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Behavioural mechanisms affecting energy regulation in mice prone or resistant to diet- induced obesity

Deike Hesse [a,b,*], Mark Dunn [c], Gerhard Heldmaier [a], Martin Klingenspor [e], Jan Rozman [d,e]

[a] Philipps-Universität Marburg, Faculty of Biology, Department of Animal Physiology, Karl-von-Frisch-Strasse 8, 35043 Marburg, Germany
[b] German Institute of Human Nutrition, Department of Experimental Diabetology, Arthur-Scheunert-Allee 114-116, 14558 Potsdam-Rehbruecke, Germany
[c] University of Southern Queensland, National Centre for Engineering in Agriculture, Toowoomba, Australia
[d] Technische Universität München, Else Kröner-Fresenius Center and ZIEL-Research Center for Nutrition and Food Science, Molecular Nutritional Medicine, Freising, Germany
[e] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Ingolstädter Landstr. 1, 85764, Neuherberg, München, Germany

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ABSTRACT

We investigated inbred SWR/J and AKR/J mice, two established models for different susceptibility to diet-induced obesity (DIO), to scrutinize the contribution of physical activity and energy assimilation to the etiology of developing obesity. Body mass gain and body composition of mice fed a high-energy (HE) or a low caloric control diet were monitored. In parallel, assimilated energy, locomotor activity and thermoregulatory behaviour were measured. Activity was continuously registered by radio telemetry and, in addition, Open Field (OF) behaviour was used as a quick screening tool for spontaneous activity before and after the feeding trial. Energy assimilation was increased in both strains on HE (AKR/J: +60.7% and SWR/J: +42.8%) but only in AKR/J, body mass (+8.1%) and fat mass (+40.7%) were significantly elevated. As a trend, total home cage activity was increased and was more scattered in SWR/J. Interestingly, HE stimulated OF activity only in SWR/J in the second trial at the end of the feeding experiment. The spatial pattern of OF activity also differed between strains with obese mice avoiding the core area. Under housing conditions, nest building behaviour was more pronounced in AKR/J. To further evaluate OF behaviour as a marker for spontaneous activity an obese mouse line was investigated. Mice lacking the leptin receptor (db/db) showed already before the onset of obesity lowest activity levels in OF.

Adjustment of energy intake, higher activity levels and energy consuming thermoregulatory behaviour are mechanisms employed by SWR/J mice to dissipate excess energy as a defence against the onset of obesity. Therefore our results deciphering mechanisms of DIO-sensitivity in mice contribute to the understanding of inter-individual differences in body weight development in an adipogenic environment.

1. Introduction

In healthy organisms energy regulation is characterized by the ability to finely tune the balance between energy intake and expenditure [1]. Already small deviations in this equilibrium can result in serious patho-physiological conditions if not corrected by compensating mechanisms. Obesity and related disorders, summarized as the metabolic syndrome, are a major global human health problem caused by a deregulated energy budget with energy intake persistently exceeding energy expenditure [2]. A positive imbalance comprises elevated energy intake, decreased energy expenditure or a combination of both. These conditions depend on aspects such as the availability of an energy dense, highly palatable and easily accessible food or reduced activity levels due to a sedentary life style. The conditions may develop to be a serious health problem as many humans exposed frequently to a high-energy diet seemingly lack effective compensatory mechanisms to stabilize energy balance. In everyday life, major sources for biological variability in daily energy expenditure occurring independently of body weight variation are different levels of physical activity and thermoregulatory expenses [3,4]. More specifically, non-exercise activity thermogenesis (NEAT) is the main component of activity [5] not including expenses during sleep, or eating or sports-like exercise. Notably, spontaneous activity-related energy expenditure was shown to be a determinant of individual body fat mass; and furthermore inter-individual variation in NEAT determined body weight gain on a high-fat diet [6,7]. But this association is not always clear as some studies were able to detect an association of diet-induced obesity (DIO) and activity in rodents [8,9] whereas others were not [10,11].

To some degree, rodent models for DIO reflect the situation in humans distinguished by consuming an energy dense diet; and therefore they are regularly used in obesity research [12,13]. These models vary in their susceptibility to DIO and also exhibit a

* Corresponding author. German Institute of Human Nutrition, Department of Experimental Diabetology, Arthur-Scheunert-Allee 114-116, 14558 Potsdam-Rehbruecke, Germany.
E-mail address: deikehesse@web.de (D. Hesse).

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considerable range of different levels of locomotor activity, corresponding with human NEAT affecting energy expenditure [13].

The challenge is to scrutinize mechanisms of energy regulation and its components. Different methods are available to quantify spontaneous locomotor activity in rodents under regular housing conditions, e. g. by implanting transponders and radio telemetry or motion detectors. We hypothesized that parameters of Open Field behaviour, a standardized behavioural test, outside the cage situation can be used as an indicator to evaluate the readiness for spontaneous locomotor activity in general [14–16] and be used as a predictor for the proneness to obesity.

As an example for inbred mouse lines displaying considerable variation in their susceptibility to diet-induced obesity, we chose AKR/J mice that are sensitive to feeding a high-caloric diet and SWR/J reported not to increase body mass and body fat when exposed to a Western style diet [17]. These differences seemingly correspond with behavioural differences mostly related to spontaneous activity with SWR/J being more vigorous than AKR/J [18–20]. In this study we dissected some components of energy balance including feeding behaviour, locomotor activity and thermoregulatory behaviour by the comparison of DIO-susceptible AKR/J and DIO-resistant SWR/J mice on a moderate high-energy diet. We investigated how these components contributed to body weight development in young male mice before the onset of obesity. To evaluate whether the Open Field trial prior to the onset of obesity could contribute significant information as a screening tool for body weight development we included a genetic model for obesity, the leptin receptor-deficient db/db mouse and C57BL/6 mice as controls.

