Effects of Acetate on Lipid Metabolism in Muscles and Adipose Tissues of Type 2 Diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) Rats

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We have reported that orally administrated acetate contributed to suppression of lipogenesis in the liver and to reduction of lipid accumulation in the adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats. The aim of this study was to investigate the effect of acetate on the skeletal muscle and adipose tissues. Treatment with acetate showed a higher rate of oxygen consumption and a smaller size of lipid droplets in white adipose and brown adipose tissues. An analysis by Northern blotting revealed that the transcripts of myoglobin and Glut4 genes in the abdominal muscle of the OLETF rats were increased by acetate treatment, while the transcripts of lipolytic genes increased in the white adipose and brown adipose tissues. It is possible that acetate has effects on lipid metabolism in the skeletal muscles and the adipose tissues, and has functions that work against obesity and obesity-linked type 2 diabetes.

Key words: acetate; lipid metabolism; obesity; Otsuka Long-Evans Tokushima Fatty (OLETF) rats; adipose tissue

Obesity causes excess fat accumulation in adipose tissue, skeletal muscles and the liver. Especially, muscle lipid accumulation causes a predisposition to decrease insulin sensitivity.1–4) We have reported that acetate had a profound anti-obese and anti-diabetic function in animal models of obesity and type 2 diabetes.5) Acetate is an endogenous metabolite of fatty acid β-oxidation in the liver mitochondria under starving conditions.6) Acetate formed in the liver is excreted into the blood stream and is utilized as a biological fuel in the skeletal muscles.7) Acetate was then absorbed immediately taken up from the intestine and excreted under fed conditions, orally administered acetate was utilized as a biological fuel in the skeletal muscles and adipose tissues. Treatment with acetate showed a higher rate of oxygen consumption and a smaller size of lipid droplets in white adipose and brown adipose tissues. An analysis by Northern blotting revealed that the transcripts of myoglobin and Glut4 genes in the abdominal muscle of the OLETF rats were increased by acetate treatment, while the transcripts of lipolytic genes increased in the white adipose and brown adipose tissues. It is possible that acetate has effects on lipid metabolism in the skeletal muscles and the adipose tissues, and has functions that work against obesity and obesity-linked type 2 diabetes.

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Materials and Methods

Acetate administration experiment. Four-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats as a non-diabetic control were obtained from Otsuka Pharmaceutical (Tokushima, Japan) and fed a normal laboratory diet (CE2, Clea, Tokyo) for 1 week to stabilize the metabolic conditions. The rats were housed individually in an air-conditioned room at approximately 25 °C with alternating 12-h periods of light and dark (light, 8:00–20:00). All the animals were allowed free access to water and the appropriate diet. The OLETF rats were randomly assigned to two groups: water-injected and acetate-injected. The water-injected group was given distilled water at 5 ml/kg of body weight, and the acetate-injected group was given 5.2 mg/kg of BW (100 l/100 g of body weight) between 9:30 and 10:30 daily 5 d a week for 6 months. The initial body weights of the LETO, water-administered OLETF, and acetate-administered OLETF rats were 84 ± 26, 126 ± 10, and 125 ± 4 g respectively. Food consumption and body weight were recorded every day. At 32 weeks of age, the rats were anesthetized by intraperitoneal injection of Nembutal, (100 μl/100 g of body weight), and muscles, white and brown adipose

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Abbreviations: OLETF, Otsuka Long-Evans Tokushima Fatty; LETO, Long-Evans Tokushima Otsuka; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; LCACD, long-chain acyl-CoA dehydrogenase; 3KACT, 3-ketoacyl-CoA thiolase; PPAR, peroxisome proliferator-activated receptor; AMPK, AMP activated protein kinase
tissues were immediately isolated, weighed, frozen in liquid nitrogen, and stored at −80°C for subsequent isolation of RNA. Part of the white adipose and brown adipose tissues of each rat was subjected to histochemical analysis. Tissue samples were taken 24 h after injection of water or acetate. The care and use of the animals in this study followed the guidelines of Okayama Prefectural University and the laws and notifications of the Japanese government.

