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Stimulation of the Lipogenic Pathway in Ginsenoside-Rb_2 Treated Rats

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In order to elucidate the effect of ginsenoside-Rb_2 on lipid metabolism, a time course experiment was carried out in rats. The first phenomenon observed was a stimulation of glucose-6-phosphate dehydrogenase activity beginning 4 h after the administration of ginsenoside-Rb_2. At this time, a slight increase of acetyl-coenzyme A carboxylase activity was observed, and a significant increase was noted 16 h after the treatment. The maximum increase in malic enzyme activity was observed at 12 h. An increase in the lipolytic activity of lipoprotein lipase was observed 4 h after the intraperitoneal administration of ginsenoside-Rb_2, reaching a maximum (262%) 16 h after the treatment, while a repressive effect was observed on hormone-sensitive lipase activity throughout the experimental period. Administration of ginsenoside-Rb_2 to rats also increased the triglyceride content in adipose tissue. These results suggest that ginsenoside-Rb_2 brings about the accumulation of triglyceride in adipose tissue as a result of its stimulating action on the lipogenic pathway.

Keywords——ginsenoside-Rb_2; acetyl-CoA carboxylase; malic enzyme; lipoprotein lipase; hormone-sensitive lipase; triglyceride; adipose tissue

In the previous paper, we reported the effect on sugar and lipid metabolism of a purified saponin from the roots of *Panax ginseng* C. A. MEYER.[1] Intraperitoneal administration of ginsenoside-Rb_2 in rats was found to stimulate the glycogenolytic and glycolytic pathways. In particular, a significant increase in the activity of phosphofructokinase, a rate-limiting enzyme of glycolysis, was observed in ginsenoside-Rb_2-treated rats. This indicated an accumulation of total lipid in adipose tissue. It may be considered that ginsenoside-Rb_2 turns the metabolic flow toward the lipogenic pathway by the degradation of sugar in the liver. The present paper deals with the effect of this compound on the activities of lipogenic and lipolytic enzymes, and on the lipid constituents in the adipose tissue and serum.

Materials and Methods

Animals and Treatments——Male rats of the JCL: Wistar strain, initially weighing 90—100 g, were used in this experiment. The rats were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 25 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chow, CE-2, purchased from CLEA Japan Inc., Tokyo, and tap water were given freely. Ginsenoside-Rb_2 (10 mg/rat) in saline was administered intraperitoneally to the rats, while control rats were treated with an equal volume of saline. At the indicated time after intraperitoneal administration of ginsenoside, rats were stunned by a sharp blow on the head, and blood samples were collected and allowed to stand for several hours in a cold room at 4 °C. Control rats were sacrificed 4 h after saline treatment. Serum was separated by centrifugation (1000 × *g*, 10 min, 4 °C). Liver and epididymal adipose tissue were removed quickly, cooled on ice, and weighed rapidly. Fresh liver and adipose tissue were homogenized in a Potter–Elvehjem type glass homogenizer with a Teflon pestle.

Saponin——Ginsenoside-Rb_2 was isolated and purified from the extract of roots of *Panax ginseng* C. A. MEYER according to the procedure of Shibata and co-workers.[2] This preparation was found to be pure by chemical and

physicochemical analyses.

Chemicals——Acetyl coenzyme A (CoA), adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide adenine dinucleotide (NAD), and reduced nicotinamide adenine dinucleotide (NADH) were purchased from P-L Biochemicals, Inc., U.S.A. β-Hydroxybutyrate dehydrogenase was obtained from Sigma Chemical Co., U.S.A. and Intralipid was from the Green Cross Corp., Osaka, Japan. ^{14}C-Sodium bicarbonate (9.2 mCi/mmol) was purchased from New England Nuclear Corp., U.S.A. All other reagents were of the highest grade commercially available.

Statistics——The significance of differences between the control and ginsenoside-treated groups was tested by means of Student's *t*-test.

Determination of Enzyme Activities in the Liver and Adipose Tissue——a) Acetyl-CoA Carboxylase: Liver was homogenized with 3 volumes of homogenizing medium (ethylenediamine tetraacetic acid (EDTA)–physiological saline solution) and then centrifuged at $105000 \times g$ for 60 min. The supernatant fraction was passed through a Sephadex G-50 column to remove endogenous substrates. After the preincubation with citrate to ensure full activation, the enzyme activity was determined by the $^{14}CO_2$–fixation assay according to the method of Nakanishi and Numa.[3]

b) Malic Enzyme: Homogenate of each liver was prepared using 19 volumes of homogenizing medium (EDTA–physiological saline solution) and then centrifuged at $105000 \times g$ for 60 min. The crude supernatant fluid was used for the enzyme assay. Malic enzyme activity was determined spectrophotometrically by measuring the extinction change due to the formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH).[4] The enzyme solution 0.10 ml, 0.25 M glycylglycine buffer (pH 7.4) 0.30 ml, 0.05 M $MnCl_2$ 0.06 ml, 0.000675 M $NADP^+$ 0.2 ml, and 0.03 M L-malate (pH 7.4) 0.05 ml were placed in a cuvette of a Hitachi spectrophotometer, model 200-20, and the increase of optical density at 340 nm was determined.

c) Lipoprotein Lipase:[5,6] Epididymal adipose tissue was passed through a grinder and defatted with acetone at room temperature. The resultant fibrous mat was cut into small pieces and extracted for 60 min at 0 °C with 10 mM NH_4OH. The insoluble residue was then removed by centrifugation and the supernatant fluid was used as the enzyme solution. The reaction mixture, containing 50 μl of active substrate [10% Intralipid (a commercial emulsion of soybean oil and lecithin) and an equal volume of rat serum incubated at 37 °C for 30 min], 350 μl of enzyme solution, 50 μl of 0.3 M Tris-HCl buffer (pH 8.5), and 50 μl of 20% bovine serum albumin, was incubated at 37 °C for 60 min. Fatty acid released was estimated by using a commercial reagent (NEFA-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan).

