Hypercagia, lower body temperature, and reduced running wheel activity preceed development of morbid obesity in New Zealand obese mice

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Jürgens, Hella S., Annette Schürmann, Reinhart Kluge, Sylvia Ortmann, Susanne Klaus, Hans-Georg Joost, and Matthias H. Tschöp. Hyperphagia, lower body temperature, and reduced running wheel activity preceed development of morbid obesity in New Zealand obese mice. Physiol Genomics 25: 234–241, 2006; doi:10.1152/physiolgenomics.00252.2005.—Among polygenic mouse models of obesity, the New Zealand obese (NZO) mouse exhibits the most severe phenotype, with fat deposits exceeding 40% of total body weight at the age of 6 mo. Here we dissected the components of energy balance including feeding behavior, locomotor activity, energy expenditure, and thermogenesis compared with the related lean New Zealand black (NZB) and obese B6.V-Lepob/J (ob/ob) strains (11% and 65% fat at 23 wk, respectively). NZO mice exhibited a significant hyperphagia that, when food intake was expressed per metabolic body mass, was less pronounced than that of the ob/ob strain. Compared with NZB, NZO mice exhibited increased meal frequency, meal duration, and meal size. Body temperature as determined by telemetry with implanted sensors was reduced in NZO mice, but again to a lesser extent than in the ob/ob strain. In striking contrast to ob/ob mice, NZO mice were able to maintain a constant body temperature during a 20-h cold exposure, thus exhibiting a functioning cold-induced thermogenesis. No significant differences in spontaneous home cage activity were observed among NZO, NZB, and ob/ob strains. When mice had access to voluntary running wheels, however, running activity was significantly lower in NZO than NZB mice and even lower in ob/ob mice. These data indicate that obesity in NZO mice, just as in humans, is due to a combination of hyperphagia, reduced energy expenditure, and insufficient physical activity. Because NZO mice differ strikingly from the ob/ob strain in their resistance to cold stress, we suggest that the molecular defects causing hyperphagia in NZO mice are located distal from leptin and its receptor.

feeding behavior; polygenic obesity; ob/ob mice; thermogenesis; locomotor activity

THE NEW ZEALAND OBESE (NZO) mouse strain represents a well-established model of morbid obesity (2). Among all polygenic mouse models of obesity, it exhibits the highest degree of adiposity (14). The development of its obesity is markedly accelerated and enhanced by exposure to a high-fat diet (24). In addition to obesity, the NZO strain presents all other characteristics of the human metabolic syndrome, such as insulin resistance progressing to overt diabetes (2, 6), hypercholesterolemia, and hypertension (23). Thus the NZO strain appears to be a most suitable model for human obesity and its secondary complications, in particular the abnormalities of glucose metabolism.

The NZO strain has been used in the search for mouse obesity and diabetes genes, and three different genome-wide scans of outcross populations with lean strains (SJL, SM, and NON) have been performed (19, 21, 25, 28, 33). These scans revealed a very complex picture of the genetic basis of their obesity: a network of multiple quantitative trait loci (QTL), in part dependent on the nongenetic, maternal environment (28), was responsible for the development of adiposity in the outcross populations. Moreover, it became apparent that not only NZO mice but also the lean strains such as SJL contributed obesity genes to the phenotype of the progeny (Giesen K, Plum L, Kluge R, Schürmann A, and Joost H-G, unpublished data).

The complexity of the polygenic obesity of the NZO mouse resembles that in humans, and it appears reasonable to expect a plethora of valuable information from the identification of its obesity genes. However, the classic approach to isolation of the critical chromosomal segments through breeding of congenic lines may fail, because the individual gene variants contribute small effects that may not be detectable on a different, lean background. Thus the identification of obesity genes may require a different approach involving the sequencing of candidate genes in critical regions (QTL) of the mouse genome. For this approach, guidance by detailed knowledge of the pathophysiology of the obesity in NZO mice is required.

