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Biotin augments acetyl CoA carboxylase 2 gene expression in the hypothalamus, leading to the suppression of food intake in mice

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ABSTRACT

It is known that biotin prevents the development of diabetes by increasing the functions of pancreatic beta-cells and improving insulin sensitivity in the periphery. However, its anti-obesity effects such as anorectic effects remain to be clarified. Acetyl CoA carboxylase (ACC), a biotin-dependent enzyme, has two isoforms (ACC1 and ACC2) and serves to catalyze the reaction of acetyl CoA to malonyl CoA. In the hypothalamus, ACC2 increases the production of malonyl CoA, which acts as a satiety signal. In this study, we investigated whether biotin increases the gene expression of ACC2 in the hypothalamus and suppresses food intake in mice administered excessive biotin. Food intake was significantly decreased by biotin, but plasma regulators of appetite, including glucose, ghrelin, and leptin, were not affected. On the other hand, biotin notably accumulated in the hypothalamus and enhanced ACC2 gene expression there, but it did not change the gene expression of ACC1, malonyl CoA decarboxylase (a malonyl CoA-degrading enzyme), and AMP-activated protein kinase α-2 (an ACC-inhibitory enzyme). These findings strongly suggest that biotin potentiates the suppression of appetite by upregulating ACC2 gene expression in the hypothalamus. This effect of biotin may contribute to the prevention of diabetes by biotin treatment.

1. Introduction

Obesity is a risk factor for developing insulin resistance and diabetes and is caused by an imbalance between energy intake in the form of food and energy expenditure. In the central nervous system, the hypothalamus is the region regulating energy homeostasis, in particular, working as an appetite center integrating peripheral hunger and satiety signals [1,2]. The regulation of appetite in the hypothalamus is controlled by hypothalamic nutrient metabolism and hormonal signaling [3–5]. Nutrient regulators of appetite include glucose, fatty acids, citrate, and malonyl CoA and hormonal regulators include ghrelin and adiponectin as orexigenic hormones and leptin, insulin, CCK, and GLP-1 as anorexigenic ones.

Malonyl CoA, the first intermediate of fatty acid biosynthesis, is synthesized from acetyl CoA by ACC and degraded to acetyl CoA and carbon dioxide by malonyl CoA decarboxylase (MCD). Malonyl CoA is also an allosteric inhibitor of carnitine palmitoyltransferase (CPT) 1, which is the first rate-limiting step in fatty acid oxidation in the mitochondria, and it acts as a satiety signal in the hypothalamus [6,7].

ACC is a biotin-dependent enzyme that has two isoforms, ACC1 and ACC2 [8,9]. ACC1 is abundant in lipogenic tissues and organs such as the adipose tissue, liver, and lactating glands and is found in the cytoplasm where fatty acids are synthesized. In contrast, ACC2 is predominantly expressed in the heart and skeletal muscle but is also found in the brain [10–12]. In the hypothalamus, ACC2 is localized to the mitochondrial membrane and increases local malonyl CoA levels to inhibit CPT-1 activity, leading to satiety [6,7,13]. On the other hand, ACC inhibits the activity of AMP-activated protein kinase (AMKP), which triggers the appetite as a downstream regulator of ghrelin and leptin signaling in the hypothalamus, resulting in a reduction in the level of malonyl CoA [5]. AMPK is a heterotrimeric protein with one catalytic (α1,2) and two regulatory subunits (beta1,2 and gamma1,2,3), and AMPK α-2 is a key target in the hypothalamus for regulating food intake [14,15].

Biotin, a soluble vitamin, acts as a cofactor of carboxylases and plays pivotal roles in the metabolism of several nutrients. In

Abbreviations: ACC, acetyl CoA carboxylase; MCD, malonyl CoA decarboxylase; CPT, carnitine palmitoyltransferase; AMKP, AMP-activated protein kinase; GLP, glucagon-like peptide; CCK, cholecystokinin.

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particular, biotin as a cofactor of ACC serves to catalyze the reaction of acetyl CoA to malonyl CoA. In addition, several studies have indicated that the biotin status affects the gene expression of biotin carboxylase and holocarboxylase synthetase, which catalyzes the transfer of biotin to carboxylase [16,17].

It is also known that biotin prevents the development of type 2 diabetes and alleviates type 1 and type 2 diabetes because of enhancements in pancreatic function and improvements in insulin sensitivity in the peripheral tissue [18–20]. A high dose of biotin increases the gene expression of glucokinase and the ATP production of pancreatic islets, which reinforces insulin secretion. Pharmacological biotin treatment also decreases the gene expression of liver phosphoenolpyruvate carboxykinase and increases the protein levels of Glut4 and insulin receptor in the skeletal muscle [21]. In addition, biotin augments the gene expression of IRS-1 and PPAR-gamma in the skeletal muscle with chromium picolinate, leading to the development of insulin receptor sensitivity [22]. It is recognized that biotin can prevent and improve diabetes by coordinated effects of all these actions. However, other effects of biotin on the prevention and alleviation of diabetes remain unclear, e.g., an anti-obesity effect, although biotin acts as a co-activator of ACC and catalyzes malonyl CoA production, leading to the suppression of food intake. In the present study, we determined whether biotin enhances ACC2 gene expression in the hypothalamus and suppresses food intake in mice.

