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Comparative Studies of Some Phenolic Compounds (Quercetin, Rutin, and Ferulic Acid) Affecting Hepatic Fatty Acid Synthesis in Mice

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The physiological activities of some phenolic compounds affecting hepatic fatty acid synthesis in mice were compared. Male ICR mice were fed an experimental diet containing 1% quercetin dihydrate, rutin, or ferulic acid or a control diet free of phenolic compounds for 15 days. Quercetin significantly lowered serum cholesterol and phospholipid levels in mice. Also, the serum triacylglycerol level was considerably lower in mice fed the quercetin-containing diet than in those fed a diet free of phenolic compounds, although the difference was not significant. Rutin and ferulic acid did not affect these parameters. Quercetin significantly reduced the activity and mRNA levels of various enzymes involved in hepatic fatty acid synthesis. Rutin reduced a few of the parameters for lipogenesis, but ferulic acid did not affect any of the parameters. It was suggested that a reduction in hepatic lipogenesis is the mechanism underlying the hypolipidemic effect of quercetin.

KEYWORDS: Quercetin; rutin; ferulic acid; hepatic fatty acid synthesis; mouse

INTRODUCTION

Phenolic compounds are ubiquitous in fruits, vegetables, and herbs; they have attracted much attention due to their potential antioxidative properties and probable role in the prevention of oxidative stress-associated diseases such as atherosclerosis and cancer. The Polygonaceae (buckwheat family) represents wild plants including many species that grow in Mongolia, where their leaves, stalks, seeds, and roots have long been used in medicines (*1*−*3*). We analyzed leaves from species of plants belonging to Polygonaceae including *Fagopyrum tataricum* (L.) Gaertn, *Polygonum angustifolium* Pall, *Polygonum aviculare* L., *Polygonum convolvulus* L., *Polygonum sibiricum* Laxm, *Polygonum viviparum* L., *Rheum undulatum* L., *Rumex acetosella* L., and *Rumex gmelinii* Turcz. ex Ledeb., and found that they are rich in phenolic compounds (20−50 mg as gallic acid/g of dry weight), and their ethanolic extracts had potent antioxidative effects (Odbayar, unpublished observation). Our analyses revealed that leaves of Polygonaceae plants contained considerable amounts of quercetin as glucoside, rhamnoside, and rutinoside (1−2 mg as quercetin/g of dry weight) (Odbayar and Tsushida, unpublished observation). We also found that some species of the genus contained a large amount of compounds having a phenylpropanoid-type structure that has yet to be specified.

Information suggests that many plant phenolic compounds not only act as antioxidants but also affect lipid metabolism in experimental animals and humans and hence exert meritorious physiological activity in organisms. Studies have demonstrated that quercetin, its glucoside, and rutinoside (*4*−*11*), as well as phenylpropanoid compounds (*12*−*14*) that are abundant in Polygonaceae plants, lower serum lipid levels in experimental animals. However, the mechanism underlying the serum lipid-lowering effect of these compounds is not known. As alterations in hepatic fatty acid synthesis are crucial to the regulation of serum lipid levels (*15*−*17*), it is possible that these compounds affect this metabolic activity and hence exert a serum lipid-lowering effect. In this context, we examined the effects of quercetin, rutin (quercetin-3-rutinoside), and ferulic acid (a phenylpropanoid compound) on the activity and mRNA abundance of hepatic lipogenic enzymes in ICR mice in the present study. Among the various enzymes involved in the regulation of hepatic fatty acid synthesis, we measured the activity of fatty acid synthase, ATP-citrate lyase, malic enzyme, and glucose 6-phosphate dehydrogenase and mRNA levels of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, and malic enzyme. In addition, we also analyzed the mRNA expression of proteins presumed to be involved in the regulation of lipogenesis including spot 14 (*18*), adiponutrin (*19*−*21*), and

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sterol regulatory element binding protein (SREBP)-1c (*22*, *23*). Cytoplasmic acetyl-CoA as a substrate to synthesize fatty acids is transported from mitochondria in the form of citrate. In the cytoplasm, citrate is converted to oxaloacetate and acetyl-CoA by an ATP-citrate lyase reaction (*24*). The synthesis of malonyl-CoA catalyzed by acetyl-CoA carboxylase is the first committed step of fatty acid synthesis (*25*). The synthesis of fatty acids from acetyl-CoA and malonyl-CoA is carried out by fatty acid synthase (*25*). Large amounts of NADPH are consumed to synthesize fatty acids. NADPH in the cytoplasm is produced by the enzymes involved in the hexose monophosphate pathway (glucose 6-phsophate dehydrogenase and 6-phosphogluconate dehydrogenase) (*25*) and malic enzyme (*25*, *26*).

