Dietary High-Dose Biotin Intake Activates Fat Oxidation and Hepatic Carnitine Palmitoyltransferase in Rat

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Summary This study investigated the effects of dietary high-dose biotin intake on fat oxidation in rats using respiratory gas analysis, and evaluated fatty-acid oxidation-related enzyme activities and gene expressions in the liver. Five-week-old male Sprague–Dawley rats were fed a control diet and three biotin-supplemented diets (additive biotin concentration: 0.05%, 0.10%, and 0.20% of diet) for 3 wk. In 2 wk, fat oxidation in the 0.20% biotin-supplemented diet group was higher than that in the 0.05% biotin-supplemented diet group; however, the energy expenditure and carbohydrate oxidation were unchanged between the dietary groups. At the end of 3 wk, body weight and epididymal white adipose tissue weight reduced in the 0.20% biotin diet group, and hepatic triglyceride levels tended to decrease. Additionally, increased plasma adiponectin concentration and hepatic mitochondrial carnitine palmitoyltransferase activity as well as decreased hepatic acetyl-CoA carboxylase 2 gene expression were observed in the 0.20% biotin-supplemented diet group compared with those in the control group. These results provide strong evidence that dietary high-dose biotin intake activated fat oxidation due to the increase in hepatic β-oxidation, which may contribute to the decrease in hepatic triglyceride concentration and white adipose tissue weight.

Key Words biotin, fat oxidation, carnitine palmitoyltransferase, acetyl-CoA carboxylase 2, adiponectin, rat liver

Biotin is a water-soluble vitamin that functions as a cofactor for four biotin-dependent carboxylases. Biotin-dependent carboxylases are involved in gluconeogenesis, fatty-acid synthesis, and branched-chain amino acid metabolism in mammals (1). Moreover, biotin regulates expression of numerous genes at the transcriptional and post-transcriptional levels (2, 3).

Recent reports have shown the effects of biotin on lipid metabolism. Plasma triglyceride concentration decreased owing to biotin supplementation in patients with type 1 diabetes (4), patients with type 2 diabetes (5) as well as nondiabetic subjects (5). The mechanisms responsible for decreases in plasma and/or hepatic triglyceride levels have been evidenced in animal experiments in which mice fed with a 0.0097% biotin-supplemented diet were compared with those fed a control diet containing 0.000176% of biotin (6–8). The feeding of a biotin-supplemented diet was associated with hypotriglyceridemic effects owing to the downregulation of lipogenic gene expression in the liver and epididymal white adipose tissue in mice (6). The intake of a biotin-supplemented diet reduced the serum and hepatic triglyceride concentrations in mice due to an increase in phosphorylated AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) 1 and a decrease in the protein expressions of sterol regulatory element-binding protein (SREBP)-1c and fatty-acid synthase (FASN) in mouse liver (7). Boone-Villa et al. indicated that phosphorylated AMPK and ACC1 expressions increased in the epididymal white adipose tissue, and the serum free fatty-acid concentration fell in biotin-supplemented mice (8). Additionally, serum and liver triglyceride concentrations decreased due to intraperitoneal biotin administration (2 mg/kg of body weight) for 4 wk in high-fructose-induced metabolic syndrome rats (9). According to these reports, high-dose biotin intake improves dyslipidemia by inhibiting fatty-acid synthesis due to the phosphorylation of AMPK in the liver and
on fat oxidation and its mechanism is unknown. We hypothesized that high-dose biotin intake activates fat oxidation in the liver (10, 11). Therefore, we hypothesized that high-dose biotin intake activates fat oxidation in the liver. However, the effect of high-dose biotin intake on fat oxidation and its mechanism is unknown. We investigated the effects of dietary high-dose biotin intake on fat oxidation in rats using respiratory gas analysis to test our hypothesis. Additionally, diet intake, body weight, liver and adipose tissue weights, biochemical parameters of plasma, hepatic triglyceride concentration, hepatic enzyme activities of β-oxidation and fatty-acid synthesis, gene expressions of fatty-acid oxidation in the liver, and biotin concentrations in the plasma and liver were evaluated.

