Biochemical and Biophysical Research Communications 473 (2016) 654–661

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Masahiro Yamasaki [a, *], Shinya Hasegawa [a], Masahiko Imai [b], Noriko Takahashi [b], Tetsuya Fukui [a, 1]

[a] Department of Health Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan
[b] Laboratory of Physiological Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan

ARTICLE INFO

Article history:
Received 22 March 2016
Accepted 23 March 2016
Available online 25 March 2016

Keywords:
Obesity
High-fat diet
Acetoacetyl-CoA synthetase (AACS)
Ketone body
Osteoclast
IL-6

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

* Corresponding author. Department of Health Chemistry, Hoshi University, 2-4-41, Ebara, Shinagawa-Ku, Tokyo, 142-8501, Japan.
E-mail address: ymskmshr@hoshi.ac.jp (M. Yamasaki).
[1] Present affiliation: College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan.

http://dx.doi.org/10.1016/j.bbrc.2016.03.115

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.

2.9. Statistical analysis

All data are presented as the means ± SD and were analyzed using unpaired Student's t-tests. p values < 0.05 were considered statistically significant.

3. Results

3.1. Ketone body utilizing enzyme was expressed in mouse bone

To investigate whether ketone bodies are utilized by AACS in bone tissues, frozen sections of mouse embryos were analyzed by in situ hybridization with an antisense AACS cRNA probe. Fig. 1 shows the results of autoradiography (Fig. 1A) of sagittal sections of the mouse embryos (E 16.5). With the probes, discrete labeling was observed in several regions of the embryo, including the brain, heart, spinal cord, lung, small intestine and backbone region, and particularly in the somite (Fig. 1A). In the developing vertebrate embryo, somites split to form skeletal muscle, cartilage and bone. Fig. 1B shows autoradiographs of sagittal sections of the mouse femurs of postnatal mice (7 weeks old). In adult mice, AACS mRNA was observed in the proximal and distal epiphysis of the femurs, while SCOT, another ketone body-utilizing enzyme, was not detected. In this region, osteocalcin and HMGCR mRNA expression were also detected. Epiphysis is known as a region between the growth plate and the extended end of the bone. Because osteocalcin is often used as a marker for the bone formation process, it is possible that ketone bodies are utilized for bone metabolism by the mevalonate pathway via AACS and HMGCR in this region.

3.2. HFD-induced obesity increased the gene expression of AACS and IL-6 mRNA in osteoblasts

Recently, several reports have suggested that diet-induced

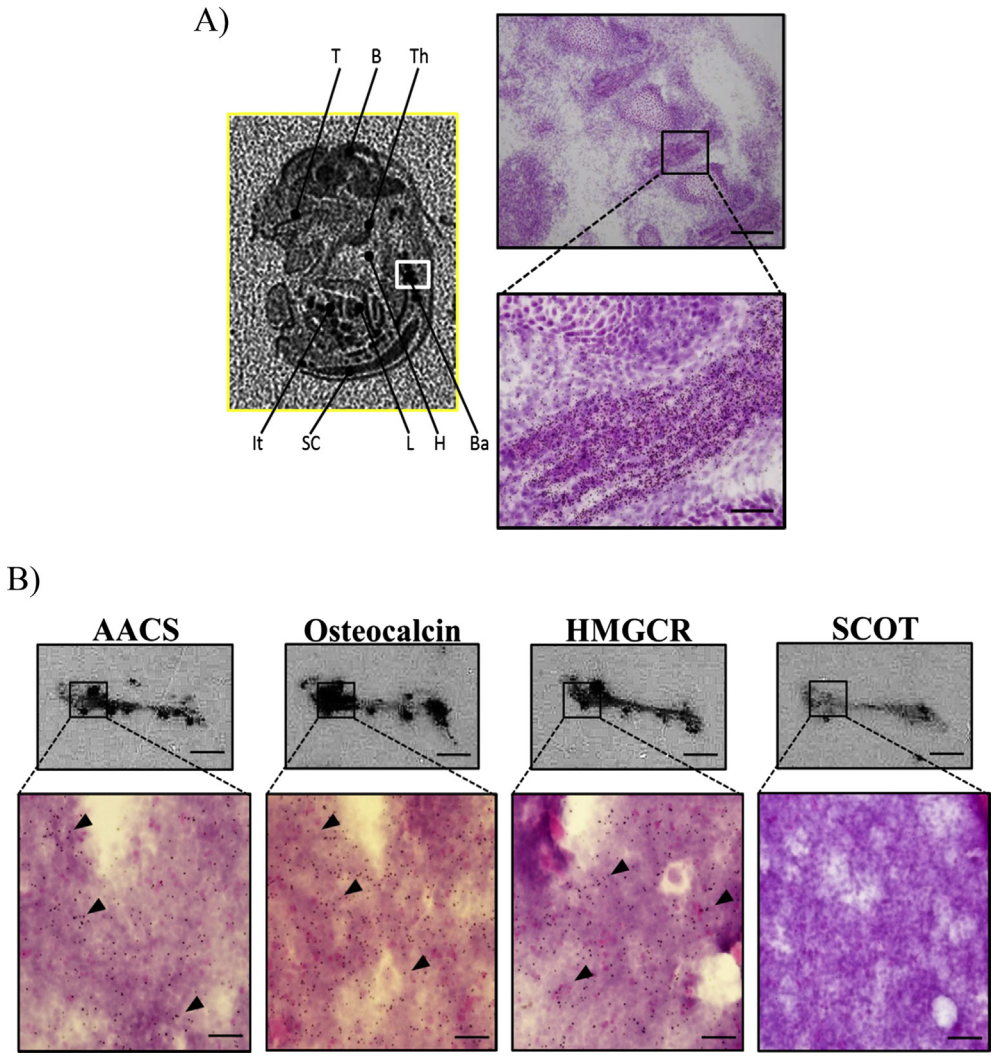
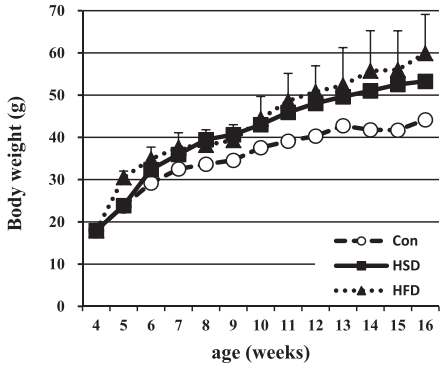