$O_2$ consumption rates of OLETF rats administered water or acetate and LETO rats. Oxygen consumption was measured with an O$_2$/CO$_2$ metabolism measuring system (model MK-5000, Muromachi, Kikai, Tokyo). Each rat was in a sealed chamber with an air flow of 3.5 l/min for 24 h at 25°C with free access to water and the diet. The consumed oxygen concentration ($O_2V$) was calculated.

Preparation of cRNA probe for Northern blotting. The cDNA fragments for rat acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), long-chain acyl-CoA dehydrogenase (LCAD), 3-ketoacyl-CoA thiolase (3KACT), myoglobin (MYO), GLUT4, KLF15, PPARα, and PPARγ were obtained by polymerase chain reaction from a cDNA library for the rat heart (Takara Shuzo, Shiga, Japan) and rat brown adipose tissue mRNA that had been isolated with an mRNA isolation library for the rat heart (Takara Shuzo, Shiga, Japan) and rat brown adipose tissue mRNA that had been isolated with an mRNA isolation library for the rat heart (Takara Shuzo, Shiga, Japan) and rat brown adipose tissue. First-strand cDNA was prepared using Superscript II transcriptase primed with oligo-dT. The PCR primers used were as follows: ACC: 5’-CCCTCCAGTAGCAGCTG-3’ and 3’-primer, 5’-GACCCCTGTGGTGTTTGAGAAC-3’; FAS: 5’-GGAGTGGCAGATGGTGCTAAAC-3’ and 3’-primer, 5’-GGTTGACACAGAGATGCCATTC-3’; 3KACT: 5’-AGAAGACTGGACACGATACGCC-3’ and 3’-primer, 5’-TATTTTTCAACCTCGAGCGCTT-3’; PPARα: 5’-GACCCCTGTGGTGTTTGAGAAC-3’; PPARγ: 5’-GGAGTGGCAGATGGTGCTAAAC-3’. The amplified products were subcloned into pGEM-T Easy vector (Promega, Madison, WI). The plasmid DNAs were used in DIG-RNA labeling by DIG labeling system (Roche), and the cRNA probes were used as probes in Northern blotting.

Northern blotting. Total RNA was extracted from each tissue using Isogen (Nippon Gene, Toyama, Japan). The RNA was dissolved in diethylpyrocarbonate-treated water and quantified by its absorbance at 260 nm. Total extracted RNA was denatured with formaldehyde and electrophoresed in 1% agarose gel containing formaldehyde; the 28 and 18 S rRNA fragments were visualized overnight at 68°C with DIG-labeled cRNA probes recognizing ACC, FAS, LCAD, 3KACT, PPARα, PPARγ, MYO, GLUT4, and KLF15 respectively. The filter was washed twice with 2 × SSC and 0.1% SDS at room temperature, further washed twice with 0.1 × SSC and 0.1% SDS at 68°C for 30 min, incubated with a blocking solution and anti-DIG antibody-conjugated alkaline phosphate for 30 min at room temperature, and then detected using CDP-Star (Roche) by exposure to an X-ray film. The amount of each transcript was quantified with an image analyzer (Gel Print 2000, Genomic Solutions, Tokyo) and was expressed as relative intensity. Each membrane was reprobed with β-actin to ensure that the changes observed did not reflect any unequal loading of the samples.

Histological analysis of white adipose and brown adipose tissues. Small samples of tissues were fixed with a 20% formalin neutral buffered solution (Wako, Osaka, Japan) and embedded in paraffin.
genes, the LCACD and 3KACT mRNA levels were not significantly different between the water- and acetate-administered OLETF rats (Fig. 3).

