Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Diversity of Thermogenic Capacity Predicts Divergent Obesity Susceptibility in a Wild Rodent

Xue-Ying Zhang [1], Wei Shen [1,2]*, Ding-Zhen Liu [2], and De-Hua Wang [1]*

Objective: The objective of the present study was to examine whether wild rodents exhibit diverse obesity susceptibility and what factors predispose subjects to this divergence in response to a high-fat diet (HFD).

Methods: Sixty male and female Brandt's voles (*Lasiopodomys brandtii*) were fed an HFD for 8 weeks, and the upper (obesity prone [OP]) and lower (obesity resistant [OR]) one-third for mass gain were selected. Energy budgets and pathologic changes were measured. Another 30 males were fed a low-fat control diet (LFD) for 10 weeks and then fed an HFD for 12 weeks. The energetic parameters of the rodents on an LFD were analyzed for the correlation with body mass of the rodents on an HFD.

Results: OP voles had higher energy intakes, higher levels of noradrenaline-induced nonshivering thermogenesis, and a greater impairment of insulin tolerance than OR voles. Unlike laboratory rodents, there were no differences in physical activity or resting metabolic rate between these groups of voles. The thermogenic capacity during LFD feeding was the strongest predictor for mass gain during HFD feeding.

Conclusions: This study suggests that a wild rodent species of Brandt's voles exhibits diverse obesity susceptibility in reaction to an HFD, providing a natural model to give insight into the mechanisms for divergent obesity susceptibility. This study also indicates that maximum thermogenic capacity has a predictive power for the development of obesity when an HFD was available.

Obesity (2018) **26**, 111-118. doi:10.1002/oby.22055

Introduction

Obesity has become a driver of an increasing number of health issues throughout most of the world because there is a direct link between obesity and greater susceptibility to many diseases and disorders, such as type 2 diabetes, hypertension, cancers, sleep apnea, and depression (1). However, in all societies and subpopulations, there are subjects whose obesity statuses vary despite living in similar environments. It has been demonstrated that these differences are the consequence of interactions between genetic and environmental factors (1,2). The environmental factors, especially unhealthy diets and sedentary lifestyles, result in the prevalence of obesity in modern society.

Obesity develops as a result of an imbalance between energy intake and expenditure that favors fat storage. Compared to obesity-resistant mice, mice with obesity induced by a high-fat diet (HFD) have higher energy intakes, lower physical activity, and/or lower thermogenic capacity (3). Increased energy intake is a main contributor to obesity both in humans and in animal models (1,3). Studies have shown that increased physical activity could drive the resistance to HFD-induced obesity (4,5). Moreover, the higher energy intake and lower physical activity during baseline control diet feeding would predispose individuals to developing obesity when fed with an HFD (6). In addition, the activation of uncoupling protein 1 (UCP1), a mitochondrial carrier protein in brown adipose tissue (BAT) (7), would increase nonshivering thermogenesis (NST) and prevent obesity induced by an HFD (8,9). On the contrary, UCP1-ablated mice or β-less mice developed obesity, due entirely to the failure of BAT thermogenesis (10,11). However, another study showed that BAT thermogenesis was essential for cold-induced thermogenesis but not for obesity resistance (12). In addition, neither diet-induced thermogenesis nor physical activity has been found to reduce in subjects with obesity (13-15). Thus, the status of BAT

[1] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China. Correspondence: De-Hua Wang (wangdh@ioz.ac.cn) and Ding-Zhen Liu (dzliu@bnu.edu.cn) [2] Key Laboratory of Biodiversity Science and Ecological Engineering of the Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing, China.

Funding agencies: This research was supported by grants from the National Natural Science Foundation of China (31270010, 31470474, and 31472006).
Disclosure: The authors declared no conflict of interest.
Author contributions: XYZ and WS conducted the experiments; XYZ, WS, and DZL analyzed the data; and XYZ and DHW conceived the experiments. All authors were involved in writing the paper.
*Xue-Ying Zhang and Wei Shen contributed equally to this work.
Additional Supporting Information may be found in the online version of this article.
Received: 31 May 2017; **Accepted:** 28 September 2017; **Published online 13 November 2017. doi:10.1002/oby.22055**

thermogenic capacity and physical activity in the contribution to obesity is still controversial.

Classical laboratory inbred strains of rodent models have generally been used and can provide important insight into the pathophysiology of obesity and diabetes under controlled conditions. However, a large percentage of human obesity and type 2 diabetes cases follow a polygenic mode of inheritance (16). Therefore, wild-derived rodents with a far greater genetic diversity are likely to provide a source of phenotypes that may not be found in conventional laboratory strains (17). Brandt's vole (*Lasiopodomys brandtii*), a typical steppe nonhibernating herbivore, is mainly distributed in the Inner Mongolian grasslands of Northern China, Mongolia, and the region of Baikal in Russia. Because of remarkable seasonal climate changes in these habitats, voles display seasonal variations in body mass and fat mass, which increase in spring and decrease in winter (18-20). An HFD caused significant increases in energy intake, digestibility, and body fat mass for the voles exposed to either a long or a short day (19). Moreover, maternal leptin treatment during peak lactation in voles did not protect against HFD-induced obesity or glucose intolerance in their offspring (21). Therefore, Brandt's vole is a good model for studying the physiological processes and mechanisms of HFD-induced obesity. However, it is still unclear whether the voles exhibit different obesity phenotypes and obesity-associated pathologies, or whether these divergences could be predisposed by the baseline characteristics associated with energy balance. We hypothesized that the baseline energy intake and thermogenic capacity on a normal diet predisposed the voles to divergent levels of HFD-induced obesity susceptibility. Baseline body mass (BM), food intake (FI), digestible energy intake (DEI), digestibility, resting metabolic rate (RMR), NST induced by noradrenaline (NE), gross activity (GA), and body temperature (Tb) were selected as potential predictors because these parameters have been found to be associated with BM regulation (6,11,13). To explore whether obesity sensitivity was caused by the reward effect of food, we also measured food preference (FP) before the voles were transferred to an HFD.

