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Research paper

Transgenic n-3 PUFAs enrichment leads to weight loss via modulating neuropeptides in hypothalamus

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HIGHLIGHTS

- The effect of fat-1 gene on body weight is achieved via regulating expression of appetite neuropeptides in hypothalamus.
- The fat-1 gene reduced the mice body weight effectively, which is related to increase of endogenous n-3 PUFAs and expression change of hypothalamic appetite neuropeptides.
- The body weight/length ratio, the serum levels of TG, CT, HDL-c, LDL-c and BG, the weight of adipose are decreased in fat-1 transgenic mice.

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ABSTRACT

Body weight is related to fat mass, which is associated with obesity. Our study explored the effect of fat-1 gene on body weight in fat-1 transgenic mice. In present study, we observed that the weight/length ratio of fat-1 transgenic mice was lower than that of wild-type mice. The serum levels of triglycerides (TG), cholesterol (CT), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and blood glucose (BG) in fat-1 transgenic mice were all decreased. The weights of peri-bowels fat, perirenal fat and peri-testicular fat in fat-1 transgenic mice were reduced. We hypothesized that increase of n-3 PUFAs might alter the expression of hypothalamic neuropeptide genes and lead to loss of body weight in fat-1 transgenic mice. Therefore, we measured mRNA levels of appetite neuropeptides, Neuropeptide Y (NPY), Agouti-related peptides (AgRP), Proopiomelanocortin (POMC), Cocaine and amphetamine regulated transcript (CART), ghrelin and nesfatin-1 in hypothalamus by real-time PCR. Compared with wild-type mice, the mRNA levels of CART, POMC and ghrelin were higher, while the mRNA levels of NPY, AgRP and nesfatin-1 were lower in fat-1 transgenic mice. The results indicate that fat-1 gene or n-3 PUFAs participates in regulation of body weight, and the mechanism of this phenomenon involves the expression of appetite neuropeptides and lipoproteins in fat-1 transgenic mice.

1. Introduction

Body weight is one index of obesity. Obesity is a vital factor to cause hypertension, type 2-diebetes, coronary heart disease, etc. [1]. To some extent, obesity refers to overweight and thick adipose layer which includes internal adipose tissue mass especially triglycerides [2]. The reason of obese occurrence is imbalance of energy metabolism that displays more calorie intake than expenditure. Hypothalamus plays a key role in maintaining the activity of energy metabolism [3]. The balance of energy is based on the regulated network which is composed of neuropeptides among hypothalamic nucleus [4–7]. Anorexigenic hormones, such as CART and POMC, inhibit intake by secreting α-melanocyte-stimulating hormone. On the contrary, several orexigenic peptides, such as NPY and AgRP, stimulate intake [1]. Wherein, nesfatin-1, a newly discovered nucleobindin 2 (NUCB2)-derived satiety neuropeptide, plays a significant role in the hypothalamic neuronal pathways regulating intake, energy expenditure and glucose homeostasis [8–14].

N-3 polyunsaturated fatty acids (PUFAs) have been reported to improve both glucose [15–17] and lipid metabolism [18–20] in obesity. Mammals have no ability to synthesize PUFAs which only come from diet. In our previous study, rats were given added n-

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3 PUFAs diet for long term. Obviously, their body weights were decreased [2]. Cintra's data had shown that the beneficial effect of n-3 PUFAs might have been induced by regulating hypothalamic neuropeptides to inhibit obesity [3].

Recently, White et al., reported that endogenous n-3 PUFAs in fat-1 transgenic mice could reduce adipose tissue weight to control body weight [21]. However, there is no report explaining how fat-1 gene controls body weight. Here we investigated endogenous n-3 PUFAs promoted weight loss, and fat-1 gene regulated expressions of appetite neuropeptide genes in hypothalamus.

2. Materials and methods

2.1. Animals grouping, feeding and measure weights/lengths

Fat-1 transgenic heterozygous mice, which came from Professor Jingxuan Kang of Harvard Medical College and Jianbo Wan of University of Macau, mated with C57BL/6 wild-type mice. The progeny mice were identified by PCR to get fat-1 transgenic mice. The male mice were divided into two groups: fat-1 transgenic group and wild-type group. Overweight or underweight mice were eliminated. Six-week-old male heterozygous fat-1(+/−) mice and their wild-type mice littermates were bred at the Qingdao University. They were allowed access to standard rodent chow as described in Refs. [9,21] and sacrificed after 8 weeks. They were housed individually in environmentally-controlled conditions (temperature 25 ± 2 °C, light cycle from 06:00 to 18:00 and dark cycle from 18:00 to 06:00) [1] and allowed ad libitum access to food and water throughout the trial. We estimated body weights and lengths of the mice weekly. The study was approved and all procedures were performed in accordance with institutional guidelines of the Animal Care and Use Committee at Qingdao University.

