Enriched Environment Moderates Obesity in Genetically Hyperphagic OLETF Rats in a Sex-dependent Manner

Mariana Schroeder, Liat Shbiro, and Aron Weller

Volume 52(e39-e45) — 2011
Abstract

The aim of the present study was to examine the effect of housing conditions on long-term voluntary food intake, body weight, and adiposity levels in the OLETF rat model of obesity. OLETF males and females develop obesity in a gradual manner and have been reported as presenting 30–40% more body weight and fat than LETO rat controls as adults. Here, rats of both sexes were provided with either customary conditions (two rats in standard-sized cages with bedding) or enriched conditions (four rats in large cages with a variety of types of bedding and toys) starting at the time of weaning. No alterations were found in any of the parameters in the LETO strain and in the OLETF females, whereas OLETF males showed decreased food intake, body mass, and adiposity as well as reduced levels of the adiposity hormone leptin. This study is the first to report that housing conditions are variables that are as critical in the study of obesity and energy balance as they are in studies examining emotionality and cognition. The standard laboratory animal environment may thus represent a confounding variable in research on obesity, producing an additive effect to the genetic predisposition that may worsen the obese phenotype (at least in males). This effect may lead to an inaccurate estimation of the direct health consequences of a specific genetic abnormality, compromising experimental results.

Key Words: adiposity; enriched environment (EE); housing condition; obesity; OLETf rat model; sex differences

Introduction

Animal models are useful in research on obesity and related metabolic disorders. But such studies do not typically account for the possible confounding influence of housing conditions such as cage size, environmental enrichment, and single versus paired or group housing. The animals are typically housed in standard cages that are relatively small, limiting the animals’ movement. In addition, inhospitable environments have been linked to abnormal brain development and behavior (Gonyou 1994; Mench 1998; Würbel 2001) and are stressful for the animal (Benefiel and Greenough 1998). Finally, rats are often singly housed, which may cause stress and have implications for the animals’ eating behavior and weight (Boggiano et al. 2008).

The Otsuka Long-Evans Tokushima fatty (OLETF) rat is an animal model of obesity currently in use (Moran and Bi 2006). Because these rats lack cholecystokinin (CCK) receptors (Moran and Bi 2006; Nakamura et al. 1998), they present hyperphagia from birth and eventually become obese (Blumberg et al. 2006; Kawano et al. 1992, 1994; Schroeder et al. 2007a,b). OLETF males develop non-insulin-dependent diabetes mellitus late in life and have been the focus of many studies that show the development of morbid obesity with complications of the metabolic syndrome (Moran et al. 1998; Moran and Bi 2006). Obesity in this strain develops differently in females (Schroeder et al. 2009c), but preobese symptoms are already evident during the suckling period in both males and females (Blumberg et al. 2006; Schroeder et al. 2006, 2007a,b, 2009b). Behavioral interventions to reduce OLETF rats’ obesity have shown significant success in moderating males’ obesity when performed in adulthood (Bi et al. 2007; Man et al. 2000; Moran 2008) and long-lasting reductions in obesity when performed early in life (Schroeder et al. 2010b,c). In contrast, OLETF females appear to be less responsive to behavioral interventions than males and usually retain their body weight and adiposity (Schroeder et al. 2009, 2010a,c).

According to accumulating evidence, exercise is one of the most effective treatments for obesity in animal models of obesity including the OLETF rat (Levin 2008; Morris et al. 2008; Schroeder et al. 2010c). Enriched environment (EE) provides the animals with entertaining activities, the opportunity to display more complex social interactions, places to hide, and sufficient space to explore without engaging in endurance training. We hypothesized that providing the animals with some entertainment in the form of additional objects of interest may not only support efforts to analyze the effects of physical activity on energy intake and expenditure but also neutralize...
possible negative effects of standard housing conditions as a confounding variable.

In the present study, we compared eating behavior and long-term obesity in animals reared in either standard or enriched conditions, in order to evaluate the effects of a relatively impoverished environment on obesity levels of young adult (90-day-old) OLETF rats.

Methods

Subjects and Housing

OLETF and Long-Evans Tokushima Otsuka (LETO) females were raised in the specific pathogen-free facility of the Gonda Brain Research Center at Bar Ilan University in Ramat-Gan, Israel. The original rats were received as a generous gift from the Tokushima Research Institute in Japan.