Methods

Subjects

All experimental procedures for animal handling and sampling complied with the *Guide for the Care and Use of Laboratory Animals* and were licensed by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Captive-outbred Brandt's voles were the F11 generation of 20 pairs trapped in the grasslands of Inner Mongolia in 1999 and rejuvenated in 2006 and 2011. Male and female Brandt's voles (3-4 mo of age) were sexually naïve and were maintained under a 16:8 light/dark cycle (lights on at 0400 h) and at 23 ± 1°C after birth. Standard rabbit pellet chow (Beijing HFK Bioscience Co., Beijing, China) and water were provided *ad libitum*. The voles were single caged (30 × 15 × 20 cm) with sawdust as bedding 2 weeks prior to the experiments.

Experimental design

Experiment 1 was designed to test the individual differences in the metabolic phenotypes in response to an HFD. Thirty male and thirty female voles (90-120 d old) were fed an HFD (22.9 kJ/g, which consisted of 27% fat [soybean oil], 18% protein, 12% crude fiber, and 23% carbohydrate; Beijing HFK Bioscience Co.) for 8 weeks. At the end of the experiment, the lower and upper one-third for BM gain were selected and defined as female obesity resistant (OR) ($n = 10$), male OR ($n = 10$), female obesity prone (OP) ($n = 10$), and male OP ($n = 10$). The middle one-third of voles for BM gain were excluded (22,23). BM and FI were measured once per week. RMR, NE-induced NST, Tb, GA, glucose tolerance, and insulin sensitivity were measured at weeks 7 and 8. The voles were sacrificed by carbon dioxide overdose between 0900 and 1100 hours in the morning after 8 weeks of HFD feeding. Serum was collected for later measurement of leptin, corticosterone (CORT), and triglyceride (TG) concentrations. Fat mass, including the masses of the mesenteric fat (MF), gonadal fat (GF), and retroperitoneal fat (RF) pads, and Oil Red O staining in liver sections to quantify lipid droplets were also measured.

Experiment 2 further explored the factors predisposing individual divergence in BM gain during HFD feeding. Adult male voles (90-120 d old; $n = 30$) were fed with a standard rabbit pellet chow (low-fat control diet [LFD]; 17.5 kJ/g, which consisted of 2.7% fat, 18% protein, 12% crude fiber, and 47% carbohydrate) for 10 weeks and were then fed with an HFD for 12 weeks. The baseline levels of BM, FI, DEI, digestibility, RMR, NE-induced NST, GA, and Tb were measured in the last 2 weeks of LFD feeding. FP was measured twice in the week before the transfer to an HFD. BM was also measured at weeks 4, 8, and 12, and FI was measured at weeks 6 and 12 of the HFD.

Experimental procedures

RMR and NST were measured in oxygen volume in milliliters per hour by using an open-flow respiratory system (LabMaster calorimetry system, TSE Systems, Bad Homburg, Germany) between 0800 and 2000 hours. RMR was measured at 30 ± 0.5°C within the thermoneutral zone and lasted for 3 hours for each animal. NST was induced by a subcutaneous injection of NE (NE [mg/kg] = 6.6 $BM^{-0.458}$) around the interscapular BAT at 25 ± 1°C (19).

Core Tb and GA were recorded telemetrically from the transmitter (Model G2 E-Mitter, Mini-Mitter Company, Inc., Bend, Oregon) implanted in the abdomen (to ± 0.1°C in the temperature range of 33°C-41°C). Individual cages were placed on the receiver board (Model ER-4000, Mini-Mitter Company, Inc.). Extended experimental procedures are included in the Supporting Information.

Statistical methods

Data were examined for normality of variance by using the Kolmogorov-Smirnov test in SPSS version 13.0 software (SPSS Inc., Chicago, Illinois). FI, RMR, and NST were analyzed by using repeated-measures analysis of covariance (ANCOVA) or by using two-way or one-way ANCOVA with BM as a covariate (24). BM and blood glucose concentrations were analyzed by using repeated-measures analysis of variance (ANOVA). Core Tb, GA, TG, fat pad mass, serum CORT, and leptin concentrations were analyzed by using two-way ANOVA, and lipid droplets in liver sections were analyzed by using independent-samples *t* tests in males. Pearson correlation was used to analyze the possible associations among serum leptin, BM, and FI, and multiple regression analysis was performed to detect the contribution of energy parameters on an LFD to individual variability in BM induced by an HFD. Results were presented as means ± SEM. $P < 0.05$ was considered to be statistically significant.