**Effects of acetate on the change in the AMP/ATP ratio and the phosphorylation of AMPK in abdominal muscle**

We determined the adenine nucleotide concentration in perchloric acid extracts of the abdominal muscle after intragastric injection of 10.5 mg/kg of BW of acetate. The AMP content of the muscle increased about 3-fold 2 min after injection of the acetate (Table 1). An increase in the AMP/ATP ratio should induce phosphorylation of AMPK, following AMPK activation.

Phosphorylated AMPK in the muscles of the water- and acetate-injected OLETF rats was determined by western blotting. In the acetate-injected OLETF rats, the protein level of phosphorylated AMPK was higher than in the water-injected rats (Fig. 4A). In a similar way, in the acetate-injected SD rats, phosphorylation of AMPK was seen 3 min after injection of acetate (Fig. 4B).

**Effects of acetate on the mRNA expression involved in lipid metabolism in white adipose and brown adipose tissues**

To determine the effects of acetate administration on fatty acid metabolism in adipose tissues, the mRNA
levels associated with lipid metabolism were measured by Northern blotting (Fig. 5). The genes associated with lipogenic enzymes did not change significantly between the water-administered and the acetate-administered OLETF rats (Fig. 5A-2), but compared to the water-administered OLETF rats, the acetate-administered ones tended to be enhanced in transcripts of the lipolytic enzymes, LCAD, 3KACT, and PPARδ, higher by about 1.7, 1.6, and 1.7 times respectively (Fig. 5A-1). Also, in brown adipose tissue, the transcripts of the LCAD, 3KACT, and PPARδ genes increased significantly (Fig. 5B).

Effects of acetate on lipid accumulation in white adipose and brown adipose tissues

Histological analysis indicated that the water-administered OLETF rats accumulated large-size droplets of lipid, although the acetate-administered OLETF rats showed smaller lipid droplets than the water-administered OLETF rats (Fig. 6A). In a similar way, lipid droplets of a smaller size were observed in brown adipose tissue in acetate-administered OLETF rats than the water-administered OLETF rats (Fig. 6B).

Discussion

We have reported that acetate has an inhibitory effect on fatty acid synthesis in the liver and that it protects against lipid accumulation in adipose tissue and ameliorates obesity and diabetes in OLETF rats. Orally administered acetate was immediately taken up from the intestine, absorbed by the liver, and this increased the AMP/ATP ratio. An increase in the AMP/ATP ratio stimulates the phosphorylation of AMPK, which regulates a number of enzymes involved in lipid homeostasis. It is possible that acetate administration inactivated carbohydrate-responsive element binding protein (ChREBP), which is involved with the transcription of lipogenic genes via phosphorylation by AMPK in the liver. Accumulation of excess lipid in the liver or skeletal muscle disturbs insulin signaling.

In this study, we focused on the effects of acetate in the skeletal muscle and adipose tissues. Acetate-administered OLETF rats showed increased oxygen consumption (Fig. 1). The transcripts of myoglobin and GLUT4 increased significantly in the abdominal muscles of the OLETF rats administered acetate as compared with the OLETF rats administrated water. The ingested acetate activated AMPK by increasing the AMP/ATP ratio in

### Table 1. Stimulation of the AMP/ATP Ratio in Abdominal Muscle after Oral Administration of Acetate

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>ATP</th>
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<td>0</td>
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<td>0.7</td>
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<td>3.4 ± 0.3</td>
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<tr>
<td>2</td>
<td>24.6 ± 3.0</td>
<td>3.9 ± 0.1**</td>
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The effects are shown of an acetate injection on the adenine nucleotide content and the AMP/ATP ratio in abdominal muscle. Acetate at concentration of 10.5 mg/kg of BW was orally injected into 10-week-old male SD rats (body weight, 350–450 g; n = 5–7 for each point). The abdominal muscle was excised at each time point indicated after the injection of 10.5 mg/kg of BW of acetate into an individual rat, then frozen and lyophilized, and the nucleotides were extracted. Each data value is expressed as the mean ± SE for five to seven rats. *p < 0.05, **p < 0.01, compared with the 0 min point for the control rats.
Fig. 5. Effects of Acetate Administration on mRNA Levels of Lipolytic and Lipogenic Genes in the White Adipose (A) and Brown Adipose (B) Tissues of OLETF Rats.
The experimental conditions were the same as in Fig. 2, except that 10-μg aliquots of total RNA were subjected to electrophoresis.

