Cardiovascular and metabolic responses to fasting and thermoneutrality are conserved in obese Zucker rats

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Overton, J. M., T. D. Williams, J. B. Chambers, and M. E. Rashotte. Cardiovascular and metabolic responses to fasting and thermoneutrality are conserved in obese Zucker rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1007–R1015, 2001.—The primary purpose of the study was to test the hypothesis that reduced leptin signaling is necessary to elicit the cardiovascular and metabolic responses to fasting. Lean (Fa/; normal leptin receptor; n = 7) and obese (fa/fa; mutated leptin receptor; n = 8) Zucker rats were instrumented with telemetry transmitters and housed in metabolic chambers at 23°C (12:12-h light-dark cycle) for continuous (24 h) measurement of metabolic and cardiovascular variables. Before fasting, mean arterial pressure (MAP) was higher (MAP: obese = 103 ± 3; lean = 94 ± 1 mmHg), whereas oxygen consumption (VO2: obese = 16.5 ± 0.3; lean = 18.6 ± 0.2 ml·min⁻¹·kg⁻⁰·⁷⁰) was lower in obese Zucker rats compared with their lean controls. Two days of fasting had no effect on MAP in either lean or obese Zucker rats, whereas VO2 (obese = −3.1 ± 0.3; lean = −2.9 ± 0.1 ml·min⁻¹·kg⁻⁰·⁷⁰) and heart rate (HR: obese = −56 ± 4; lean = −42 ± 4 beats/min) were decreased markedly in both groups. Fasting increased HR variability both in lean (+1.8 ± 0.4 ms) and obese (+2.6 ± 0.3 ms) Zucker rats. After a 6-day period of ad libitum refeeding, when all parameters had returned to near baseline levels, the cardiovascular and metabolic responses to 2 days of thermoneutrality (ambient temperature 29°C) were determined. Thermoneutrality reduced VO2 (obese = −2.4 ± 0.2; lean = −3.3 ± 0.2 ml·min⁻¹·kg⁻⁰·⁷⁰), HR (obese = −46 ± 5; lean = −55 ± 4 beats/min), and MAP (obese = −13 ± 6; lean = −10 ± 1 mmHg) similarly in lean and obese Zucker rats. The results indicate that the cardiovascular and metabolic responses to fasting and thermoneutrality are conserved in Zucker rats and suggest that intact leptin signaling may not be requisite for the metabolic and cardiovascular responses to reduced energy intake.

thermogenesis; obesity; caloric restriction; blood pressure; indirect calorimetry

CALORIC DEPRIVATION INVOKES a constellation of homeostatic, physiological responses including: increased appetite (57), reduced metabolic rate (49, 55), altered pituitary function (13, 54), reduced sympathetic nervous system activity to heart and brown adipose tissue (42, 59), decreased heart rate (HR), and blood pressure (55, 60), and increased sympathetic activity to white adipose tissue (33). The regulation of these divergent responses is likely to involve a complex array of neurohumoral pathways. Several lines of evidence suggest that decreased serum leptin levels and subsequent signaling within the hypothalamus are crucial mediators of the response to starvation (16, 17, 47). If reduced leptin signaling is a critical mechanism mediating the physiological responses to reduced caloric availability, then animals with disrupted leptin-signaling capabilities should display suppressed homeostatic responses to starvation.

The obese phenotype of the fatty Zucker rat is due to a point mutation (Gln269Pro) in the portion of the leptin receptor gene coding for the extracellular domain of all isoforms of the leptin receptor (10, 12, 38). The mutation leads to reduced receptor expression, reduced binding affinity for leptin, and defective leptin-mediated signal transduction (12). Thus the obese Zucker rat is markedly resistant to the physiological effects of leptin administration (3, 11, 14, 21, 23, 31, 48, 50). The leptin-resistant, obese Zucker rat exhibits many characteristics of animals exposed to caloric deprivation, including hyperphagia (19), reduced metabolic rate (27), hyperthermia (36), reduced sympathetic activity (29, 41), and decreased HR (4). Therefore, we speculated that these animals would display minimal physiological responses to starvation. Thus the first purpose of this study was to test the hypothesis that the obese Zucker rat would display blunted cardiovascular and metabolic responses to 48 h of caloric deprivation.

