Brief communication

MOLECULAR METABOLISM

Sex differences in obesity development in pair-fed neuronal lipoprotein lipase deficient mice

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ABSTRACT

Objective: Compared to men, postmenopausal women suffer from a disproportionate burden of many co-morbidities associated with obesity, e.g. cardiovascular disease, cancer, and dementia. The underlying mechanism for this sex difference is not well understood but is believed to relate to absence of the protective effect of estrogen through the action of estrogen receptor alpha (ERα) in the central nervous system. With the recently developed neuron-specific lipoprotein lipase deficient mice (NEXLPL−/−) (Wang et al., Cell Metabolism, 2011 [15]), we set to explore the possible role of lipid sensing in sex differences in obesity development.

Methods: Both male and female NEXLPL−/− mice and littermate WT controls were subjected to pair feeding (pf) where daily food amount given was adjusted according to body weight to match the food intake of ad libitum (ad) fed control WT mice. Food intake and body weight were measured daily, and pair feeding was maintained to 42 wk in male mice and to 38 wk in female mice. Various brain regions of the mice were harvested, and ERα gene expression was examined in both male and female NEXLPL−/− and WT control mice under both ad- and pf-fed conditions.

Results: Although both male and female NEXLPL−/− mice developed obesity similarly on standard chow, male NEXLPL−/− mice still developed obesity under with pair feeding, but on a much delayed time course, while female NEXLPL−/− mice were protected from extra body weight and fat mass gain compared to pair-fed WT control mice. Pair feeding alone induced extra fat mass gain in both male and female WT mice, and this was mostly driven by the reduction in physical activity. LPL deficiency resulted in an increase in ERα mRNA in the hypothalamus of ad-fed female mice, while pair feeding alone also resulted in an increase of ERα in both female WT control and NEXLPL−/− mice. The effect on increasing ERα by pair feeding and LPL deficiency was additive in pair-fed female NEXLPL−/− mice. ERα mRNA levels were not significantly modified in other brain regions examined, nor in the hypothalamus of male NEXLPL−/− mice compared to control mice.

Conclusions: These results suggest that the mechanism underlying ERα regulation of body weight interacts with the LPL-dependent lipid processing in the hypothalamus in a sex specific way. ERα could provide the link between brain lipid sensing and sex differences in obesity development. This study has the potential important clinical implication to provide better management for women who suffer from obesity and obesity-related co-morbidities.

Keywords Lipoprotein lipase; Pair feeding; Sex-differences; Estrogen receptor; Obesity

1. INTRODUCTION

Energy homeostasis is known to be regulated by a complex neuro-endocrine system involving both central and peripheral signals. In the central nervous system (CNS), neuronal signals in the hypothalamus cooperatively regulate energy intake and energy expenditure in a co-ordinate manner with circulating hormones to ensure the balance of energy storage during periods of food scarcity and/or food abundance [1−3]. Sex differences are well known to exist in the regulation of energy balance in response to calorie shortage or excess [4−6]. Historically, this is likely due to the different roles played by males and females in survival of the species; for example, males are more responsible for hunting and gathering, while females are responsible for gestation, lactation, and caregiving. Moreover, the reproductive capability of females is importantly related to adipose tissue fuel deposition. Below a critical level of body fat, menarche is absent or delayed, and, when menstruation begins, oligomenorrhea, anovulatory cycles, and subsequent amenorrhea often follow [7]. More recently, various studies have shown that there might be sex-specific molecular mechanisms underlying the sex differences in the regulation of body composition [8], insulin resistance [9,10] and energy homeostasis [11,12].

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Lipoprotein lipase (LPL) is a gate-keeping enzyme that hydrolyzes triglyceride-rich lipoproteins to release triglyceride (TG) free fatty acids (FFA) for uptake and oxidation in a tissue specific manner [13,14]. In CNS, LPL has been implied to play a role in regulating lipid uptake and possibly providing important signals in different brain regions to regulate energy balance [14–18] as well as behavior including learning and memory [14,19]. Specifically, male neuron-specific LPL deficient mice (NEXLPL−/−) developed obesity on standard chow by 16 wk, with a food intake increase between 12 and 22 wk, and a reduction in energy expenditure at 24 wk and older. Neuropeptides AgRP and NPY, that are known to regulate food intake and energy expenditure, were upregulated in the hypothalamus of NEXLPL before the onset of obesity, and the increase was persistent after obesity developed. Female NEXLPL−/− mice developed obesity in a similar time course as males but gained a higher percentage of excess body weight than the male counterparts by 6 mo [15].

To understand the importance of earlier food intake in obesity development, and to explore the possible sex differences in obesity development, both male and female NEXLPL−/− mice were subjected to pair feeding conditions in which the daily food intake for the experimental mice was limited to the average daily food intake for control ad libitum fed mice. Interesting sex differences in obesity development in response to food scarcity were discovered and reported here.

