

Fasting-induced reductions in cardiovascular and metabolic variables occur sooner in obese versus lean mice

Jason M Tanner^{1,2}, Devin T Kearns¹, Bum Jun Kim², Crystal Sloan^{2,3}, Zhanjun Jia⁴, Tianxin Yang⁴, E Dale Abel^{2,3} and J David Symons^{1,2}

¹College of Health, University of Utah, Salt Lake City, UT 84132; ²Division of Endocrinology, Metabolism and Diabetes; ³Program in Molecular Medicine; ⁴Division of Nephrology and Hypertension, University of Utah School of Medicine, Salt Lake City, UT 84112, USA
Corresponding author: J David Symons, College of Health, and Division of Endocrinology, Metabolism and Diabetes, University of Utah School of Medicine, Building 585, Room 168, 30 N 2030 E, Salt Lake City, UT 84112, USA. Email: j.david.symons@hsc.utah.edu

Abstract

It is not uncommon for laboratory animals to be fasted prior to experimentation. Fasting evokes marked reductions in heart rate (HR), blood pressure (BP), heat production and oxygen consumption (VO_2) in rodents. Mice with diet-induced obesity exhibit elevated HR and BP, and lower VO_2 and heat production in the fed condition versus their lean counterparts. It is unknown whether body composition alters the tempo of response to fasting. We tested the hypothesis that cardiovascular and metabolic responses to fasting are delayed in obese versus lean male C57BL/6J mice. In the fed condition, mice that consumed high-fat (HF, 45% fat) chow for 98 ± 5 days had elevated ($P < 0.05$) body fat percentage (DEXA), serum leptin (ELISA), HR and BP (72-h biotelemetry), and lower ($P < 0.05$) heat production and VO_2 (72-h metabolic chamber) versus animals that consumed standard chow (CON, 10% fat; $n = 16$ per group). HR, BP, VO_2 , heat production and serum leptin decreased (all $P < 0.05$) in response to a 16-h fast (16:00–08:00 h) in both groups. Although the overall fold changes in cardiovascular and metabolic parameters were similar in magnitude among animals, fasting-induced reductions in cardiovascular and metabolic variables occurred ~ 4 and ~ 7 h earlier ($P < 0.05$), respectively, in HF versus CON mice. These findings indicate that while metabolic and cardiovascular stress evoked by a 16-h fast at 22°C is not different between HF and CON mice, fasting-induced responses occur sooner in obese animals.

Keywords: starvation, blood pressure, heart rate, metabolic rate, animal models

Experimental Biology and Medicine 2010; 235: 1489–1497. DOI: 10.1258/ebm.2010.010171

Introduction

It is not uncommon for laboratory animals to be fasted prior to experimentation. While this might be done to minimize risk to the animal during procedures such as endotracheal intubation,^{1,2} to lessen physiological responses associated with digestion prior to protocols that involve physical exercise,^{3,4} and/or to maximize agonist (e.g. insulin)-induced stimulation of tissue signaling pathways,⁵ fasting represents a substantial challenge to an organism. For example, fasting evokes marked reductions in heart rate (HR) and blood pressure (BP) in rodents.^{6–9} These reductions result from the organism entering into a state of torpor as it attempts to conserve energy.^{8,10–13} Support for this statement is that fasting suppresses oxygen consumption, heat production, locomotion and body temperature in rodents.^{8,10–16} Factors contributing to the severity of these reductions might include the animal species, species strain, gender,

ambient and core body temperature, length of fast, and/or composition of the previously consumed diet.

Our laboratory investigates mechanisms responsible for cardiovascular complications (e.g. systemic hypertension and endothelial dysfunction) that exist in mice with diet-induced obesity.⁵ We recently confirmed previous findings that HR and BP are elevated in obese versus lean mice during both the light (06:00–17:59 h) and dark (18:00–05:59 h) phases of their 24-h cycle.^{5,17} Furthermore, our preliminary data and results from others¹⁸ indicate that heat production, oxygen consumption and locomotion are less in obese versus lean animals. It is unknown whether differences exist between lean and obese mice specifically concerning the severity and timing of cardiovascular and metabolic responses to fasting. Addressing this issue is important to studies of vascular function because BP can significantly impact arterial function,^{19,20} and vascular reactivity is typically determined *in vitro* using vessels

obtained from mice that have completed a 16-h fast (i.e. 16:00–08:00 h).^{5,17}

We hypothesized that fasting-induced reductions in cardiovascular parameters (e.g. HR and BP) and metabolic function (e.g. oxygen consumption and heat production) would be less severe and/or delayed in obese versus lean mice. The rationale for this hypothesis was that because adipose tissue stores are more abundant in obese versus lean animals, fasting should pose less of a stress. Biotelemetry and metabolic chamber studies showed that HR, BP and heat production were higher, and oxygen consumption and locomotion were lower in obese versus lean mice in the fed condition. As anticipated, each of these variables decreased in response to fasting. Interestingly, the tempo of these changes was distinct, occurring earlier in the obese model. However, after 16 h of fasting, cardiovascular and metabolic adaptations had decreased to a similar extent overall. These data indicate that a 16-h fast is a suitable period after which to study vascular function in models of diet-induced obesity. However, shorter-term periods of fasting could be confounded by differential rates of metabolic and vascular adaptations.

Materials and methods

Animals and diet

All protocols were approved by the Animal Use and Care Committee at the University of Utah. Ten-week-old male C57BL/6J mice were purchased commercially (Jackson Laboratories, Bar Harbor, ME, USA), and housed under controlled temperature (22°C), light (06:00–17:59 h), and dark (18:00–05:59 h) conditions. Animals were allowed *ad libitum* access to water and standard chow containing (kcal%) 13% fat, 53% carbohydrate and 34% protein (#8656, Harlan Teklad, Denver, CO, USA; CON, $n = 32$). After a one-week quarantine period, mice continued the CON diet ($n = 16$) or were switched to chow containing (kcal%) 45% fat, 35% carbohydrate and 20% protein (D12451, Research Diets Inc; HF, $n = 16$). After 98 ± 5 days on the respective diets, cardiovascular variables were assessed in the fed and fasted condition in one cohort of seven CON and seven HF mice, while blood sampling, body composition, metabolic variables and urine collection procedures were performed in a second cohort of five CON and five HF mice.