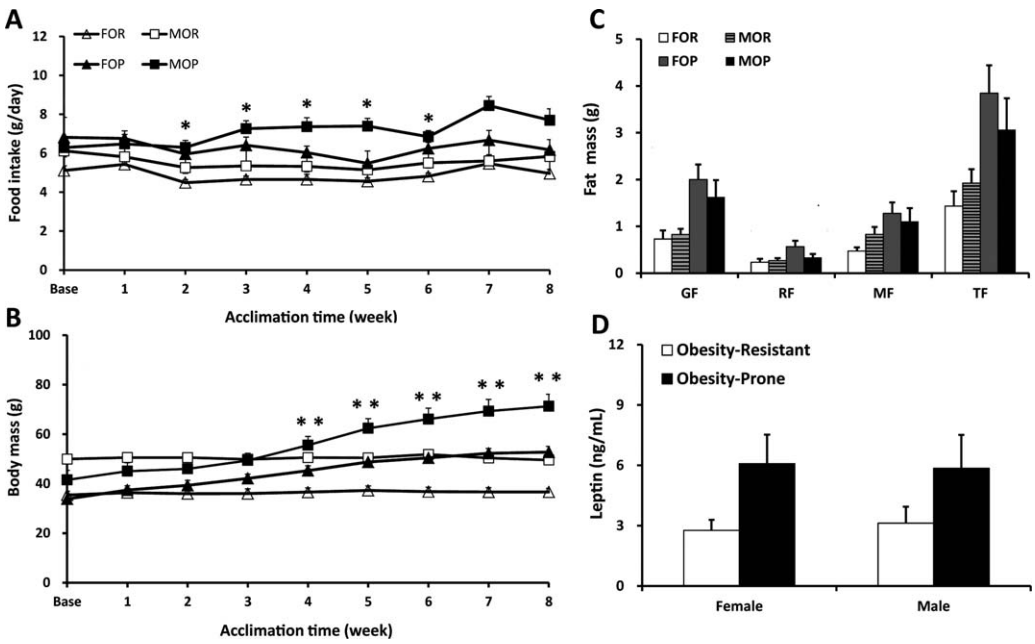

Figure 1 (**A**) Food intake, (**B**) body mass, (**C**) body fat, and (**D**) serum leptin in OP and OR voles. The OP voles had higher food intakes, body mass, body fat mass, and serum leptin concentrations than OR voles. Values are means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$, OP versus OR (two-way ANCOVA). F, female; M, male; TF, total fat.

Results

FI, BM, and serum leptin concentration

The FI of OR voles slightly decreased by 15.2% ($P > 0.05$) and then stayed constant after the introduction of an HFD, whereas the FI of OP voles remained stable during the course of the experiment ($P > 0.05$). FI was not affected by sex or by the interaction between obesity and sex but was affected by phenotype; that is, the FI of OP voles was significantly higher than that of OR voles from weeks 2 to 6 of HFD feeding ($P < 0.05$) (Figure 1A). We also measured the caloric value of food and feces at week 6 and week 12 of HFD feeding and calculated the gross energy intake, DEI, and digestibility. HFD feeding led to the decrease in gross energy intake ($P < 0.05$) (Supporting Information Figure S1A). OP voles obtained 30% more DEI than OR voles at week 6 of HFD feeding, but this difference did not reach statistical significance ($P > 0.05$) (Supporting Information Figure S1B). The digestibility at week 6 of HFD feeding was higher than that on LFD ($P < 0.05$), but there was no significant difference between OP and OR voles ($P > 0.05$) (Supporting Information Figure S1C).

The BM of OP voles increased ($P < 0.05$), reached maximal levels after 6 weeks of HFD feeding, and then remained stable ($P > 0.05$); whereas the BM of OR voles remained at baseline level until the voles were sacrificed ($P > 0.05$). The BM of OP voles was significantly higher than those of OR voles from 4 weeks of HFD feeding to the end of acclimation (Figure 1B).

The OP voles had higher masses in MF ($F_{1,35} = 6.14$, $P < 0.05$), GF ($F_{1,35} = 14.71$, $P < 0.001$), RF ($F_{1,35} = 6.71$, $P < 0.05$), and total fat ($F_{1,35} = 11.19$, $P < 0.01$) than OR voles (Figure 1C). Visceral fat mass (MF and GF) in OP voles was about 2.2 times that in OR voles, whereas subcutaneous fat mass (RF) in OP voles was only 1.8 times that in OR voles. The serum leptin concentration in OP voles was significantly higher than that in OR voles ($F_{1,35} = 6.69$, $P < 0.05$) (Figure 1D). At week 8 of the HFD, serum leptin concentration was positively correlated with BM ($r = 0.40$, $P < 0.05$) but not with FI ($r = 0.12$, $P = 0.51$).

GA, Tb, RMR, and NST

No significant differences were found in GA between OP and OR voles at most time points ($P > 0.05$), except at 2000 hours ($F_{1,13} = 5.027$, $P < 0.05$), or between males and females ($P > 0.05$) (Figure 2A-2B). The mean photophase GA did not show any differences between OR and OP voles ($F_{1,13} = 0.394$, $P = 0.541$) or between male and female voles ($F_{1,13} = 0.918$, $P = 0.356$), but the mean scotophase GA of OP voles was significantly higher than that of OR voles ($F_{1,13} = 5.271$, $P < 0.05$) and did not show a difference between male and female voles ($F_{1,13} = 0.307$, $P = 0.589$). There was no significant difference in average core Tb between OP and OR voles or between males and females ($P > 0.05$) (Figure 2C-2D). RMR was affected by neither phenotype ($F_{1,27} = 0.78$, $P = 0.38$) nor sex ($F_{1,27} = 0.44$, $P = 0.51$) (Figure 2E). OP voles had significantly heavier interscapular BAT ($F_{1,34} = 15.025$, $P < 0.001$) (Figure 2F) and higher NE-induced NST ($F_{1,13} = 4.674$, $P < 0.05$) (Figure 2G) than OR voles. NST was measured only in males.