MATERIALS AND METHODS

Animals and Diets. Male ICR mice purchased from Charles River Japan, Kanagawa, Japan, at 5 weeks of age were housed individually in a room with controlled temperature (20−22 °C), humidity (55−65%), and lighting (lights on from 7:00 a.m. to 7:00 p.m.) and fed a commercial nonpurified diet (type NMF, Oriental Yeast). After 7 days of acclimation, the mice were divided into four groups with the same mean body weights (33.1−33.6 g) and were fed purified experimental diets containing 1% quercetin dihydrate (Tokyo Chemical Industry Co., Tokyo, Japan), rutin (Nacalai Tesque, Inc., Kyoto, Japan), or ferulic acid (Wako Pure Chemical, Osaka, Japan) or a control diet free of phenolic compounds for 15 days. The basal composition of the purified experimental diets was as follows (in g/kg): casein, 200; palm oil, 100; cellulose, 20; mineral mixture (*27*), 35; vitamin mixture (*27*), 10; DL-methionine, 3; choline bitartrate, 2; and sucrose to 1 kg. Phenolic compounds were added to experimental diets instead of sucrose. This study was approved by the review board of animal ethics of our institute, and we followed the institute's guidelines in the care and use of laboratory animals.

Analysis of the Activity of Hepatic Lipogenic Enzymes. At the end of the experiments, the mice were anesthetized using diethyl ether and killed by bleeding from the interior vena cava, after which the livers were quickly excised. Approximately 0.6 g of each liver was homogenized with 6 mL of 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris-HCl (pH 7.2). The homogenates were centrifuged at 200000*g* for 30 min. The activities of enzymes involved in fatty acid synthesis were measured spectrophotometrically using the 200000*g* supernatant of the liver homogenate. Fatty acid synthase activity represents the rate of malonyl-CoA-dependent oxidation of NADPH in the presence of acetyl-CoA (*28*). ATP-citrate lyase activity, as the rate of CoA-dependent oxidation of NADH in the presence of potassium citrate, ATP, and malate dehydrogenase, was analyzed according to the method of Takeda et al. (*29*). Malic acid-dependent reduction of NADP was analyzed to measure malic enzyme activity (*30*). The rate of glucose 6-phosphate-dependent reduction of NADP in the presence of 6-phosphogluconate dehydrogenase was taken as the activity of glucose 6-phosphate dehydrogenase (*28*).

RNA Analysis. Hepatic RNA was extracted using the acid guanidium thiocyanate−phenol−chloroform method (*31*), and mRNA abundance was analyzed by a quantitative real-time PCR using an Applied Biosystems Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) as detailed elsewhere (*32*). mRNA abundance was calculated as a ratio to the mRNA abundance of β-actin in each cDNA sample and expressed as a percentage, assigning a value of 100 for mice fed a control diet free of phenolic compounds. Nucleotide sequences of forward and reverse primers and probes to detect the respective mRNAs were the same as described previously (*32*).

Analyses of Serum Lipids and Insulin. Serum triacylglycerol (triglyceride E-test Wako), cholesterol (cholesterol E-test Wako), and phospholipid (phospholipid E-test Wako) concentrations were assayed using commercial enzyme kits purchased from Wako Pure Chemicals. Serum insulin levels were analyzed with a commercial ELISA kit (Ultra Sensitive Insulin ELISA Kit/Rat, Morinaga, Kanagawa, Japan) using mouse insulin as a standard.

Statistical Analyses. StatView for Macintosh (SAS Institute Inc., Cary, NC) was used for the statistical analysis. The data were analyzed with a one-way ANOVA, followed by a Tukey−Kramer post-hoc analysis to detect significant differences of the means at the level of $P < 0.05$.

Table 1. Effect of Phenolic Compounds on Serum Lipid and Insulin Levels

	group			
	control	quercetin	rutin	ferulic acid
lipids (μmol/dL)				
triacylglycerol	209 ± 32[a]	146 ± 10	219 ± 27	252 ± 26
cholesterol	726 ± 33	529 ± 53*[b]	610 ± 31	699 ± 40
phospholipid	578 ± 43	476 ± 39*	486 ± 22	601 ± 28
insulin (ng/dL)	815 ± 94	782 ± 168	603 ± 111	574 ± 151

[a] Data are means ± SEM; $n = 7–8$. [b] An asterisk (*) indicates a significant difference ($P < 0.05$) from the value in mice fed a control diet free of phenolic compounds.