OLETF and LETO offspring were housed together with their dams and litters until weaning. Food (2018S Teklad Global, Harlan, Israel; 5% fat) and water were freely available. The animals were on a 12:12-hour light:dark cycle, with lights on at 6:00 AM. Room temperature was maintained at 22±2°C. We used 11 or 12 animals, randomly chosen from six to eight litters per strain, for each housing condition, with males and females from each litter evenly split between the two experimental conditions. We were thus able to assess males and females, LETO and OLETF rats, and the effects of standard versus enriched housing.

For the standard housing condition, littermates of the same sex were housed in pairs after weaning, beginning on postnatal day (PND) 22. Standard-sized polycarbonate cages (23.5 cm height × 26.5 cm width × 43 cm length) were covered with stainless steel wire lids, with wood shavings as bedding material.

For the enriched condition, rats of the same sex and strain, but from two or three different litters, were housed in groups of four after weaning. The polycarbonate cages for this group were large (20 cm height × 33 cm width × 60 cm length) for both LETO and OLETF rats until 2 months of age. For the third month, OLETF rats were moved to slightly larger cages (25 cm height × 40 cm width × 65 cm length) to allow them more space in accordance with their larger size and to achieve similar space for each individual animal. The cages were equipped with plastic tunnels, little plastic balls, small bells, different types of bedding material, paper towels, wood, hammocks, chains, and tubes. These elements were changed every week to different ones, but the original food remained the same.

The research protocol adhered to the guidelines of the American Psychological Association and the Society for Neuroscience and was approved by the Bar Ilan University Institutional Animal Care and Use Committee.

Experimental Procedure

Body Weight and Intake

All rats were weighed every fifth day from weaning until PND 90. Intake was assessed daily from cages containing either two or four same-sex, same-strain rats and was divided by the number of animals per cage.

Locomotor Activity

The activity of each individual animal was assessed in the open-field paradigm, which consisted of an arena (62 cm × 62 cm) enclosed by walls (30 cm high). The floor and walls were built from green polygal (opaque polycarbonate). Four lines were drawn on the floor to create nine identical squares (approx. 20 × 20 cm each). During a 5-minute trial, two activity measures (total number of line crossings and number of rearings) were counted. Videotape recordings were analyzed using the public domain Etholog program.

Statistical Approach

Group differences (male and female controls vs. EE rats) in body weight (BW) and intake were analyzed by repeated measures analysis of variance (ANOVA) comparing the four groups (independent variable) over the days of measurements. Group differences at particular days of measurement were followed by one-way ANOVA with post hoc Duncan’s tests (p < 0.05). Similar one-way ANOVA with post hoc Duncan’s tests were performed to compare the four groups (control vs. EE within each sex and strain) on the study variables.

Tissue Collection

Rats were weighed and then sacrificed in a separate room on PND 90. Interscapular brown adipose tissue (BAT), retroperitoneal (Retro), inguinal (IAT) and visceral adipose tissues as well as the animals’ livers were collected and weighed. Body mass index (BMI = body weight in grams/body length in cm²) and waist circumference (WC) were also examined as additional obesity parameters. Trunk blood for leptin, insulin, and adiponectin analysis was collected in chilled heparinized vacutainer tubes coated with ethylenediaminetetraacetic acid. Plasma was stored at −80°C until assayed (3–4 months).

Leptin, Insulin, Adiponectin, and Corticosterone

Plasma leptin, insulin, adiponectin, and corticosterone levels were assessed using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, Minnesota, for the first two; and for the last two, respectively, Linco and Assaypro, both in St. Charles, Missouri) according to the manufacturers’ instructions. For leptin, intra- and interassay variance values were 2.7 and 5.76, respectively, with a minimum detectable amount of 22 pg/ml. For insulin, intra- and interassay variances were 1.91 and 7.63, respectively, with a minimum detectable amount of 0.2 ng/ml. For adiponectin, intra- and interassay coefficients of variation were 4.1% and 7.2%, respectively, and
the minimum detectable dose was 0.5 ng/ml. For corticosterone, intra- and interassay coefficients of variation were 4.8% and 7.2%, respectively, and the minimum detectable dose was 40 pg/ml.

For corticosterone, intra- and interassay coefficients of variation were 4.8% and 7.2%, respectively, and the minimum detectable dose was 40 pg/ml.