CORT and TG concentrations and liver pathologic changes

The liver mass in OP voles was significantly heavier than that in OR voles ($F_{1,35} = 6.68$, $P < 0.05$) and was also significantly heavier in male voles than in female voles ($F_{1,35} = 4.15$, $P < 0.05$)

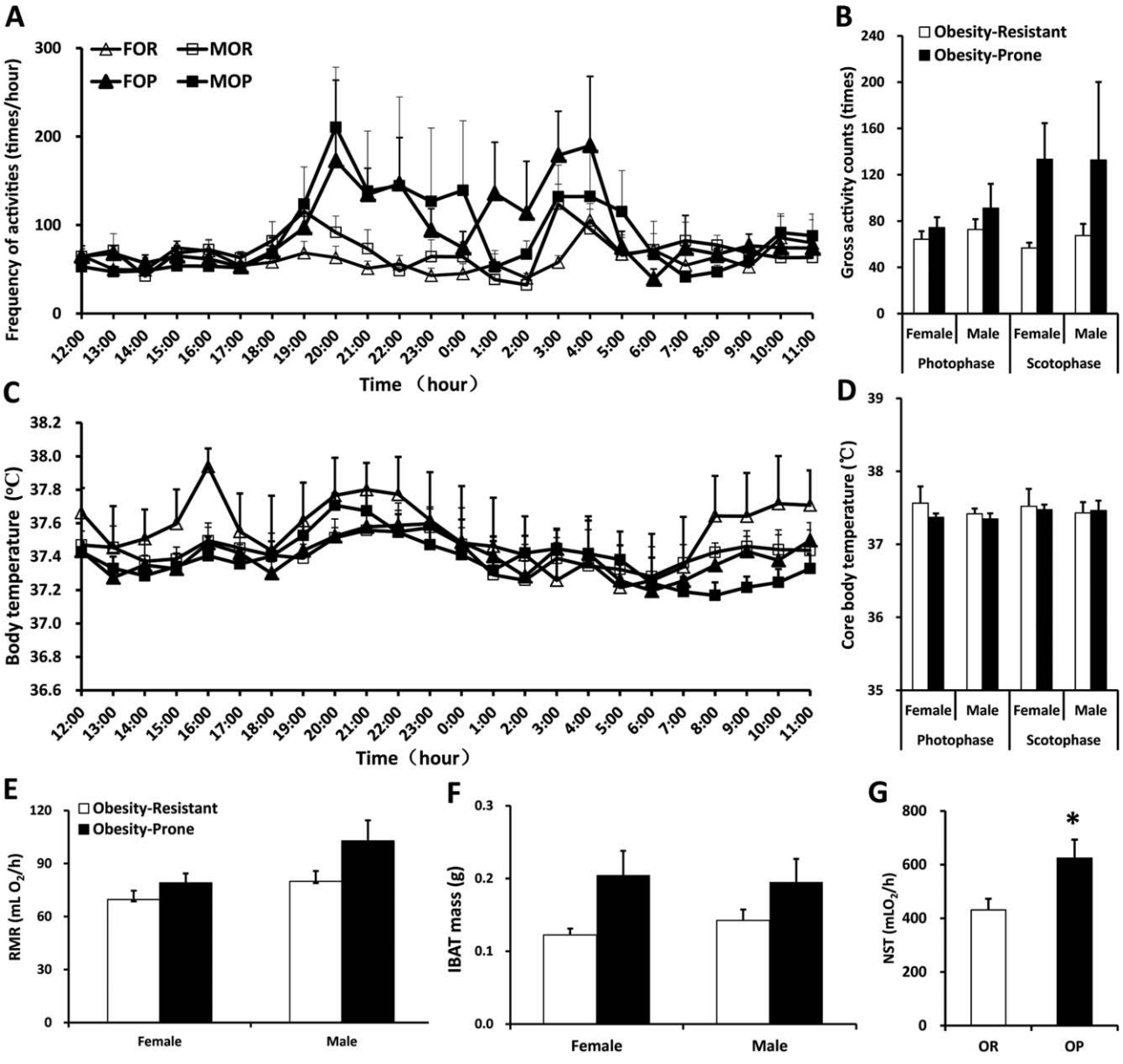

Figure 2 (**A**) Gross activity per hour and (**B**) photophase and scotophase gross activity (expressed as average value of gross activity counts per 0.1 h). (**C**) Average core body temperature and (**D**) photophase and scotophase core body temperature in OR and OP voles. (**E**) RMR, (**F**) interscapular brown adipose tissue mass (IBAT), and (**G**) NST in OR and OP voles. OP voles had higher IBAT mass and NST compared to OR voles. Values are means ± SEM. $^{*}P < 0.05$. F, female; M, male.

(Figure 3A). No significant difference in serum CORT concentrations was detected between OP and OR voles ($F_{1,35} = 1.84$, $P = 0.18$), but males had higher serum CORT than females ($F_{1,35} = 9.45$, $P < 0.01$) (Figure 3B). Although serum and liver TG concentrations were significantly higher in OP voles than in OR voles (serum: $F_{1,35} = 4.82$, $P < 0.05$; liver: $F_{1,35} = 4.61$, $P < 0.05$), they showed no significant difference between male and female voles (serum: $F_{1,35} = 0.27$, $P = 0.61$; liver: $F_{1,35} = 1.56$, $P = 0.22$) (Figure 3C-3D). Furthermore, the lipid droplet levels in the hepatocytes of OP voles were significantly higher than those in OR voles ($t = 2.21$, $df = 14$, $P < 0.05$) (Figure 3E-3G).