A-1

<table>
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<td>β-actin</td>
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A-2

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B

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<tr>
<td>Relative intensity</td>
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Fig. 6. Histological Sections of White Adipose (A) and Brown Adipose (B) Tissues from LETO, Water-Administered OLETF, and 5.2 mg/kg of BW of Acetate-Administered OLETF Rats.
A. White adipose tissue (×100 magnification). B. Brown adipose tissue (×200 magnification). Sections were cut and stained with hematoxylin and eosin. Scale bars: A, 50 μm; B, 30 μm.
abdominal muscle, as in the liver. Acetate is converted to acetyl-CoA by a catalytic activity of acetyl-CoA synthetase in the cytosol. In our previous study, cytosolic type acetyl-CoA synthetase (AceCS1) was widely distributed in tissues containing skeletal muscle. After acetate administration, the AMP content increased within 5 min of injection (Table 1), then AMPK was phosphorylated in the muscle by stimulation of a higher AMP/ATP ratio. Myoglobin is a cytosolic hemoprotein expressed selectively in cardiac and skeletal myocytes, where it functions to facilitate the diffusion of oxygen and to maintain mitochondrial respiration during muscle contraction. In adult mammals, myoglobin expression is modulated by environmental stimuli, including chronic hypoxia and endurance exercise training.

Myocyte enhancer factor 2 (MEF2) is a transcription factor involved in skeletal muscle differentiation and the regulation of myoglobin transcription. Activation of MEF2 in skeletal muscle is regulated via parallel intracellular signaling pathways, including activation of AMPK. It has been reported that MEF2 plays an important role in GLUT4 expression in skeletal muscle and that GLUT4 gene regulation is associated with AMPK activation. Increases in the transcripts of the myoglobin and GLUT4 genes by acetate-treatment might be mediated by activation of MEF2 via AMPK activation. Transcription of the KLF15 gene tended to increase in the muscle with the administration of acetate. KLF15 has been reported to regulate transcription of the GLUT4 gene. The physiological relationship between KLF15 and AMPK is now under investigation.

The important role of AMPK is also shown by its association with adiponectin. Adiponectin enhances insulin sensitivity and increases fatty-acid oxidation. That function was shown to involve the activation of AMPK. The anti-diabetic drugs metformin and rosiglitazone can also activate AMPK, suggesting that AMPK plays an important role in the regulation of glucose and lipid metabolism.

On the other hand, acetate treatment had an effect in protecting against hypertrophy of the adipocytes. Lipid accumulation in brown adipose tissue was also inhibited by acetate treatment. In white adipose tissue, mRNA expression of fatty acid oxidizing enzymes was stimulated in the OLETF rats administered acetate. Activation of AMPK in rodent adipocytes was reported to lead to decreased lipogenic flux, decreased triglycerides synthesis, and an increase in fatty acid oxidation. Activation of AMPK also had an inhibitory action on free fatty acid release and decreased the availability of fatty acid in the plasma, and consequently it is beneficial in insulin-resistant states such as type 2 diabetes. Activation of AMPK in adipocytes has been found to lower the expression and secretion of pro-inflammatory cytokines TNF-α and interleukin-6 (IL-6). Whether acetate treatment activates AMPK in white and brown adipose tissues is now under investigation.

The data obtained here prompt the suggestion that acetate has anti-obese and anti-diabetic functions in the skeletal muscles and adipose tissues of an animal model of obesity and type 2 diabetes.

Acknowledgments

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References