In several previous studies the pathobiochemistry of insulin resistance, glucose metabolism, and lipogenesis in NZO mice was investigated (1, 10, 29, 31, 36). Surprisingly, hardly any published information is available regarding the pathophysiology of energy balance in the NZO strain. In a longitudinal study it was shown previously that treatment of NZO mice with the β3-receptor agonist CL-316,243 increased energy expenditure and reduced adiposity (20). No data are available comparing parameters of energy balance in NZO mice with those in lean, related strains. Thus we here carefully dissected feeding behavior, locomotor activity, thermogenesis, and energy expenditure in the NZO mouse at an early age (8 wk) compared with the New Zealand black (NZB) strain, its closest relative (3). In addition, we performed a direct comparison with the B6.V-Lepob/J (ob/ob) strain as an obese reference strain with a monogenic defect in the control of feeding behavior and thermogenesis. Our data indicate a significant hyperphagia, a reduced body temperature but a functional adaptive thermogenesis on cold exposure, and a reduced voluntary running activity in NZO mice. Thus we conclude that obesity in NZO mice at least in part results from defects in the central control...
of feeding and body temperature, presumably localized distal from the leptin receptor, and from a behavioral abnormality.

**METHODS**

**Animals.** Measurements of core body temperature, home cage activity, gas exchange (indirect calorimetry), and food intake were performed in 7- to 9 wk-old male adult NZO/H1Bom mice from our own colony (R. Kluge) and NZB/OlaHsd (Harlan Winkelmann, Paderborn, Germany), C57BL/6J (B6), and B6.V-Lepob/J (Charles River, Sulzfeld, Germany) mice. The different strains were studied in matched pairs wherever possible to exclude seasonal variations; all experiments were performed at the Potsdam lab. Mice were singly housed at a temperature of 22°C with a 12:12-h light-dark cycle (lights on at 6:00 AM) in nonenriched type II Macrolon cages with soft wood bedding. Animals had ad libitum access to water and food (maintenance diet for rats and mice, art. no. 1324, Altromin, Lage, Germany). The standard chow contained 11,825 kJ/kg digestible energy with 19% (wt/wt) protein, 4% fat, and 50.5% carbohydrates. The animals were kept in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and all experiments were approved by the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State of Brandenburg, Germany).

**Analysis of body composition.** Body composition (fat and lean mass) was measured by NMR with a Bruker Minispec instrument (Echo Medical Systems, Houston, TX) as described previously (18). Conscious mice were placed in an applied static magnetic field for 0.9 min (34).

**Feeding behavior.** Food intake rates of singly housed mice were recorded over a period of 5–6 days with an automated drinking and feeding monitor system (TSE, Bad Homburg, Germany) consisting of Macrolon type III cages equipped with baskets connected to weight sensors. The baskets contained standard chow pellets and were freely accessible to the mice. Each time food was removed from the basket, the computer recorded the duration of the event, the amount of food retrieved, and the time at which the event occurred. Weight variations were monitored every 6 s. Mice were habituated to the test cages for 2 days before trials. Recorded food consumption was normalized for metabolic body mass [MBM, food intake/(body wt in kg)0.75]. A detailed meal pattern analysis was performed, adopting a minimum intermeal interval separating two meals of 5 min.

For determination of assimilation efficiency, animals were singly housed in metabolic cages (Tecniplast, Buguggiate, Italy) that allowed collection of feces and urine separately. Over a 3-day period, food consumption and feces and urine production were recorded. Feces and food samples were dried to constant weight, and energy content was measured by bomb calorimetry (IKA Werke, Staufen, Germany). Food samples were dried to constant weight, and energy content was measured by bomb calorimetry (IKA Werke, Staufen, Germany). The standard chow contained 11,825 kJ/kg digestible energy with 19% (wt/wt) protein, 4% fat, and 50.5% carbohydrates.

Assimilated energy was calculated as the difference of the total energy consumed and the energy content of feces and was presented as percentage of total energy intake. Energy loss through urine was calculated by dividing TEE (kJ/day) by the MBM of the animal. Energy loss through urine was calculated by dividing TEE (kJ/day) by the MBM of the animal and expressed as kilojoules per kilogram of MBM per day.

