Life Sciences 179 (2017) 30–36

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

The effects of calorie-matched high-fat diet consumption on spontaneous physical activity and development of obesity

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ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 15 April 2017
Accepted 24 April 2017
Available online 25 April 2017

Keywords:
Spontaneous physical activity
Energy homeostasis
High-fat diet
Obesity
Hypothalamus

ABSTRACT

Aims: To characterize the effects of a calorie matched high-fat diet (HFD) on spontaneous physical activity (SPA), body weight, inflammatory status and expression of genes related to energy homeostasis in hypothalamus of mice.

Main methods: C57Bl/6 mice (n = 5 per group) were fed a control diet (16.5% calories from fat) - control group (C), or a calorie matched HFD (60% calories from fat). We evaluated, periodically, body weight and SPA by infrared beam sensors and, at the end of the 12th week, we verified blood glucose levels, fat pads weight, plasma insulin, TNF-α and IL-6 by ELISA and the hypothalamic expression of 84 genes related to energy homeostasis, by quantitative real-time PCR array.

Key findings: Isocaloric HFD reduced SPA already in the first 48 h and SPA was kept lower in the HFD compared to C throughout. These changes resulted in an increase in body weight, adiposity, TNF-α and IL-6, blood glucose and hyperinsulinemia in the HFD group when compared to the C group. Expression of the Agrp, Bdnf, Adra2b and Pyy genes were altered in the hypothalamus of HFD-fed mice, highlighting the downregulation of Bdnf, key regulator of energy homeostasis.

Significance: Dietary macronutrient distribution plays an important part in energy homeostasis that goes beyond its energy content. Despite calorie-matched, the HFD led to increased body weight and adiposity due to decreased SPA, highlighting the key role of SPA on energy balance. The changes in hypothalamic gene expression seem to underlie the reduction in SPA caused by HFD.

1. Introduction

About 30% of adults world-wide are physically inactive, reaching 43% in the Americas [1]. This sedentary behavior has been associated with the increase of chronic degenerative diseases [2–5] and exorbitant costs with public health [1]. Coupled with the increased energy intake, the decrease in energy expenditure as a result of reduced levels of physical activity in the last decades also greatly contributed to the obesity epidemic and its related comorbidities [6].

Physical activity can be classified as volitional exercise (in humans: sports and fitness-related activities; in rodents: running wheel) and spontaneous physical activity, or SPA (in humans: activities other than volitional exercises such as fidgeting, non-specific ambulatory behavior, transportation, activities of daily living, occupation; in rodents: all activity in home-cage) [7]. The SPA itself and consequently its energy cost varies substantially between people by up to 2000 kcal per day and thus has a considerable impact on energy balance and weight gain [8–12]. However, despite the health and economic burden of the lack of physical activity, the biological mechanism controlling it is poorly understood.

A growing body of evidence from studies with humans using family and twin models as well as studies with animals has demonstrated a significant genetic influence on physical activity. Importantly, most candidate genes are proposed to act centrally [13], and hypothalamus seems to play a key role [8]. Among the most studied regulators of SPA in rodents are neuropeptide Y, leptin, agouti-related protein, orexins and ghrelin [8]. Nevertheless, the discovery of neuromodulators of SPA is still at its beginning, and new candidates and mechanisms of SPA modulation are emerging.

High-fat diet (HFD) decreases SPA in rodents, reducing energy expenditure and contributing to the increase in body and fat mass [14–16]. HFD also causes hypothalamic inflammation and neuron injury [17]. Of note, icv insulin injection increases SPA in lean but not in diet-induced obese (DIO) mice, probably as a consequence of central insulin resistance [18], known to arise from inflammation [19–21]. Another candidate to regulate SPA is Bdnf [22,23]. Hypothalamic Bdnf has been

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http://dx.doi.org/10.1016/j.lfs.2017.04.017
0024-3205/

shown to positively modulate SPA [22,23]. Interestingly, DIO resistant animals have higher basal levels of hypothalamic Bdnf whereas HFD further reduce Bdnf in DIO prone mice [24].

Thus, due to the high energy density and high-fat content of our western diet [25] it becomes evident the need to better understand the impact of HFD on hypothalamus, the central regulator of energy homeostasis, and on SPA, especially when considering the low levels of physical activity in our society and its consequences. In this sense our goal was to characterize the effects of a HFD offered isocalorically to mice on SPA, inflammatory process and the expression of genes related to energy homeostasis in the hypothalamus. We hypothesize that several genes known to affect locomotor activity could be modulated by HFD.

2. Material and methods

2.1. Animals and diets

The experiments were approved by the Institutional Ethics Committee on Animal Use (CEUA n. 5346190814). Male C57Bl/6 mice (8 weeks-old) were obtained from the Center for Development of Animal Models for Medicine and Biology (CEDEME), Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil. They were housed 5 per cage in a temperature-controlled room (22 °C) with a 12:12-h light-dark cycle and free access to water. Mice were distributed into two groups (n = 5 mice per group) and maintained during 12 weeks on either a control diet with a caloric composition of 16.5% fat, 65.7% carbohydrate, and 17.7% protein, and a caloric density of 3.82 kcal/g (C group) or fed a high-fat diet (HFD group) with a caloric composition of 60.2% fat, 26% carbohydrate, and 13.8% protein, with a caloric density of 5.23 kcal/g (Table 1) [26].