In addition to energy deprivation, exposure to thermoneutral ambient temperatures (Tₐ ≈ 29°C) also lowers oxygen consumption, HR, and blood pressure in rodents compared with levels observed at typical laboratory conditions of Tₐ = 21–23°C (22, unpublished results). These observations are consistent with the idea that standard housing temperatures for rats represent a form of mild cold stress requiring ongoing activation of sympathetically mediated nonshivering thermogenesis that tonically elevates HR and blood pressure.

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pressure. Obese Zucker rats exhibited reduced capacity for nonshivering thermogenesis on exposure to cold stress (7, 41). Given the reduced sympathetic outflow at rest and the reduced capacity for nonshivering thermogenesis in obese Zucker rats, the second purpose of this study was to examine the metabolic and cardiovascular responses to removal of mild cold stress by exposure to thermoneutrality. This additional protocol allowed for the comparison of the cardiovascular responses to two distinct interventions that reduce energy expenditure (caloric deprivation and thermoneutrality) in lean and obese Zucker rats.

**METHODS**

Male obese Zucker (fa/fa) rats (n = 8; 7–12 wk of age) and lean Zucker (Pa; n = 7) rats (12 wk of age; Harlan; Indianapolis, IN) were anesthetized (pentobarbital sodium, 50 mg/kg) and instrumented with a catheter in the descending aorta and coupled with a sensor and transmitter (TA11PA-C40; Data Sciences; St. Paul, MN) for telemetric monitoring of blood pressure. During recovery from surgery, rats were housed individually with ad libitum access to powdered rodent chow (Purina 5001; physiological energy value = 3.3 kcal/g) and deionized water in acclimation cages as described previously (55). During the initial phase of the experiment, rats were housed in an ambient temperature of 23 ± 0.1°C and maintained on a 12:12-h light-dark schedule.

**Indirect calorimetry.** The apparatus and procedures used for environmental control and indirect calorimetry have been described previously (40, 55). Oxygen consumption (V\text{\textsubscript{O2}}) and carbon dioxide production (V\text{\textsubscript{CO2}}) were determined every 2.5 min by open-circuit respirometry and stored on a floppy disk. V\text{\textsubscript{O2}} was adjusted for mass (ml min\textsuperscript{-1} kg\textsuperscript{-0.75}). Respiratory quotient [RQ (V\text{\textsubscript{CO2}}/V\text{\textsubscript{O2}})] was calculated for the entire 12-h dark phase and for 10 h of the light phase.

**Telemetry monitoring.** A telemetry receiver (RPC-1; Data Sciences) was positioned under the experimental cage within the metabolic chamber. Mean arterial blood pressure (MAP), HR, and the standard deviation of the interbeat interval (SDIBI) were calculated for each 30-s period of the day and stored on a floppy disk as described previously (55).

**Locomotor activity monitoring.** Some of the metabolic chambers were instrumented to record locomotor activity, which was measured in meters, accumulated in 30-s periods and stored with a 1-mm resolution as described previously (55). This resulted in n = 4 for activity measures both in the lean and in the obese groups.

**Protocol.** After the rats’ recovery from surgery and acclimation to the housing conditions, 4 days of baseline cardiovascular, metabolic, and behavioral data were obtained at T\textsubscript{a} = 23°C. Food and deionized water were available ad libitum. Food intake, water intake, and body weight were determined during a daily maintenance period that occurred 1–2 h before lights off at 10:00 AM. We then determined the cardiovascular, metabolic, and behavioral effects of food deprivation for 48 h. Food was removed at the maintenance period (just before lights off), and the deionized water was replaced with an electrolyte solution containing 78 meq/l NaCl and 15 meq/l KCl to provide sodium and potassium. After 48 h, food and deionized water were returned to the cages just before lights off to ensure that the rats would resume food consumption at a time consistent with normal circadian feeding behavior. In the refeeding period, food was available ad libitum for 6 days, during which recovery kinetics of all parameters was measured. We then examined the cardiovascular, metabolic, and behavioral responses to acute exposure to thermoneutrality. T\textsubscript{a} within the environmental chambers was increased to 29 ± 0.1°C over a period of 20 min beginning 1 h before the onset of the dark phase. Cardiovascular and metabolic responses to T\textsubscript{a} = 29°C were examined for 48 h, and the temperature in the chamber was returned to T\textsubscript{a} = 23°C to verify that the responses to thermoneutrality were reversible.