We observed that EE decreased caloric intake in LETO males on many measurement days throughout the study (Figure 2A) but not overall (Figure 2C). Changes in EE females were less frequent and in both directions, and did not reach overall significance ($p < 0.05$; Duncan’s tests at specific ages, $p < 0.05$; Figure 2A,C). On days when EE LETO males had decreased intake, they displayed a compensatory increase in intake the next day. In the OLETF strain, EE decreased intake in 30% of the days in the males and in 24% of the days in the females, and reached overall significance in both sexes (Duncan’s tests at specific ages, $p < 0.05$; Figure 2B,D).

Group differences in fat pad mass, expressed as a percentage of BW, are presented in Figure 3. The weight of the inguinal fat pad of OLETF EE males was significantly lower than in controls (Duncan’s tests, $p < 0.05$), whereas EE access did not significantly affect OLETF EE females or LETO EE rats of either sex (Figure 3B). Similarly, only the OLETF EE males showed reductions in the retroperitoneal fat pad ($F(3,28) = 3.29, p < 0.05$; Figure 3A), visceral fat ($F(3,28) = 14.01, p < 0.001$; Figure 3C), and total white fat ($F(3,28) = 6.57, p < 0.01$; Figure 3D). The raw weight of every fat pad is presented in Tables 1 (LETO) and 2 (OLETF).

Brown adipose tissue and liver weights were not affected by EE (raw weights are shown in Tables 1 and 2). The body mass index score of the OLETF EE males was significantly lower than that of the OLETF controls ($F(3,38) = 15.13, p < 0.001$), and waist circumference was surprisingly increased in LETO EE females ($F(3,49) = 56.97, p < 0.001$; Tables 1 and 2) (Duncan’s tests, $p < 0.05$).

The level of plasma leptin of the OLETF EE males was significantly lower than that of controls ($F(3,18) = 6.14, p < 0.01$; no EE effects were evident in leptin levels in the other groups (Figure 4A). Insulin levels were not affected in any of the groups (Figure 4B), and adiponectin levels were higher in OLETF EE females compared with controls (Figure 4C) (Duncan’s tests, $p < 0.05$). In contrast to the other hormones, corticosterone levels, analyzed for all groups together and then for each strain separately, were similar among the strains, although ANOVA revealed that they were higher in the EE groups compared with controls ($F(3,36) = 4.41, p < 0.01$). When analyzed by strain, only OLETF EE males displayed higher corticosterone levels than LETO male controls, whereas the other groups showed only a tendency to significance (Duncan’s tests, $p < 0.05$; Figure 4D). We did not find a correlation (in any of the groups) between adiposity measures and the corticosterone levels of the individual animals.

The enriched environment had no effect on activity levels (line crossing and rearing frequency) in the LETO strain (Figure 5A,C) but produced an overall increase in rearing frequency in the OLETF strain ($F(3,41) = 4.46, p < 0.01$). Specifically,
EE OLETF females displayed increased rearing frequency compared with OLETF female controls (Duncan’s tests, \( p < 0.05 \); Figure 5D). We detected no significant effect on line cross frequency in the OLETF strain (Figure 5B).

**Discussion**

The results of this study suggest a significant contribution of housing conditions to the OLETF males’ hyperphagia and obesity.

**Effects of Exercise versus Enriched Environment**

In a previous study in which rats had RW access for 23 days, starting at the time of weaning, we observed significant long-lasting changes in adult (PND 90) OLETF males and in LETO males and females (Schroeder et al. 2010c). The OLETF males reduced their overall voluntary intake in similar proportions in the RW study and the current EE study (~15%). However, at termination (PND 90), the reduction in body weight was approximately 8.5% in the RW study and only 3.3% in the EE study. The adiposity measures yielded the most interesting
results: RW OLETF males showed reductions of 31% retroperitoneal, 33% inguinal, and 39% visceral fat, whereas among the EE animals the reductions were 19%, 21%, and 13%, respectively. Leptin levels also were reduced, by 33% in the RW group versus 18% in the EE group. While less impressive than the results observed after early short-term exercise, it is clear that exercise unquestionably reduced obesity levels and improved health.

Furthermore, the moderation in adiposity among the EE animals—even the hypolocomotive OLETF rats—suggests that standard, nonenriched rodent housing conditions may be partly responsible for the obesity of these rats. Thus, in addition to increased physical activity, the enrichment properties of exercise may account significantly for reductions in adiposity and, conversely, the sedentary and monotonous conditions provided to male OLETF rats in standard housing may exacerbate the animals’ strong genetic tendency toward hyperphagia and obesity.