Glucose and insulin tolerance tests

The blood glucose concentrations of OP voles were significantly lower than those of OR voles only at 30 minutes ($F_{1,28} = 6.16$, $P < 0.05$) in glucose tolerance tests (GTTs, Figure 4A). The glucose concentrations of OP voles were significantly higher at 30 ($F_{1,28} = 6.32$, $P < 0.05$), 60 ($F_{1,28} = 12.11$, $P < 0.01$), and 90 minutes ($F_{1,28} = 10.62$, $P < 0.01$) in insulin tolerance tests (ITTs) (Figure 4B). The glucose area under the curve showed no significant difference in GTT results ($F_{1,28} = 0.35$, $P = 0.56$) (Figure 4C), but the value was significantly higher in OP voles than in OR voles in ITT results ($F_{1,28} = 10.93$, $P < 0.01$) (Figure 4D). There was no

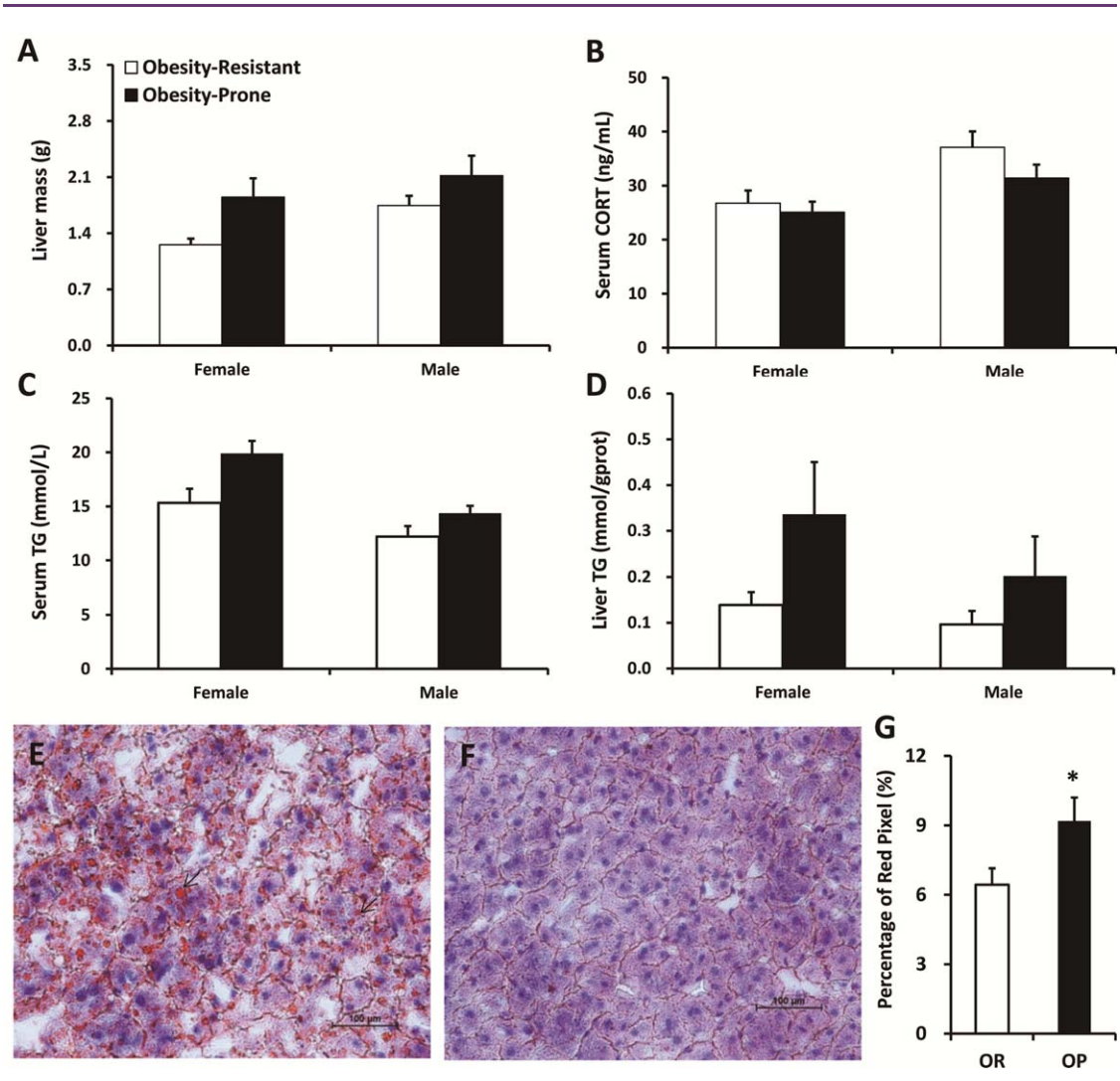

Figure 3 (**A**) Liver mass, (**B**) serum CORT, and (**C**) serum and (**D**) liver TG concentrations in OP and OR voles. (**E**-**F**) Lipid droplet (arrows) by Oil Red O (ORO) staining in liver sections (scale bars, 100 μm; magnification is × 400). (**G**) Quantitative assessment of ORO staining from OP and OR voles, expressed as the percentage of red pixels. OP voles had higher liver mass and serum and liver TG concentrations and more lipid droplets than OR voles. Values are means ± SEM. *$P < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

significant difference between males and females in GTT or ITT results ($P > 0.05$).

Multiple regression analyses on the prediction of individual variability in BM

The mass gains during both the LFD and HFD showed large variations and accorded with normal distribution ($P > 0.05$). The mass gain during the LFD was not correlated with that during the HFD ($R^2 = 0.02$, $P = 0.47$).

Three prediction models were developed for the mass gain at 4, 8, and 12 weeks of HFD feeding by using the energetic parameters on the LFD as predictors (Table 1). According to the overall model at 12 weeks, 58.2% of the individual variability could be predicted, with NE-induced NST on the LFD and Tb as the significant predictors ($P < 0.05$) (Table 1). Furthermore, in the stepwise multiple regression of BM during the HFD against the energetic parameters on the LFD, only one variable of NST entered the model (Week 4: $r_{t\sim t+1} = 0.07\mathrm{NST}_t + 37.88$, $R^2 = 0.40$, $F_{1,29} = 18.26$, $P < 0.001$; Week 8: $r_{t\sim t+1} = 0.09\mathrm{NST}_t + 37.24$, $R^2 = 0.37$, $F_{1,29} = 16.31$, $P < 0.001$; Week 12: $r_{t\sim t+1} = 0.07\mathrm{NST}_t + 43.02$, $R^2 = 0.34$, $F_{1,29} = 14.57$, $P < 0.001$). Regression analysis also showed that NE-induced NST on the LFD was positively correlated with FI on the LFD ($R^2 = 0.25$, $P < 0.01$) and at week 12 of the HFD ($R^2 = 0.27$, $P < 0.01$).

