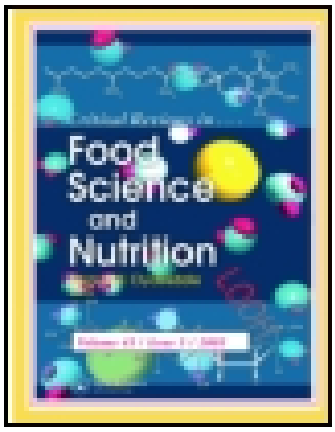

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Biological Function of Acetic Acid- Improvement of Obesity and Glucose Tolerance by Acetic Acid in Type 2 Diabetic Rats

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Biological function of acetic acid -Improvement of obesity and glucose tolerance by acetic acid in type 2 diabetic rats

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Abstract

Fatty acids derived from adipose tissue are oxidized by □-oxidation to form ketone bodies as final products under the starving condition. Previously we found that free acetic acid was formed concomitantly with the production of ketone bodies in isolated rat liver perfusion, and mitochondrial acetyl CoA hydrolase was appeared to be involved with the acetic acid production. It was revealed that acetic acid was formed as a final product of enhanced □-oxidation of fatty acids and utilized as a fuel in extra hepatic tissues under the starving condition. Under the fed condition,

-oxidation is suppressed and acetic acid production is decreased. When acetic acid was taken daily by obesity-linked type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats under the fed condition, it protected OLETF rats against obesity. Furthermore, acetic acid contributed to protect from the accumulation of lipid in the liver as well as abdominal fat of OLETF rats. Transcripts of lipogenic genes in the liver were decreased, while transcripts of myoglobin and Glut4 genes in abdominal muscles were increased in the acetic acid–administered OLETF rats. It is indicated that exogenously-administered acetic acid would have effects on lipid metabolisms both in liver and skeletal muscles and have function that work against obesity and obesity-linked type 2 diabetes.

Key Words

acetic acid; AMPK; lipid metabolism; obesity; type 2 diabetes

Abbreviations

AceCS; acetyl CoA synthetase

AMPK; AMP-activated protein kinase

OLETF; Otsuka Long-Evans Tokushima Fatty

LETO; Long-Evans Tokushima Otsuka

OGTT; oral glucose tolerance test

ChREBP; carbohydrate-responsive element-binding protein

ACC; acetyl CoA carboxylase

ME; malic enzyme

G6PD; glucose 6-phosphate dehydrogenase

L-PK; liver pyruvate kinase

FAS; fatty acid synthetase

LCACD; long-chain acyl CoA dehydrogenase

3KACT; 3-ketoacyl-CoA thiolase

PPAR□□ peroxisome proliferator-activated receptor□□

SREBP-1; sterol regulatory element-binding protein-1

The liver is the principal organ responsible for the catabolism and anabolism of fatty acid depending on the physiological conditions. Under the starving condition, fatty acids derived from adipose tissue are oxidized by □-oxidation to form ketone bodies as final products. Ketone bodies produced are excreted to blood stream and utilized as physiological fuel in extrahepatic tissues. Previously we found that free acetic acid that is detected in ruminant as a compound produced by bacterial fermentation (Annison and Armstrong 1970; Ballard 1972; Knowles et al. 1974) and also usually found in high concentration in mammalian blood plasma after administration of alcohol (Lundquist 1962; Lundquist et al. 1962; Suokas et al. 1984) was formed concomitantly with the production of ketone bodies in isolated rat liver perfusion (Yamashita et al. 2001). Possible reaction forming free acetic acid in cells was considered to be catalyzed by acetyl CoA hydrolase. In order to investigate the involvement of mitochondrial acetyl CoA hydrolase in the regulation of acetyl CoA metabolism and acetic acid production, we isolated an acetyl CoA hydrolase to apparent homogeneity from rat liver mitochondria, and examined its kinetic