Fig. 1. Distribution of AACS mRNA in mouse bone. a) Distribution of AACS mRNA in mouse embryo. Sagittal sections were prepared from C57Bl/6 mouse embryos. The sections were examined using in situ hybridization with ^{35}S-labeled AACS probes. E16.5 embryos were hybridized with antisense AACS probes. Photomicrographs of emulsion-dipped sections are shown. Left: Embryonic backbone. Right: Magnified view of the region indicated in the square in the left panel. Scale bar = 30 μm. B: brain, T: tongue, Th: thymus gland, H: heart, SC: spinal cord, L: lung, It: small intestine, Ba: backbone. b) Distribution of AACS mRNA in adult mouse femur. Sagittal sections were prepared from adult male C57Bl/6 mice (8 weeks old). The sections were examined using in situ hybridization with ^{35}S-labeled each probe. The sections were dipped in liquid emulsion and counter-stained with hematoxylin and eosin. Macroscopic and microscopic views are shown in the upper and lower panels, respectively. Box indicates the magnified area. Scale bar = 2 μm (upper panels) and 30 μm (lower panels).

obesity triggers bone metabolic disorder [1–3]. Moreover, diabetes mellitus, one of the obesity-related diseases, causes bone loss and impaired bone healing [18–20]. We have shown that the expression level of AACS mRNA was significantly affected by diet-induced obesity and STZ-induced diabetes [21–23]. Because the serum level of ketone bodies is increased by both diabetes and high-fat feeding, it is possible that ketone bodies are used as metabolic substrates in the bone by AACS and are related to dysfunctional bone metabolism in high-fat diet-induced obese states. To clarify the effect of obesity on the ketone body metabolism of bone, we investigated whether the two different types of obesity affect the gene expression of these enzymes in mouse femurs.

First, we investigated the effects of obesity on the gene expression of ketone body-utilizing enzymes, using high-fat diet (HFD)- and high-sucrose diet (HSD)-induced obese mice. Twelve weeks of feeding the mice with the HFD or the HSD resulted in

A) Body weight



B) Blood parameter

Plasma concentration	Control	HFD	HSD
glucose (mg/dl)	212±71	276±87	297±134
total ketone bodies (μmol/l)	420±185	1036±114	744±122