Cardiovascular variables

Mice were anesthetized using 2–5% isoflurane. Using aseptic techniques, the abdomen was opened and a transmitter (model PA-C10, Data Sciences International, St Paul, MN, USA) was inserted and secured in place to allow ambulation with minimal hindrance. A catheter attached to the transmitter then was advanced subcutaneously and inserted into the right common carotid artery. Thirty second averages of systolic, diastolic and mean arterial blood pressure (MAP; mmHg) and HR (beats/min) were recorded every 15 min for four consecutive 24-h periods starting ~10 days postsurgery.^{5,17,21–23}

MAP and HR from the first three days were combined to represent fed values from the HF and CON mice. Fed values then were compared to the fourth day that contained the fasting segment from 16:00 to 08:00 h.

Blood sampling, body composition, metabolic variables and urinary catecholamines

Blood was obtained from a tail clip at ~08:00 h from fed, conscious CON and HF mice to quantify serum leptin (Millipore/Linco, St Charles, MO, USA) and triglycerides (Sigma-Aldrich, St Louis, MO, USA). At least 24 h later, body composition (total body mass, fat mass and lean mass [g]) was assessed in fed CON and HF mice using dual energy X-ray absorptiometry (DEXA; pDEXA Sabre Bone Densitometer, Norland Medical Systems, Fort Atkinson, WI, USA) on lightly anesthetized mice (0.015–0.02 mL avertin/g body mass).⁵ Three days later, VO_2 (mL/kg h), heat production (kcal/kg h), respiratory exchange ratio (RER), locomotor activity (beam breaks; X + Y + Z planes), food ingestion (g) and water intake (mL) were measured in mice housed individually in metabolic chambers (Oxymax; Columbus Instruments, Columbus, OH, USA) for 72 h. This duration consisted of familiarization (24 h) and data collection (24 h) periods wherein mice consumed their respective chow *ad libitum*, and a 24-h period that contained the fasting segment from 16:00–08:00 h. Body mass and rectal temperature (model TH-5; Physitemp, Clifton, NJ, USA) were measured prior to chamber entry (~08:00 h on day 1), body mass was assessed before the fasting segment (~16:00 h on day 2), and both variables again were recorded upon completion of the fasting period (~08:00 h on day 3). Mice were returned to their original housing conditions and resumed their respective feeding regimen.

Ten days later, mice were housed individually for 72 h in cages designed specifically to collect urine.^{22,23} This duration consisted of a familiarization (24 h) and data collection (48 h) period. Urine that accumulated in a vial containing 10 μL of 6 mol/L HCl was recovered at ~08:00 h on days 1–3, and was stored at -80°C until all samples could be analyzed simultaneously. Urinary epinephrine (ng/ μL) and norepinephrine (ng/ μL) were measured using the 2-CAT ELISA kit (Labor Diagnostika Nord, Nordhorn, Germany). Identical procedures were followed for all collections, except that food was removed at 16:00 h on day 3. When mice were removed from the urine collection chamber at the end of the fasting period (i.e. ~08:00 h on day 3), blood samples were obtained from a tail clip to assess leptin and triglycerides. Next, mice were anesthetized deeply (3–5% isoflurane), their chest was opened, heart excised, and gonadal fat pad mass was assessed.

Data analysis and statistics

Comparisons were made: (1) within the CON and HF group (light phase versus dark phase and FED versus FAST conditions); (2) between the CON and HF groups at identical time points in the FED and FAST conditions; and (3) between the CON and HF groups concerning the

magnitude of the fasting-induced response. To calculate the latter, FED values were subtracted from FAST values at identical time points. This number was divided by the FED value and represents the percent change due to fasting. Comparisons were made using a two-way analysis of variance with replication (Microsoft Excel 2007, Redmond, WA, USA). Significance was accepted when $P < 0.05$. All values in Figures 1–7 and Tables 1 and 2 are presented as mean $\pm$ standard error.

Results

Animal characteristics

Body mass, gonadal fat pad mass and total fat mass were greater, while lean mass was less in HF versus CON mice (Table 1; Figure 1a and b). Fasting triglycerides (mg/dL) were higher in HF (39.8 ± 2.4) versus CON (23.2 ± 2.9) mice. Previously we showed this duration of HF feeding to similarly aged C57Bl6 mice impairs glucose tolerance, evokes hyperinsulinemia, increases free fatty acids and causes endothelial cell but not vascular smooth muscle cell dysfunction.^{5,17}

Cardiovascular responses

Twenty-four hour HR and MAP were elevated in HF versus CON mice during the FED period (Figure 2a and b). While HR and MAP decreased in response to fasting in both groups, the onset was ~ 4 h sooner in HF (Figure 2c and d) versus CON mice (Figure 2e and f). The percent reduction induced by fasting (Δ) was similar between groups for HR (Figure 3a) and MAP (Figure 3b), although responses occurred sooner in HF animals.

Metabolic responses

Total 24-h VO_2 and heat production were lower in HF versus CON mice during the FED period (Figure 4a and b; Table 1). While heat production and VO_2 decreased in response to fasting in both groups, the onset was ~ 7 h sooner in HF (Figure 4c and d) versus CON mice (Figure 4e and f). The overall percent reduction induced

by a 16-h fast was similar between groups for heat production (Figure 5a) and VO_2 (Figure 5b). As expected, RER was lower in HF versus CON mice in both FED and FAST conditions, regardless of diurnal phase, and fasting precipitated decreases of RER in all animals (Table 1; Figure 6a and b). Fasting-induced percent reduction of RER was greater in CON versus HF mice, which was not surprising considering the lower baseline (i.e. FED) RER in HF versus CON animals (Figure 6c). No differences were observed in the tempo of fasting-induced decreases of RER, indicating that the fasting-induced shift of substrate usage occurred similarly in lean and obese animals (Figure 6a and b). Average values for these variables for both groups during the light and dark phases are shown in Table 1.

Body temperature, food consumption, body mass and locomotion

Rectal temperatures ($^{\circ}\text{C}$) during the FED (36.2 ± 0.3 and 36.3 ± 0.4) and at completion of the FAST (36.4 ± 0.3 and 36.5 ± 0.5) period were similar between HF and CON animals, respectively. Fasting-induced reductions in body temperatures of ~ 2 – 5°C have been observed after six hours of fasting.^{12,14,24} Therefore, it is curious that we did not observe differences after 16 h of food removal. One explanation is that temperature reductions were minimized/abolished because of increases in skeletal muscle activity secondary to animal restraint that was required to insert the rectal probe.