properties and its regulatory role for acetic acid production in liver mitochondria (Yamashita et al. 2006). The purified enzyme was 43-kDa in molecular mass by SDS-PAGE. Internal amino acid sequencing of this enzyme revealed that it was identical with mitochondrial 3-ketoacyl CoA thiolase. 3-ketoacyl CoA thiolase is the enzyme for catalyzing the 4th step reaction of □-oxidation and production of acetyl CoA. It was suggested that this enzyme has two kinds of activities, 3-ketoacyl CoA thiolase activity and acetyl CoA hydrolase activity. Kinetic study clearly indicated that this enzyme controlled those activities depending on each substrate level. When □-oxidation is promoted excessively and production of acetyl CoA is enhanced in mitochondria, following 3-ketoacyl CoA thiolase activity is inhibited by acetyl CoA, while it turns on the hydrolase activity, following the production of acetic acid is accelerated (Yamashita et al. 2006).

Free acetic acid produced in the liver was found to be hardly metabolized in liver mitochondria, and provided to extrahepatic tissues as a fuel in a similar way to the physiological role of ketone bodies (Yamashita et al. 2001). In peripheral tissues, acetic acid is found to be activated by acetyl CoA synthetase 2 (AceCS2) to produce

acetyl CoA and it oxidized in the TCA cycle for fuel production (Fujino et al. 2001).

Respiratory activity of liver mitochondria with acetic acid was sluggish regardless of dietary conditions. This rate was similar to that of acetoacetic acid and about one tenth to that of succinate. Ketone bodies are not oxidized in liver cells due to the lack of 3-ketoacylCoA transferase. However, in contrast with liver, heart mitochondria oxidized actively all kinds of the respiratory substrates examined including acetic acid. In the case of the heart mitochondria, oxidation rate of each substrate was faster in the starved condition than that in the fed condition (Yamashita et al. 2001). Based on those results, it is indicated that acetic acid is formed as a final product of enhanced □-oxidation of fatty acids in liver and is utilized as a fuel in extra hepatic tissues.

Under the fed condition, □-oxidation is suppressed and acetic acid production is decreased. When we take acetic acid exogenously under fed condition, it had not been known how the acetic acid would act physiologically in the body. So we investigated the physiological role of exogenous acetic acid taken under fed condition (Yamashita et al. 2007). When acetic acid is orally taken up, it may be

readily absorbed into the blood stream and then it would be incorporated into tissues. Acetic acid absorbed is converted to acetyl CoA with the formation of AMP by the catalytic action of cytosolic acetyl CoA synthetase (AceCS1) and it may lead to an increase of AMP/ATP ratio in the cytosol. An increase of AMP/ATP ratio in the cytosol leads to an activation of AMP-activated protein kinase (AMPK). AMPK is a multi-subunit protein kinase which acts as a key metabolic master switch, and regulates a number of enzymes involved in lipid homeostasis. We investigated whether orally administered acetic acid would contribute to the lipid metabolism through the activity of AMPK. When we injected acetic acid to SD rats, we found that acetic acid had an effect of lower weight gain for the SD rats. So we investigated the function of acetic acid using OLETF rats that are a genetic model of animals showing hyperglycemic obesity with hyperinsulinemia and insulin resistance (Shima 1999).

Four-week-old male OLETF rats and, LETO as non-diabetic control rats were used for this study. Those rats were fed on a normal laboratory diet (CE2, Clea, Tokyo, Japan) for 1 week to stabilize the metabolic conditions. 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 acetic acid-injected. The water-injected group was given distilled water at 5ml/kg of body weight and the acetic acid-injected group was given 1 vol% acetic acid of 5ml/kg of body weight, which is equivalent to 52.5mg/kg body weight in the amount of acetic acid for human as well as rats, each daily 5 days a week for 6 months. The initial body weights of the three groups of rats were not significantly different. The food consumption and body weight were recorded every day.

