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Acute physical exercise increases the adaptor protein APPL1 in the hypothalamus of obese mice

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ABSTRACT

Adiponectin is considered an adipokine that has essential anti-inflammatory and insulin-sensitivity actions. The adaptor protein containing the pleckstrin homology domain, the phosphotyrosine-binding domain, and leucine zipper motif 1 (APPL1) is a protein involved in adiponectin signaling that plays a role in many physiological and pathophysiological processes. In the central nervous system, adiponectin can potentiate the effects of leptin in the arcuate proopiomelanocortin (POMC) neurons. However, the role of APPL1 in the hypothalamus is not well understood. Therefore, in this study, we explored the effects of acute physical exercise on APPL1 protein content in the hypothalamus and food intake control in leptin stimulated-obese mice. Here we show that acute exercise increased serum adiponectin levels and APPL1 content in the hypothalamus, which were followed by reduced food intake in obese mice. Further, at the molecular level, the exercised obese mice increased the protein kinase B (Akt) signaling in the hypothalamus and attenuated the mammalian homolog of Drosophila tribbles protein 3 (TRB3) levels. In conclusion, the results indicate physical exercise is capable of increasing APPL1 protein content in the hypothalamus of leptin stimulated-obese mice and modulating food intake.

1. Introduction

Obesity is a disease characterized by abnormal or excessive body fat accumulation and has a worldwide prevalence [1]. Acquired adiposity is the consequence of a chronic positive energy balance, which the hypothalamus plays a crucial role. The hypothalamus receives peripheral stimuli during the food intake and thermogenesis regulation [2]. Among these stimuli, we can highlight the action of adipokines and hormones (adiponectin and leptin) for adequate energy metabolism regulation [2,3].

Adiponectin performs important peripheral roles such as insulin-sensitivity, anti-inflammatory properties, participation in fat oxidation, and glucose metabolism [4,5]. It is known that APPL1 is the most important protein in the adiponectin signaling pathway [5]. When associated with adiponectin receptors (AdipoRs), APPL1 becomes active and mediates the intracellular signaling of adiponectin [6]. Despite its relevant peripheral role, the central actions of this adipokine are not completely clear in the literature. Recently, at the central level, it was observed that adiponectin could potentiate the effects of leptin in the arcuate POMC neurons [7]. However, the role of APPL1 in this mechanism was not reported. The APPL1 was able to mediate leptin signaling in hepatocellular carcinoma HepG2 cell and breast cancer MCF-7 cell [8]. Furthermore, it was shown that APPL1 can bind to the leptin receptor (LepR) and to signal transducer and activator of transcription 3 (STAT3) [8]. However, the molecular mechanism responsible for controlling the central actions of adiponectin is not known. Thus, we hypothesize that APPL1 may be a crucial protein in the food intake control by leptin stimulus.

APPL1 is a protein highly conserved in different species and expressed in several tissues such as heart, brain, and skeletal muscle. This

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protein has a PTB (phosphotyrosine-binding) domain, which is an essential molecule in insulin sensitivity [6]. In fact, APPL1 can associate with substrates of insulin receptor 1 and 2 (IRS-1 and IRS-2), Akt, and the P110 subunit of the phosphoinositide 3-kinase (PI3K) protein, which improves insulin sensitivity [9–11]. Also, APPL1 can prevent Akt from being inactivated by the TRB3 protein [12]. Furthermore, obesity is associated with a reduction in serum adiponectin levels, in the AdipoR1 (adiponectin 1 receptor) as well as in the APPL1 protein content in peripheral tissues [13–15]. On the other hand, APPL isoform 2 (APPL2) performs a counteraction in APPL1 regulation [6]. The overexpression of APPL2 in C2C12 cells results in the adiponectin pathway negative regulation and lower APPL1 activation [16]. Also, in humans, weight excess and obesity are correlated with polymorphisms in the APPL2 gene [17].

Physical exercise has been recommended as an important non-pharmacological strategy to treat and prevent obesity [18]. Studies have shown that exercised obese rodents presented an increase in AdipoR1, AdipoR2, and APPL1 protein contents in the liver, skeletal muscle, and white adipose tissue (WAT), which can restore glycemic homeostasis [13,19]. However, Coope and colleagues showed that AdipoR1 mediates the anorectic and insulin/leptin-like actions of adiponectin in the hypothalamus of Wistar rats [2]. In parallel, a previous study indicated other central effects of adiponectin on energy balance by regulating locomotor activity in rats [3]. Therefore, the molecular pathway underlying the anorectic effect of adiponectin is not yet fully elucidated.

Although the role of adiponectin in the central nervous system (CNS) has been previously explored [2,3], the effects of obesity and exercise on hypothalamic APPL1 protein levels are not known. Here we hypothesized that APPL1 might be crucial in the control of food intake. Thus, this study aimed to investigate the effects of an acute physical exercise session on hypothalamic APPL1 protein content and food intake control in obese mice induced by a high-fat diet.