**Indirect calorimetry.** Total energy expenditure (TEE) was measured at 22 and 4°C for 23 h with an open circuitry calorimetry system (Hartmann & Braun, Frankfurt/Main, Germany; VO2 analyzer Magnos 16, VCO2 analyzer Uras 14) as described previously (18). Air-tight respiratory cages with a flow rate of ~30 l/h were placed in climate chambers (Vöttsch Industrietecn, Reiskirchen-Lindenstruth, Germany) to maintain constant temperatures. Rate of oxygen consumption (VO2) and rate of carbon dioxide production (VCO2) were recorded in 6-min intervals for each animal, and TEE was calculated with the equation (11) TEE = 16.17VO2 + 5.03VCO2 + 5.98N, where TEE is expressed in kilojoules per day, VO2 is expressed in liters per day, and VCO2 is expressed in liters per day. N is excreted nitrogen and was assumed to be 0.1 g/day. MBM-specific TEE of each animal was calculated by dividing TEE (kJ/day) by the MBM of the animal and expressed as kilojoules per kilogram of MBM per day.
animals had free access to the running wheels as well as to food and water. The system recorded each quarter-revolution of the wheel, and data were expressed as total number of revolutions per hour.

Statistical analysis. All statistical analyses were performed with the software package SPSS 11.5 (SPSS, Chicago, IL). Differences were considered significant when $P < 0.05$. Core body temperature and energy expenditure of NZO vs. NZB and ob/ob vs. B6 were compared by a mixed linear model with subject as random factor and strain and time as fixed factors. For comparison of body weight, lean mass, and fat mass a hypothesis-based one-way ANOVA multicomparison post hoc test (least significant difference) with predefined contrasts was used.

RESULTS

Development of body weight and adiposity. To characterize the development of obesity in the NZO strain, we compared total body weight, fat mass, and lean mass in B6, ob/ob, NZO, and NZB strains at ages of 8 and 23 wk. As illustrated in Fig. 1, NZO mice developed a marked adiposity until 8 wk of age that continued to increase thereafter; at 23 wk the relative fat mass was 3.6-fold higher in NZO mice than in NZB mice (40.2 ± 3.2% vs. 11.3 ± 1.9%). At both time points, adiposity in NZO mice was lower then in the ob/ob strain (fat mass: 24.5 ± 1.3% in NZO vs. 62.4 ± 0.5% in ob/ob at 8 wk, 40.2 ± 3.2% vs. 65.0 ± 0.9% at 23 wk). In contrast, lean mass in NZO mice was significantly higher than in ob/ob mice at both time points (8 wk: NZO 25.8 ± 0.5 g, ob/ob 18.3 ± 0.3 g ($P < 0.001$); 23 wk: NZO 32.0 ± 1.0 g, ob/ob 27.5 ± 0.4 g ($P < 0.001$)). In addition, it should be noted that lean body mass of NZO mice was significantly higher than in NZB mice (Fig. 1), suggesting that obesity of NZO mice is associated with an increased overall body size.

Food consumption and meal pattern. Compared with B6 and NZB mice, food consumption of NZO and ob/ob mice was markedly increased at wk 8 (Fig. 2A). Normalization of the data for the MBM of the animals attenuated the effect in NZO mice (Fig. 2B), but a significant hyperphagia was still apparent. A small portion of the feed consumed by the animals was not ingested (spillage), but no significant difference between strains was detected (% of total feed consumed: NZB 8.7 ± 1.3, NZO 5.4 ± 0.7, B6 7.4 ± 0.6, ob/ob 9.5 ± 1.5). NZO and NZB mice assimilated the consumed food, as determined by bomb calorimetry of feces, somewhat more efficiently than ob/ob and B6 mice. However, no significant difference between NZB and NZO or B6 and ob/ob mice was observed (% of consumed calories: NZB 76.8 ± 0.5, NZO 75.5 ± 0.6%, B6 73.1 ± 0.3%, ob/ob 73.7 ± 1.2%; $P < 0.05$ for differences NZO vs. B6 and NZB vs. B6).

A detailed analysis of the meal pattern (Figs. 3–5) confirmed the hyperphagia of NZO mice: NZO mice consumed larger portions (0.4- to 0.7-g meals) more frequently than NZB mice (Fig. 4) and showed an extended meal duration (420 – 600 s; Fig. 5). In contrast, hyperphagia in ob/ob mice was characterized by a markedly increased number of small-sized meals (Fig. 4). Therefore, feeding behavior in NZO and ob/ob mice differed in meal size and meal duration.