The body weight change was lower in LETO rats than in the water-administered OLETF rats. After administering acetic acid to the OLETF rats from 5 weeks of age, they began to show a lower weight gain than that of the water-injected group, and at the age of 30 weeks, the average body weight was significantly lower in the acetic acid-injected OLETF rats as well as LETO rats than in the water-injected OLETF rats.

From the total food intakes of three groups of rats, the food efficiencies were calculated. The food efficiency was calculated as the body weight gain divided by

total food intake. The food efficiency was not significantly different between the water-administered OLETF rats and LETO rats, while it was significantly lower in the food efficiency for acetic acid-injected group as compared with the water-injected group.

At 32 weeks of age, the abdominal fat contents of the LETO and acetic acid-administered OLETF rats were also lower by about 80% and 70%, respectively, than that in the water-administered OLETF rats.

The oral glucose tolerance test (OGTT) was performed on fasted rats by administering orally 2g/kg-BW of glucose. Blood was collected from the tail vein immediately before and 30, 60, 90 and 120 min after the administration to determine the plasma glucose levels. The plasma glucose levels in the LETO and acetic acid-administered OLETF rats were significantly lower than that in the water-administered OLETF rats.

The plasma glucose, triglyceride, cholesterol, insulin levels were significantly lower in the LETO and acetic acid-administered OLETF rats than in the water-administered OLETF rats.

We examined about the lipogenesis in the liver. The mRNA levels of lipogenic genes in the liver were analyzed. Compared to water-administered OLETF rats, the acetic acid-administered group was lower in transcripts of the ACC, ME, G6PD, L-PK, and FAS genes, which are lipogenic genes, by about 30%, 60%, 30%, 30%, and 60%, respectively. In respect of the lipolytic genes, the LCACD, 3KACT, and SREBP-1 mRNA levels were not significantly different between the water- and acetic acid-administered OLETF rats.

Histological analysis indicated that the water-administered OLETF rats accumulated higher levels of hepatic lipid, while the acetic acid-administered OLETF rats had a lower hepatic lipid accumulation than the water-administered OLETF rats.

When acetic acid is orally administered, it would be immediately taken up from the intestine, excreted into the blood stream, and be absorbed by tissues. Then acetic acid would be activated to acetyl CoA with the concomitant formation of AMP by the catalytic activity of cytosolic AceCS. A dose of acetic acid at each concentration to SD rats stimulated the plasma acetic acid concentration within 1 min after the injection, although its concentration returned to the basal level within 10 min after

the injection. The AMP content in the liver increased about 3-fold within 30 sec after injection of acetic acid. An increase in the AMP/ATP ratio would induce phosphorylation and activation of AMPK (Moore et al. 1991; Woods et al. 1996; Winder and Hardie 1996; Dyck et al. 1999; Salt et al. 1998; Hardie et al. 1998; Winder and Hardie 1999; Hardie 2003). Phosphorylated AMPK in each liver in water- or acetic acid-injected OLETF rats was analyzed by western blotting. In the acetic acid-injected group, the protein level of phosphorylated AMPK was higher than that in the water-injected group.

AMPK acts as the key metabolic “master switch” and regulates a number of enzymes involved in lipid homeostasis. Activation of AMPK leads to the inactivation of acetyl-CoA carboxylase by phosphorylation, blocking fatty acid synthesis, and permitting the activation of fatty acid oxidation by decreasing intracellular malonyl CoA to generate energy and enhance energy expenditure system. It has been reported that carbohydrate-responsive element-binding protein (ChREBP) was also phosphorylated by AMPK (Kawaguchi et al. 2002). ChREBP, a transcription factor, plays an essential role in glucose-induced gene transcription of liver pyruvate kinase