Food consumption, total caloric intake and calories consumed from fat, carbohydrate and protein were similar regardless of the diurnal phase (i.e. light versus dark) in HF mice. In contrast, all of these variables were greater in the dark versus light cycle in CON mice (Table 2). While HF mice consumed less food and fewer calories than CON mice during the dark phase, a larger portion of their calories consumed over 24 h was provided from fat, and a smaller portion from carbohydrates and protein (Table 2). No changes in body mass were observed after two days in the metabolic chamber (e.g. *ad lib* feeding; data not shown), but fasting (16:00–08:00 h on day 3) reduced body mass by $6.0 \pm 0.4\%$ and $12.5 \pm 0.6\%$ in HF versus CON mice,

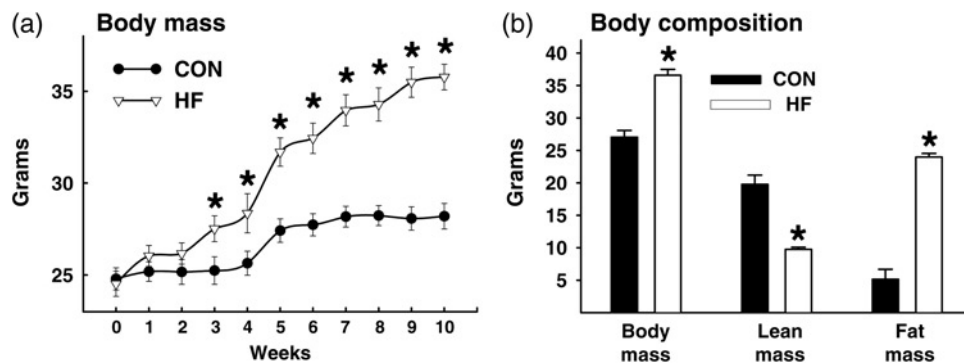


Figure 1 Body mass and composition of random-fed CON and HF mice. Body mass was greater ($P < 0.05$) in HF versus CON mice after three weeks of feeding. (a) Body mass and fat mass were greater and lean mass was less in HF versus CON mice (all $P < 0.05$; (b) Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

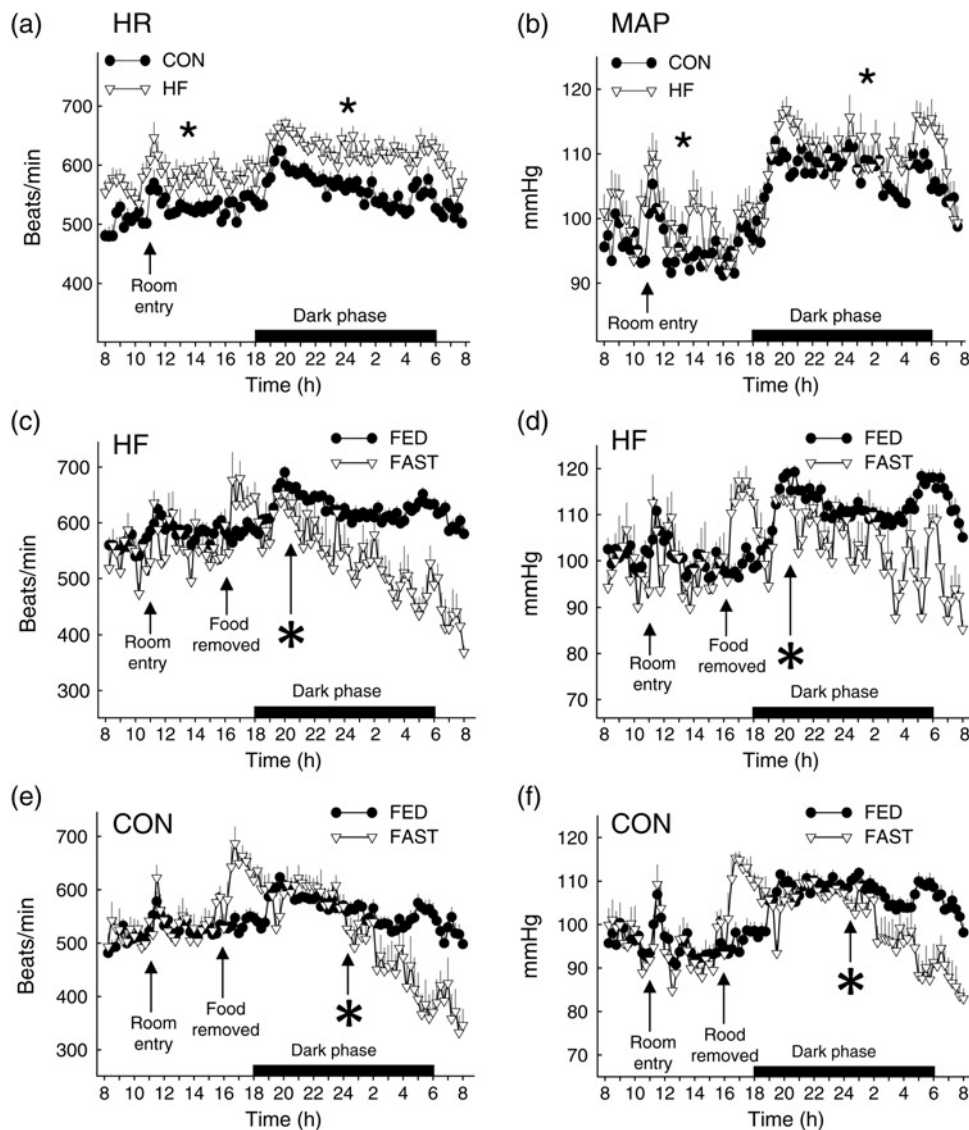


Figure 2 Heart rate (HR) and mean arterial pressure (MAP) from CON and HF mice during the FED and FAST conditions. HR (a) and MAP (b) were elevated ($P < 0.05$) in HF versus CON mice during the light and dark phases of the FED period. The fasting-induced reduction in HR (c, e) and MAP (d, f) occurred sooner ($P < 0.05$) in HF versus CON mice. FED values represent mean data from 3×24 h periods (i.e. days 1–3); FAST values represent an 1×24 period (i.e. day 4) that contains a fasting segment from ~16:00 to ~08:00. Spikes in HR and MAP evoked by room entry for cleaning (~1100) and food removal/cage change (~1600) were not included in the analyses. The “*” indicates the time at which a sustained statistical difference between FED and FAST values was first observed. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