C) Gene expression level

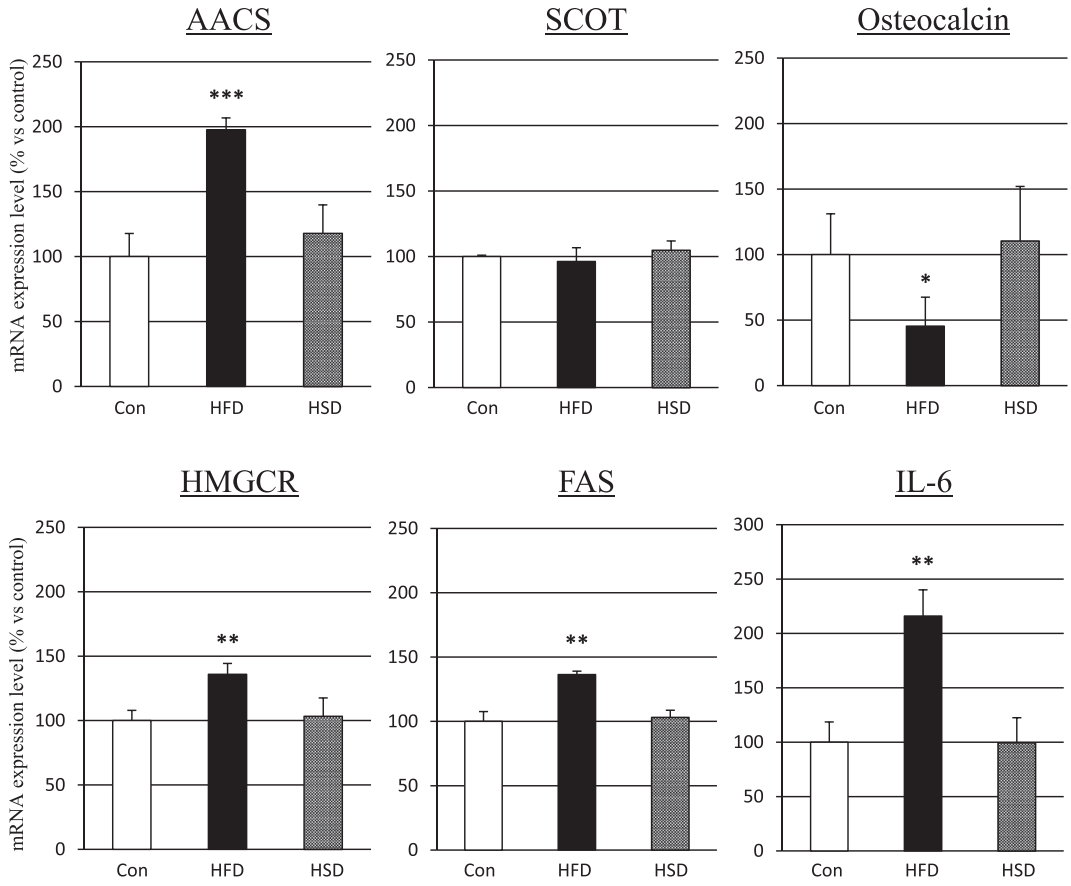


Fig. 2. Effect of high-fat diet- and high-sucrose diet-induced obesity on mRNA expression of ketone body-utilizing enzymes in mouse femurs. Four weeks old ddY mice were fed with a normal diet (Con) or a high-fat diet (HFD) or a high-sucrose diet (HSD) for 12 weeks (n = 4 mice per group). a) Body weights of control, HFD and HSD fed mice were measured of each time. b) Serum concentration of glucose and total ketone bodies. c) Gene expression levels of ketone body-utilizing and lipogenic enzymes. Total RNAs were prepared from the femurs of control mice, HFD and HSD fed mice, and subjected to RT-PCR analysis. Each expression level was normalized to the expression level of β-actin. The mean ± SD (n = 4) is shown. Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with the control.

almost the same gain in total body weight (Fig. 2A) and an approximately 1.5-fold (HFD) or 0.7-fold (HSD) increase in the blood ketone body level (Fig. 2B) compared with feeding the normal diet. In contrast, no differences were observed in the blood glucose level (Fig. 2B).

Fig. 2C shows the gene expression levels of two ketone body-utilizing enzymes, AACS and SCOT, in the femur. AACS mRNA expression was higher in HFD-fed mice than in normal diet-fed mice. However, such a difference was not observed in HSD-induced obese rats. These HFD-specific increases were observed in the HMGCR, fatty acid synthase (FAS) and interleukin (IL)-6 genes, but no differences in SCOT gene expression were observed among the three diet groups. In contrast, the gene expression level of osteocalcin was significantly decreased only in HFD-fed mice. Previous studies have demonstrated that obesity and diabetes, which is also characterized as high concentration of serum ketone bodies, causes bone marrow adiposity and bone resorption and increases the risk of developing osteoporosis [24]. On the other hand, chronic inflammation causes a reduction in the bone mineral density (BMD), which leads to osteopenia and osteoporosis [25]. Taken together, our results suggest that the HFD decreases bone formation via inflammation of the bone marrow and also up-regulates ketone body utilization for lipid synthesis in the bone tissues.