(L-PK) which catalyzes final step of glycolysis (Yamashita et al. 2002; Kawaguchi et al. 2001). Furthermore, ChREBP has been revealed to activate the transcription of genes involving lipogenesis, including ACC, ME, G6PD, FAS, ACL as well as L-PK (Iizuka et al. 2004; Ishii et al. 2004). ChREBP is inhibited by the phosphorylation through the action of AMPK (Kawaguchi et al. 2002). The phosphorylation of ChREBP by AMPK resulted in the inhibition of its DNA-binding activity and decreased transcriptional activity for lypogenic genes, which associate with fatty acid synthesis from excess carbohydrate in the liver. The ingested acetic acid activates AMPK by an increase of the AMP/ATP ratio in the liver, and it would lead to the decrease of transcripts of lipogenic genes through the phosphorylation of ChREBP.

Intake of acetic acid protected the OLETF rats against obesity. Acetic acid contributed to lowering the accumulation of abdominal fat and protected from the accumulation of lipid in the liver. It has been reported that continuous intake of vinegar (750mg~1.5g acetic acid) had effect on reductions of body weight, body fat accumulation, and waist circumference in human study with obese Japanese subjects (Kondo et al. 2009). Under lipogenic conditions, the synthesis of several lipogenic

enzymes, including ACC, FAS, ACL, ME G6PD and LPK, are induced. Transcripts of lipogenic genes in the liver were decreased in the acetic acid–administered OLETF rats. An accumulation of excess lipid in the liver or skeletal muscle disturbs insulin signaling. So the effect of acetic acid on the marked reduction of lipid content in the adipose tissue and liver would contribute to improving of glucose tolerance and insulin resistance on OLETF rats (Yamashita et al. 2007).

The skeletal muscle is one of the most important insulin-responsive organs in the body, and accumulation of locally derived fat metabolites in it generates a risk that contributes to insulin resistance (Kim et al. 2001; Baron et al. 1988). In order to investigate whether intake of acetic acid contributes to improvement of energy metabolism in skeletal muscle and adipose tissues, we examined effects of acetic acid on those tissues (Yamashita et al. 2009). Oxygen consumptions of OLETF rats administered acetic acid or water as well as LETO rats were determined for whole animals. Acetic acid-administered OLETF rats had higher rates of oxygen consumption than that of water-administered group, and the rate was significantly higher in the active period.

The effects of acetic acid administration on mRNA induction related with energy metabolism in the muscle were examined. Expressions of genes involved with fatty acid □-oxidation in abdominal muscle were not changed between water- and acetic acid administered groups. However, the acetic acid administered group was two times higher in transcripts of the myoglobin and GLUT4 genes in abdominal muscle as compared to those of water administered group. As for the forelegs, in a similar way, the transcripts of the myoglobin and GLUT4 genes were stimulated by the injection of acetic acid, while lipolytic genes were not significantly different between the water- and acetic acid-administered OLETF rats.

We determined concentrations of adenine nucleotides in abdominal muscle of SD rats after injection of 2 vol% acetic acid. The AMP content in muscle increased by three-fold in 2 min after administration of acetic acid. In the acetic acid-administered SD rats, phosphorylation of AMPK was enhanced in 3 min after injection of acetic acid, and that was also seen in acetic acid administered OLETF rats at 32 weeks of age.

To determine the effects of acetic acid administration on fatty acid metabolism

in adipose tissues, the mRNA levels associated with lipid metabolism were measured. The genes associated with lipogenic enzymes did not change significantly between the water- and the acetic acid-administered groups, but transcripts of the lipolytic and those related genes such as LCACD, 3KACT, and peroxisome proliferator-activated receptor PPAR□□genes, were increased with 1.6 to 1.7 times in the acetic acid-administered group as compared to those in the water-administered group. Also, in brown adipose tissue, the transcripts of the LCACD, 3KACT, and PPAR□ genes, which are involved with lipid catabolism, increased significantly in the acetic acid-administered group as compared with water-administered group.