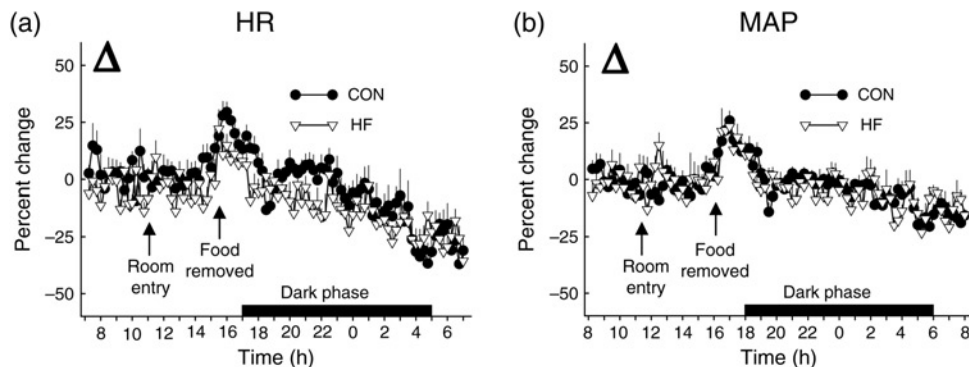


Figure 3 Fasting-induced decreases in heart rate (HR) and mean arterial pressure (MAP) compared between HF and CON mice. The overall fasting-induced percent change (Δ) in HR (a) and MAP (b) observed from 16:00 to 08:00 h was similar between CON and HF mice. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

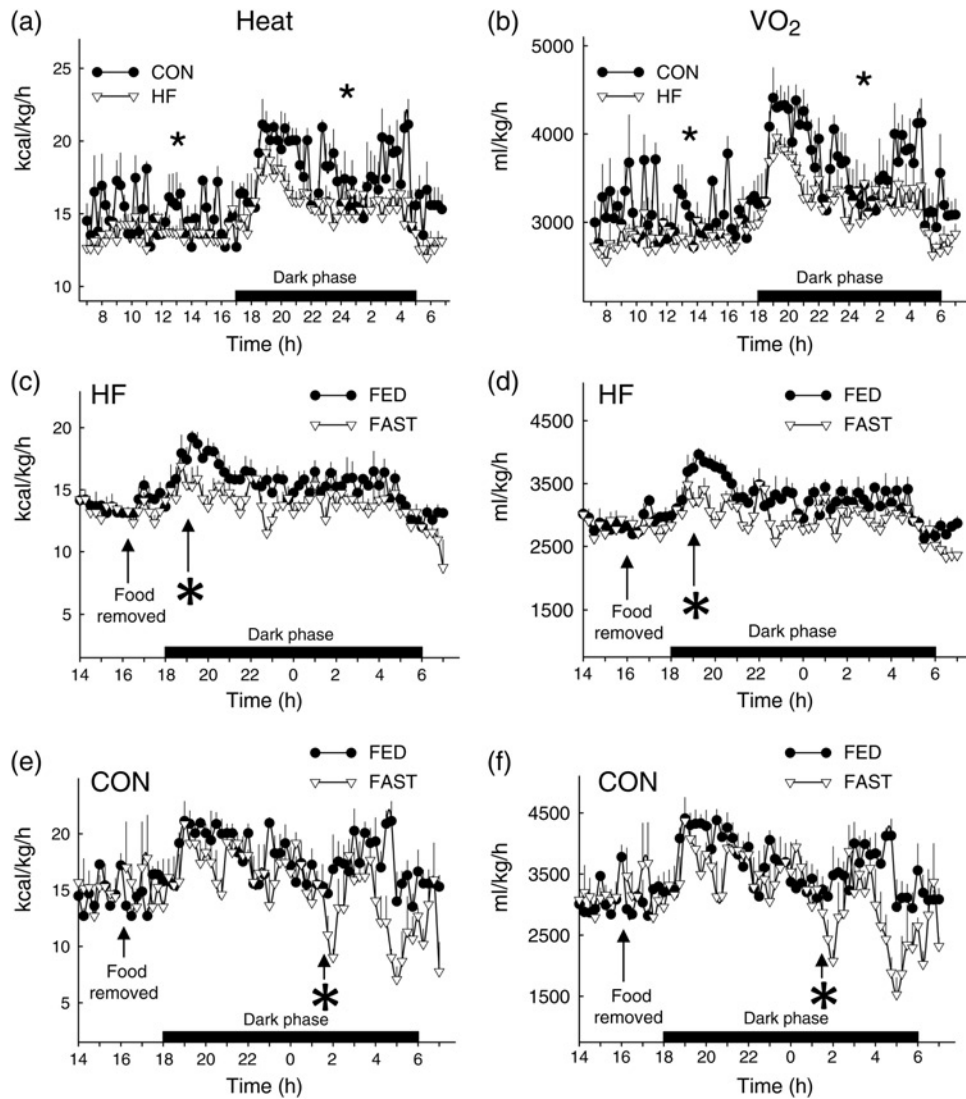


Figure 4 Heat production (HEAT) and oxygen consumption (VO₂) from CON and HF mice during the FED and FAST conditions. Heat production (a) and VO₂ (b) were lower ($P < 0.05$) in HF versus CON mice during the light and dark phases of the FED period. The fasting-induced reduction in heat production (c, e) and VO₂ (d, f) occurred sooner ($P < 0.05$) in HF versus CON mice. FED values represent mean data from 2×24 -h periods (i.e. days 1–2); FAST values represent an 1×24 -h period (i.e. day 3) that contains a fasting segment from ~16:00 to ~08:00. The '*' indicates the time at which a sustained statistical difference between FED and FAST values was first observed. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

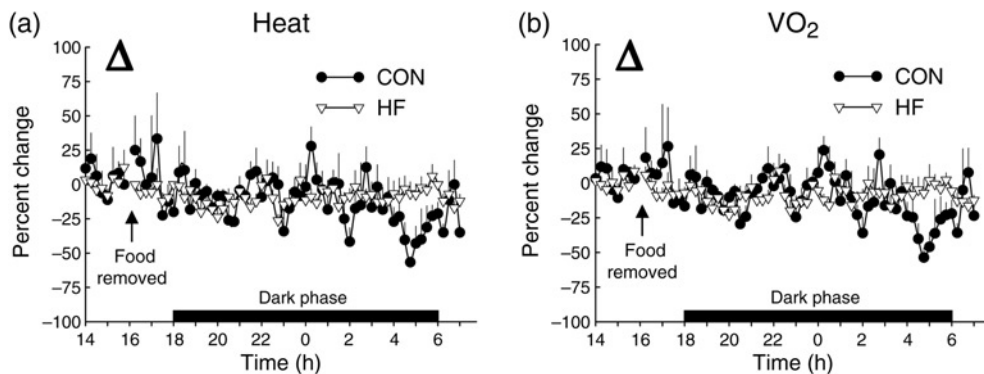


Figure 5 Fasting-induced decreases in heat production (HEAT) and oxygen consumption (VO₂) compared between HF and CON mice. The overall fasting-induced percent change (Δ) in heat production (a) and VO₂ (b) observed from 16:00 to 08:00 h was similar between CON and HF mice. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