3.3. HFD-induced inflammation induced AACS gene expression in osteoclasts

Bone homeostasis is a highly coordinated process responsible for bone resorption and formation [26]. Two main types of cells are responsible for bone metabolism: osteoblasts (which secrete new bone), and osteoclasts (which break bone down). To investigate the cell specificity of AACS gene expression in bone tissues, we examined AACS gene expression in two cell lines, RAW 264 osteoclasts

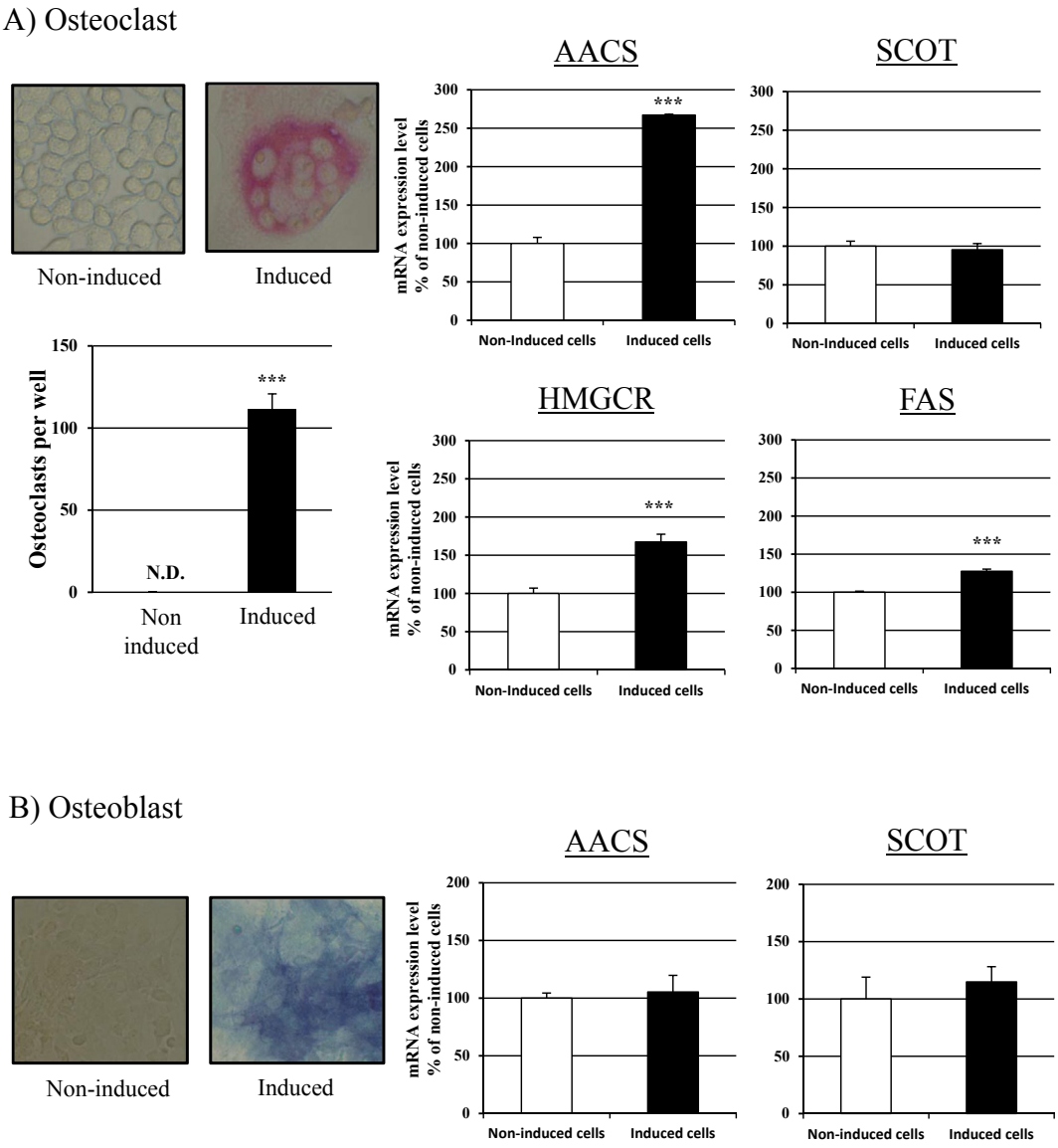


Fig. 3. Gene expression of ketone body-utilizing enzymes in osteoclasts and osteoblasts. A) Left: Number of TRAP-positive cells. RAW 264 cells were stimulated with (Induced cells) or without (Non-induced cells) RANKL. TRAP-positive cells were counted as multinuclear osteoclasts. Right: Gene expression of AACS and SCOT. Total RNAs were prepared from non-induced or induced RAW 264 cells and subjected to RT-PCR analysis. B) Left: ALP staining of differentiation-induced MC3T3-E1 cells. Right: Gene expression of AACS and SCOT. Total RNAs were prepared from non-induced or induced MC3T3-E1 cells and subjected to RT-PCR analysis. Each expression level was normalized to the expression level of β-actin. The mean ± SD is shown. (n = 4) Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with the control.

and MC3T3-E1 osteoblasts. TRAP staining was used as the differentiation marker of osteoclasts. AACS mRNA expression was detected in undifferentiated cells and increased in differentiated RAW 264 cells (Fig. 3A). Alterations in HMGCR and FAS gene expression were also observed during osteoclast differentiation. SCOT mRNA expression levels were not changed by the differentiation. In MC3T3-E1 cells, no significant changes were detected in the gene expression of AACS and SCOT during osteoblast differentiation (Fig. 3B). These results suggest that ketone bodies are actively utilized in differentiated osteoclasts for the lipogenic pathway via AACS, rather than in osteoblasts.

Human and animal experiments have implicated pro-inflammatory cytokines, including IL-1, tumor necrosis factor-α (TNF-α), and IL-6, as primary mediators of accelerated bone loss [27]. Our data demonstrated that not only AACS but also IL-6 mRNA expression was increased in femur bones by HFD feeding (Fig. 2C). These results suggest that the HFD causes inflammatory responses in bone, such as the up-regulation of IL-6 production, and one of these responses might be the activation of ketone-lipid metabolism. To clarify this possibility, we examined the effect of the IL-6 protein on AACS gene expression in osteoclast and osteoblast cell lines (Fig. 4). After IL-6 treatment, AACS mRNA expression was