MATERIALS AND METHODS

Animals and diets. Four-week-old male Sprague–Dawley (SD) rats were obtained from CLEA Japan, Inc. (Osaka, Japan). The animals were housed with laboratory chow (CE-2; CLEA Japan, Inc.) to acclimate them to the animal room for one week. They were housed individually using stainless steel cages with a meshed bottom under controlled conditions (22±1°C, 55±5% humidity, and 08:00–20:00 light and dark cycle). They were divided into four dietary groups, namely, a control group (n=8), 0.05% biotin group (n=8), 0.10% biotin group (n=9), and 0.20% biotin group (n=9). The control group was fed the CE-2 diet (containing 0.0000441% biotin), and the experimental groups were fed the CE-2 diet supplemented with 0.05%, 0.10%, or 0.20% biotin. A previous study reported that a diet with more than 0.04% of biotin supplementation leads to some physiological effects (e.g., biotin accumulation in tissues and body weight loss) (12); thus, as to the best of our knowledge, this was the first experiment to assess the effects of high-dose biotin supplementation on fat oxidation, biotin doses with physiological effects (0.05%, 0.10%, and 0.20%) were selected in this study. The animals were given experimental diets and distilled water ad libitum throughout the experimental period. The average intake of biotin in the control, 0.05% biotin group, 0.10% biotin group, and 0.20% biotin group were 0.00874, 9.85, 20.0, and 37.9 mg/d, respectively. The rats were given experimental diets from 5-wk of age for 3 wk, and body weights and diet intakes were measured every 2 d. The rats were anesthetized using isoflurane and killed at 8-wk of age. Their blood was collected directly from the abdominal aorta using blood collection tubes containing ethylenediaminetetraacetic acid dipotassium salt. Additionally, liver, intercapsular brown adipose tissue, and white adipose tissues (epididymal, perirenal, and mesenteric) were sampled and weighed. Liver samples for measuring triglyceride concentration, enzyme activity, gene expression, and biotin concentration were collected from the left lateral lobe. Plasma was prepared from whole blood by centrifugation (3,000×rpm for 10 min at 4°C). The tissues and plasma were stored at −80°C before the analysis. The experimental procedure was approved by the animal use committee of the University of Nagasaki (Notification No. 28-21; August 26, 2016).

Respiratory gas analysis. On the day before the experiment began (week 0) and 2 wk after the experiment began (week 2), respiratory gas analysis was performed. The systems (Arco Systems Inc., Chiba, Japan) consisted of acrylic metabolic chambers, a mass spectrometer (ARCO-2000), a gas sampler (ARCO-2000-GS-16), and a software (ARCO-2000-RAT). The rats were placed individually in a metabolic chamber for 24 h to measure the volumes of oxygen consumption (VO2) and carbon dioxide production (VCO2). The animals were given experimental diets and distilled water ad libitum during respiratory gas analysis. Room air was pumped every 5 min through the chambers at a rate of 1.0 L/min. Expired air was directed to a mass spectrometer, and the resulting data were recorded using a spreadsheet. The respiratory quotient (RQ), energy expenditure, and fat and carbohydrate oxidation were calculated using the mathematical formula stated below: RQ=VCO2/VO2, energy expenditure (Lusk’s formula) (13) = 3.816×VO2+1.231×VCO2, fat oxidation (Frayn’s formula) (14) = 1.67×(VO2−VCO2), and carbohydrate oxidation (Frayn’s formula) (14) = 4.51×VCO2−3.18×VO2. RQ was expressed as the average of values per day and hour. Total values of energy expenditure measured daily and hourly, fat oxidation, and carbohydrate oxidation were divided by body weight (BW, kg). These values were expressed as kcal/d or h/kg BW, g/d or h/kg BW, and g/d or h/kg BW, respectively.

Biochemical analysis in plasma and liver. Plasma glucose, triglyceride, and free fatty-acid concentrations were measured using commercially available assay kits as follows: Glucose CII test Wako (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), Triglyceride E test Wako (FUJIFILM Wako Pure Chemical Corporation), and NEFA C test Wako (FUJIFILM Wako Pure Chemical Corporation), respectively. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using Transaminase C II-test Wako (FUJIFILM Wako Pure Chemical Corporation). Plasma insulin, leptin, and adiponectin levels were determined using enzyme-linked immunosorbent assay (ELISA) kits as follows: Rat Insulin ELISA Kit (Mercodia AB, Uppsala, Sweden), Mouse/Rat Leptin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), and Rat Total Adiponectin/Acrp30 Quantikine ELISA Kit (R&D Systems, Inc., Minnesota, U.S.A.), respectively. The index of insulin resistance was calculated using the mathematical formula stated below (15).