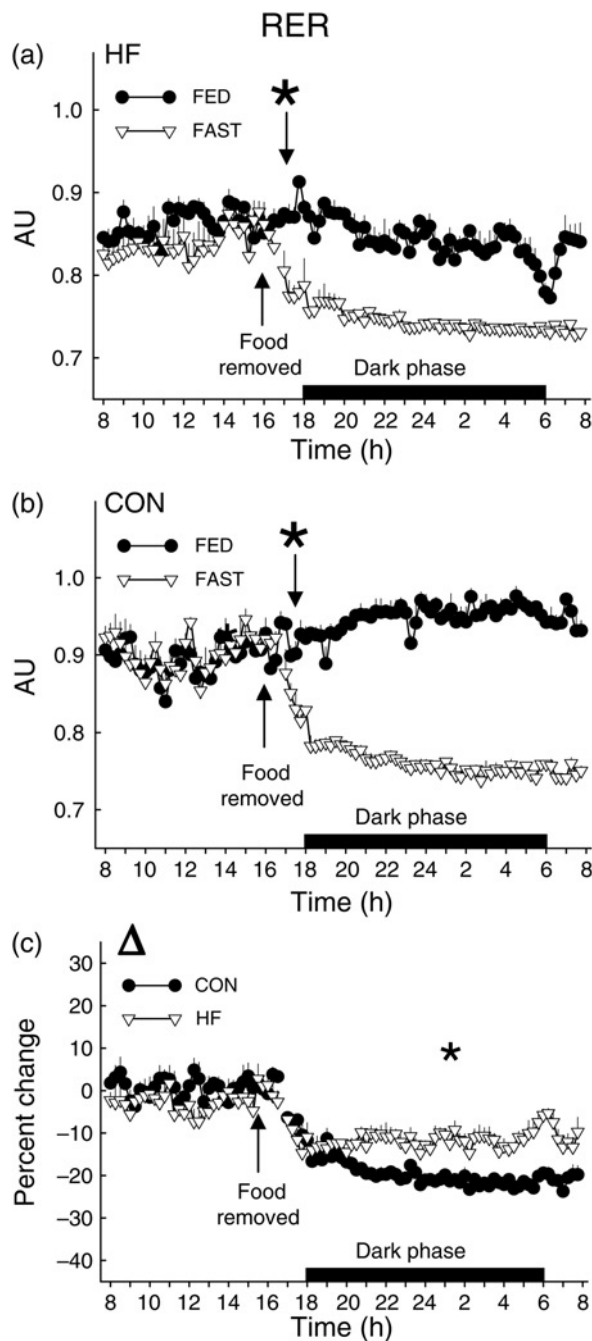


Figure 6 Respiratory exchange ratio (RER) from CON and HF mice during the FED and FAST conditions. The tempo of fasting-induced reduction of RER was not different between HF and CON mice (a, b). Noting the lower baseline (i.e. FED) RER in HF versus CON mice (a, b), it is not surprising that the overall fasting-induced percent change (Δ) in RER observed from 16:00 to 08:00 h on day 3 was greater in CON versus HF mice (c). FED values represent mean data from 2×24 -h periods (i.e. days 1–2); FAST values represent an 1×24 -h period (i.e. day 3) that contains a fasting segment from ~16:00 to ~08:00. The “*” indicates the time at which a sustained statistical difference between FED and FAST values was first observed. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

respectively (Figure 7a). HF mice accumulated more fat and body mass versus CON animals during their respective 14-week feeding regimens (Figure 1a and b) even though data obtained during the 72-h ‘snapshot’ of metabolic variables at 14 weeks indicated HF mice had less food

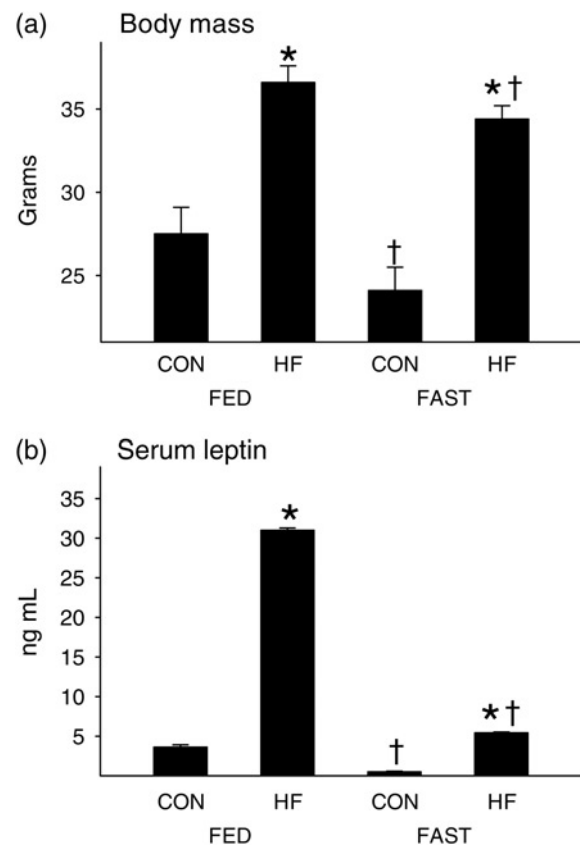


Figure 7 Body mass and serum leptin in CON and HF mice during the FED and FAST conditions. Body mass (a) and serum leptin (b) were elevated ($^*P < 0.05$) in HF versus CON mice during the FED and FAST periods. While fasting reduced ($^{\dagger}P < 0.05$) leptin concentrations similarly between HF and CON mice, the fold reduction in body mass was ~two-fold greater in CON versus HF mice. Values represent mean $\pm$ SEM. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

consumption and caloric intake. While this paradox might partially be explained by decreased metabolic rate and activity in HF versus CON mice (Table 1), these findings are consistent with previous work done by Lin *et al.*²⁵ In that study, even though less energy was consumed by fat-fed versus lean mice at four weeks, body mass and adipose tissue mass continued to increase in the former group for an additional 11 weeks. Regarding locomotion, nocturnal (i.e. dark cycle) activity was lower in HF mice upon fasting, whereas fasting did not alter nocturnal activity in lean animals (Table 1).