Calorie intake in the C group, which had ad libitum access to food, was estimated weekly, as follows: Step 1) the difference between the weight of the given and the remaining food, taking into account spillage, was calculated in a 48 h period and then divided by 2, in order to obtain the daily food consumption (in g/mouse); Step 2) this value was next corrected by the body weight, to achieve the food consumption in g/g of mice, which was subsequently multiplied by the caloric content of the diet (3.82 kcal/g), to find the calorie intake in kcal/g of mouse. As mice were separated in individual cages every weekend for measurements of activity or other analysis not included in this study, it was possible to calculate the individual calorie intake.

To determine the amount of food to be given each day to the HFD group, the average calorie consumption (kcal/g of mice/day) of the C group was divided by the caloric content of the high-fat diet (5.33 kcal/g) and then multiplied by the body weight (b.w.) of each mouse (e.g. average consumption of the C group was 0.68 kcal/g of mice/day; then 0.68 / 5.33 × 24.5 b.w. = 3.11 g of high-fat diet/day).

Table 1
Composition of the experimental diets.

Components (g)	Control	High-fat
Casein (84% protein)[a]	202	200
Starch	397	115.5
Dextrin	130.5	132
Sucrose	100	100
L-cystine	3	3
Fiber (microcellulose)	50	50
Soybean oil	70	40
Fat (lard)	–	312
Salt mix AIN93G[b]	35	35
Vitamins mix AIN93G[b]	10	10
Choline hydrochloride	2.5	2.5
Calories/kg	3818.72	5230

[a] Values corrected for protein content of casein.
[b] Detailed composition given by Reeves et al. 1993 [27].

When HFD mice were housed in collective cages, the amount of food given was the sum of food for each animal.

As the HFD group received a precise amount of food and mice remained most of the time in collective cages, the ratio of the maximum to minimum body weight was determined at the beginning and end of the experiment for each group, to ensure that no relevant difference in energy consumption occurred from that stipulated from the control group. If this was the case, the ratio between the maximum to minimum body weight would necessarily increase considerably along the experiment as a higher-than-stipulated calorie consumption by one mouse would necessarily imply in less calories consumed by the others. This ratio was 1.14 in the C and 1.10 in the HFD group in the beginning of the study. At the end, the ratio was 1.24 in the C (which had ad libitum access to food intake) and 1.17 in the HFD group.

2.2. Spontaneous physical activity (SPA)

To monitor the spontaneous physical activity of mice, an IR Actimeter system (Panlab-Harvard Apparatus, Barcelona, Spain) was used. This consisted of a cage surrounded by a 25 × 25 cm square frame, containing a total of 16 × 16 infrared beams located on the sides and spaced at 1.3 cm intervals.

SPA was determined using ActiTrack v2.7 software (Panlab-Harvard Apparatus, Barcelona, Spain). SPA included the sum of stereotypes (the number of samples where the position of the subject is different from its position during the previous sample and equal to its position during the second sample back in time) and locomotion (the number of samples where the position of the subject is different from its position during the previous sample and different to the position of the second sample back in time). The software also allowed for measurements of time spent resting/moving, distance covered and average speed. SPA was measured individually for 48 h starting at week 1 and then every 2 weeks (at weeks 1, 3, 5, 7, 9 and 11). At week 1, activity count is shown separately for every dark (19:00–7:00) and light (7:00–19:00) cycles. For the following weeks results are shown as the mean of the activity counts during the two dark cycles of the 48 h period. Mice were allowed an acclimation period of 2 h in the activity cages before the beginning of the analysis.

2.3. Euthanasia

At the end of the experimental period, fed mice were killed by decapitation, and perigonadal and retroperitoneal fat pads were weighted. Blood was collected in tubes containing heparin anti-coagulant, centrifuged for the plasm separation and stored for biochemical analysis. Hypothalamus was removed from skull, washed with saline solution to minimize a possible contamination with blood products, and stored for gene expression profile analysis, as described in the following sessions.

2.4. Biochemical analysis

Blood glucose levels were measured using a glucose analyzer (Accu-Check Advantage II, Roche, Basel, Switzerland). Plasma from fed mice was obtained by centrifugation (18,000 rpm for 10 min) and stored at −80 °C for determination of insulin (EZRMI-13K|Rat/Mouse Insulin ELISA - Merck Millipore), TNF-α (EZMTNFA Mouse TNF-α ELISA - Merck Millipore) and IL-6 (EZMIL6 Mouse IL-6 ELISA - Merck Millipore), according to the manufacturer's instructions.

2.5. Quantitative real-time PCR array

RNA was extracted from the hypothalamus of control and high-fat diet fed mice (RNeasy Microarray Tissue Mini Kit, catalog no. 73304, SABiosciences). RNA concentrations were determined using a spectrophotometer (Nanodrop 2000, Thermo Scientific). cDNA was synthesized