RESULTS

Mean body weights at the time of killing were comparable among the groups (39.6−42.1 g). The dietary phenolic compounds affected neither growth (6.3−8.4 g/15 day) nor food intake (5.2−5.6 g/day). Diets containing phenolic compounds compared to a control diet free of phenolic compounds did not affect the liver weight of mice (5.1−5.6 g/100 g of body weight).

The diet containing quercetin, compared to the control diet, significantly decreased the serum concentrations of cholesterol and phospholipids (**Table 1**). Although the difference was not significant, serum triacylglycerol levels were also lower in mice fed the quercetin-containing diet than in those fed the control diet. The diets containing rutin and ferulic acid compared with the control diet free of phenolic compounds did not affect serum lipid levels. None of the dietary phenolic compounds affected the concentration of insulin in the serum.

We measured activity levels of heptic lipogenic enzymes including fatty acid synthase, ATP-citrate lyase, malic enzyme, and glucose 6-phosphate dehydrogenase (**Figure 1**). The diet containing quercetin compared to the control diet caused significant 40, 53, 36, and 52% decreases in the activity of the respective enzymes. Rutin also caused significant 27% decreases in the activity of ATP-citrate lyase and malic enzyme. However, this compound was ineffective in reducing the activity of fatty acid synthase and glucose-6-phosphate dehydrogenase. Ferulic acid did not affect the activity of any of the lipogenic enzymes.

The mRNA levels of lipogenic enzymes were expressed as percentages, assigning the value in ICR mice fed a control diet free of phenolic compound as 100 (**Figure 2**). Consistent with the results obtained for enzymatic activity, quercetin caused significant 42, 49, 55, and 44% decreases in the mRNA abundance of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase, and malic enzyme. Rutin significantly lowered the mRNA level of malic enzyme but not that of other enzymes. Ferulic acid modified none of these parameters. Spot 14 and adiponutrin are proteins presumed to be involved in the regulation of lipogenesis (*18*−*21*, *32*). The diet containing quercetin compared to the control diet caused a significant 55% decrease in the mRNA abundance of spot 14. However, rutin and ferulic acid did not affect this parameter. Quercetin caused a strong decrease in the mRNA expression of adiponutrin (84% decrease). Rutin also caused a significant 45% decrease in this parameter. However, ferulic acid had no effect on this parameter. Sterol element binding protein-1c (SREBP-1c) is a transcription factor that regulates the gene expression of lipogenic enzymes (*17*, *22*, *23*). Despite quercetin's ability to significantly lower

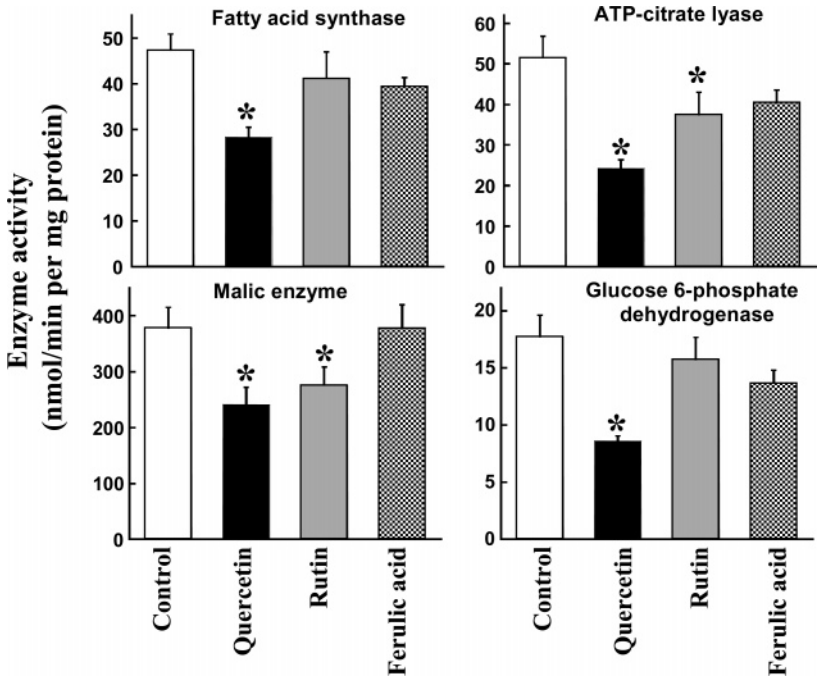

Figure 1. Effect of phenolic compounds on the activity of hepatic enzymes involved in fatty acid synthesis. Data are means ± SEM; $n = 7–8$. An asterisk indicates a significant difference ($P < 0.05$) from the value in mice fed a control diet free of phenolic compounds.