Water consumption, urine excretion and urinary catecholamines

In general, HF mice consumed less water and produced less urine per 24 h than CON animals during the FED and FAST period. Water consumption and urine production were less during the FAST versus FED period for both groups (Table 2). Urinary epinephrine and norepinephrine excretion were similar between HF and CON mice in the FED state. Urinary excretion of both catecholamines was lower in HF versus CON animals at the end of the FAST period (Table 1).

Table 1 Urinary catecholamines and metabolic averages

	CON	HF
Age (days)	160 ± 5	162 ± 6
Gonadal fat (g)	0.26 ± 0.1	1.60 ± 0.2*
Urinary epinephrine (ng/μL)		
FED	30 ± 6	18 ± 2
FAST	22 ± 4	8 ± 2*†
Urinary norepinephrine (ng/μL)		
FED	240 ± 61	211 ± 33
FAST	178 ± 24	82 ± 21*†
Heat production during the FED state (kcal/kg h)		
Light phase	15.0 ± 0.2	13.7 ± 0.1*
Dark phase	18.0 ± 0.2†	15.7 ± 0.2*†
Heat production during the FAST state (kcal/kg h)		
Light phase	14.7 ± 0.3	13.1 ± 0.1*†
Dark phase	15.4 ± 0.3†	14.1 ± 0.1*††
VO ₂ during the FED state (mL/kg h)		
Light phase	3099 ± 37	2837 ± 18*
Dark phase	3628 ± 45†	3253 ± 29*†
VO ₂ during the FAST state (mL/kg h)		
Light phase	3095 ± 42	2814 ± 22*
Dark phase	3174 ± 66†	2935 ± 28*††
RER during the FED state (AU)		
Light phase	0.900 ± 0.003	0.864 ± 0.002*
Dark phase	0.951 ± 0.002†	0.840 ± 0.002*†
RER during the FAST state (AU)		
Light phase	0.893 ± 0.004	0.831 ± 0.004*†
Dark phase	0.758 ± 0.001††	0.741 ± 0.001*††
Activity during the FED state, beam breaks (X + Y + Z)		
Light phase	311 ± 30	225 ± 24*
Dark phase	1662 ± 119†	1194 ± 83*†
Activity during the FAST state, beam breaks (X + Y + Z)		
Light phase	411 ± 48	235 ± 29*
Dark phase	1842 ± 122†	931 ± 73*††

Values are means ± SE. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

**P* < 0.05 HF versus CON

†*P* < 0.05 FED versus FAST

††*P* < 0.05 light phase versus dark phase

Leptin

Serum leptin was elevated in HF versus CON mice in both the FED and FAST conditions (Figure 7b). Compared to values obtained in the FED condition, fasting-induced reductions in leptin were similar between HF (82.4 ± 0.2%) and CON (86.0 ± 3%) mice.

Discussion

The independent effects of fasting^{8,10,13,16,26} and diet-induced obesity^{17,27–32} on cardiovascular and metabolic variables in rodents are well known. To our knowledge, specific comparisons of the tempo of fasting-induced reductions in cardiovascular and/or metabolic variables between lean and obese mice have not been made. We hypothesized that fasting would induce a smaller and/or delayed reduction in HR, BP, VO₂ and heat production in obese versus lean mice because the absence of food would pose less of a stress in the former group of animals. Biotelemetry and metabolic chamber studies confirmed

Table 2 Food and water intake, and urine output

	CON	HF
Food consumption (g)		
Light phase	2.3 ± 0.3	1.4 ± 0.1*
Dark phase	4.9 ± 0.3†	1.6 ± 0.1*
Total caloric intake (kcal)		
Light phase	6.7 ± 0.8	6.8 ± 0.4
Dark phase	14 ± 0.8†	7.7 ± 0.6*
Calories consumed from fat (kcal)		
Light phase	0.9 ± 0.1	3.0 ± 0.2*
Dark phase	1.9 ± 0.1†	3.4 ± 0.3*
Calories consumed from carbohydrate (kcal)		
Light phase	3.6 ± 0.4	2.4 ± 0.1
Dark phase	7.6 ± 0.4†	2.7 ± 0.2*
Calories consumed from protein (kcal)		
Light phase	2.3 ± 0.3	1.4 ± 0.1*
Dark phase	4.9 ± 0.3†	1.5 ± 0.1*
Water consumption (mL 24 h ⁻¹)		
FED	4.8 ± 0.3	2.5 ± 0.2*
FAST	2.4 ± 0.5†	1.6 ± 0.2†
Urine excretion (mL 24 h ⁻¹)		
FED	1.7 ± 0.3	0.84 ± 0.1*
FAST	1.1 ± 0.2†	0.22 ± 0.02*†

Values are means ± SE. CON, mice that consumed standard chow; HF, mice that consumed high-fat chow

**P* < 0.05 HF versus CON

†*P* < 0.05 FED versus FAST

††*P* < 0.05 light phase versus dark phase

that HR and BP were higher, while oxygen consumption, heat production and locomotion were lower in obese versus lean mice in the fed condition. Further, each of these variables decreased in response to a 16-h fast at 22°C. In contrast to our hypothesis, the overall severity of the respective decreases was similar between groups. These data are important for investigators who use murine models of diet-induced obesity because they indicate that the degree of metabolic and cardiovascular adaptations to fasting were similar after 16 h in lean and obese mice.

Cardiovascular and metabolic data were collected at ~15-min intervals and averaged hourly. These procedures allowed us to determine the time required for the onset of fasting-induced reductions to become evident, and whether differences existed between groups. Compared to the fed condition, fasting-induced reductions in HR and BP occurred ~4 h earlier in obese versus lean mice. Likewise, VO₂ and heat production became statistically lower ~7 h earlier in HF versus CON animals. While the mechanisms responsible for these unexpected findings are unclear, several possible explanations including differences in gender, differential activation of the autonomic nervous system and altered leptin signaling will be discussed.