Discussion

Wild-derived rodents are increasingly popular mammalian models for seasonal BM (17). For example, the field vole (*Microtus agrestis*) was a useful BM and adiposity regulation model for understanding the process of leptin resistance (25), and Brandt's vole was a

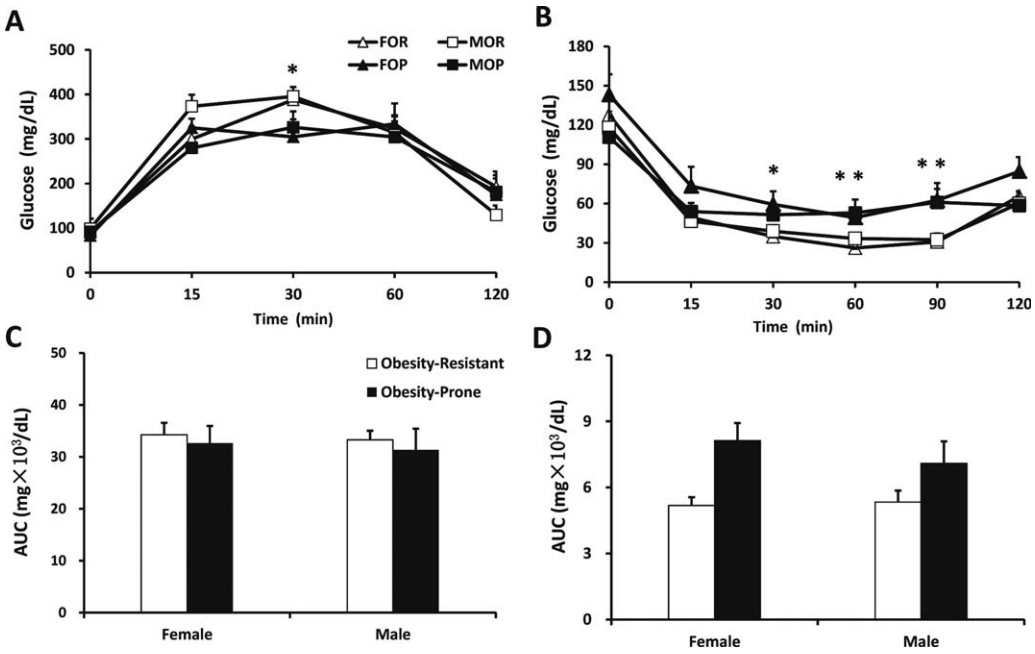

Figure 4 (**A**-**B**) Blood glucose concentrations and (**C**-**D**) area under the curve (AUC) during glucose tolerance and insulin sensitivity tests in OR and OP voles. Values are means ± SEM ($n = 10$). *$P < 0.05$. **$P < 0.01$, OP versus OR (two-way ANOVA). F, female; M, male.

useful model for understanding the "healthy obesity" induced by a long-day photophase (26). The present study demonstrated that Brandt's voles displayed significant interindividual differences in mass gain when fed with an HFD. More energy intake (rather than less GA, lower RMR, and lower rates of NE-induced NST) contributed to more fat accumulation and lower insulin sensitivity in OP voles. The data further indicated that the thermogenic capacity before the exposure to the HFD was a significant predictor for BM during HFD feeding.

TABLE 1 Multiple regression analysis predicting the contribution of energy parameters on the LFD to individual variability in BM induced by feeding an HFD in male Brandt's voles

		BM,4 wk	BM, 8 wk	BM, 12 wk
Models	R^2	0.597	0.680	0.582
	P model	0.013	0.002	0.017
Predictors	BM_0	n.s.	0.006	0.018
	FI	n.s.	n.s.	n.s.
	DEI	n.s.	n.s.	n.s.
	Digestibility	n.s.	n.s.	n.s.
	RMR	0.028	0.012	n.s.
	NST	0.061	0.050	0.047
	GA	n.s.	n.s.	n.s.
	Tb	0.062	0.007	0.030
	FP	n.s.	0.018	n.s.

n.s., not significant.

Obesity results from a chronic imbalance between energy intake and energy expenditure, but it is unclear which factors have contributed more to the obesity epidemic. Our data showed that the FI of OP voles was significantly higher than that of OR voles prior to and during HFD feeding, which was a finding supported by previous studies in Pima Indians (15) and in animal models (27). The FI of OR voles slightly decreased, but that of OP voles remained stable after the introduction of the HFD. The OP voles obtained 30% more digestible energy than OR voles at week 6 of HFD feeding. These results indicated that OR voles likely compensated for the difference in dietary energy density and that excess energy intake was a main factor contributing to more fat accumulation in the voles with obesity. Leptin has been implicated as one of the peripheral signals in regulation of body fat reserves and energy intake in mammals (28). Our data showed that body fat mass and serum leptin concentrations were significantly higher in the OP voles than in the OR voles, but leptin concentration did not show a significant correlation with FI. This suggests that leptin may not function correctly in regulating energy intake in subjects with obesity (29).