![Fig. 2. Daily food consumption in NZO, NZB, B6, and ob/ob mice. Food consumption was monitored at the age of 8 wk for 5– 6 days. Means ± SE of the average daily food consumption (A) and average daily food consumption normalized for metabolic body mass (MBM) (B) are given. Data represent means of the indicated numbers of animals. Significance of the difference to the respective control strain: **$P < 0.01$, ***$P < 0.001$.](http://physiolgenomics.physiology.org/)

![Fig. 3. Meal frequency in NZO, NZB, B6, and ob/ob mice. Daily meal frequency was calculated from the data shown in Figs. 4 and 5, and means ± SE were calculated. Significance of the difference to the respective control strain: ***$P < 0.001$.](http://physiolgenomics.physiology.org/)
Body temperature and thermogenesis. To obtain precise measurements of the core body temperature, implanted sensors were used that allowed a continuous telemetric monitoring. As illustrated in Fig. 6A, the core body temperature exhibited a diurnal variation, with ~1°C higher readings during the time of activity (dark phase). During both phases, the curves of the core body temperature exhibited a significantly lower temperature in NZO than NZB mice (Fig. 6A). The average core body temperatures of the four strains during dark and light phases are given in Fig. 6, B and C. The body temperature of ob/ob mice was 2°C lower than that of the B6 control strain. Average body temperature in NZO mice was ~1°C lower than in B6 mice and also lower than in NZB mice, in particular during the period of inactivity (0.7°C).

To determine the thermogenic ability of the NZO strain, mice were subjected to a prolonged cold exposure (4°C; Fig. 7). As described previously (8), core body temperature in ob/ob mice dropped rapidly to 33–34°C. Subsequently, some of the ob/ob mice were unable to maintain that temperature for longer periods and had to be removed from the cold chamber. In contrast, NZO mice were able to maintain their core body temperature within the range of 36–37°C for at least 20 h.

Energy expenditure. TEE was assessed by indirect calorimetry. Figure 8 illustrates the diurnal pattern of TEE. Absolute TEE was higher in NZO than NZB mice in both light and dark phases (data not shown). Because TEE is a function of body weight, data were normalized for MBM (Fig. 8) and revealed a somewhat lower TEE in NZO mice during the dark phase. Normalization per total body weight (not shown) further increased the difference between NZO and NZB mice. No statistically significant difference in the thermoneutral zone, as determined by measurement at different temperatures between

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**Fig. 4.** Distribution of meal size in NZO, NZB, B6, and ob/ob mice. Mice were monitored at the age of 8 wk for 5–6 days, and means of 12 (NZO), 12 (NZB), 8 (B6), and 8 (ob/ob) animals were calculated.

**Fig. 5.** Distribution of meal duration in NZO, NZB, B6, and ob/ob mice. Mice were monitored at the age of 8 wk for 5–6 days, and means of 12 (NZO), 12 (NZB), 8 (B6), and 8 (ob/ob) animals were calculated.
22°C and 37°C, was detected between strains (30.61–31.02°C; data not shown).

***Locomotor and running wheel activity.*** No statistically significant differences in spontaneous locomotor activity in a home cage environment as determined with implanted transponders were detected among the four strains (data not shown). In contrast, marked differences in motivated physical activity were observed when mice had access to a running wheel. Both NZO and ob/ob mice (Fig. 9, B and D) exhibited markedly lower voluntary running wheel activities compared with the respective control strains [NZB (Fig. 9A) and B6 (Fig. 9C)].

**DISCUSSION**

The present data suggest that obesity in the NZO mouse is due to significant hyperphagia and reduced thermogenesis, resulting in a positive energy balance. In addition, NZO mice exhibit a behavioral anomaly in that their voluntary running activity is markedly reduced. With this pattern, the obesity syndrome in NZO mice closely resembles that of the ob/ob mouse, with an important exception: in contrast to ob/ob mice, NZO mice were able to maintain their regular body temperature during a 20-h period of cold exposure, whereas ob/ob mice would not survive at 4°C. Thus we conclude that the primary defects in NZO mice affect the central circuits governing orexigenic drive and body temperature but do not interrupt essential mechanisms of cold-induced thermogenesis.

This study is the first comprehensive characterization of all components of energy balance in NZO mice compared with their closest relatives, NZB mice, and with a standard model of obesity, the ob/ob strain. With the resulting data, we intended to provide guidance for the search of the obesity genes in NZO mice. We (19) and others (28, 33) have used a genetic approach to localize these genes and have described several chromosomal segments harboring adipogenic alleles. The detailed characterization of the pathophysiology of NZO will help to set priorities when candidate genes within the susceptibility loci are studied. Specifically, the present data point to genes that are involved in the regulation of the orexigenic drive, energy expenditure, and behavioral parameters such as voluntary running activity.

