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Diet-induced obesity resistance of adult female mice selectively bred for increased wheel-running behavior is reversed by single perinatal exposure to a high-energy diet

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ABSTRACT

Female mice from independently bred lines previously selected over 50 generations for increased voluntary wheel-running behavior (S1, S2) resist high energy (HE) diet-induced obesity (DIO) at adulthood, even without actual access to running wheels, as opposed to randomly bred controls (CON). We investigated whether adult S mice without wheels remain DIO-resistant when exposed - via the mother - to the HE diet during their perinatal stage (from 2 weeks prior to conception until weaning on post-natal day 21). While S1 and S2 females subjected to HE diet either perinatally or from weaning onwards (post-weaning) resisted increased adiposity at adulthood (as opposed to CON females), they lost this resistance when challenged with HE diet during these periods combined over one single cycle of breeding. When allowed one-week access to wheels (at week 6–8 and at 10 months), however, tendency for increased wheel-running behavior of S mice was unaltered. Thus, the trait for increased wheel-running behavior remained intact following combined perinatal and post-weaning HE exposure, but apparently this did not block HE-induced weight gain. At weaning, perinatal HE diet increased adiposity in all lines, but this was only associated with hyperleptinemia in S lines irrespective of gender. Because leptin has multiple developmental effects at adolescence, we argue that a trait for increased physical activity may advance maturation in times of plenty. This would be adaptive in nature where episodes of increased nutrient availability should be exploited maximally. Associated disturbances in glucose homeostasis and related co-morbidities at adulthood are probably pleiotropic side effects.

1. Introduction

Obesity is a risk factor for impairment of sustainable health, as it increases susceptibility for developing type-2 diabetes, cardiovascular diseases, cancer, and neurodegenerative diseases [1, 2]. Although the energetics underlying obesity are rather straightforward - it results from a mismatch between intake and expenditure - the underlying mechanisms probably include a multitude of interactions between environmental and genetic factors [3]. Sensitivity to obesity in humans has long ago been reported to have a high degree of inter-individual variation [4, 5], yet the underlying mechanisms are still unclear [4]. The increasing incidence of childhood obesity [6] points towards early stages of life as a critical window for acquisition of predisposition to gain weight later in life [7–9]. Indeed, several animal studies have shown that over-nutrition by a high-energy (HE) diet during pregnancy and lactation predisposes offspring to energy-balance disorders and cardio-metabolic derangements later in life [10–15]. Early nutritional influences on long-term health outcomes have been named "fetal programming" [7, 16] or developmental plasticity [17]. From an evolutionary point of view, tendency for weight gain is regarded as an adaptive strategy to secure nutrients in order to survive periods of famine [18].

Besides energy intake, another variable component affecting energy balance is metabolic rate (MR). Metabolic rate increases with physical

Abbreviations: CON, not selected control line; DIO, diet induced obesity; HE, high energy; LF, low fat; MEO, milk energy output; MR, metabolic rate; OGTT, oral glucose tolerance test; PA, physical activity; RMR, resting metabolic rate; RQ, respiratory quotient; S, mice selectively bred for increased voluntary wheel-running behavior.

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activity (PA), which can account for 20–40% of total MR in humans [19]. PA greatly varies among individuals within species as well as between species [20]. One possible factor underlying this variation is that certain personality traits are associated with different levels of voluntary PA in humans [21, 22], as well as in other animals [20, 23, 24]. One approach to study the neurobiology of "innate" PA and its role in energy balance and adiposity is to investigate these regulations in mice from lines selectively bred for high voluntary wheel-running behavior [20, 25]. In previous studies, it was found that - relative to mice from non-selected control lines - activity-selected mice have reduced body mass, reduced body fat content, increased mass-adjusted food consumption, increased daily MR, and a high maximal oxygen consumption during forced treadmill exercise [26–28]. These traits are expressed even if mice do not have access to running wheels [29, 30]. When exposed to a high energy (HE) diet in adulthood, the hyperactivity trait appears to protect female mice from diet-induced obesity (DIO), despite the fact that they are markedly hyperphagic compared to CON mice that do become obese [26]. Resistance in highly active females is, in part, attributable to diet-induced augmentation of voluntary PA, as well as several metabolic and endocrine changes that stimulate fuel metabolism [26]. Whether these mice are also protected against DIO and development of metabolic derangement when subjected to developmental programming effects by a HE diet is unknown. For this reason, male and female mice from a non-selected control line (CON) and from two of the selectively bred high-activity lines (S1 and S2) were subjected to combinations of perinatal and/or post-weaning HE diet exposure (45% fat and 13% added sucrose) or a low-fat diet (and lower in energy content) consisting of 13% fat and no added sugars. The consequences of these combinations for weight gain, adiposity levels, fuel homeostasis, voluntary PA, and wheel-running behavior were investigated. Based on the robustness of the high-activity trait in several other studies (see [18] for comprehensive review), we hypothesized that the S1 and S2 mice, unlike the CON mice, would resist combinations of perinatal and post-weaning HE diet-induced changes in the aspects of energy balance mentioned above.