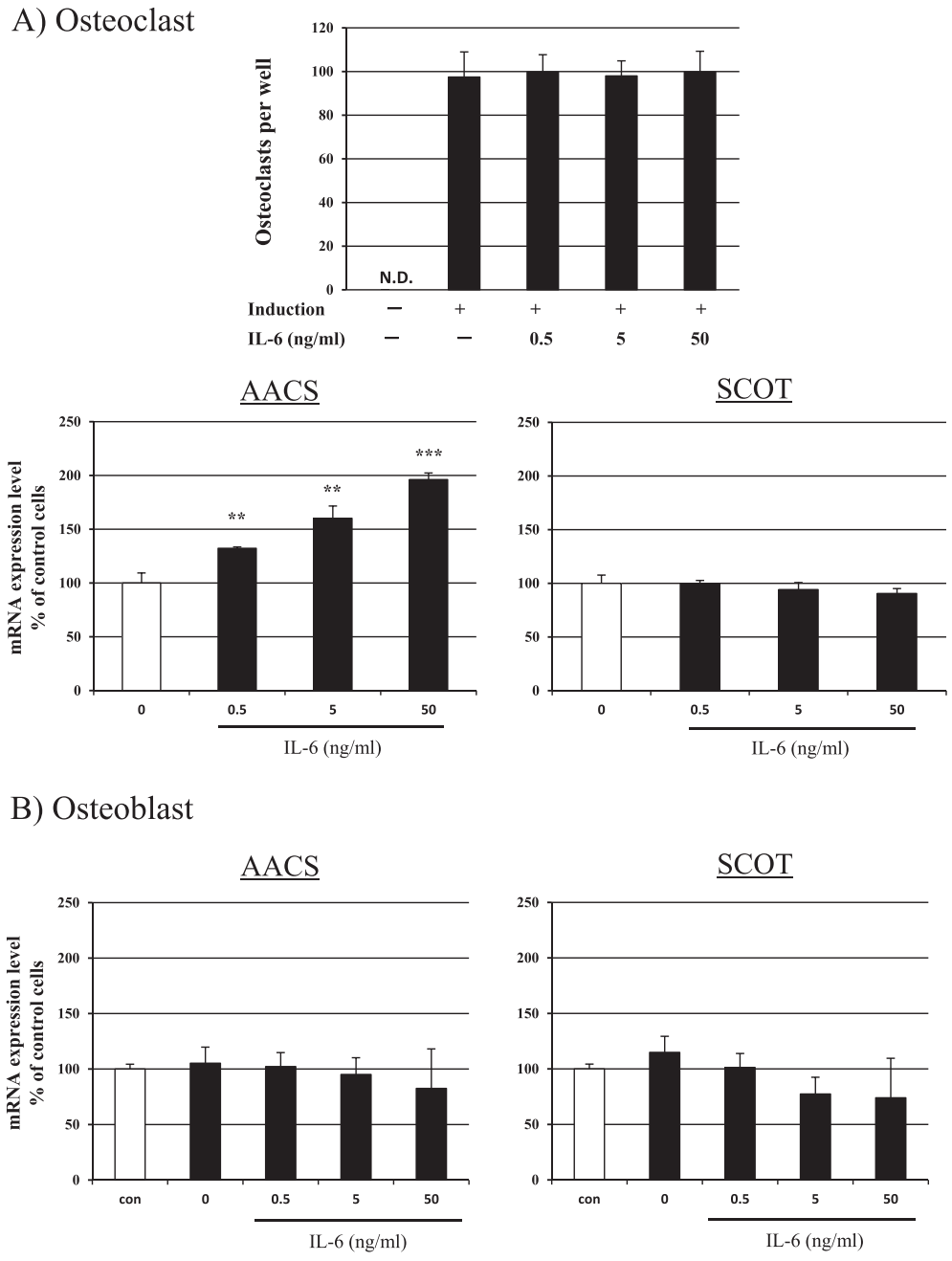


Fig. 4. Effect of inflammatory cytokine on the gene expression of ketone body-utilizing enzymes in osteoclasts and osteoblasts. A) Effect of IL-6 on the number of TRAP-positive cells (upper panel) and the gene expression of AACS and SCOT (lower panels). After IL-6 treatment for 24 h, total RNAs were prepared from RAW 264 cells and subjected to RT-PCR analysis. B) Effect of IL-6 on the gene expression of AACS and SCOT. After IL-6 treatment for 24 h, total RNAs were prepared from MC3T3-E1 cells and subjected to RT-PCR analysis. Con indicates the SCOT expression level in non-induced cells. Each expression level was normalized to the expression level of β-actin. The mean ± SD is shown. (n = 4). Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with the control.

increased in differentiated RAW 264 cells (Fig. 4A). The TRAP-positive cell number and SCOT mRNA expression level were not changed by IL-6 treatment. On the other hand, in MC3T3-E1 cells, no increase was observed in either AACS or SCOT (Fig. 4B). These results suggest that inflammatory cytokines up-regulate AACS gene expression in differentiated osteoclasts but not in osteoblasts.

4. Discussion

In the present study, we showed that the AACS gene was expressed in bone, was mainly present in the epiphysis region, and was induced by high-fat diet-induced obesity. This mRNA expression profile was similar to that of HMGCR, which is known as the rate-limiting enzyme for cholesterol synthesis; but such a profile was not observed for SCOT, which is known as the enzyme responsible for the activation of ketone bodies for energy generation. These results suggest that HFD-induced obesity stimulates ketone body utilization for the mevalonate pathway rather than using ketone bodies as an energy source.

Moreover, we revealed that AACS gene expression was significantly up-regulated by IL-6 treatment only in osteoclasts, but the number of TRAP-positive RAW 264 cells was not affected (Fig. 4A). These facts suggest that the increase in AACS expression in the femurs of HFD-induced obese mice is due to the inflammatory activation of osteoclasts. Previous studies have demonstrated that the HFD contributes to obesity in association with a state of chronic inflammation [28]. Thus, an increase in IL-6 expression in whole femurs (Fig. 2C) was due to the inflammatory response in the bone caused by HFD feeding. Because abnormal activation of osteoclasts by IL-6 causes an imbalance between bone resorption and bone formation, IL-6 is one of the factors in osteoporosis [29]. HFD-induced obesity correlates with a decrease in BMD [2,18]. Therefore, an activation of ketone body utilization by IL-6 might stimulate bone resorption, and AACS likely plays an important role for the relationship between osteoporosis and diet-induced obesity.

