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High-fat diet-induced obesity stimulates ketone body utilization in osteoclasts of the mouse bone

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ABSTRACT

Previous studies have shown that high-fat diet (HFD)-induced obesity increases the acetoacetyl-CoA synthetase (AACS) gene expression in lipogenic tissue. To investigate the effect of obesity on the AACS gene in other tissues, we examined the alteration of AACS mRNA levels in HFD-fed mice. In situ hybridization revealed that AACS was observed in several regions of the embryo, including the backbone region (especially in the somite), and in the epiphysis of the adult femur. AACS mRNA expression in the adult femur was higher in HFD-fed mice than in normal-diet fed mice, but this increase was not observed in high sucrose diet (HSD)-induced obese mice. In addition, HFD-specific increases were observed in the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and interleukin (IL)-6 genes. Moreover, we detected higher AACS mRNA expression in the differentiated osteoclast cells (RAW 264), and found that AACS mRNA expression was significantly up-regulated by IL-6 treatment only in osteoclasts. These results indicate the novel function of the ketone body in bone metabolism. Because the abnormal activation of osteoclasts by IL-6 induces bone resorption, our data suggest that AACS and ketone bodies are important factors in the relationship between obesity and osteoporosis.

1. Introduction

High-fat diet (HFD)-induced obesity has now emerged as a major risk factor for lifestyle-related disease, such as cardiovascular disease, stroke, and non-insulin-dependent diabetes mellitus (NIDDM). These disorders induce unusual metabolism of carbohydrates, lipids, and ketone bodies [1]. Because fatty acids and these metabolites are increased in the circulating serum of obese animals and owing to the onset of lifestyle-related disease, it is now widely recognized that fatty acid metabolism lies at the heart of a complex network that participates in the regulation of a variety of quite diverse biological functions.

Recently, several reports have suggested that diet-induced obesity triggers bone metabolic disorders. For example, HFD-fed mice had lower serum levels of bone formation markers, such as procollagen type 1 N-terminal propeptide (P1PN) and osteocalcin, and palmitic acids reduced osteoblast mineralization activity [2]. In obese humans, ectopic and serum lipid levels are positively correlated with bone marrow fat [3]. On the other hand, statin therapy to lower hyperlipidemia is associated with increased bone-mineral density and decreased fracture risk [4]. These facts indicate that a high level of serum lipids can be a risk factor for bone disease, such as osteoporosis. However, more research is required to explore the association between obesity and bone loss.

Lipid oxidation produces ketone bodies, D(−)-β-hydroxybutyrate and acetoacetate as byproducts [5]. Thus, elevation of the serum ketone level is a sign that a tissue is using fat for energy instead of using glucose under conditions of insulin resistance, fasting or high-fat feeding. These metabolites have been regarded as energy sources, and mitochondrial succinyl-CoA: 3-oxoacid CoA-transferase (SCOT, EC 2.8.3.5) is known to be the enzyme responsible for the activation of acetoacetate for energy generation [6]. On the other hand, in the cytosol, acetoacetate is known to be directly activated through the ligase reaction catalyzed by acetoacetyl-CoA synthetase (acetoacetate-CoA ligase, EC 6.2.1.16; AACS) [7,8] for the synthesis of biologically important lipogenic substances, such as cholesterol and fatty acids [9]. In fact, we have previously shown

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that AACS is a key enzyme for fatty acid accumulation in adipocytes and cholesterol production in the liver [10,11]. These facts suggest that AACS plays important roles in lipid biosynthesis in lipogenic tissues. However, the pathological and physiological roles of AACS in adipose tissues have not yet been fully clarified.

We previously found that the expression of AACS mRNA in subcutaneous white adipose tissue was increased in high-fat diet-induced obese rodents [12]. Morbid obesity causes a metabolic disorder, which includes unusual lipogenesis and ketogenesis [1]. We have also demonstrated that feeding rats pravastatin markedly increased the hepatic AACS activity and decreased the level of plasma ketone bodies in the diabetic rats [13]. Statins are commonly prescribed drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and decrease hepatic cholesterol biosynthesis. Mundy et al. revealed that statins enhance new bone formation in rodents [14]. Because AACS might be related to obesity-induced disorders of energy metabolism, such as lipid and ketone body utilization, we thought that AACS might also be related to bone formation via the ketone-cholesterol metabolic pathway. To clarify the relationship between AACS and bone metabolism in obesity, we investigated whether the obesity affects AACS gene expression in the femurs of mice.

2. Materials and methods

2.1. Animals

Mice of the ddY strain were purchased from the Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

2.2. In situ hybridization

Mouse embryos (E16.5) and adult mouse femurs (7 weeks old) were frozen in powdered dry ice. The sagittal sections were cut at a thickness of 16 μm using a cryostat and thaw-mounted onto poly-L-lysine-coated slides. In situ hybridaization was performed as described previously [15].