**Data analysis and statistics.** Cardiovascular data and locomotor activity data were collected and stored in 30-s bins. Metabolic data were collected and stored in 2.5-min bins. Before further analysis, all data were averaged or cumulated into 10-min bins. The final 2 h of the light phase (during which daily chamber-maintenance procedures were performed) was excluded from analysis, resulting in 12-h averages for the dark phase and 10-h averages for the light phase. With the use of these values, baseline levels of variables were calculated as a 3-day average. Simple two-way ANOVA was used to determine whether there were significant circadian and group effects on baseline parameters before intervention. One-way ANOVA (within circadian phase, across days) was then performed to determine the effects of fasting, refeeding, and exposure to thermoneutrality on dependent variables. Finally, the effects of fasting, refeeding, and exposure to thermoneutrality were expressed as changes from baseline levels for between-group comparisons using ANOVA. Tukey’s post hoc tests were used to determine significant differences between means. Significance levels of P < 0.05 were accepted.

**RESULTS**

**Food/fluid intake and body weight.** During baseline, obese Zucker rats exhibited greater body weight, food intake, and fluid intake compared with lean controls (Fig. 1, A-C). Baseline sodium intake was also significantly greater (P < 0.05) in obese Zucker (5.9 ± 0.2 mmol/day) compared with lean controls (4.0 ± 0.1 mmol/day). Two days of fasting significantly reduced body weight both in lean (−33 ± 1 g) and in obese groups (−47 ± 2 g). Fasting was associated with marked reduction of fluid intake (electrolyte solution) in obese Zucker rats but with only a slight decrease in lean Zucker animals (Fig. 1B). Sodium intake during caloric deprivation was lower in the obese Zucker rats (0.6 ± 0.1 mmol/day) than the lean controls (1.7 ± 0.3 mmol/day). On the first day of refeeding, lean (baseline intake = 77 ± 1 kcal; refeeding intake = 97 ± 4 kcal; P < 0.05), but not obese, (baseline caloric intake = 114 ± 4 kcal; refeeding intake = 108 ± 3 kcal) rats displayed a significantly increased caloric intake. In addition, both groups drank significantly more water during the first day of refeeding.

Exposure to thermoneutrality was associated with significant reductions in food intake both in lean (−10 ± 3 kcal) and in obese (−21 ± 3 kcal) animals, with no effect on body weight or water intake (Fig. 1, A-C). One day of normalization of T\textsubscript{a} to 23°C restored food intake to levels not significantly different from baseline conditions.

**Blood pressure and HR.** MAP was elevated at baseline in obese Zucker rats (dark: 106 ± 3 mmHg; light: 103 ± 3 mmHg) compared with lean Zucker controls (dark: 99 ± 1 mmHg; light: 94 ± 1 mmHg) (Fig. 2A).
Fasting significantly reduced dark-phase MAP in lean Zucker rats but had no effect on light-phase MAP in lean Zucker rats and had no effect on MAP in obese Zucker rats (Fig. 2, A-C). After transient elevations in MAP during the initial refeeding period, MAP returned to baseline levels both in lean and in obese animals. Exposure to thermoneutrality (Ta = 29°C) produced reductions in MAP of 7–12 mmHg in lean and obese Zucker rats (Fig. 2C). These reductions in MAP were statistically significant with the exception of the decrease in MAP observed during the dark phase in obese Zucker rats (Fig. 2C). Restoration of Ta to standard conditions (23°C) normalized MAP within 24 h (Fig. 2, A and B).

Baseline dark-period HR was significantly lower in obese Zucker rats compared with lean controls (obese: 379 ± 7; lean: 410 ± 6 beats/min; P < 0.05), whereas there was no significant difference between groups in light-phase HR (obese: 354 ± 7; lean: 363 ± 6 beats/min; Fig. 2D). Fasting significantly reduced HR both in lean and in obese Zucker rats during the first 12-h dark period of food deprivation. The magnitude of the bradycardia increased as the duration of the fast was extended from 1 to 2 days (Fig. 2, D-F). The obese Zucker rats displayed greater bradycardia during the light phases of both the first (lean: −24 ± 3 beats/min; obese: −43 ± 2 beats/min, P < 0.05) and the second (lean: −42 ± 4 beats/min; obese: −56 ± 4 beats/min, P < 0.05) days of fasting (Fig. 2F). Refeeding promptly increased HR toward control levels, although HR tended to remain below baseline levels for the first several days of the refeeding period (Fig. 2, D-E). Increasing Ta to thermoneutrality promptly reduced HR both in lean and in obese Zucker rats. The response was generally similar, although the lean Zucker rats displayed a greater bradycardia during the dark phase of the second day of exposure to thermoneutrality (Fig. 2F; lean: −60 ± 6 beats/min; obese: −40 ± 5 beats/min, P < 0.05). Decreasing Ta back to 23°C promptly restored HR toward baseline levels (Fig. 2, D and E).