Index of insulin resistance

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\text{Index of insulin resistance} = \frac{\text{plasma glucose (mg/dL)} \times \text{plasma insulin (ng/mL)}}{405}
\]

Hepatic total lipids were extracted according to the
method reported by Folch et al. (16). One hundred milligrams of the liver was homogenized with 6 mL methanol, followed by 12 mL chloroform. This homogenate was incubated at 40°C for 30 min with occasional vortexing. The homogenate was filtered through filter paper (No. 2, Advantech Co., Ltd., Osaka, Japan), and 3.6 mL of distilled water was added to the sample and mixed. Then, the supernatant was removed from the sample after centrifugation at 3,000 rpm for 5 min. The lower layer was dried using nitrogen at 40°C, and the dried sample was dissolved in 5 mL of chloroform. Subsequently, 1 mL of dissolved sample was dried using nitrogen at 40°C, and the dried sample was dissolved in 50 µL of Triton X-100 : methanol (1 : 1 by wt : vol) mixture. This dissolved sample was dried at 105°C for 20 min, and the dried sample was then dissolved in 100 µL of distilled water, and resultant sample was used for hepatic triglyceride concentration measurement. The hepatic triglyceride concentration was measured using the Triglyceride E test Wako (FUJIFILM Wako Pure Chemical Corporation), with minor modifications.

The protein concentration of hepatic mitochondria, peroxisomes, and cytosolic fractions were determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific K.K., Tokyo, Japan).

**Hepatic enzyme activities.** Hepatic mitochondria, peroxisomes, and cytosolic fractions were prepared as described previously (17). The liver was homogenized with six times the volume of 10-mmol/L Tris-HCl buffer (pH 7.4) containing 0.25-mol/L sucrose and 1-mmol/L ethylenediamine-N,N,N′,N′-tetraacetic acid (EDTA) and centrifuged at 3,000 × rpm at 4°C for 10 min after which the supernatant was collected. Next, the supernatant was centrifuged at 11,000 × rpm at 4°C for 20 min, and the pellet was suspended in the 10-mmol/L Tris-HCl buffer (pH 7.4) containing 1-mmol/L sucrose and 1-mmol/L EDTA and collected (mitochondria and peroxisome fractions). Finally, the supernatant was centrifuged at 40,000 × rpm at 4°C for 60 min, after which the supernatant was collected (cytosol fraction). These fractions were stored at −80°C before the analysis.

The mitochondrial carnitine palmitoyltransferase (CPT) activity was measured spectrophotometrically by monitoring the release of CoA-SH from palmitoyl-CoA using 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (18). In the reaction cuvette at 27°C, the carnitine-dependent reaction was performed by adding mitochondria and peroxisome fractions to a 52-mmol/L Tris-Cl (pH 8.0) containing 1.25-mmol/L EDTA, 0.1% Triton X-100, 0.25-mmol/L DTNB, 37.5-mmol/L palmitoyl-CoA, and 1.25-mmol/L L-carnitine, and the reaction was followed at 412 nm. The carnitine-independent reaction was measured by following a similar procedure without 1.25-mmol/L L-carnitine, and the difference between the L-carnitine-dependent and -independent reaction rate was considered as CPT activity.

The peroxisomal acyl-CoA oxidase (ACOX) activity was measured spectrophotometrically by monitoring the palmitoyl-CoA-dependent H₂O₂ production (19). In the reaction cuvette at 30°C, the palmitoyl-CoA-dependent reaction was performed by adding mitochondria and peroxisome fractions to a 58-mmol/L potassium phosphate buffer (pH 7.4) containing 0.82-mmol/L 4-aminoantipyrine, 10.6-mmol/L phenol, 10-mmol/L flavin adenine dinucleotide, 4-units of peroxidase, 0.1-mmol/L palmitoyl-CoA, and 0.2 mg/mL bovine serum albumin, and the reaction was followed at 500 nm.