GA, RMR, and thermogenic capacity are the most variable components of energy expenditure. Several studies have suggested that increases in RMR and GA would prevent obesity in humans (30-32) and rats (13). In addition, the increase in thermogenic capacity, indicated by the activation of UCP1 in BAT (7), has been shown to prevent obesity induced by an HFD (8,9). A previous study, in which it was shown that a short day resulted in increased BAT thermogenesis and less mass gain than a long day when the voles were fed with an HFD, supported this idea (19). In addition, the transgene mice with UCP1 deficiency or the three known βAR deficiencies developed obesity (10,11). Therefore, BAT has become a potential target for preventing and treating obesity, especially because beige adipocytes

were found in adult humans (33). The current study showed no differences in GA or RMR at times when marked mass differences had already developed between OP and OR voles. This finding was similar to findings in humans with obesity, who have not been shown to display reduced GA or diet-induced thermogenesis (13-15). Moreover, we found that the OP voles had higher thermogenic capacity than OR voles. Although the increased thermogenesis in a short day could surely alleviate obesity (19), our findings indicated that the development of obesity in the natural animal and human models was not associated with decreased physical activity, RMR, or thermogenic capacity.

Obesity is accompanied by many metabolic diseases, such as type 2 diabetes and fatty liver disease (1,2). Our results showed that excess fat was accumulated, especially as visceral fat, that lipid droplets and TG were accumulated in the liver, and that serum TG concentrations were also significantly increased in OP voles. There was no significant difference in glucose tolerance indicated by area under the curve data between OP and OR voles, and an early rise in glucose concentration after 30 minutes implied a rebound of liver gluconeogenesis in OP voles. However, ITT data showed that OP voles with HFD-induced obesity had impaired insulin sensitivity compared with OR voles. These data suggest that OP voles had developed some pathophysiological features, such as fat deposition in the liver and impaired insulin sensitivity. In contrast, long-day–induced obesity was not associated with the impairment of glucose homeostasis in Brandt's voles (26). More exploration is needed to distinguish the mechanism for diet- or photophase-induced pathways for the development of obesity. CORT, one of the important modulators of energy balance, was necessary for the stimulation of hypertriglyceridemia and insulin resistance in response to the HFD (34). However, serum CORT levels did not differ between OP and OR voles in the present study, which contradicts the findings of studies in HFD rats and mice (34,35). The differences between studies in the fat content of each diet and the duration of HFD exposure may have resulted in different responses in some physiological, behavioral, and hormonal parameters. Further testing in Brandt's voles is needed to determine whether CORT levels change with prolonged exposure to an HFD and whether such changes are necessary for the development of obesity and insulin resistance.

As stated earlier, Brandt's voles, like mouse and human models, exhibited divergent metabolic phenotypes under both LFD and HFD feeding. The mass gain under LFD feeding was not positively correlated with that under HFD feeding, suggesting that the high mass gain during development at normal LFD feeding does not predict future additional mass gain during HFD feeding. Further study showed that the baseline FI, RMR, GA, Tb, and FP on the LFD were not significantly correlated with divergent phenotypes in BM induced by the HFD. Similar results were also found in a study of C57BL/6J mice (6), which showed that baseline RMR, FI, and the reward effect of an HFD were not the significant factors determining the mass gain during HFD feeding. However, the baseline GA, especially during the dark phase, has been shown to have a negative impact on mass gain during HFD feeding in mice (6). Among all the parameters measured in our study, the thermogenic capacity of Brandt's voles on the LFD was the strongest predictor for mass gain during the HFD. Although the increase in BAT thermogenesis by physiological and/or pharmacological activation in mice, voles, and humans could partly counteract HFD-induced obesity (8,9,19,36), we observed in the present study that OP voles had a higher thermogenic capacity but developed obesity in response to the HFD, compared with OR voles. In humans with obesity, studies have also shown that diet-induced thermogenesis did not decrease (13,14). Moreover, we observed that the higher thermogenic capacity of voles on an LFD was associated with higher energy intake both on the LFD and after long-term HFD feeding. The increased energy expenditure in thermogenesis, together with the reward effect of food (37) and impaired leptin sensitivity (29), may induce the animals to overfeed, resulting in a high risk of developing obesity. Therefore, the role of BAT thermogenesis in maintaining energy balance is still controversial. Our data indicated that high maximum thermogenic capacity may be a strong predictor for the development of obesity when an HFD is available.

The response of wild-derived Brandt's voles to an HFD exhibited divergent obesity susceptibility and associated pathophysiological features. Excess energy intake, rather than reduced RMR, GA, or BAT thermogenesis, was a main factor contributing to more fat accumulation in the voles with obesity. Additionally, OP voles with HFD-induced obesity showed impaired insulin sensitivity compared with OR voles, whereas long-day–induced obesity did not impair glucose homeostasis in Brandt's voles (26). Therefore, Brandt's vole is a natural model for understanding the variability in individual risk for the development of obesity. This study further demonstrated that the voles with high maximum thermogenic capacity during LFD feeding had a risk of developing HFD-induced obesity. This implies that the concept of BAT naturally maintaining a low BM is surely a matter of discussion and that the idea of diet-induced thermogenesis can be paradoxical per se. O

Acknowledgments

The authors would like to thank the three anonymous reviewers for their constructive comments and suggestions and John R. Speakman at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, for the critical suggestions on this research. Thanks to Wei Liu for the technical assistance in multiple regression analysis and to the other members of the Animal Physiology Ecology Group for the helpful discussion.