Baseline HR variability, as quantified by the SDIBI, was significantly lower in obese Zucker rats (Fig. 2G) during both the light (lean: 5.7 ± 0.4 ms; obese: 4.7 ± 0.3 ms, P < 0.05) and the dark (lean: 6.2 ± 0.4 ms; obese: 4.6 ± 0.2 ms, P < 0.05) phases. Fasting significantly increased HR variability in both lean and obese Zucker rats, with the magnitude of this increase being greater during the dark phase of the second fasting day in the obese Zucker rats compared with lean controls (Fig. 2, H and I). Refeeding rapidly normalized HR variability in both strains. In contrast to fasting, the marked bradycardia associated with exposure to thermoneutrality was not accompanied by altered HR variability in either lean or obese Zucker rats (Fig. 2, H and I).

\( VO_2 \) and locomotor activity. Baseline \( VO_2 \), normalized for body mass, was significantly reduced in the obese Zucker rats compared with lean controls in the dark phase (lean: 18.6 ± 0.2; obese: 16.5 ± 0.3 ml·min\(^{-1}\)·kg\(^{-0.75}\), P < 0.05) but not the light phase (Fig. 3A). Fasting produced similar reductions in \( VO_2 \) in lean and obese Zucker rats (Fig. 3, B and C). Refeeding restored \( VO_2 \) toward baseline levels over the course of a few days. Exposure to thermoneutrality resulted in rapid and sustained reductions in \( VO_2 \) in both lean and obese rats (Fig. 3, A-C), although the magnitude of the decrease was greater in lean compared with obese rats in both the light (lean: −3.3 ± 0.2; obese: −2.4 ± 0.3 ml·min\(^{-1}\)·kg\(^{-0.75}\), P < 0.05) and the dark phase (lean: −3.4 ± 0.2; obese: −2.2 ± 0.4 ml·min\(^{-1}\)·kg\(^{-0.75}\), P < 0.05). One day of return to standard Ta = 23°C increased \( VO_2 \) toward baseline levels.

RQ was greater at baseline in obese Zucker rats compared with lean controls (Fig. 3D). Because fasting reduced RQ in both groups to 0.75–0.80, the magni-
The magnitude of the reduction was greater in obese Zucker rats than in lean controls on both fasting days (Fig. 3, E and F). Refeeding rapidly restored RQ to baseline levels. Exposure to thermoneutrality had no effect on RQ in lean Zucker rats but produced slight, yet significant, reductions in RQ in obese rats.

Locomotor activity at baseline was reduced in the dark phase in obese (77 ± 12 m) compared with lean (108 ± 8 m) Zucker rats (Fig. 3G). Fasting generally increased locomotor activity both in lean and in obese Zucker rats (Fig. 3, G-I), although the increases were not statistically significant (n = 4 for activity due to equipment limitations). Exposure to T_\text{a} = 29°C produced significant increases in activity in obese but not lean Zucker rats. In particular, dark-phase locomotor activity was markedly increased in obese rats both on the first (+101 ± 10 m) and on the second (+53 ± 7 m) days of T_\text{a} = 29°C. Locomotor activity was rapidly restored to baseline when T_\text{a} was reduced back to 23°C.