The cytosolic fatty-acid synthase (EASN) activity was measured spectrophotometrically by monitoring the malonyl-CoA-dependent β-nicotinamide-adenine dinucleotide phosphate (NADPH) oxidation (20). In the reaction cuvette at 30°C, the EASN reaction was performed by adding the cytosol fraction to a 0.10-mmol/L potassium phosphate buffer (pH 7.0) containing 0.20-mmol/L EDTA, 0.05-mmol/L acetyl-CoA, 0.30-mmol/L NADPH, and 0.20-mmol/L malonyl-CoA, and the reaction was followed at 340 nm. The malonyl-CoA-
Table 2. Effect of dietary high-dose biotin intake on growth parameters and tissue weights.

| Dietary groups | Control | 0.05% | 0.10% | 0.20% |
|---------------|---------|-------|-------|-------|
| Body weight (g) |         |       |       |       |
| Initial       | 154±4   | 154±8 | 155±5 | 155±5 |
| Final         | 308±14a | 310±11a| 308±14a| 285±20b|
| Diet intake (g/d) | 19.8±1.0 | 19.7±0.9 | 20.0±0.9 | 18.9±1.2 |
| Food efficiency (g BW gain/g total intake of diet) | 0.370±0.021a | 0.377±0.021a | 0.364±0.018a | 0.325±0.014b |
| Tissue weight (g/100 g BW) |         |       |       |       |
| Liver         | 3.97±0.20 | 3.92±0.39 | 4.04±0.31 | 3.78±0.28 |
| Interscapular brown adipose tissue | 0.112±0.014 | 0.105±0.017 | 0.112±0.017 | 0.110±0.013 |
| White adipose tissue |         |       |       |       |
| Epididymal    | 0.728±0.096a | 0.651±0.108ab | 0.594±0.137ab | 0.542±0.101b |
| Perirenal     | 0.624±0.179 | 0.587±0.218 | 0.551±0.174 | 0.451±0.119 |
| Mesenteric    | 0.571±0.116 | 0.616±0.122 | 0.569±0.119 | 0.493±0.077 |
| Total         | 1.93±0.29  | 1.85±0.43  | 1.71±0.40  | 1.49±0.22 |

BW, body weight. Values are shown as the mean±SD (n=8–9). a,b p<0.05 (Tukey–Kramer test or Steel–Dwass test), compared between dietary groups in each result.

RESULTS

Energy metabolism

The energy metabolism of the rats at 0 and 2 wk is...
shown in Fig. 1A–H. At week 0, energy metabolism parameters did not differ between the dietary groups (Fig. 1A, C, E, and G). By week 2, RQ decreased (p < 0.05) and fat oxidation increased (p < 0.05) in the 0.20% group compared with that in the 0.05% group (Fig. 1A, B, E, and F). Moreover, fat oxidation in the 0.20% group tended to increase compared with that in the control groups (Fig. 1E and F). By week 2, some hourly total energy expenditure and carbohydrate oxidation values were changed due to dietary biotin intake (Fig. 1D and H); however, daily total energy expenditure and carbohydrate oxidation levels were unchanged (Fig. 1C and G).

**Biochemical parameters in the plasma and liver**

Concentrations of lipids, glucose, and hormones in the plasma and liver are shown in Table 3. In the 0.20% group, the adiponectin concentration in plasma was higher than that in the other groups (p < 0.05). The hepatic triglyceride concentration exhibited a dose-dependent decrease according to the level of biotin intake; the hepatic triglyceride concentrations in the 0.20% group tended to be lower than those in the control group (p > 0.05). The free fatty-acid concentration in plasma was tended to increase in the 0.20% group compared with those in the other groups (p > 0.05). Glucose and leptin concentrations in the plasma of all biotin-supplemented groups tended to increase (p < 0.05). Plasma insulin level in the 0.20% group tended to be lower than those in the control group (p > 0.05). No difference was observed in the triglyceride, AST, and ALT concentrations between the dietary groups in each result.
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levels in the plasma and the index of insulin resistance between the dietary groups.

**Hepatic enzyme activities**

Figure 2 shows the hepatic enzyme activities. Hepatic mitochondrial CPT activities in all biotin supplement groups increased compared to those in the control group ($p < 0.05$). Hepatic peroxisomal ACOX activity did not differ between the dietary groups. Cytosolic FASN activity exhibited a dose-dependent decrease in the level of biotin intake, and that in the 0.20% group was significantly lower than that in the control group ($p < 0.05$).

**Hepatic gene expressions**

Hepatic gene expression is shown in Fig. 3. The hepatic gene expressions of *Acc2* and *Pgc-1α* exhibited a dose-dependent decrease according to the level of biotin intake, and those in the 0.20% group were significantly lower than those in the control group ($p < 0.05$). However, the expression of other genes did not differ between the groups.