2. Materials and methods

2.1. Animals

All AKR/J and SWR/J ($n=9$–10 per treatment group) mice were supplied by our own breeding colony after weaning on day 21. All experiments were performed in the animal housing facility of the Faculty of Biology, Philipps-Universität Marburg. Mice were singly housed at a temperature of 22–23 °C with a 12:12-h light–dark cycle (lights on at 6:00 am) in type II Macrolon cages (width × length × height 17 cm × 23 cm × 13.5 cm) with two-ply filter paper as bedding material to simplify faeces and food spillage collection. Animals had ad libitum access to food and water. The standard chow (C, breeding diet for rats and mice, art. no. 1314, Altromin, Lage, Germany, 4.8% fat by weight) contained 17.9 kJ/kg gross energy (per dry weight, own measurement) whereas the high-energy diet (art. no. 1314, 4.8% fat by weight modified with additional 12% of coconut fat containing high amounts of saturated palmitic acid, Altromin, Lage, Germany) consisted of 20.7 kJ/kg gross energy. Animals were weight-matched before the assignment to a dietary treatment group.

All genotypes of db/db animals (wildtype +/+ $n=11$, heterozygous db/+ $n=13$ and homozygous db/db $n=6$) were obtained from our own breeding facility, whereas C57BL/6 mice ($n=6$) were purchased from Harlan (Harlan Winkelmann, Borchen, Germany) at the age of 4 weeks. These mice were used for the evaluation of Open Field tests and subjected to the same handling as animals used in the feeding trial but without diet intervention.

All animal experiments were conducted according to the German animal welfare law.

2.2. Design of the study

Male mice subjected to the experimental treatment were weaned on day 21 of life, singly housed and when assigned to the HE group exposed to a dietary switch on day 35 after birth (= experimental day 0/ED 0). Open Field (OF) activity was registered shortly prior to ED 0. After 10 days of feeding a HE diet (ED 10, 45 days after birth), Open Field performance was measured a second time. Body weight and assimilated energy were determined every 2 to 3 days starting 5 days prior to the dietary switch on ED 0. In a second independent experimental group ($n=6$ each) spontaneous locomotor activity before and during HE feeding was obtained by implanted radio transmitters under home cage conditions. To minimize the health risk of an early implantation, surgery was performed on day 30 after birth. Therefore, the experimental protocol had to be modified and the diet switch for implanted animals was done on day 40 of life. Body composition was determined on day 50 (see below). Chow control animals were treated accordingly in both experimental setups.

Locomotor activity of db/db and C57BL/6 mice was investigated by exposure to the OF on days 35 and 45 of life. Body weight was recorded after trials.

2.3. Feeding behaviour/energy intake

Food intake (FI), corrected for spillage, of singly housed mice was recorded every 2 to 3 days over a total time period of 15 days, starting 5 days before the diet switch. For the determination of assimilation efficiency all excreted faeces (Faec) were collected every 2 to 3 days. Food and faeces samples were dried to constant weight in a drying oven (Memmert, Schwabach, Germany) at 60 °C and energy contents of food (EFI) and faeces (EFaec) were measured by bomb calorimetry (C7000, IKA Werke, Staufen, Germany). Assimilation efficiency (AssEff) was calculated as follows

$$AssEff\,(\%) = 100-(100 \times (Faec \times EFaec) / (FI \times EFI)).$$

Assimilated energy was calculated as total amount of energy consumed minus energy loss via faeces excretion. Energy loss through urine could not be measured directly and was assumed to be small (2% of energy uptake; [21]) and equal between groups. To correct cumulative assimilated energy for the different digestibility of the diets, total assimilated energy was calculated by multiplying total energy intake by assimilation efficiency.

Body weight gain efficiency (g/MJ) was calculated as the increase in body weight (g) over the experimental period divided by total assimilated energy (MJ).

2.4. Open Field

The Open Field test is an established tool to determine spontaneous activity measured as distance and speed as well as exploratory and anxiety-like behaviours by investigating the time spent in an open core area [15,22–24]. To measure Open Field (OF) activity a test trial of 3 min duration each was given to single animals during the morning (8 am to 9 am) before and after 10 days of high-energy feeding. The OF arena was a 50 × 50 × 50 cm white wooden box with a black removable abraded plastic floor, following the EUMORPHIA standard operation procedures (www.eumorphia.org). Between trials the box was wiped with 50% ethanol to remove scent marks. The device was shielded by a black curtain to detain interfering visual impulses. Constant illumination was obtained by four light bulbs in the corners 1.9 m above the arena and light intensity was adjusted at 400 Lux at ground level. A webcam was located ~1.9 m above the floor so that the mouse could be tracked automatically during each trial. Each mouse was placed in the middle of the arena and released facing the opposite wall with the trial starting immediately after the closure of the curtain. For analysis of data the total area of the arena was virtually divided into a core area (30 × 30 cm) and a peripheral area (10 cm on each side) to assess exploratory behaviour separately. Total distance (cm), velocity while ambulating (cm/s), time of inactivity (s) and time spent in the core area (s) were calculated from coordinates of the mouse which were collected 5 times per