2. Experimental procedures

2.1. Experimental animals

Four-week-old Swiss mice were used from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science (CEMIB) – University of Campinas (UNICAMP). All experiments were approved by the Ethics Committee in the use of Animal (CEUA) of the Institute of Biological Sciences, UNICAMP - Campinas-SP, protocol no. 3946-1.

After six weeks of age, the mice were divided into two groups: Control Group (CTL) = mice fed with a commercial diet (NUVILAB®) and Obese group (OB) = mice fed with a high-fat diet. The high-fat diet was modified to contain approximately 35% of lipids (31.2% of lard and 4.0% of soybean oil), and the diet composition was the same as previously described [**Control Diet**: 42.75% Corn Starch, 20% Casein, 13.2% Sucrose, 10% Dextrinated Starch, 4% Soybean Oil, 5% Cellulose, 3.5% Mineral Mix, 1% Vitamin Mix, 0.3% L-Cysteine, 0.25% Choline; **High-Fat Diet**: 11.55% Corn Starch, 20% Casein, 13.2% Sucrose, 10% Dextrinated Starch, 4% Soybean Oil, 31.2% Lard, 5% Cellulose, 3.5% Mineral Mix, 1% Vitamin Mix, 0.3% L-Cysteine, 0.25% Choline] [20,21]. This intervention lasted eight weeks until the mice become glucose intolerant. Further, the OB group was distributed in the following subgroups: High-fat Diet (HFD) = animals fed with a high-fat diet and not submitted to the physical exercise protocol; High-fat Diet Exercised (HFD-EXE): animals fed with a high-fat diet and submitted to an acute exercise protocol (Fig. 1A). Body mass was analyzed in the beginning and at the end of the experimental period using the Gehaka® analytical balance (BK3000).

2.2. Treadmill adaptation and incremental load test

The animals were adapted to the treadmill to minimize their stress during the incremental load test and acute exercise session. The animals were adapted for 5 days, 10 min/day at a speed of 3 m/min, as previously standardized [22]. The incremental load test was performed two days after the adaptation period. The initial velocity of the test was 6 m/min, with 0% of inclination and increments of 3 m/min every 3 min until exhaustion, which occurred when the mice touched five times the end of the treadmill without an interval of 1 min. The velocity (m/min) of exhaustion (EV) was used to prescribe the intensity of the acute exercise session. After two days of the incremental load test, the acute physical exercise protocol was performed.

2.3. Acute aerobic physical exercise protocol

The acute aerobic physical exercise protocol consisted of a single session of treadmill running exercise without inclination. The mice ran three bouts of 45 min with 15 min of passive recovery between them, totalizing 2 h and 45 min for the total protocol. The intensity used was 60% of the EV (Fig. 2). For the physiological analysis and tissue extraction, the animals remained eight hours fasting. The HFD-EXE group started the fasting concomitant to the physical exercise protocol.

2.4. Glucose tolerance test (GTT)

After 8 h fasting, it was performed a distal puncture in the tail of the animals for basal glucose analysis, equivalent to time zero (t0) in the protocol. An intraperitoneal (IP) injection of glucose solution [50%] in a dose of 2 g/kg body weight was applied. Subsequently, blood samples were collected at 30, 60 and 120 min for serum glucose levels. Then, the area under the curve was calculated for each experimental group.

2.5. Evaluation of food intake

Firstly, the food intake was determined to measure the difference between the weight of the diet offered to the animal and the weight of the remaining diet after 24 h (6:00 p.m. to 6:00 p.m.). This procedure was performed with the animals in individual cages without previously fasting period. This procedure was performed again after 24 h of the exercise protocol (6:00 p.m. to 6:00 p.m.). In this second analysis, there was a previous 8-hour fasting period for all experimental groups (Fig. 4A), which was adopted to match with the fasting period and time after the exercise that was utilized for the euthanasia of the animals, in which the hypothalamus was extracted for molecular analysis. The weight measurement was accessed by Gehaka® analytical balance (BK3000).

2.6. Serum adiponectin concentration

Before the tissue extraction, the blood was collected from the tail, centrifuged (Eppendorf® Centrifuge 5804 R) at 3500 rpm during 15 min and the serum stored at −80 °C. Subsequently, the concentrations of adiponectin were determined by the enzyme immunoassay (ELISA) method using the commercial kit (Mouse Adiponectin/Acrp30 R & D Systems® – #DY1119)

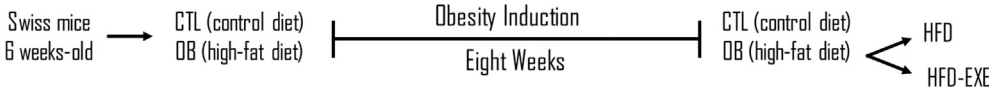

Fig. 1. Experimental design. The Swiss mice were divided into two groups: control (CTL), fed a chow diet; and obese (OB), fed a high-fat diet for eight weeks. After obesity induction, the OB animals were divided into two subgroups: HFD and HFD-EXE.