2. Materials and methods

2.1. Animals and diets

Six-week-old C57BL/6J male mice were purchased from Clea Japan (Tokyo, Japan). The mice were housed in a temperature-controlled room with a 12-h light–dark cycle and placed in individual cages. The control group was fed a normal diet (CE-2, Clea Japan) and water *ad libitum*. The biotin group was given *ad libitum* access to the CE-2 diet supplemented with 1.0% biotin during an experimental period of 8 days. Body weights were measured every 2 days. Food intake for 2 days (48 h) was recorded during the experimental period. At the end of the experiment, all mice were killed by decapitation after 4 h of fasting. Blood samples were collected for measurements of plasma hormones. The stomach, duodenum, liver, epididymal fat, skeletal muscle, and brain were removed from the mice. The cortex, hypothalamus, and hippocampus were dissected from the brain under a microscope. Blood and tissues were preserved at −80 °C until assay. All animal experiments were approved by the Animal Ethics Committee of the University of Niigata Prefecture and conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978) and the Institutional Guidelines for the Care and Use of Animals of University of Niigata Prefecture.

2.2. Measurement of biotin level in tissues

Biotin level was microbiologically measured using *Lactobacillus plantarum* (ATCC8014), as described previously, with minor modifications [23]. In brief, tissues were homogenized in 0.04 M PBS (pH 7.2). Homogenates were acid hydrolyzed using 4.5 M HCl and then neutralized with 4.5 M NaOH prior to assay. The medium for biotin (Nissui pharmaceutical Co., LTD., Tokyo, Japan) and bacterial solution were dispensed in a 96-well plate. Standard and sample solutions were added to wells. After 24 h of incubation at 37 °C, absorbance was measured at 655 nm using a spectrophotometer, and the biotin level was calculated using a standard curve.

2.3. Measurements of blood glucose and plasma hormone levels

Before decapitation, mouse blood samples were obtained from the tip of the tail to measure blood glucose levels. Immediately after bleeding, blood glucose levels were measured using a blood glucose meter (Nipro, Osaka, Japan). Plasma hormone levels were measured using a commercial EIA kit for ghrelin (Bertin Pharma, Montigny-le-Bretonneux, France), insulin, leptin (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), adiponectin, glucagon-like peptide (GLP) 1 (Shibayagi Co. Ltd., Gunma, Japan), and cholecystokinin (CCK) (Phoenix Pharmaceuticals, Inc., Cal, USA), in accordance with the manufacturers' protocols.

2.4. Measurement of gene expression by quantitative real-time RT-PCR

Gene expression of leptin in the epididymal fat and that of ACC1, ACC2, MCD, and AMPK α-2 in the hypothalamus were measured by real-time RT-PCR. Total RNA was extracted from these tissues using Trizol reagent (Thermo Fisher Scientific, MA, USA) and prepared in accordance with the manufacturer's protocol. First-strand cDNA was synthesized by reverse transcription for 50 min at 50 °C using Superscript III First-strand Synthesis SuperMix (Thermo Fisher Scientific, MA, USA) with oligo dT primer. Real-time PCR was performed using an SYBR Premix EX Taq™ (Perfect Real Time; Takara Bio Inc., Shiga, Japan) and the PicoReal 96 Real-Time PCR System (Thermo Fisher Scientific, MA, USA), with 40 cycles at 95 °C for 5 s and extension at 60 °C for 30 s. Amplification primers for ACC1, ACC2, MCD, NPY, POMC, leptin, and β-actin were as follows: ACC1: 5′-GCAACTGACAGAGGAAGATGG-3′/5′-TGGAAGGGGAATCCA-TAGTG-3′, ACC2: 5′-ACAGCACTGGTCAGCCAAG-3′/5′-AGCAGCT-GAGCCACCTGTAT-3′, MCD: 5′-ACTCCATCAGCCTGACCCAG-3′/5′-ACCCCTTGAGGCTCTCGTGA-3′, AMPK α-2: 5′-CATGGCTGA-GAAGCAGAAGCAC-3′/5′-CTTAACTGCCACTTTATGGCCTG-3′, leptin: 5′-GACATTTCACACACGCAGTCG-3′/5′-AGCCCAGGAATGAAGTCCAA-3′, and β-actin: 5′-CTTGGGTATGGAATCCTGTGG-3′/5′-GTACTTGCGCTCAGGAGGAG-3′. The specificity of amplification was confirmed by melting curve analysis. The relative amount of each gene transcript was normalized with respect to β-actin transcripts present in the same cDNA.

2.5. Statistical analysis

All data analyses were conducted using StatView 5.0 (SAS Institute, NC, USA). Data are shown as the mean ± SEM. Statistical analysis was performed using the Mann–Whitney *U* test. The data on body weight and food intake were also analyzed by two-way repeated measures ANOVA coupled to Bonferroni's *post hoc* test. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Body weight and food intake

Growth curves for the control and biotin groups are shown in Fig. 1a. Body weight gradually increased in both these groups but did not differ between the groups during the experimental period.

The food intake in the control and biotin groups is shown in Fig. 1b and Table 1. The food intake in the biotin group was significantly lower than that in the control group from day 3 onward. The food intake in the biotin group was decreased by 15% in comparison with that in the control group. This indicates that biotin can potentiate the suppression of food intake.