Histological analysis indicated that the water-administered OLETF rats accumulated large-size lipid droplets in white adipose tissue. On the other hand, acetic acid-administered rats showed smaller lipid droplets than those in the white adipose tissue of water-administered rats. In a similar way, lipid droplets of smaller size were observed much more in brown adipose tissues of acetic acid-injected rats than those of water-injected rats.

It is summarized that treatment with acetic acid results in a higher rate of

oxygen consumption and a smaller size of lipid droplets in white adipose and brown adipose tissues on OLETF rats. An analysis of mRNA level revealed that transcripts of myoglobin and Glut4 genes in abdominal muscles of OLETF rats were increased by the treatment of acetic acid, while transcripts of some lipolytic genes tends to be increased in white adipose and increases significantly in brown adipose tissues (Yamashita et al. 2009). It is possible that acetic acid has effects on lipid metabolisms both in skeletal muscles and adipose tissues and has function that work against obesity and obesity-linked type 2 diabetes.

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Figure 1

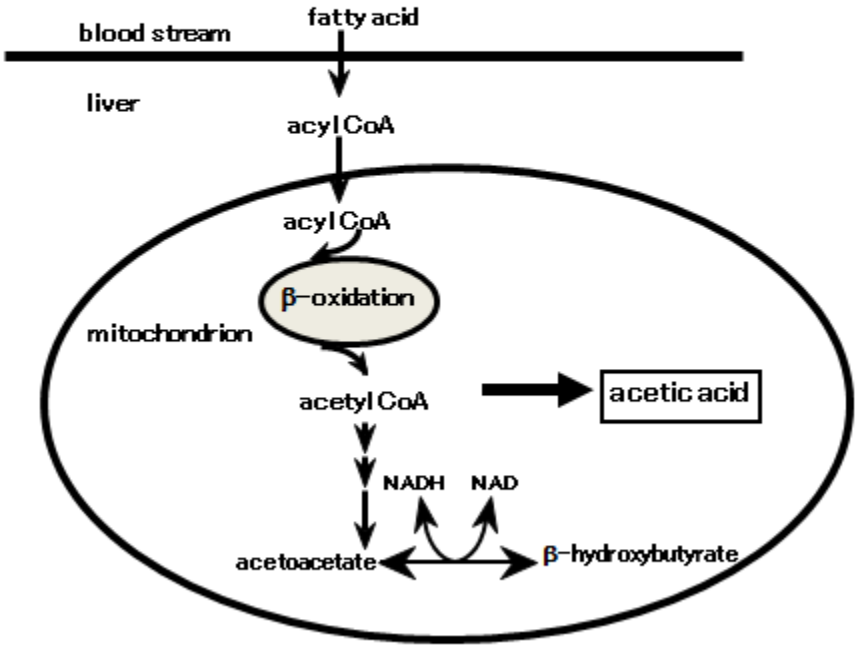

Fig. 1 Generation of ketone bodies and acetic acid through the □-oxidation of fatty acid in liver mitochondria

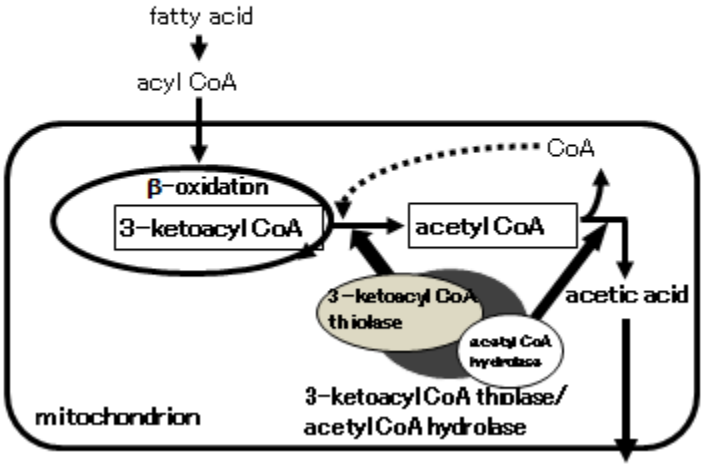

Fig. 2 Production of acetic acid by the activity of acetyl CoA hydrolase in liver mitochondria