2.2. Measure TG, CT, HDL-c, LDL-c and BG

The serum levels of TG, CT, HDL-c, LDL-c and BG were measured twice separately in 4th and 6th week of the trial using corresponding kits (BG kit, ELISA, Bio-Rad Laboratories, Hercules, CA; CT kit, RongSheng Company, Shanghai; TG kit, HDL-c kit and LDL-c kit, DongOU Biological Company, Zhejiang). The data are presented as an average of the two measurements [21].

2.3. Hypothalamus histology harvesting

All mice were decapitated in the period of 7–9 PM [1] in order to minimize the variation of circadian mRNA expression. Each mouse's hypothalamus was excised after decapitation under deep anesthesia and kept in RNA Stabilization Reagent (Qiagen Sciences Inc., Germantown, MD) until RNA extraction [9,21]. Then they were stored in −80 °C refrigerator for the post-study.

2.4. Adipose histology harvesting

Adipose tissues include peri-bowels fat, perirenal fat and peritesticular fat. All the adipose tissues were obtained from the rest mice's body. The weights of the adipose tissues were estimated as they were got.

2.5. Real time PCR

Total RNA was extracted from hypothalamus using the Trizol (TaKaRa) kit according to the protocol. Then reverse-transcription was performed by Transcriptor First Strand cDNA Synthesis Kit. The primers of CART, POMC, NPY, AgRP, ghrelin and nesfatin-1 were derived from the software of primer premier 5 as described below and in the table. No sequences bearing significant homology to the designed premiers were found in the Gene Bank (NCBA).

Primer sets used for quantitative RT-PCR.

Gene	Reverse	Forward
CART	5′-AAGTCCAGCACCATGGAGAG-3′	5′-CCCCTTCACAAGCACTTCAA-3′
NPY	5′- GCTCTGCGACACTACATCAA-3′	5′-TGGTTTCAGGGGATGAGATG-3′
POMC	5′- CTCCTGCTTCAGACCTCCA-3′	5′-TTTTCAGTCAGGGGCTGTTC-3′
AgRP	5′-AACTCTGACCAAATCCACCCC-3′	5′-TGAGGTGCCTCCCATTGTGT-3′
Nesfatin-1	5′-GACCCTGCAGAGAAGAAC-3′	5′-CTTGCACAGATGAGGCCACT-3′
Ghrelin	5′-AGGAGCTGGAGATCAGGTTCA-3′	5′-GCCTGTCCGTGGTTACTTGT-3′
Gapdh	5′- GGTGAAGGTCGGTGTGAACG-3′	5′-CTCGCTCCTGGAAGATGGTG-3′

Each PCR contained 3.0 ng of reverse-transcribed RNA, 200 nM of each specific primer, SYBR Premix Ex Taq master mix, and RNase free water added to a 25 μL final volume. Real-time PCR were performed using the Roche LightCycler 480. GAPDH was used as a control to normalize the amount of sample of RNA. Amplification conditions were as follows: 95 °C 30 s initial denaturation, then 40 cycles of 95 °C 5 s denaturation, 60 °C 30 s annealing, with a 72 °C 30 s final extension. The relative fold changes were determined by the method of $2^{-\Delta\Delta Ct}$ as described in Ref. [1].

2.6. Statistical analysis

For semi-quantitative analyses, the relative levels of CART, POMC, AgRP, NPY, ghrelin and nesfatin-1 mRNA in the hypothalamus from fat-1 transgenic mice and wild-type mice were estimated with RT-PCR as described in Ref. [26]. Mean values ± SEM obtained from real-time PCR measurements, mice's body weighs and lengths, the data of hematic parameters and adipose weight were compared using StudentTranscriptor First Strand's *t* test and $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Effect of fat-1 gene on body weight/length ratio

The fat-1 transgenic mouse pups at birth are essentially indistinguishable in size from wild-type mouse. As shown in Fig. 1B, the body weight/length ratio of fat-1 transgenic group was significantly lower than that of wild-type group. There was a significantly difference in body weight/length ratios between fat-1 transgenic mice and wild-type mice at 14th week (fat-1: 2.285 ± 0.003, wild-type: 2.350 ± 0.005 g/cm, $n = 6$ animals per group, $P < 0.05$, Fig. 1A).

3.2. Effect of fat-1 gene on the serum levels of TG, CT, HDL-c, LDL-c and BG

The level of TG, CT, HDL-c, LDL-c and BG was presented as an average of the two measurements in 4th and 6th week of the trial. According to Fig. 2, there were significant differences in the serum levels of TG, CT, HDL-c, LDL-c and BG between fat-1 transgenic group and wild-type group. The data suggested that fat-1 transgenic group possesses better biochemistry data.

3.3. Effect of fat-1 gene on adipose tissue

Three kinds of adipose tissues were examined as the mice were killed. The weights of the adipose tissues from two different groups were analyzed by Student's *t* tests. The results are presented in Table 1. According to Table 1, peri-bowels fat, perirenal fat and peritesticular fat decreased in fat-1 transgenic mice. Therefore, the data suggested that weight loss was promoted in fat-1 transgenic mice.