**Biotin concentrations of plasma and liver**

Figure 4 shows biotin concentrations of the plasma and liver in the rats. Biotin concentrations in the plasma and liver exhibited a dose-dependent increase according to the level of biotin intake. Biotin concentration in the plasma was higher in all the experimental groups than those in the control group ($p < 0.05$). Biotin concentration in the liver increased in the 0.10% and 0.20% groups compared to the control and 0.05% groups ($p < 0.05$).

**DISCUSSION**

Previous studies have shown that high-dose biotin intake reduces triglyceride concentration in the serum and liver by inhibiting fatty-acid synthesis owing to an increase in phosphorylated AMPK in the liver (7); however, AMPK induces a decrease in lipogenesis and an increase in fat oxidation in the liver (10, 11). Therefore, we hypothesized that high-dose biotin intake might activate fat oxidation in the liver. Therefore, we investigated the effects of dietary high-dose biotin intake on fat oxidation in rats using respiratory gas analysis and evaluated the fatty-acid oxidation-related enzyme activities and gene expressions in the liver. Subsequently, we found that dietary high-dose biotin intake activates fat oxidation due to increased hepatic β-oxidation, which may contribute to the decrease in hepatic triglyceride concentration and white adipose tissue weight.
In this study, fat oxidation assessed using respiratory gas analysis (Fig. 1E, and F) and hepatic mitochondrial CPT activity (Fig. 2A) increased in the 0.20% group compared with those in other groups. As a result, in the 0.20% group, BW and epididymal white adipose tissue weight decreased (Table 2), and hepatic triglyceride concentration tended to reduce (Table 3). Additionally, hepatic cytosolic FASN activity in the 0.20% group was lower than that in the control group (Fig. 2C). In previous studies, BW reduction in rats by feeding them with a 0.08–1.00% biotin-supplemented diet for 4–6 wk was observed (12, 25). Intake of a 0.0097% biotin-supplemented diet for 8 wk decreased epididymal white adipose tissue weight (6), hepatic triglyceride level (7), and liver FASN protein expression (7) in mice. In the high-fructose-induced metabolic syndrome rats, serum and liver triglyceride concentrations were decreased owing to intraperitoneal biotin administration (2 mg/kg of BW) for 4 wk (9). Thus, the present and previous research results show that the intake or administration of high-doses of biotin leads to decreased BW, white adipose tissue weight, and hepatic triglyceride concentration owing to the activation of fat oxidation and inhibition of fatty-acid synthesis in the liver.

In the 0.20% group, triglyceride concentration tended to decrease in the liver, but triglyceride concentration was unchanged in the plasma (Table 3). These results might be caused by short-term biotin-supplemented period compared with several reports. Several reports have demonstrated that triglyceride concentration in the serum in mice decrease owing to the intake of a biotin-supplemented diet for 8 wk (6, 7). Free fatty-acid concentration in plasma tended to rise in the 0.20% group (Table 3), but the weight of epididymal
white adipose tissue decreased (Table 2). However, previous report showed that 0.0097% biotin-supplemented diet for 8 wk reduced serum free fatty-acid level in mice (8). Furthermore, 0.0097% biotin-supplemented diet increased the inactive form (phosphorylated) ACC2 protein expressions in epididymal white adipose tissue in mice (8). Taken together, dietary high-dose biotin intake may result in an increase in free-fatty-acid concentration in the plasma due to activated fat oxidation in the white adipose tissue; however, the free fatty-acid level in the plasma may be elevated only for a short duration, and this value may decrease with the longer biotin-supplemented period.