**DISCUSSION**

We hypothesized that obese Zucker rats would exhibit diminished cardiovascular and metabolic responses to caloric deprivation. Instead, fasting reduced V\text{O}_2 and HR, while increasing HR variability, both in lean and in obese Zucker rats. There was no evidence of either a reduced magnitude of response or a delay in...
the activation of the homeostatic cardiovascular and metabolic responses to reduced caloric intake in obese Zucker rats. Interestingly, the fasting-induced decrease in HR and VO₂ was not accompanied by lower MAP in the obese Zucker rats but was associated with a modest decrease in dark-phase MAP in the lean Zucker rats. Nonetheless, it is clear that obese Zucker rats, which display a starvation-like phenotype in the ad libitum-fed condition due to dysfunctional leptin signaling, exhibit a vigorous homeostatic response to caloric deprivation. Thus our findings suggest that reductions in circulating leptin and central nervous system leptin signaling are not requisite for the metabolic and cardiovascular responses to caloric deprivation.

Increasing Tₐ from standard conditions of mild cold (23°C) to thermoneutrality (29°C) elicited concurrent reductions in VO₂, HR, and MAP both in lean and in obese Zucker rats. The responses are similar to those we have recently observed in spontaneously hypertensive rats (SHR) and Sprague-Dawley rats (unpublished results) and suggest that the decreased requirement for thermogenesis at thermoneutrality is accompanied by systemic cardiovascular adaptations including reductions in HR and MAP. The magnitude of the bradycardia was similar to that produced by caloric deprivation (~50 beats/min). In contrast to fasting, this bradycardia was accompanied by significantly lower MAP both in lean and in obese animals but was not associated with increased HR variability. This sug-
gests that different autonomic and cardiovascular mechanisms are involved in the responses to fasting and exposure to thermoneutrality. Together, the findings reveal that both lean and obese Zucker rats display concurrent cardiovascular and metabolic responses to reduced energy expenditure produced by exposure to thermoneutrality.

Cardiovascular and metabolic responses to fasting and thermoneutrality. Whereas the major focus of our study was not obesity-induced hypertension, our work confirms other reports indicating that obese Zucker rats have elevated blood pressure and lower resting HR compared with their lean counterparts (4, 28, 61). In this model of obesity, the mechanism for the elevated MAP appears to be enhanced angiotensin II responsiveness and not augmented sympathetic outflow (4, 61). In contrast, the obese diabetic Zucker rat exhibits hypertension that is accompanied by greater sympathetic support of blood pressure (8).

We are not aware of any previous reports of the effects of fasting or thermoneutrality on cardiovascular function in lean and obese Zucker rats, although pair feeding did not reduce blood pressure in obese Zucker rats (28). Whereas we hypothesized that obese Zucker rats would display attenuated cardiovascular responses to fasting, we observed greater fasting-induced bradycardia during the light phase in the obese Zucker rats. The mechanisms producing this bradycardia are not completely understood but are likely to be due to a combination of increased vagal tone and reduced sympathetic tone. As in prior studies with other rat strains (55), we observed that fasting-induced bradycardia in lean and obese Zucker rats is accompanied by increased HR variability. We have also recently observed that chronic beta-1-receptor blockade has no effect on this measure of HR variability (unpublished observations). Thus we suggest that fasting-induced increases in HR variability are indicative of increased vagal tone that contributes to fasting-induced bradycardia. In the current study, it is interesting to note that the obese Zucker rats display lower HR variability at baseline. This observation is somewhat consistent with reduced baroreflex control of HR that has been reported in obese Zucker rats (5). At this time, we have no information concerning the role of altered arterial baroreflex function on the modulation of the cardiovascular responses to caloric deprivation.

Caloric deprivation produced a modest (6–7 mmHg) reduction in dark-phase MAP in lean rats with no effect on MAP in obese Zucker rats or during the light phase in lean rats. We have recently observed a similar pattern of modest, dark-phase specific reductions in MAP in Sprague-Dawley rats (unpublished observations) but have previously observed much more significant fasting-induced reductions in MAP in SHR (55). There are a number of mechanisms that might explain the variable fasting effects on MAP between obese and lean Zucker rats. Given that the obese Zucker rats exhibited greater bradycardia, it is unlikely that the explanation is directly related to a failure to invoke homeostatic responses to fasting. One possibility is that compensatory responses to reduced sodium and fluid intake may be involved. During fasting, lean rats consumed normal levels of an electrolyte solution, whereas obese Zucker rats had dramatic reductions in fluid intake (Fig. 1). It is possible that reduced fluid and sodium intake in obese Zucker rats during fasting activated the renin-angiotensin-aldosterone system that served to defend arterial pressure to a greater extent in obese than in the lean Zucker rats. We have no direct support for this hypothesis, but it is consistent with the trend for MAP to be elevated during the first few days of refeeding in obese but not lean rats.