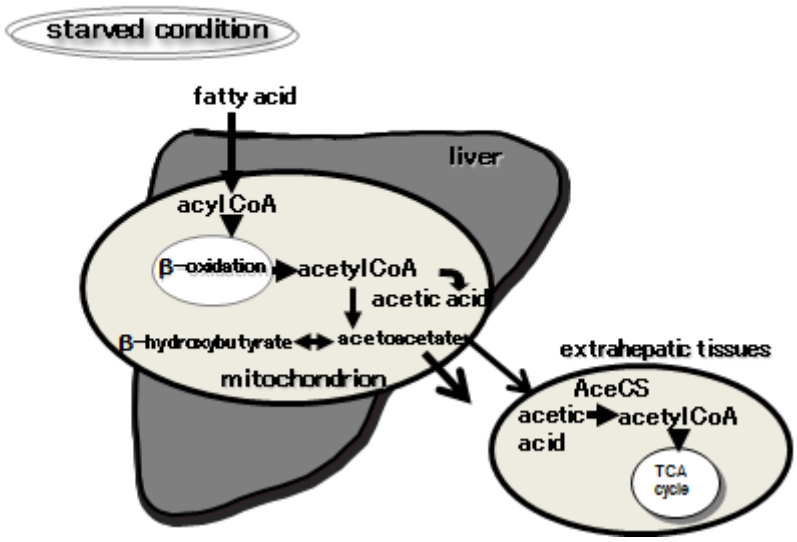

Fig. 3 production of acetic acid in liver and its utilization in extrahepatic tissues under starved condition

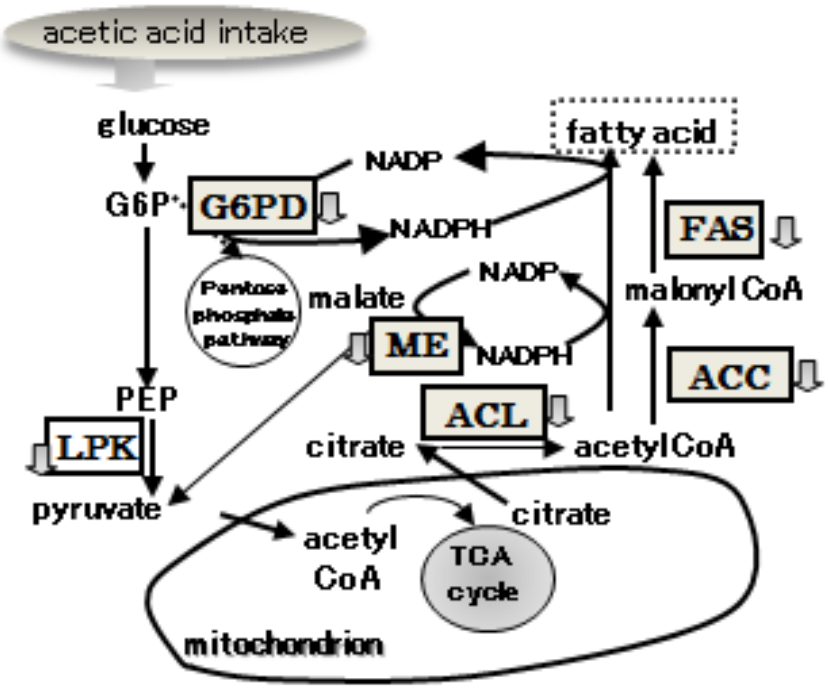

Fig. 4 Administered acetic acid suppresses lipogenesis in the liver

Fig. 5 Activation of AMPK and inactivation of ChREBP via metabolism of acetic acid