First, differences in sex may modulate metabolic responses to fasting. For example, Swoap *et al.*¹² reported that female mice are more likely to enter a state of fasting-induced torpor than male mice. However, gender cannot contribute to our findings because all animals were male. A second possible explanation involves the generalized fasting-induced depression of the sympathetic limb of

the autonomic nervous system thought to exist in rodents.^{13,33–37} We assessed overall activation of the sympathetic nervous system by measuring urinary epinephrine and norepinephrine following the fed and fasting periods. Fasting-induced reductions of urinary catecholamines occurred in HF but not in lean animals. While these data are consistent with the notion that overall sympathetic withdrawal existed to a greater degree in HF versus CON mice, the exact time when sympathetic activity became lower in HF versus CON animals cannot be discerned from our data. To accomplish this would have required serial blood sampling throughout the fasting period (rather than urine collection), which is difficult to do in an organism with a small blood volume, i.e. 4% of body weight,³⁸ without influencing baroreceptor function. As such, without accurate serial assessment of sympathetic and/or parasympathetic activity, we cannot state with certainty whether differential activation of the two limbs of the autonomic nervous system contributed to earlier reductions in cardiovascular and metabolic variables in obese versus lean mice.

A third possibility that merits consideration for why reductions in cardiovascular and metabolic variables occurred sooner in HF versus CON mice is that central and/or peripheral leptin resistance might have developed in the former group of animals. The hormone leptin is secreted in proportion to fat stores. Decreased food intake (e.g. fasting or starvation) results in decreased serum leptin concentration and subsequent decreases in energy expenditure.³⁹ This effect is mediated primarily via leptin signaling in the hypothalamus (i.e. centrally).⁴⁰

We do not believe central leptin resistance existed in HF versus CON mice in the present study. Support for this statement is our observation that HF mice, while continuing to gain weight, consumed fewer calories (Figure 1a and b; Table 2). These observations are similar to those reported by Lin *et al.*²⁵ In that study even though less energy was consumed by fat-fed versus lean mice after four weeks of feeding, body mass and adipose tissue mass continued to increase in the former group for an additional 11 weeks. The authors concluded that hypophagia in the high-fat-fed group likely resulted from an attempt to regulate the rate of adipose tissue accumulation via intact hypothalamic/central leptin signaling. Accelerated decreases in HR and BP in HF versus CON mice could also be the result of an attempt to regulate energy expenditure via leptin signaling, and thus also suggests intact leptin sensitivity centrally and/or peripherally. However, because neither central nor peripheral leptin signaling was assessed directly in the present study, we cannot state with certainty that the tempo of reductions in VO_2 , heat production, BP and HR in obese mice was hastened by factors related to leptin resistance.

The ability to make accurate comparisons between lean and obese mice after they have been fasted might be jeopardized if fasting poses a greater/lesser stress on one group versus another. Our findings indicate that the severity of cardiovascular and metabolic stress evoked by a 16-h fast at 22°C is similar among lean and obese mice. These data indicate that a 16-h fast is a suitable period after which to study vascular function in models of diet-induced obesity.

We also observed that the tempo of fasting-evoked reductions in cardiovascular and metabolic variables is hastened in obese versus lean animals, although shifts in substrate usage (i.e. from carbohydrate to fat) were not. As such, shorter-term periods of fasting could be confounded by differential rates of metabolic and vascular adaptations, and should be taken into consideration when designing experimental protocols.

Author contributions: JMT, JDS and DTK designed the research; JMT, JDS, DTK and BJK conducted the research; CS, ZJ, TY, EDA and JDS provided essential materials for leptin and catecholamine analyses; JMT and JDS analyzed data and performed statistical analysis, wrote the paper and had primary responsibility for final content.

ACKNOWLEDGEMENTS

The assistance of Dr Robert Cooksey and Deborah Jones (metabolic chambers), and Dr Andreas Rohrwasser and Elaine Hillas (arterial telemetry) is greatly appreciated. Student support was provided, in part, by the Western States Affiliate of the American Heart Association Summer Student Research Program, the American Physiological Society and the University of Utah Undergraduate Research Opportunities Program. This work was funded, in part, by the University of Utah College of Health, the University of Utah Research Foundation, American Heart Association Western States Affiliate Grant-In-Aid 06-55222Y, NIH HL 091493-01, and American Diabetes Association Research Grant 7-08-RA-164 (JDS).

REFERENCES

- 1 Symons JD, Gunawardena S, Kappagoda CT, Dhond MR. Volume overload left ventricular hypertrophy: effects on coronary microvascular reactivity in rabbits. *Exp Physiol* 2001;**86**:725–32
- 2 Symons JD, Rendig SV, Fu LW, Longhurst JC. Endothelin-1 limits increases in blood flow to native and collateral dependent myocardium. *Am J Physiol* 1997;**273**:R41–8
- 3 Symons JD, Firoozmand E, Longhurst JC. Repeated dipyridamole administration enhances collateral-dependent flow and regional function during exercise. A role for adenosine. *Circ Res* 1993;**73**:503–13
- 4 Symons JD, Longhurst JC, Stebbins CL. Response of collateral-dependent myocardium to vasopressin release during prolonged intense exercise. *Am J Physiol* 1993;**264**:H1644–52
- 5 Symons JD, McMillin SL, Riehle C, Tanner J, Palionyte M, Hillas E, Jones D, Cooksey RC, Birnbaum MJ, McClain DA, Zang Q-J, Gale D, Wilson LJ, Abel ED. Contribution of insulin and Akt1 signaling to eNOS in the regulation of endothelial function and blood pressure. *Circ Res* 2009;**104**:1085–94
- 6 Duffy PH, Feuers RJ, Hart FW. Effect of chronic caloric restriction on the circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. *Chronobiol Int* 1990;**7**:291–303
- 7 Overton JM, Williams TD, Chambers JB, Rashotte ME. Cardiovascular and metabolic responses to fasting and thermoneutrality are conserved in obese Zucker rats. *Am J Physiol* 2001;**280**:R1007–15
- 8 Williams TD, Chambers JB, Henderson RP, Rashotte ME, Overton JM. Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6j mice. *Am J Physiol* 2002;**282**:R1459–67
- 9 Williams TD, Chambers JB, May OL, Henderson RP, Rashotte ME, Overton JM. Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. *Am J Physiol* 2000;**278**:R255–62