Importantly, the significant decrease in blood pressure observed on exposure to thermoneutrality demonstrates that MAP can respond to reduced energy expenditure both in lean and in obese Zucker rats. Increasing Ta from standard conditions of mild cold (23°C) to thermoneutrality (29°C) produced similar reductions in HR compared with fasting yet reduced MAP by 7–12 mmHg in lean and obese rats. Two points concerning the bradycardia produced by thermoneutrality should be noted. First, the magnitude of the bradycardia during the dark phase was greater in lean than obese Zucker rats. We noted that obese Zucker rats exhibited an intriguing increase in locomotor activity when Ta was increased to 29°C. This raises the question of the selection of 29°C as thermoneutral temperature for both lean and obese Zucker rats, which differ in body and fat mass. Both lean and obese Zucker rats exhibit a maximum of rapid eye movement sleep at 29°C (32), which may be a more precise estimate of thermoneutrality than resting metabolic rate (53). Thus, whereas we acknowledge that the selection of 29°C as thermoneutrality for both lean and obese rats may be a limitation of the current study, it is clear that exposure to this Ta reduces food intake, HR, and VO₂ in both strains of rats compared with 23°C. The observations are consistent with the possibility that 29°C is within or near the zone of thermoneutrality both for lean and for obese Zucker rats.

The second key point concerning the bradycardia associated with thermoneutrality is that unlike fasting, the reduction in HR is not associated with an increase in HR variability. We suspect this pattern of response reflects a reduction in cardiac sympathetic activity with no increase in vagal tone. Experiments are currently underway to test this hypothesis. Nonetheless, the results of this study are consistent with our recent findings from SHR and Sprague-Dawley rats indicating that thermogenic mechanisms related to the regulation of body temperature have important effects on cardiovascular function (9). We speculate that the reduction in MAP due to thermoneutrality reflects some combination of a generalized reduction in thermogenic sympathetic outflow, reduced metabolic vasodilation in peripheral tissues, reduced cardiac output, and perhaps altered blood flow distribution.

Evidence that leptin is a key starvation signal. Results of studies performing exogenous leptin administration during fasting support the concept that reduced circulating leptin, and thus reduced central nervous...
system leptin signaling, is a critical mediator of the homeostatic responses to reduced energy availability. Leptin administration by twice daily intraperitoneal injections during fasting attenuated (but did not prevent) the reductions in thyroxine, luteinizing hormone, and testosterone levels, as well as the increase in corticosterone, that accompanied 48 h of fasting in mice (2). More recently, the same group reported that physiological peripheral leptin replacement using osmotic pumps prevented fasting-induced reductions in thyroxine and testosterone but not fasting-induced increases in corticosterone (1). Furthermore, leptin replacement prevented fasting-induced increases in neuropeptide Y (NPY) mRNA and decreases in cocaine- and amphetamine-related transcript and proopiomelanocortin (POMC) mRNA (1). The finding is consistent with others indicating that exogenous leptin administration blunts the hypothalamic gene-expression responses to starvation (46) and the development of starvation-induced anorexia (58). These studies provide convincing evidence that leptin plays a critical role in many aspects of the homeostatic adaptive response to reduced caloric availability. Indeed, we have recently completed studies that indicate that continuous central leptin infusion during fasting virtually abolishes the reduction in HR and VO₂ that accompanies 48 h fasting in Sprague-Dawley rats (37). Thus there is compelling evidence that leptin replacement prevents the hypothalamic, neuroendocrine, metabolic, and cardiovascular responses to fasting. The logical conclusion from these studies is that reduced leptin signaling represents a requisite signal for activation of homeostatic responses to starvation.