In mice, only a small portion of consumed energy is stored as triglycerides: NZO mice gain excess adipose tissue at a rate of $0.14 \text{ g/day} (5.6 \text{ kJ/day})$, corresponding with only $\approx 7\%$ of the total energy consumed daily ($79.2 \text{ kJ/day at age of 8 wk}$). The difference in food intake between NZO and NZB mice is also much higher ($26.6 \text{ kJ/day}, 2.3 \text{ g food}$) than the energy stored as adipose tissue, reflecting the higher energy expenditure of the heavier NZO mouse. This disparity renders it very difficult to detect differences in energy expenditure contributing to the development of obesity. Absolute values of TEE were higher in NZO than in NZB mice, although NZO mice had a lower body temperature. To correct for the dependence of TEE on body weight, data are usually normalized per total
or per metabolic body weight (9). The present data indicate that TEE was somewhat lower in NZO than NZB mice when normalized per metabolic body weight; this difference was enhanced when data were normalized per total body weight. Therefore, given that the present data show a reduction of the body temperature in NZO mice, we believe that basal energy expenditure in NZO mice is lower than in lean mice. This conclusion is consistent with previous data from Koza et al. (20) indicating that treatment of NZO mice with the β3-receptor agonist CL-316,243 increased energy expenditure and reduced adiposity.

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ob/ob mice are caused by the lack of leptin action in the hypothalamus. Thus it is reasonable to assume that leptin controls the thermogenesis in brown adipose tissue via a central regulation, presumably through the sympathetic nervous system (30, 37). Because NZO mice, in contrast to ob/ob mice, were totally resistant to cold exposure, the molecular defects causing hyperphagia in NZO mice might be located distal from the leptin receptor, i.e., in a pathway that is not involved in the metabolic control of brown fat cells. Alternatively, the genetic background of NZO mice might specifically rescue the thermogenic response to cold exposure in impaired leptin action; such a scenario has been described for the BALB/cJ background (26).

Previous data have suggested that leptin action may be affected in NZO mice. NZO mice show hyperleptinemia and are resistant to subcutaneously injected leptin (15). This leptin resistance appeared to result from an impaired transport of the hormone through the blood-brain barrier, because central application of leptin produced the expected anorexigenic effect (13). NZO mice carry a leptin receptor variant with four amino acid exchanges (15). The signaling capability of this variant was slightly reduced, and it was concluded that the variant might contribute to the obesity syndrome. However, the genetic evidence indicated that its contribution to obesity was minor and was only visible in combination with other adipogenic alleles (19). Thus leptin resistance in NZO mice can be explained only to a small extent by the receptor variant, and additional, more distally located defects are entirely possible.

No significant differences in spontaneous locomotor activity were detected in the home cage environment among the investigated mouse strains, although lower levels of activity were previously described in ob/ob mice (7). This lack of difference is probably due to a low activity in the lean strains and to their housing in isolation and in small cages during the course of the experiment. Our study design also deviated from earlier experiments in which older ob/ob mice were studied. However, marked difference were observed when mice had access to running wheels: both obese strains showed a lower activity compared to the lean strains (4, 5, 17) as well as in obese humans (16). Future studies are on the way to test whether the reduced running activity is associated with further behavioral anomalies.

Human obesity reflects the complex interaction between a polygenic predisposition and environmental factors such as a high-calorie environment and an increasingly sedentary lifestyle (35). To identify the molecular components of this complex interaction, mouse strains with polygenic obesity represent the most suitable models. The morbid obesity syndrome in Pima Indians, like that in NZO mice, is based on a polygenic predisposition and an exposure to a high-calorie environment (thrift genetic hypothesis) (27). Ravussin and colleagues (32) have shown that decreased energy expenditure precedes weight gain in morbidly obese Pima Indians. Furthermore, human obesity is significantly associated with low levels of physical exercise (12, 16). It was also been proposed recently that nonexercise activity thermogenesis is an important component in human energy balance (22). With its increased caloric intake, decreased body temperature, and reduced voluntary running activity, the NZO mouse therefore represents the major pathophysiological characteristics of human obesity. Thus, on the basis of the findings presented here, the NZO mouse might be the animal model that most closely resembles the obesity syndrome observed in humans.

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REFERENCES