In the 0.20% group, higher hepatic mitochondrial CPT activity (Fig. 2A) and lower Acc2 gene expression (Fig. 3) were demonstrated; these might be associated with high levels of fat oxidation. Additionally, peroxisomal ACOX activity (Fig. 2B) and Acox1 gene expression (Fig. 3) in the liver were unchanged between the dietary groups. These results indicated that biotin supplementation activated mitochondrial β-oxidation in the liver but not peroxisomal β-oxidation. Intake of a 0.0097% biotin-supplemented diet for 8 wk increased the expression of phosphorylated AMPK in mice liver (7). Phosphorylated AMPK and ACC2 expression with fatty-acid oxidation were elevated in pharmacological biotin-supplemented 3T3-L1 cells (26). The reduction of malonyl-CoA production due to decreased ACC2 activity through increased phosphorylated AMPK expression elevates CPT1 activity in the mitochondrial outer membrane; thus, β-oxidation is enhanced in the liver (10, 11). Taken together, fatty-acid oxidation in the liver might increase owing to AMPK activation because of high-dose biotin intake. Furthermore, phosphorylated AMPK inhibits cholesterol synthesis due to the inactivation of 3-hydroxy-3-methylglutaryl-CoA reductase (10, 11). Therefore, the effect of high-dose biotin intake on cholesterol synthesis should be demonstrated in future studies.

We showed that the plasma concentration of adiponectin increased in the 0.20% biotin group (Table 3). It is well-known that adiponectin elevates fat oxidation, downregulates lipogenesis, and improves insulin sensitivity in the liver and muscle through activated AMPK (27, 28). Recent studies have indicated that a high level of phosphorylated AMPK expression and the reduction of lipogenesis in the liver and epididymal white adipose tissue were observed in the mice when fed with a 0.0097% biotin diet compared with that when fed with a control diet (6–8). Thus, it is strongly suggested that increased fatty-acid oxidation and decreased lipogenesis owing to biotin supplementation are caused due to a high level of plasma adiponectin. In contrast, adiponectin is produced and released depending on the guanylate cyclase/protein kinase G (PKG) phosphorylation cascade in adipose tissue (29). Furthermore, it is indicated that feeding of a 0.0097% biotin-supplemented diet elevated the cGMP concentration of epididymal white adipose tissue in mice comparing control group; thus, the guanylate cyclase/PGK phosphorylation cascade might be activated in adipose tissue (8). Therefore, high levels of plasma adiponectin concentration in the 0.20% group might be affected by the activated production and release of adiponectin in white adipose tissue.

Sawamura et al. indicated that the total biotin concentration in the liver was elevated in rats fed with a 0.04% biotin-supplemented diet for 28 d (12). The hepatic free biotin levels of rats on a 0.01% biotin-supplemented diet for 21 d were 44 times higher than those in the control group (30). Previous reports and our results suggest that a biotin-supplemented diet intake for more than 3 wk results in the accumulation of a high concentration of biotin in rat liver. Although biotin regulates gene expression by covalently binding to histone lysine residues (histone biotinylation), this effect depends on the biotin level in cells (31, 32). Sone et al. showed that feeding a 1.0% biotin-supplemented diet to the mice for 8 d changed the Acc2 gene expression due to a high level of biotin accumulation and the histone biotinylation event in the hypothalamus (33). Biotinylation increased on the histones coiling around DNA at the promoter region for Acc2 in the hypothalamus (33). Therefore, a high-dose dietary biotin intake deposits a high level of biotin in some tissues, which may bind to histone and change gene expression. In the liver of the 0.20% group, biotin concentration increased (Fig. 4B), and Acc2 gene expression decreased (Fig. 3) in this study. Thus, decreased hepatic Acc2 gene expression might be caused by histone biotinylation. However, feeding of a 0.01% biotin-supplemented diet decreased biotinylated (holo form) ACC2 protein expression in rat liver; thus, liver ACC2 enzyme activity might reduce due to high-dose biotin intake (30). Aguiller-Méndez and Fernández-Mejía reported that the expression of phosphorylated AMPK was elevated in mice liver due to the intake of a 0.0097% biotin-supplemented diet for 8 wk (7). Previous studies and our results suggest that ACC2 activity in the liver may be inhibited due to dietary supplementation with high doses of biotin through levels of the biotinylation, phosphorylation, and downregulation of gene expression.