Our previous studies have demonstrated that knockdown of AACS in vivo decreases total blood cholesterol, inhibits the differentiation of 3T3-L1 cells and suppresses the expression of the adipocyte markers, peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) [11,12]. These results suggest that ketone body metabolism via AACS activity plays an important role not only in cholesterol homeostasis but also in adipogenic differentiation. Motyl et al. demonstrated that the C/EBP family enhances bone marrow adiposity and bone resorption [30]. Mirzaei et al. reported that serum IL-6 was significantly higher in an osteopenic group than a non-osteopenic group, and over expression of PPARγ in obese humans may have a critical role in relationship between obesity and bone loss [31]. Taken together, it is possible that AACS plays a key role in bone marrow adiposity during obese state via the activation of adipogenic transcriptional factors, such as C/EBP family and PPARγ. Furthermore, cholesterol biosynthesis, which is controlled by HMGCR activity, is a key factor in isoprenoid-dependent IL-6 mediated inflammation [32], and anti-HMGCR drug statins enhance new bone formation in rodents [14]. Because the AACS expression pattern was similar to that of HMGCR in the bones of HFD-induced obese mice and IL-6-induced inflammatory osteoclasts, ketone body utilization via AACS may contribute to the ossification effect of statins. Further studies are recommended to clarify the role of AACS in osteoclasts and bone metabolism.

Conflict of interest

The authors in this article indicated no potential conflicts of interest.

Acknowledgment

This work was supported in part by grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (JSPS KAKENHI Grant Number 15K08048). We are grateful to Dr. Michiaki Kiryu, Department of Health Chemistry, University of Toho for his important contributions to the experiments with RAW264 cells.

References

[1] C.J. Bailey, On the physiology and biochemistry of obesity, Sci. Prog. 65 (1978) 365–393.
[2] A. Alsahli, K. Kiefhaber, T. Gold, M. Muluke, H. Jiang, S. Cremers, U. Schulze-Späte, Palmitic Acid Reduces Circulating Bone Formation Markers in Obese Animals and Impairs Osteoblast Activity via C16-ceramide Accumulation, Calcif Tissue Int, 2016.
[3] M.A. Bredella, C.M. Gill, A.V. Gerweck, M.G. Landa, V. Kumar, S.M. Daley, M. Torriani, K.K. Miller, Ectopic and serum lipid levels are positively associated with bone marrow fat in obesity, Radiology 269 (2013) 534–541.
[4] C.J. Edwards, D.J. Hart, T.D. Spector, Oral statins and increased bone-mineral density in postmenopausal women, Lancet 355 (2000) 2218–2219.
[5] N. Auestad, R.A. Korsak, J.W. Morrow, J. Edmond, Fatty acid oxidation and ketogenesis by astrocytes in primary culture, J. Neurochem. 56 (1991) 1376–1386.
[6] G.A. Mitchell, S. Kassovska-Bratinova, Y. Boukaftane, M.F. Robert, S.P. Wang, L. Ashmarina, M. Lambert, P. Lapierre, E. Potier, Medical aspects of ketone body metabolism, Clin. Invest Med. 18 (1995) 193–216.
[7] T. Fukui, M. Ito, K. Tomita, Purification and characterization of acetoacetyl-CoA synthetase from Zoogloea ramigera I-16-M, Eur. J. Biochem. 127 (1982) 423–428.