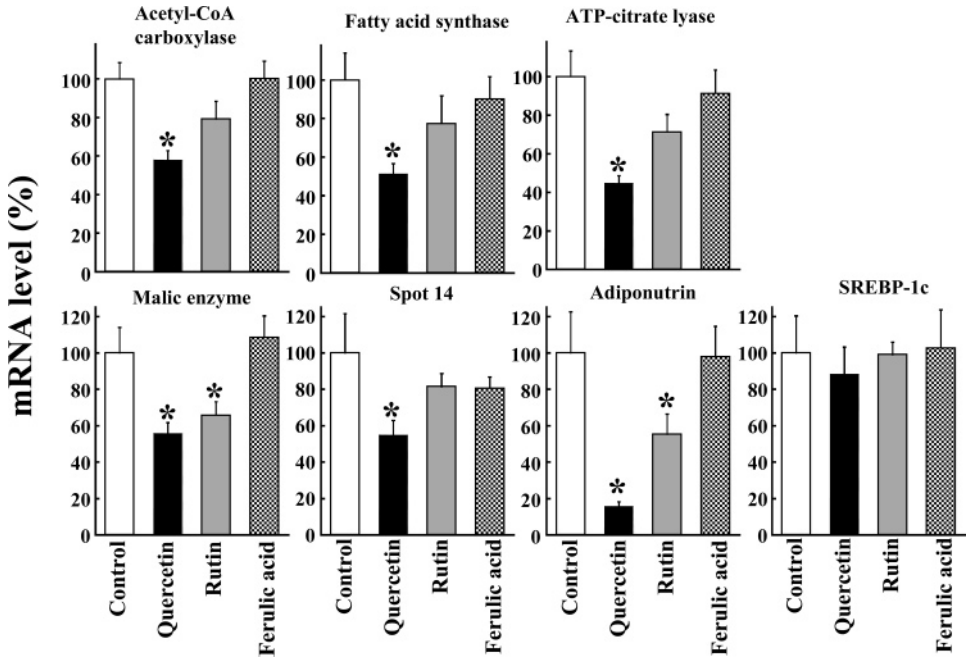

Figure 2. Effect of phenolic compounds on mRNA abundance of hepatic enzymes involved in fatty acid synthesis, spot 14, adiponutrin, and sterol regulatory element binding protein (SREBP)-1c. Data are means ± SEM; $n = 7–8$. An asterisk indicates a significant difference ($P < 0.05$) from the value in mice fed a control diet free of phenolic compounds.

mRNA levels of various lipogenic enzymes, no significant differences were seen in mRNA levels of SREBP-1c among groups.

DISCUSSION

The leaves of Polygonaceae found in Mongolia abundantly contain quercetin derivatives. Also, some species of this family contain compounds having a phenylpropanoid-type structure in their leaves. It has been demonstrated that quercetin (*10*, *33*, *34*) and phenylpropanoid compounds such as caffeic acid (*12*) and ferulic acid (*35*) act as antioxidants in vivo. The physiological activity of quercetin or its glucoside (*4*, *7*−*10*), and phenylpropanoid compounds (*14*) in reducing the progression of atherosclerosis has been ascribed to their antioxidative effects. In addition, these compounds reduce serum lipid levels (*4*−*14*), which may also contribute to their antiatherogenic effect. Quercetin or its glucoside reduce serum lipid levels in various experimental animals including mice (*4*), rats (*5*, *6*), hamsters (*7*), and rabbits (*8*−*10*). Consistent with these findings, we observed that quercetin lowered serum concentrations of cholesterol and phospholipids in mice. Although the difference was not significant, the serum triacylglycerol concentration was lower in mice fed a diet containing quercetin than in those fed a diet free of phenolics. Information on the effect of rutin

(quercetin-3-rutinoside) and ferulic acid on serum lipid levels is scarce. Krishna et al. (*6*) reported that rutin reduced serum cholesterol and triacylglycerol levels in rats with streptozotocin-induced diabetes. Santos et al. (*11*) reported that rutin reduced serum triacylglycerol levels in normal rats. Sri Balasubashini et al. (*13*) showed that ferulic acid decreased serum concentrations of triacylglycerol, cholesterol, and phospholipid in rats with streptozotocin-induced diabetes. Also, Wang et al. (*14*) showed that it reduced serum concentrations of triacylglycerol in rabbits fed a high-fat diet. No further information on the effect of rutin and ferulic acid on serum lipid levels is available. In the present study, neither rutin nor ferulic acid significantly modified serum concentrations of triacylglycerol, cholesterol, and phospholipid. Apparently, further studies are required to clarify the physiological activity of rutin and ferulic acid in affecting serum lipid levels.