Evidence of homeostatic responses to starvation independent of leptin. We interpret the observations of fasting-induced reductions in HR and VO₂ along with increased HR variability in obese Zucker rats as strong evidence that reductions in central leptin signaling are not requisite for recruitment of homeostatic mechanisms to starvation. Indeed, it is clear that various responses to starvation occur in ob/ob mice, db/db mice, and Zucker rats. For example, long-term energy restriction reduces metabolic rate in obese Zucker rats (27, 52). Furthermore, the ob/ob mouse exhibits an appropriate reduction in body temperature in response to fasting (20). Thus there is some prior physiological evidence indicating that animals genetically lacking leptin signaling respond appropriately to reduced energy availability. In addition, hypothalamic NPY and agouti-related protein (AGRP) mRNA are further increased, and POMC mRNA is further decreased by fasting in the db/db mouse (34, 35). Thus hypothalamic neuropeptide systems regulated by leptin can be activated by other mechanisms during fasting in mice devoid of leptin signaling. In obese Zucker rats, there are conflicting reports showing that fasting either increases (43) or does not increase hypothalamic NPY mRNA (25). In addition, 24 h of fasting the ob/ob mouse increases both melanin-concentrating hormone mRNA and NPY mRNA (39). It should be noted that others have failed to demonstrate fasting-induced increases in AGRP mRNA in ob/ob or db/db mice (15, 56). Together, these findings provide a body of evidence that physiological and hypothalamic responses to starvation are engaged in the absence of a reduction in tonic leptin signaling. We suggest that there are redundant mechanisms capable of engaging homeostatic hypothalamic pathways in the absence of leptin signaling.

At this point, we can only speculate as to the mechanisms that may engage energy conservation mechanisms in the absence of leptin signaling. A number of lines of evidence supports a role for insulin in the regulation of energy balance (6). However, obese Zucker rats are resistant to the anorexics effects of central insulin administration (24). In addition, insulin hyperpolarizes hypothalamic glucose-responsive neurons in lean but not obese Zucker rats (51). Finally, insulin replacement during fasting does not prevent an increase in hypothalamic NPY mRNA in obese Zucker rats (45). Thus it seems unlikely that reductions in circulating insulin and hypothalamic insulin binding are likely to explain the potent physiological response to fasting in obese Zucker rats.

An alternative explanation is the possibility that peripheral and/or central mechanisms that respond to metabolic fuel availability represent a key component to the homeostatic response to reduced energy intake. Metabolic fuels are important regulators of food intake (18, 26). Dramatic reductions in food intake without the appropriate compensatory increase in hypothalamic NPY mRNA were recently produced by central and peripheral administration of fatty acid-synthesis inhibitors (30). Furthermore, there is evidence that the reproductive axis response to decreased energy availability is regulated by metabolic fuel signals (54) and that the actions of leptin on this axis are through effects on fuel oxidation (44). Thus future work in our laboratory will examine the concept that metabolic fuel availability may represent a key component of the afferent signaling mechanisms regulating the cardiovascular responses to reduced energy availability.

In summary, we combined cardiovascular telemetry with indirect calorimetry to test the hypothesis that obese Zucker rats, which are virtually unresponsive to leptin, would exhibit attenuated responses to starvation and thermoneutrality compared with their lean controls. Caloric deprivation decreased HR, VO₂, and RQ while increasing HR variability both in lean and in obese Zucker rats. Acute exposure to thermoneutrality reduced HR, MAP, and VO₂ with no effect on RQ or HR variability. The magnitude and timing of these responses were, for the most part, indistinguishable between obese and lean Zucker rats. We conclude that intact leptin signaling is not requisite for the cardiovascular and metabolic responses to caloric deprivation or thermoneutrality.

Perspectives

The role of leptin signaling in the regulation of thermogenesis has yet to be clarified. Reduced food availability rapidly engages multiple energy-conservation
mechanisms in many species. Whereas it is well known that circulating leptin levels are generally proportional to adipose tissue mass, it is also clear that reduced caloric intake is associated with a rapid suppression of circulating leptin levels before reductions in fat mass. The observations that animals devoid of leptin signaling display several aspects of a “starvation” phenotype, in concert with a number of studies indicating that exogenous leptin administration attenuates or prevents many adaptive responses to fasting, provide a compelling case for the concept that a primary evolutionary role for leptin is energy conservation. Yet, it is clear that this concept requires additional examination. Our findings add to a body of evidence indicating that animals devoid of leptin signaling display vigorous physiological responses to reduced caloric availability. It is our view that animals devoid of leptin signaling will provide an excellent model for the identification of the mechanisms allowing appropriate physiological responses to negative energy balance in the absence of leptin. Careful studies will then be required to dissect the relative importance of leptin-dependent and -independent pathways in the control of the homeostatic responses to reduced caloric availability.

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LEPTIN-INDEPENDENT RESPONSES TO FASTING

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