**OLETF Phenotypic Characteristics**

OLETF males have been reported to display a hypoactive phenotype with respect to their large (rather than small) movements (Sei et al. 1999). We have also found in a previous study that this hypolocomotion cannot be improved by changes in the early environment for either sex (Schroeder and Weller 2010). Although activity measures obtained from open-field analysis obviously provide limited information about overall activity levels in the home cage, it appears that hypolocomotion in the OLETF strain is innate and apparently inalterable. Indeed, our findings in the open field confirm that OLETF rats are hypolocomotive even when reared in EE conditions, probably implying that increased activity levels are unlikely to be the main reason that males became leaner but rather that their reduction in BW results directly from their spontaneous decrease in food intake.

While RW access had significant effects on the intake and body composition of LETO rats (Schroeder et al. 2010c), EE had no significant effects on these measures. These findings
support the idea that standard conditions may have stronger effects on the knockout animals used in this study than on genetically intact rats, whose capacity to regulate energy homeostasis is normal and much more resistant to environmental manipulations.

Finally, OLETF females, whose resistance to weight loss has been tested with different early interventions such as food restriction (Schroeder et al. 2010a), exercise (Schroeder et al. 2010c), and even cross fostering (Schroeder et al. 2009a), also showed no benefit from an enriched environment. Despite transitory reduction in body weight, some increase in activity (rearing frequency), and overall moderation in intake throughout the study, they remained as obese as the control group, in contrast to the OLETF males. This pattern of sex differences is in general accordance with reports showing that other effects of enrichment appear to be greater for males than for females (Chen et al. 2005; Elliott and Grunberg 2005), although the opposite pattern of effects (females more affected by EE than males) has also been reported (Klein et al. 1994).

Possible Biological Mechanism

We propose a biological mechanism that may underlie the adiposity-reducing effect of EE on the OLETF males, but not the females, in the current study.

Brain-derived neurotrophic factor (BDNF) expression is well known to increase after EE and to be affected by estrogens. Chen and colleagues (2005) further showed significant, region-specific gender differences in BDNF expression after EE. BDNF plays a role in the hypothalamic pathway that controls body weight and energy homeostasis and also regulates energy metabolism in peripheral organs. In fact, low levels of BDNF are characteristic of obesity. Pedersen and colleagues (2009) suggest that BDNF mediates some of the effects of exercise, while others (Martin et al. 2007; Stranahan et al. 2009) report that it is increased by food restriction. A combination of EE and the resulting decrease in voluntary intake in the males may have led to increased BDNF and improved regulation of energy metabolism. Thus, we recommend that future research examine the potential mediating role of BDNF in the adiposity-attenuating effects of EE in male OLETF rats.

Corticosterone levels were increased in all animals housed in enriched conditions, which we believe reflects the rats’ excitement over the improved conditions rather than the stress produced by the environment. Tamashiro and colleagues (2004) have proposed that when males are group housed with females, some social hierarchies develop that may alter their intake routines as well as their body weight and body composition (Benaroya-Milshtein et al. 2004). We do not believe that this is the case in the present study, given that these animals were group housed since weaning, all animals in the same condition presented similar corticosterone and activity levels, and only OLETF males (not LETO males) showed reduced adiposity and BW. In addition, given that group-housed males without the presence of females do not develop a social hierarchy (Tamashiro et al. 2004), the potential factor of dominance was discarded as an intervening variable in the changes observed in the EE OLETF males.

Conclusion

This study is the first to demonstrate that housing conditions are variables that are as critical in research on obesity and energy balance as they are in studies of memory, emotionality, and cognition (Benaroya-Milshtein et al. 2004; Laviola et al. 2008). Although in the case of OLETF rats, and presumably other genetic animal models of obesity, the problem extends beyond the environment and the animals will be obese under most circumstances, we urge researchers to consider providing experimental animals with housing conditions that will enable isolation of the genetic problem from confounding environmental variables. Standard housing conditions may exacerbate the phenotype (particularly in males, the sex most often used in studies) and lead to inaccurate estimations of the direct consequences of a genetic mutation.

Acknowledgments

The authors thank Dr. Kazuya Kawano of the Tokushima Research Institute (Otsuka Pharmaceutical, Japan) for the generous gift of the OLETF and LETO rats. This research was performed as part of the first author’s PhD dissertation in the Faculty of Life Sciences at Bar Ilan University in Ramat Gan, Israel. Schroeder and Shbiro were supported by President’s Fellowships from Bar Ilan University. This study was supported by a grant from the US National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (RO1 DK57609).

References


Mench JA. 1998. Why it is important to understand animal behavior. ILAR J 39:20-26.