In the present study, we found that quercetin profoundly decreased activity and mRNA levels of various hepatic lipogenic enzymes. It has been well documented that a reduction in hepatic fatty acid synthesis reduces the availability of fatty acids for the synthesis of triacylglycerol and, in turn, decreases the assembly and secretion of triacylglycerol-rich lipoproteins and hence decreases serum lipid levels (*15*−*17*). Therefore, it is plausible that a reduction in hepatic fatty acid synthesis is the mechanism underlying the hypolipidemic effect of quercetin. We also showed that dietary quercetin down-regulated the mRNA expression of spot 14, a nuclear protein presumed to be involved in the regulation of fatty acid synthesis (*18*), and of adiponutrin. Adiponutrin has been cloned as a protein of unknown function specifically expressed in adipose tissue (*19*). Recent studies (*20*, *21*) indicated that adiponutrin is a member of the calcium-independent phospholipase A2 family that also possesses acylglycerol transacylase activity, utilizing monoacylglycerol as an acyl donor, which, in the presence of monoacylglycerol or diacylglycerol acceptors, results in the synthesis of diacylglycerol and triacylglycerol, respectively. The gene for adiponutrin is also expressed in liver, although at a much lower level than in adipose tissue (*32*). We recently found that dietary conjugated linoleic acid- and fish oil-dependent changes in the mRNA abundance of adiponutrin paralleled those of many enzymes involved in fatty acid synthesis in the liver of mice (*32*). In the present study, we showed that dietary quercetin reduced the mRNA abundance of adiponutrin in mouse liver accompanying the reduction in the activity and mRNA expression of various lipogenic enzymes. These observations indicated that adiponutrin is a protein involved in the regulation of lipogenesis in the liver. Although the reductions were attenuated, the rutin-containing diet compared to the control diet free of phenolic compounds significantly reduced the activity of ATP-citrate lyase and malic enzyme and mRNA levels of malic enzyme and adiponutrin. Therefore, this compound may have the propensity to cause a moderate reduction in hepatic fatty acid synthesis. It is reasonable to consider that rutin has physiological activity to reduce hepatic lipogenesis, because quercetin, which has a potent suppressive effect on lipogenesis, is the aglycon of this compound. It has been reported that quercetin is less bioavailable in the form of rutin than in the form of free quercetin, presumably because rutin must be hydrolyzed by the cecal microflora before quercetin aglycon can be absorbed (*36*). In addition, the level of quercetin aglycon in the diet containing 1% rutin was about half that in the diet containing 1% quercetin dihydrate in the present study. Therefore, it is possible that rutin exerts a more clear-cut effect in reducing lipogenesis at dietary levels higher than 1%. It has been demonstrated that about 50% of ingested ferulic acid was recovered in urine in rats fed a purified diet containing this compound (*37*). Therefore, ferulic acid is absorbed quite efficiently, and the difference in bioavailability may not account for the divergent effect of quercetin and ferulic acid on hepatic lipogenesis.

It is well documented that insulin is a hormone affecting hepatic lipogenesis (*38*). As dietary quercetin did not affect the concentration of insulin in serum in the present study, it is difficult to consider that insulin is involved in the quercetin-dependent changes in hepatic lipogenesis. SREBP-1c is a transcription factor involved in the regulation of gene expression of many enzymes involved in fatty acid synthesis (*17*, *22*, *23*). This transcription factor is synthesized as an approximately 1150 amino acid precursor bound to the endoplasmic membrane and nuclear envelope. For SREBP-1c to be active, an NH_2-terminal sequence approximately 500 amino acids long must be released by a sequential two-step proteolytic cleavage process. The liberated NH_2-terminal, mature form of SREBP-1c then enters the nucleus and activates the gene. Although quercetin did not affect the mRNA level of SREBP-1c in the present study, it is still possible that this flavonoid affects the proteolytic cleavage of the immature form of this transcription factor to convert it to the mature form and hence affect hepatic lipogenesis. In relation to this consideration, it has been demonstrated that fish oil at a low dietary level did not affect the mRNA expression of this transcription factor but caused the inhibition of its proteolytic cascade and hence down-regulated the mRNA expression of lipogenic enzymes (*39*). An analysis of the nuclear contents of the mature form of SREBP-1c is required to clarify this point.

In conclusion, we confirmed the serum lipid-lowering effect of quercetin abundant in Mongolian medicinal plants of the family Polygonaceae, using mice. In addition, we demonstrated that quercetin significantly lowered the activity and mRNA levels of hepatic lipogenic enzymes. Therefore, a reduction in hepatic lipogenesis is considered to be the mechanism underlying the hypolipidemic effect of this flavonoid.

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