- 10 Himms-Hagen J. Food restriction increases torpor and improves brown adipose tissue thermogenesis in ob/ob mice. *Am J Physiol* 1985;**248**:E531–9
- 11 Hudson JW, Scott IM. Daily torpor in the laboratory mouse, *mus musculus* var. albino. *Physiol Zool* 1979;**52**:205–18
- 12 Swoap SJ, Gutilla MJ. Cardiovascular changes during daily torpor in the laboratory mouse. *Am J Physiol* 2009;**297**:R769–74
- 13 Williams TD, Chambers JB, Roberts LM, Henderson RP, Overton JM. Diet-induced obesity and cardiovascular regulation in C57BL/6J mice. *Clin Exp Pharmacol Physiol* 2003;**30**:769–78
- 14 Gavrilova O, Leon LR, Marcus-Samuels B, Mason MM, Castle AL, Refetoff S, Vinson C, Reitman ML. Torpor in mice is induced by both leptin-dependent and -independent mechanisms. *Proc Natl Acad Sci USA* 1999;**96**:14623–8
- 15 Koizumi A, Tsukada M, Wada Y, Masuda H, Weindruch R. Mitotic activity in mice is suppressed by energy restriction-induced torpor. *J Nutr* 1992;**122**:1446–53
- 16 Swoap SJ. Altered leptin signaling is sufficient, but not required, for hypotension associated with caloric restriction. *Am J Physiol* 2001;**281**:H2473–9
- 17 Symons JD, Holland W, Tanner J, Kearns D, Cahoon J, Palionyte M, Duncan B, Losee J, Narra K, Hillas E, Rohrwasser A, Abel ED, Summers S. Inhibiting vascular ceramide synthesis prevents arterial dysfunction and hypertension in mice with diet-induced obesity. *FASEB J* 2008;**22**:743.5
- 18 Makimura H, Mizuno TM, Beasley J, Silverstein JH, Mobbs CV. Adrenalectomy stimulates hypothalamic proopiomelanocortin expression but does not correct diet-induced obesity. *BMC Physiol* 2003;**3**:4
- 19 Jalili T, Carlstrom J, Kim S, Freeman D, Jin H, Wu TC, Litwin SE, Symons J. Quercetin-supplemented diets lower blood pressure and attenuate cardiac hypertrophy in rats with aortic constriction. *J Cardiovasc Pharmacol* 2006;**47**:531–41
- 20 Carlstrom J, Symons JD, Wu TC, Bruno RS, Litwin SE, Jalili T. A quercetin supplemented diet does not prevent cardiovascular complications in spontaneously hypertensive rats. *J Nutr* 2007;**137**:628–33
- 21 Bek MJ, Wang X, Asico LD, Jones JE, Zheng S, Li X, Eisner GM, Grandy DK, Carey RM, Soares-da-Silva P, Jose PA. Angiotensin II type I receptor-mediated hypertension in D4 dopamine receptor-deficient mice. *Hypertension* 2007;**47**:288–95
- 22 Wang N, Yang G, Jia Z, Zhang H, Aoyagi T, Soodvilai S, Symons JD, Schnermann JB, Gonzalez FJ, Litwin SE, Yang T. Vascular PPAR γ controls circadian variation in blood pressure and heart rate through Bmal1. *Cell Metab* 2008;**8**:1–10
- 23 Wang N, Symons JD, Zhang H, Jia Z, Gonzalez FJ, Yang T. Distinct functions of vascular endothelial and smooth muscle PPAR γ in regulation of blood pressure and vascular tone. *Toxicol Pathol* 2009;**37**:21–7
- 24 Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA. Endocrine regulation of the fasting response by PPAR α -mediated induction of fibroblast growth factor 21. *Cell Metab* 2007;**5**:415–25
- 25 Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57BL/6J mice. *Int J Obes Relat Metab Disord* 2000;**24**:639–46
- 26 Williams TD, Chambers JB, Gagnon SP, Roberts LM, Henderson RP, Overton JM. Cardiovascular and metabolic responses to fasting and thermoneutrality in Ay mice. *Physiol Behav* 2003;**78**:615–23
- 27 Abel ED, Litwin SE, Sweeney G. Cardiac remodeling in obesity. *Physiol Rev* 2008;**88**:389–419
- 28 Chiang BN, Perlman LV, Epstein FH. Overweight and hypertension: a review. *Circulation* 1969;**39**:403–21
- 29 Davy KP, Hall JE. Obesity and hypertension: two epidemics or one? *Am J Physiol* 2004;**286**:R803–13
- 30 Dustan HP. Mechanisms of hypertension associated with obesity. *Intern Med* 1983;**98**:860–4
- 31 Kotsis V, Stabouli S, Bouldin M, Low A, Toumanidis S, Zakopoulos N. Impact of obesity on 24-hour ambulatory blood pressure and hypertension. *Hypertension* 2005;**45**:602–7
- 32 Wood JE, Cash JR. Obesity and hypertension: clinical and experimental observations. *Ann Intern Med* 1939;**13**:81–90
- 33 Grassi G, Servalle G, Colombo M, Bolla G, Cattaneo BM, Cavagnini F, Mancia G. Body weight reduction, sympathetic nerve traffic, and arterial baroreflex in obese normotensive humans. *Circulation* 1998;**97**:2037–42
- 34 Ikeda T, Gomi T, Hirawa N, Sakurai J, Yoshikawa N. Improvement of insulin sensitivity contributes to blood pressure reduction after weight loss in hypertensive subjects with obesity. *Hypertension* 1996;**27**:1180–6
- 35 Kushiro T, Kobayashi F, Osada H, Tomiyama H, Satoh K, Otsuka Y, Kurumatani H, Kajiura N. Role of sympathetic activity in blood pressure reduction with low calorie regimen. *Hypertension* 1991;**17**:965–8
- 36 Overton JM, VanNess JM, Casto RM. Food restriction reduces sympathetic support of blood pressure in spontaneously hypertensive rats. *J Nutr* 1997;**127**:655–60
- 37 Overton JM, Williams TD, Chambers JB, Rashotte ME. Central leptin infusion attenuates the cardiovascular and metabolic effects of fasting in rats. *Hypertension* 2001;**37**:663–9
- 38 Hoff J. Methods of blood collection in the mouse. *Lab Anim* 2000;**29**:47–53
- 39 Rahmouni K, Haynes WG. Leptin and the cardiovascular system. *Recent Prog Horm Res* 2004;**59**:225–44
- 40 Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;**395**:763–70

(Received May 27, 2010, Accepted August 9, 2010)