2. Material and methods

2.1. Animals and housing

Mice from one Control (CON, lab-designated line 2) and from two lines (here S1 and S2, lab-designated, respectively, line 7 and 8) obtained from the same ancestral line but selectively bred for high wheel-running activity were used (starting population for all lines was outbred Hsd:ICR mice). They were 53rd-generation offspring that were obtained from T. Garland Jr., Riverside, CA, USA [31]. At generation 45, ten pairs from CON, S1, and S2 lines were shipped to Groningen, the Netherlands, and maintained at the University of Groningen animal facility and further selected for high wheel-running activity. We studied two selected lines since by artificial selection phenotypic changes in one trait can impact on the expression of other traits. Hence, mice in different lines may have different adaptations to sustain the behavior they are selected for.

Three cohorts of CON, S1 and S2 mice were bred each using 20 virgin females per line, of which half received a low-fat diet (LF; 15.9 kJ/g, 13% fat, 63% starch 24% protein, RMH-B 2181, HopeFarms BV, Woerden, NL) and the other half a high-energy diet (HE; 19.7 kJ/g, 45% fat, 18% starch, 13% sucrose, 24% protein). After being on diets for two weeks, each female was paired for two weeks with a male from the same line (and thus ate from the same diet as the female). After delivery, litters were not culled and kept unaltered after birth, because there is no way of knowing whether culling itself would interact with the effect of line and/or diet on several parameters. We nevertheless only used offspring in litters of six or larger, and that consisted of at least two males and at least two females.

All mice were generally housed in Plexiglas cages (Macrolon Type II, UNO Roestvaststaal BV, Zevenaar, NL), with food and water ad libitum, and nesting material (EnviroDry®) as bedding. They were kept on a 12:12 light-dark cycle (lights on: 9 am) at 22 ± 1 °C. All experiments were approved by the Animal Experimental Committee of the University of Groningen.

2.2. Perinatal effects of diet

In breeding cohort 1, offspring was sacrificed at PND21, and the perinatal (i.e., here defined as the period from ~2 weeks before pregnancy until PND 21) effects of HE diet vs LF diet on several parameters were assessed. Half the mice were used for carcass analysis, in which liver, retroperitoneal and gonadal fat pads, gastrointestinal tract, kidneys, and skin including subcutaneous fat were removed and weighed. Carcasses and organs were weighed and then dried for 4 h at 103 °C. Fat was removed from carcasses and organs using petroleum ether extraction. In the other half of cohort 1, gonadal adipose tissue was submerged in 4% paraformaldehyde solution for fixation and embedded in paraffin for histology. In total, 103 white adipose tissue samples were sliced (thickness 4 μm) using a microtome and placed on adhesive glasses each containing 2–4 slices. After deparaffinization by xylol, alcohol, and deionized water, slices were stained for hematoxilin and covered using Kaiser's glycerin. Pictures were taken using microscope software Leica Qwin V3, creating an image in which areas of one pixel corresponds with 0.1971 μm^2. Three pictures were taken from representative adipose tissue areas, and analyzed using ImageJ (http://rsbweb.nih.gov). From each section, exactly 620 cells were randomly chosen and sizes were determined. We excluded objects > 240 μm and we measured shapes with a circularity between 0.3 and 1. Due to the skewness of distribution, all adipocytes of all mice irrespective of group were pooled and ranked according to size from small to large and then reassigned to groups. Rank numbers where averaged for each group.

2.3. Post-weaning effects of diet

In cohorts 2 and 3, two males and two females from each litter were weaned at PND21 and singly housed. To control for maternal effects, one male and female from each litter were subjected to LF diet whereas the other male and female of the same litter were subjected to HE diet. Remaining mice were used to continue the breeding lines. In each gender and line, this resulted in four dietary groups: LF-LF, LF-HE, HE-LF and HE-HE (first abbreviation: perinatal diet; second abbreviation: post-weaning diet). Cohort 2 had no access to running wheels and underwent several tests as described below. Only cohort 3 was studied for wheel-running activity between 6 and 8 weeks and at 10 months of age according to methodologies described elsewhere [31].

2.4. Food intake, bomb calorimetry, and home-cage activity

Food intake over seven days in cohort-2 offspring was recorded at four and eight months of age. After receiving clean cages, amount of food consumed (corrected for spillage) was determined (to the nearest 0.05 g). Furthermore, feces were collected and its energy content was measured by a bomb calorimeter (CBB 330, standard benzoic acid 26.44 kJ/g). Absorbed energy was calculated from differences between energy intake and fecal energy content. At four months of age, home-cage activity was measured by passive infrared sensors (Optex Wonderex FX-35) over four days [26].

2.5. Indirect calorimetry

Directly after food intake and home-cage activity measurements, cohort 2 offspring were placed in indirect calorimeter chambers for 24 h (with food and water available). Home-cage bedding was added to minimize stress of novelty. Gas exchange measurements were performed according to methodologies explained in detail elsewhere [32]. As described above, physical activity was measured by passive infrared sensors. Respiratory quotient (RQ) and metabolic rate (MR, kJ h^{-1}) were