[8] M. Ito, T. Fukui, M. Kamokari, T. Saito, K. Tomita, Purification and characterization of acetoacetyl-CoA synthetase from rat liver, Biochim. Biophys. Acta 794 (1984) 183–193.
[9] G. Endemann, P.G. Goetz, J. Edmond, H. Brunengraber, Lipogenesis from ketone bodies in the isolated perfused rat liver. Evidence for the cytosolic activation of acetoacetate, J. Biol. Chem. 257 (1982) 3434–3440.
[10] S. Hasegawa, K. Noda, A. Maeda, M. Matsuoka, M. Yamasaki, T. Fukui, Acetoacetyl-CoA synthetase, a ketone body-utilizing enzyme, is controlled by SREBP-2 and affects serum cholesterol levels, Mol. Genet. Metab. 107 (2012) 553–560.
[11] S. Hasegawa, Y. Ikeda, M. Yamasaki, T. Fukui, The role of acetoacetyl-CoA synthetase, a ketone body-utilizing enzyme, in 3T3-L1 adipocyte differentiation,, Biol. Pharm. Bull. 35 (2012) 1980–1985.
[12] M. Yamasaki, S. Hasegawa, T. Kitani, K. Hidai, T. Fukui, Differential effects of obesity on acetoacetyl-CoA synthetase gene in rat adipose tissues, Eur. J. Lipid Sci. Technol. 109 (2007) 617–622.
[13] H. Sato, N. Takahashi, M. Nakamoto, M. Ohgami, M. Yamazaki, T. Fukui, Effects of streptozotocin-induced diabetes on acetoacetyl-CoA synthetase activity in rats, Biochem. Pharmacol. 63 (2002) 1851–1855.
[14] G. Mundy, R. Garrett, S. Harris, J. Chan, D. Chen, G. Rossini, B. Boyce, M. Zhao, G. Gutierrez, Stimulation of bone formation in vitro and in rodents by statins, Science 286 (1999) 1946–1949.
[15] M. Ohnuki, N. Takahashi, M. Yamasaki, T. Fukui, Different localization in rat brain of the novel cytosolic ketone body-utilizing enzyme, acetoacetyl-CoA synthetase, as compared to succinyl-CoA:3-oxoacid CoA-transferase, Biochim. Biophys. Acta 1729 (2005) 147–153.
[16] I. Miwa, J. Okudo, K. Maeda, G. Okuda, Mutarotase effect on colorimetric determination of blood glucose with -D-glucose oxidase, Clin. Chim. Acta 37 (1972) 538–540.
[17] Y. Harano, K. Kosugi, T. Hyosu, S. Uno, Y. Ichikawa, Y. Shigeta, Sensitive and simplified method for the differential determination of serum levels of ketone bodies, Clin. Chim. Acta 134 (1983) 327–336.
[18] S. Botolin, L.R. McCabe, Bone loss and increased bone adiposity in spontaneous and pharmacologically induced diabetic mice, Endocrinology 148 (2007) 198–205.
[19] Y.C. Shyng, H. Devlin, P. Sloan, The effect of streptozotocin-induced experimental diabetes mellitus on calvarial defect healing and bone turnover in the rat,, Int. J. Oral Maxillofac. Surg. 30 (2001) 70–74.
[20] J.C. Krakauer, M.J. McKenna, N.F. Buderer, D.S. Rao, F.W. Whitehouse, A.M. Parfitt, Bone loss and bone turnover in diabetes, Diabetes 44 (1995) 775–782.
[21] M. Ohgami, N. Takahashi, M. Yamasaki, T. Fukui, Expression of acetoacetyl-CoA synthetase, a novel cytosolic ketone body-utilizing enzyme, in human brain, Biochem. Pharmacol. 65 (2003) 989–994.
[22] R. Narishima, M. Yamasaki, S. Hasegawa, S. Yoshida, S. Tanaka, T. Fukui, Leptin controls ketone body utilization in hypothalamic neuron, Neurosci. Lett. 490 (2011) 185–190.
[23] S. Kim, I. Sohn, J.I. Ahn, K.H. Lee, Y.S. Lee, Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model, Gene 340 (2004) 99–109.