2.3. Preparation of probes for in situ hybridization

The cDNA fragment of mouse AACS, SCOT, HMGCR and osteocalcin were amplified from mouse liver or femur cDNA. The oligonucleotide primers used for amplification were: forward (tccgcaaccatgtccaagct) and reverse (atcacatgcacagctggatg) for mouse AACS, forward (cgaagatggcggctctcaaa) and reverse (gatgcttcaagttgaaatct) for mouse SCOT, forward (agagtttgaccgccttccga) and reverse (gtcagccagacttcttcaga) for mouse for HMGCR, and forward (ctcctgaaggtctcacaatc) and reverse (agggttaagctcacactgct) for mouse for osteocalcin. The fragments were cloned into the pGEM-T vector (Promega Co., WI, U.S.A.). ^{35}S-labeled cRNA probe was transcribed from the cDNA as template using SP6 or T7 RNA polymerase (Takara Bio, Shiga, Japan) in the presence of 5′-α-[^{35}S] thiotriphosphate (−30 TBq/mmol) (Perkin Elmer, MA, U.S.A.). The probe was shortened to an average length of 200 bases by alkaline hydrolysis.

2.4. Diet-induced obese mouse

Four-week-old male mice of the ddY strain (Tokyo Laboratory Animals Science Co.) were used after acclimatization for at least 3 days. They were given food and tap water *ad libitum* and maintained on a light–dark cycle of 12 h (light on at 8 a.m.). To induce obesity nutritionally, the mice were given a high-fat chow (type F2HFD2, Oriental Yeast Co., Tokyo, Japan; 60.0% fat, 24.5% protein, and 7.5% carbohydrate) or high-sucrose chow (type F2HScD, Oriental Yeast Co.; 2.5% fat, 12.0% protein, and 77.0% carbohydrate) for 12 weeks. The control mice were fed a regular chow (type MF, Oriental Yeast Co.; 5% fat, 24% protein, and 54% carbohydrate) for the same period. Then, the animals were killed; the femur bones were excised for in situ hybridization and RT-PCR.

2.5. Preparation of RNA

RNA was prepared from the mouse femurs and cell cultures using ISOGEN (Nippon gene, Tokyo, Japan). The RNA integrity was confirmed by electrophoresis on a denaturing agarose gel containing formaldehyde.

2.6. Measurement of plasma glucose and total ketone bodies

The plasma glucose concentration was determined using a glucose assay kit (Glucose CII-Test Wako, Wako Pure Chemical Industries, Tokyo, Japan), which was developed from the mutarotase-glucose oxidase method [16]. Determination of plasma ketone bodies [17] was carried out using ketone body assay kit (Ketone Test Sanwa, Sanwa Kagaku Co., Tokyo, Japan).

2.7. Cell culture

Mouse RAW 264 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 4.5 mg/ml glucose and supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen), then maintained at 37 °C in an atmosphere of 5% CO_2. When the cells reached confluence, they were induced to differentiate into osteoclasts by changing to a differentiation medium consisting of standard culture medium supplemented with 30 ng/ml RANKL (R & D systems, MN, U.S.A.) for 48 h. The medium was changed twice and replaced with DMEM containing 10% FBS. After 6 h, cells were treated with 0.5, 5, or 50 ng/ml IL-6 (R & D systems) for 24 h.

MC3T3-E1 cells were cultured in α-modification minimal essential medium (α-MEM; Sigma, MO, U.S.A.) supplemented with 10% FBS in a humidified 5% CO_2 incubator at 37 °C. The cells were cultured in a differentiation medium consisting of standard culture medium supplemented with 5 mM β-glycerol phosphate (Sigma) and 50 μg/ml L-ascorbic acid (Sigma). After 4 days, the cells were treated with 0.5, 5, or 50 ng/ml IL-6 (R & D systems) for 24 h. Tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP) staining was carried out using a TRAP/ALP Stain kit (Wako Pure Chemical Industries).

2.8. RT-PCR

Aliquots of total RNA (4 μg) were isolated from B16 cells and subjected to RT-PCR analysis of the antioxidant enzyme expression using a set of primers specific for each enzyme, then analyzed with Lumivision imager (AISIN SEIKI Co., Tokyo, Japan). The oligonucleotide primers used for amplification were: forward (tccgcaaccatgtccaagct) and reverse (atcacatgcacagctggatg) for mouse AACS, forward (cgaagatggcggctctcaaa) and reverse (gatgcttcaagttgaaatct) for mouse SCOT, forward (agagtttgaccgccttccga) and reverse (gtcagccagacttcttcaga) for mouse HMGCR, forward (ccatggaggaggtggtgata) and reverse (cgtctcgggatctctgctaa) for mouse FAS, and forward (tgcaagagacttccatccag) and reverse (ttgccgagtagatctcaaag) for mouse IL-6.