In this study, the Pgc-1α gene expression was decreased in the liver of the 0.20% group compared with that of the control group (Fig. 3). Sugita et al. demonstrated that intraperitoneal administration of 1.0 mg/kg BW of biotin decreased mRNA expressions of the hepatocyte nuclear factor 4, Pgc-1α, and gluconeogenesis enzymes in streptozotocin-induced diabetic rats (34). It was unclear whether decreased Pgc-1α gene expression affected hepatic gluconeogenesis in the 0.20% group. Nevertheless, findings of low levels of Pgc-1α gene expression in the liver due to biotin supplementation were consistent in previous and present studies. Recent studies have suggested that PGC-1α enhances fatty-acid oxidation and mitochondrial biogenesis (35, 36). Therefore, dietary supplementation with high doses of biotin enhances fatty-acid oxidation in the liver, but this observation may not be associated with activated hepatic PGC-1α and/or mitochondrial biogenesis.
Glucose and leptin levels in the plasma of all biotin-supplemented groups tended to increase (Table 3). Moreover, plasma insulin concentration in the 0.20% group tended to be lower than those in the control group (Table 3). However, the changes in these parameters were not statistically significant. A previous study revealed that feeding mice a 1.0% biotin-supplemented diet for 8 d did not affect the plasma glucose, leptin, and insulin concentrations (37). However, in rats fed a high-fat diet, serum leptin concentration, fasting blood glucose, and insulin level decreased by drinking biotin-supplemented water for 12 wk (37). Thus, for a brief time of biotin supplementation, dietary high-dose biotin intake may have no effect on the plasma glucose, leptin, and insulin level. However, the presence of a metabolic disorder or a long-term biotin-supplemented period may cause these characteristics to change with high-dose biotin supplementation.

Plasma ALT and AST activities did not differ between the dietary groups in this study (Table 3). Intake of a 1.0% biotin-supplemented diet for 6 wk did not result in high serum ALT, AST, creatinine, and blood urea nitrogen levels (25). These results suggest that the intake of a 0.20% biotin-supplemented diet does not have toxic effects on the liver and kidney. However, the histopathological analysis of these tissues obtained from biotin-supplemented model rats has not been shown in previous and present studies. The diets that damage the liver, such as high-fat and/or high-fructose diet, may exaggerate the toxic effects on the liver due to a high-dose biotin intake. Therefore, toxicity experiments of high-dose biotin intake on the liver and/or other tissues are needed in future studies.

We examined the effects of dietary high-dose biotin intake on fat oxidation over a short duration. Thus, although mitochondrial CPT activity was increased in the 0.05% and 0.10% groups, no difference was observed in fat oxidation. However, we characterized the effects of biotin on fatty-acid oxidation of the liver; however, the other tissues (e.g. interscapular brown adipose tissue and white adipose tissue) responsible for fatty-acid oxidation were not investigated. Additionally, biotin-supplemented levels in this study were higher than those in previous studies, and protein expressions, phosphorylated form expressions, and enzyme activities of ACC2 and AMPK in the liver were not demonstrated. Future studies should demonstrate the effects of long-term and nutritional dietary biotin supplementation on fat oxidation, β-oxidation in other tissues, and protein and phosphorylated form expression of hepatic ACC2 and AMPK.

Our results suggested that the intake of a normal diet with high-dose biotin activated fat oxidation in rat liver. Several reports have demonstrated that serum and liver triglyceride concentrations decreased in mice that were fed a normal diet with high-dose biotin due to the inhibition of fatty-acid synthesis (6, 7). In high-fructose-induced metabolic syndrome rats, triglyceride concentrations in the serum and liver decreased following intraperitoneal biotin administration (9). Furthermore, in rats fed a high-fat diet, serum triglyceride concentration decreased by drinking of biotin-supplemented water (37). Therefore, high-dose biotin intake or administration activates fat oxidation and inhibition of fatty-acid synthesis in both normal conditions and metabolism disorder, which may prevent and improve metabolic syndrome and dyslipidemia. However, our results only showed the effect of biotin on a low-fat diet, and its effects on fatty-acid oxidation when included in a high fructose or high-fat diet is unknown. Future studies should demonstrate the effects of biotin supplementation on fat oxidation and under high fructose or high-fat diet.

For the first time, our data indicated that high-dose biotin intake elevated fat oxidation through increased plasma adiponectin concentration and liver mitochondrial CPT activity and decreased hepatic ACC2 gene expression. In addition, high-dose biotin intake reduced white adipose tissue weight and tended to decrease hepatic triglyceride concentration. These results provide strong evidence that dietary high-dose biotin intake activates fat oxidation due to increased hepatic β-oxidation, which may contribute to the decrease in hepatic triglyceride concentration and white adipose tissue weight.

**Authorship**

Research conception and design: MY; experiments: MY and KoK; statistical analysis of the data: MY; interpretation of the data: MY, MU, KaK, HS, and TW; writing of the manuscript: MY. All authors read and approved the final manuscript.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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