[24] C.J. Rosen, M.L. Bouxsein, Mechanisms of disease: is osteoporosis the obesity of bone? Nat. Clin. Pract. Rheumatol. 2 (2006) 35–43.
[25] C.A. Lima, A.C. Lyra, R. Rocha, G.O. Santana, Risk factors for osteoporosis in inflammatory bowel disease patients, World J. Gastrointest. Pathophysiol. 6 (2015) 210–218.
[26] L.J. Raggatt, N.C. Partridge, Cellular and molecular mechanisms of bone remodeling, J. Biol. Chem. 285 (2010) 25103–25108.
[27] G.R. Mundy, Osteoporosis and inflammation, Nutr. Rev. 65 (2007) S147–S151.
[28] L. Zhao, S. Zhong, H. Qu, Y. Xie, Z. Cao, Q. Li, P. Yang, Z. Varghese, J.F. Moorhead, Y. Chen, X.Z. Ruan, Chronic inflammation aggravates metabolic disorders of hepatic fatty acids in high-fat diet-induced obese mice, Sci. Rep. 5 (2015) 10222.
[29] S.L. Teitelbaum, Bone resorption by osteoclasts, Science 289 (2000) 1504–1508.
[30] K.J. Motyl, M. Raetz, S.A. Tekalur, R.C. Schwartz, L.R. McCabe, CCAAT/enhancer binding protein β-deficiency enhances type 1 diabetic bone phenotype by increasing marrow adiposity and bone resorption, Am. J. Physiol. Regul. Integr. Comp. Physiol. 300 (2011) R1250–R1260.
[31] K. Mirzaei, A. Hossein-Nezhad, S.M. Eshaghi, H. Ansar, A. Najmafshar, The relationship between obesity and bone mineral density: evidence for a role of peroxisome proliferator-activated receptor gamma, Minerva Endocrinol. 40 (2015) 177–185.
[32] S. Omoigui, Cholesterol synthesis is the trigger and isoprenoid dependent interleukin-6 mediated inflammation is the common causative factor and therapeutic target for atherosclerotic vascular disease and age-related disorders including osteoporosis and type 2 diabetes, Med. Hypotheses 65 (2005) 559–569.