlargement in human obesity is well documented,$^{2,4}$ with normotensive individuals displaying eccentric LV hypertrophy (normal or decreased relative wall thickness) as an adaptation to the expanded intravascular volume and low peripheral vascular resistance caused by excess adipose tissue. The presence of LV systolic dysfunction is still debated, with conflicting studies showing either decreased$^{4,23,26,27}$ or normal$^{4,24,25}$ LV systolic performance. High end-diastolic volumes and use of Starling reserve have been postulated as the mechanisms for preservation of function.$^{4}$ Diastolic function by Doppler-derived filling indexes appears to be impaired in obese individuals.$^{28}$ Circulatory dysfunction is also present,$^{18}$ with increases in blood volume and cardiac output necessary to meet the higher metabolic requirements.

The cardiac morphological consequences of hypertension and obesity are the net result of the opposing hemodynamic patterns. Systemic hypertension is associated with contracted intravascular volume, high total peripheral resistance, and normal cardiac output. LV hypertrophy becomes more severe and shows a more concentric pattern when systemic hypertension coexists with obesity.$^{4,18,24}$ Although cardiac function examined at rest may remain normal, the double burden of increased preload and afterload greatly enhance the risk of developing heart failure.

Because an accurate assessment of intrinsic contractility is problematic in humans, rodent models of obesity may offer several advantages in this regard. Nonetheless, few studies are available.

**Previous Studies in Animal Models of Obesity**

In genetically obese Zucker rats, Segel et al$^8$ found that whereas resting isolated heart function at 19 weeks was similar to that of controls, the obese rat showed reduced tolerance to hypoxia. Paradise et al,$^7$ studying the same model at 11 to 13 months, found diminished values of unnormalized wall stress, suggesting either reduced intrinsic contractility or...
dilation, whereas LV chamber compliance was not different from that of controls. LV hypertrophy was suggested by an increased ratio of LV mass to tibial length. It is worth noting that in the Zucker rat, obesity is typically associated with systemic hypertension and hyperleptinemia due to a mutation of the leptin receptor. Using JCR:LA obese, insulin-resistant rats, Lopaschuk and Russel found greater metabolic vulnerability in isolated hearts because they required high levels of insulin and buffer calcium to maintain mechanical function. In vivo analysis of cardiac structure and function was not performed in these earlier rodent investigations.

Hearts in Mice With Reduced Brown Fat
In UCP-DTA mice, high anatomic preload was documented by elevated end-diastolic volumes, while systemic hypertension imposed an afterload excess (30% increase in aortic blood pressure compared with wild-type controls). The consequent 135% increase in LV mass represents a greater extent of hypertrophy than in other rodent obesity models, whereas the increased lean body mass is also at variance with other obese rodents but is a known feature of human obesity. The structural and functional abnormalities displayed by the UCP-DTA mice reflect the dominant impact of obesity rather than hypertension, with eccentric rather than concentric remodeling. UCP-DTA mice also exhibited high cardiac output and stroke volume, and total peripheral resistance index was slightly decreased in UCP-DTA mice, all of which occur in obesity, not hypertension.

Although preload, afterload, and cardiac output were increased, systolic function was within normal limits, whether measured by ejection phase indexes (endocardial and midwall fractional shortening) or isovolumic wall stress, an accurate
The obese hyperinsulinemic UCP-DTA mouse has increased leptin levels and is resistant to exogenous leptin administration even before developing obesity. Although UCP-DTA mice are resistant to the weight- and food intake–reducing effect of leptin, they appear to be sensitive to other actions of leptin, including the regulation of hypothalamic NPY expression and the activity of the CRH-ACTH-adrenal axis. Therefore, hyperleptinemia may provide a novel mechanism by which hypertension develops, in addition to the well-known link between hyperinsulinemia and insulin resistance and hypertension. Specifically, it is possible that the hypertension and volume overload displayed by UCP-DTA mice may be secondary to the renal long-term chronic sympathetic activation with subsequent sodium retention induced by hyperleptinemia, because kidneys appear to be leptin sensitive. Whether hyperleptinemia with resistance to its neural effects is the primary cause of obesity because of the decrease of energy expenditure or is a compensatory mechanism for other unknown pathogenetic factors remains an open issue.

**Special Considerations and Study Limitations**

The absence of systolic dysfunction in UCP-DTA mice at 12 weeks does not exclude the possibility that overt cardiac dysfunction would appear later in life. In fact, the enlarged and fibrotic hearts of young UCP-DTA mice work at a distinct metabolic and mechanical disadvantage, as shown by the impaired recovery after global ischemia, which aging and/or additional disease states can only exacerbate. Our data are cross-sectional, however, and future longitudinal research is needed.

In the present study, the assessment of systemic blood pressure under anesthesia was not ideal, because it lowers blood pressure 20% to 30%. However, both groups were handled similarly, and results most likely reflect actual intergroup differences.

The possibility of “leaky” DTA expression in cardiac or surrounding tissues theoretically exists in this transgene. However, considering that UCP, which was used to drive the expression of DTA, is BAT-specific and that the clinical phenotype of UCP-DTA mice is very different from the one observed after DTA exposure, this possibility appears very unlikely.

**Clinical Implications**

As characterized, UCP-DTA mice represent a novel and faithful model of human obesity on the basis of on their phenotypic characteristics. The physiological/clinical charac-
teristics of this strain, i.e., hyperinsulinemia, hyperleptinemia, obesity, diabetes, hyperlipidemia,\textsuperscript{10–16,36} and now hypertension and cardiac abnormalities, make this strain relevant for the study of the development of cardiac abnormalities in humans. Future longitudinal assessment of the molecular basis for cardiac changes in this model is likely to provide additional insight into the critical links between obesity and cardiovascular disease.

Acknowledgments

This work was supported in part by NIH grants HL-31117 and HL-511307-01 (Dr Morgan), DK-46930 (Dr Flier), and RR-01032-22S2 (Dr Mantzoros).

References


Cardiovascular Abnormalities in Transgenic Mice With Reduced Brown Fat: An Animal Model of Human Obesity


Circulation. 1999;100:2177-2183
doi: 10.1161/01.CIR.100.21.2177

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/100/21/2177

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 Circulation is online at:
http://circ.ahajournals.org/subscriptions/