2. MATERIAL AND METHODS

2.1. Mice and pair feeding conditions

NEXLPL−/− mice and littermate controls were generated as described before [15]. At 10 wk of age, male and female mice were individually caged for a week before being subjected to different pair feeding conditions. All diets were standard chow. To establish the pair feeding conditions, a separate group of wildtype (WT) littermate control mice were first individually caged but fed ad libitum. Food intake was measured daily between 3 and 5 PM for a week, and the daily food intake (FI) normalized per body weight (BW) was calculated for each mouse. The average daily FI/BW was calculated from all control WT ad libitum fed mice (wt-ad), and this was used to determine the amount of food given daily to each individually caged pair-fed mouse. The average daily FI/BW was adjusted every week based on the actual food intake and body weight measured in the previous week for the control wt-ad mice. Food for pair feeding mice (both WT and NEXLPL−/− mice) was given daily between 3 and 5 PM, and the leftover food from previous day was measured and removed from the cage before the new food was given. For the long term pair-feeding experiment, all male mice were fed up to 42 wk of age to observe the significant difference in BW between genotypes, and female mice were fed up to 38 wk of age with no sign of differences in BW. For the short term pair feeding experiment, all mice were fed up to 20 wk of age, at which point no BW differences were observed for all groups.

2.2. Measurement of body weight, body composition, and plasma metabolic parameters

Body weights were monitored on a daily basis between 3 and 5 PM. Body compositions were measured on anesthetized mice by dual-energy X-ray absorptiometry using a mouse densitometer (PIXImus2, Lunar Corp., Madison, WI) at the end of feeding when terminal blood and tissues were collected for analysis. Blood was collected by cardiac puncture, and plasma was stored at −20 °C until further analysis. Plasma glucose was measured using the Cayman Glucose Colorimetric Assay Kit (Caymen Chemical, San Diego CA). TG and FFA were measured using enzymatic, colorimetric assays (Sigma, St. Louis, MO and Wako Chemicals USA, Richmond, VA, respectively), and insulin was measured using a RIA kit (Linco Research, St. Charles, MO). Plasma leptin and adiponectin were measured using specific enzyme-immunoassay kits (ELISA) designed for quantitative determination of mouse plasma samples (Alpco Diagnostics, Salem, NH).

2.3. Indirect calorimetry and physical activity measurements

An open-ended indirect calorimetry system was used to measure oxygen consumption (O_2) and carbon dioxide (CO_2) production in mice for the calculation of metabolic rate and respiratory quotient (RQ) [20]. Animals at the end of feeding periods were placed in 4 metabolic chambers for measurements taken over three days with free access to food and water. The differential O_2 and CO_2 concentrations, flow rate, RQ, and metabolic rate (Weir equation) were calculated and stored in a computer configured with data acquisition hardware (Analogic, Wakefield, MA) and software (Labtech, Wilmington, MA). The metabolic rate will be reported in the unit of kcal/day/gram of LBM, where LBM stands for lean body mass. In addition, measurements of physical activity were carried out using the Columbus Instruments Opto M3, a multi-channel activity monitor that utilizes infrared beams to monitor an animal's movement in the X, Y and Z axis. The total physical activity during the last 24 h of calorimetry experiment was determined by adding all the ambulatory counts in the X direction.

2.4. Quantitative real-time PCR

Tissue was collected into RNAlater (Qiagen) from anesthetized mice after a 4 h fast and stored at 4 °C. Total RNA was extracted and reverse transcribed as previously described [15]. Quantitative PCR was performed using primer sets for genes of interest, two reference genes (GAPDH and Ubc) and iQ Supermix or iQ SYBR Supermix (Bio-Rad) following the manufacturer's protocols.

2.5. Statistical analyses

Results are presented as mean ± SEM. One way repeated measure ANOVA was performed for body weight and cumulative food intake data. Two tail, unequal variance t-tests were performed for all the other statistical analysis using the Data Analysis Tool pack in Excel 2010 (Microsoft). A $p < 0.05$ was considered significant.

3. RESULTS

3.1. Pair feeding delays the obesity development in male NEXLPL−/−mice

Three separate groups of littermate wild type (WT) and NEXLPL−/− mice were subjected to the following feeding conditions: WT ad libitum feeding (wt-ad), WT pair feeding (wt-pf), and NEXLPL−/− pair feeding (ko-pf). When male mice were allowed to remain on these feeding conditions for up to 42 wk of age, there was no significant difference in body weight for wt-pf mice compared to wt-ad mice (Figure 1A,B), although a trend for higher body weight, higher fat mass and higher % fat mass was consistently observed (Figure 1B). On the other hand, the expected weight gain in ad-fed NEXLPL−/− mice [15] was significantly delayed by pair feeding; however, ko-pf mice eventually experienced additional weight gain starting at 34 wk (Figure 1A). Moreover, both body weight and % fat mass were significantly higher than in wt-pf mice at 42 wk (Figure 1B). It is interesting to note that although the portion of food given daily to pair-fed male mice (both WT and NEXLPL−/−) was based on the average daily food intake normalized to body weight from the wt-ad group, both pair-fed groups appeared to consistently leave food behind, resulting in less FI/BW for both pair-fed