References

1. Speakman JR. Obesity: the integrated roles of environment and genetics. *J Nutr* 2004;134:2090S-2105S.
2. Barsh GS, Farooqi IS, O'Rahilly S. Genetics of body-weight regulation. *Nature* 2000;404:644-651.
3. Levin BE. Factors promoting and ameliorating the development of obesity. *Physiol Behav* 2005;86:633-639.
4. Teske JA, Billington CJ, Kotz CM. Mechanisms underlying obesity resistance associated with high spontaneous physical activity. *Neuroscience* 2014;256:91-100.
5. Perez-Leighton CE, Boland K, Billington CJ, Kotz CM. High and low activity rats: elevated intrinsic physical activity drives resistance to diet-induced obesity in nonbred rats. *Obesity (Silver Spring)* 2013;21:353-360.
6. Zhang LN, Morgan DG, Clapham JC, Speakman JR. Factors predicting nongenetic variability in body weight gain induced by a high-fat diet in inbred C57BL/6J mice. *Obesity (Silver Spring)* 2012;20:1179-1188.
7. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277-359.
8. Ma S, Yu H, Zhao Z, et al. Activation of the cold-sensing TRPM8 channel triggers UCP1-dependent thermogenesis and prevents obesity. *J Mol Cell Biol* 2012;4:88-96.
9. Liew CW, Boucher J, Cheong JK, et al. Ablation of TRIP-Br2, a regulator of fat lipolysis, thermogenesis and oxidative metabolism, prevents diet-induced obesity and insulin resistance. *Nat Med* 2013;19:217-226.

10. Bachman ES, Dhillon H, Zhang CY, et al. Beta AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 2002;297:843-845.
11. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab* 2009;9:203-209.
12. Stefl B, Janovská A, Hodný Z, et al. Brown fat is essential for cold-induced thermogenesis but not for obesity resistance in aP2-Ucp mice. *Am J Physiol* 1998; 274:E527-E533.
13. Weinsier RL, Nelson KM, Hensrud DD, Darnell BE, Hunter GR, Schutz Y. Metabolic predictors of obesity - contribution of resting energy-expenditure, thermal effect of food, and fuel utilization to 4-year weight-gain of post-obese and never-obese women. *J Clin Invest* 1995;95:980-985.
14. Luke A, Dugas LR, Ebersole K, et al. Energy expenditure does not predict weight change in either Nigerian or African American women. *Am. J Clin Nutr* 2009;89: 169-176.
15. Tataranni PA, Harper IT, Snitker S, et al. Body weight gain in free-living Pima Indians: effect of energy intake vs expenditure. *Int J Obesity (Lond)* 2003;27:1578-1583.
16. Bouchard C, Perusse L. Gentics of obesity. *Annu Rev Nutr* 1993;13:337-354.
17. Guenet JL, Bonhomme F. Wild mice: an ever-increasing contribution to a popular mammalian model. *Trends Genet* 2003;19:24-31.
18. Li XS, Wang DH. Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (*Microtus brandtii*). *Horm Behav* 2005;48:321-328.
19. Zhao ZJ, Chen JF, Wang DH. Diet-induced obesity in the short-day-lean Brandt's vole. *Physiol Behav* 2010;99:47-53.
20. Zhang XY, Wang DH. Energy metabolism, thermogenesis and body mass regulation in Brandt's voles (*Lasiopodomys brandtii*) during cold acclimation and rewarming. *Horm Behav* 2006;50:61-69.
21. Liu XY, Wang DH. Effects of leptin supplementation to lactating Brandt's voles (*Lasiopodomys brandtii*) on the developmental responses of their offspring to a high-fat diet. *J Comp Physiol B* 2011;181:829-839.
22. Levin BE, Keesey RE. Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol* 1998;274:R412-R419.
23. Swierczynska MM, Mateska I, Peitzsch M, et al. Changes in morphology and function of adrenal cortex in mice fed a high-fat diet. *Int J Obes (Lond)* 2015;39: 321-330.
24. Tschöp MH, Speakman JR, Arch JR, et al. A guide to analysis of mouse energy metabolism. *Nat Methods* 2011;9:57-63.
25. Krol E, Speakman JR. Regulation of body mass and adiposity in the field vole, *Microtus agrestis*: a model of leptin resistance. *J Endocrinol* 2007;192:271-278.
26. Liu XY, Yang DB, Xu YC, Gronning MO, Zhang F, Wang DH, Speakman JR. Photoperiod induced obesity in the Brandt's vole (*Lasiopodomys brandtii*): a model of 'healthy obesity? *Dis Model Mech* 2016;9:1357-1366.
27. Jeffery RW, Harnack LJ. Evidence implicating eating as a primary driver for the obesity epidemic. *Diabetes* 2007;56:2673-2676.
28. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763-770.
29. Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol* 2002;283:R941-R948.
30. Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 1999;283:212-214.
31. Teske JA, Levine AS, Kuskowski M, Levine JA, Kotz CM. Elevated hypothalamic orexin signaling, sensitivity to orexin A, and spontaneous physical activity in obesity-resistant rats. *Am J Physiol* 2006;291:R889-R899.
32. Ortmeyer HK, Goldberg AP, Ryan AS. Exercise with weight loss improves adipose tissue and skeletal muscle markers of fatty acid metabolism in postmenopausal women. *Obesity (Silver Spring)* 2017;25:1246-1253.
33. Sidossis L, Kajimura S. Brown and beige fat in humans: thermogenic adipocytes that control energy and glucose homeostasis. *J Clin Invest* 2015;125:478-486.
34. Mantha L, Palacios E, Deshaies Y. Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity. *Am J Physiol* 1999;277:R455-R464.
35. Chi QS, Wang DH. Thermal physiology and energetics in male desert hamsters (*Phodopus roborovskii*) during cold acclimation. *J Comp Physiol* 2010;181:91-103.
36. Tseng YH, Cypess AM, Kahn CR. Cellular bioenergetics as a target for obesity therapy. *Nat Rev Drug Discov* 2010;9:465-481.
37. Sinha R. Role of addiction and stress neurobiology on food intake and obesity. *Biol Psychol* 2017 [published online May 4, 2017]. doi:10.1016/j.biopsycho.2017.05.001