d) Hormone-Sensitive Lipase:[6,7] Homogenization and centrifugation of each adipose tissue were carried out in a cold room at 4 °C. Extracts of fat pads were prepared by homogenizing the tissue in 3 ml of 0.25 M sucrose per g of tissue in a glass chamber with a Teflon pestle. The homogenate was centrifuged at $12000 \times g$ for 10 min. The fat cake accumulated at the top of the tube was discarded. The remaining supernatant was saved for assay of hormone-sensitive lipase activity. Lipolytic activity was measured by placing 0.1 ml of an extract in a glass-stoppered tube with 0.1 ml of 2% Intralipid, 0.02 ml of 20% extracted albumin (pH 7.4), 0.2 ml of 0.03 M phosphate buffer (pH 7.4), and sufficient water to make a final volume of 2.5 ml. After 60 min of incubation at 37 °C, fatty acid released was estimated by using a commercial reagent (NEFA-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Determination of Protein——Protein was determined by the method of Itzhaki and Gill, with bovine serum albumin as a standard.[8]

Determination of Triglyceride, Phospholipid, and Non-esterified Fatty Acid in the Adipose Tissue——Epididymal adipose tissue was quickly removed after decapitation of the rat, cooled on ice, and weighed. The adipose tissue was mixed with 20 ml of $CHCl_3$–MeOH (2 : 1, v/v). The $CHCl_3$–MeOH solution was used for the estimation of triglyceride, phospholipid, and non-esterified fatty acid. Determinations were performed by using commercial reagents (TG-Five Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan; Phospholipids-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan; NEFA Kainos obtained from Kainos Laboratories, Inc.).

Determination of Triglyceride, Non-esterified Fatty Acid, Total Cholesterol, 3-Hydroxybutyrate, and Acetoacetate in the Serum——Triglyceride, non-esterified fatty acid, and total cholesterol were determined by using commercial reagents (TG-Five Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan; NEFA Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan; Cholesterol B-Test Wako obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan). 3-Hydroxybutyrate was determined spectrophotometrically by measuring the increase of optical density at 340 nm, which was based on the oxidation of 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase and NAD.[9] The determination of acetoacetate was based on the decrease in extinction at 340 nm due to the oxidation of NADH.[10]

Results

Time Course of the Effect of Ginsenoside-Rb_2 on the Lipogenic Enzyme Activities in the Liver

The activities of lipogenic enzymes in the liver are shown in Table I. The activity of

TABLE I. Effect of Ginsenoside-Rb_2 on the Enzyme Activities in the Liver

Time after treatment (h)	Acetyl-CoA carboxylase (nmol/min/mg protein)	Malic enzyme (ΔOD/h/mg protein)
Control	2.24±0.45 (100)	625± 25 (100)
4	3.12±0.76 (139)	613± 31 (98)
8	3.02±0.58 (135)	838± 59[a] (134)
12	3.26±0.62 (146)	968±132[a] (155)
16	4.26±0.66[a] (190)	770± 22[b] (123)
24	1.46±0.11 (65)	681± 35 (109)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$.

acetyl-CoA carboxylase, a rate-limiting enzyme of fatty acid synthesis, was altered by treatment of the animals with ginsenoside-Rb_2. As shown in Table I, an increase in the activity of this enzyme was observed 4 h after the intraperitoneal administration of ginsenoside-Rb_2 and this tendency continued until 12 h after the treatment. However, there was no statistically significant difference between the control and ginsenoside-Rb_2 treated groups. A significant increase in the acetyl-CoA carboxylase activity was observed 16 h after the treatment but this activity was 35% below the control level at 24 h. On the other hand, the treatment with ginsenoside-Rb_2 altered the NADPH-generating enzyme activities, which is of interest in view of the importance of the NADPH/$NADP^+$ ratio *in vivo* in the control mechanism of fatty acid biosynthesis.[11] As reported previously,[1] a change in the activity of glucose-6-phosphate dehydrogenase was observed 2 h after the intraperitoneal administration of ginsenoside-Rb_2. An increase was noted at 4 h in the present work (Fig. 1), but there was no statistically significant difference between the control and ginsenoside-Rb_2 treated groups. The present data further confirmed that the intraperitoneal administration of ginsenoside-Rb_2 modified the malic enzyme activity. As shown in Table I, the maximum increase in this enzyme activity was observed 12 h after the treatment. Eight and 16 h after the administration of ginsenoside-Rb_2, there were increases of 34 and 23% in the enzyme activity, respectively, but the increase was no longer significant after 24 h.

Time Course of the Effect of Ginsenoside-Rb_2 on the Lipolytic Enzyme Activities in the Adipose Tissue

Table II shows the lipolytic activity of lipoprotein lipase and hormone-sensitive lipase. An increase in the lipolytic activity of lipoprotein lipase was observed 4 h after the intraperitoneal administration of ginsenoside-Rb_2 and reached the maximum (162%) at 16 h. In contrast, administration of ginsenoside-Rb_2 to rats tended to decrease the activity of hormone-sensitive lipase.

Time Course of the Effect of Ginsenoside-Rb_2 on Lipid Constituents in the Adipose Tissue

As shown in Table III, a striking change of triglyceride content in the adipose tissue was observed 4 h after the intraperitoneal administration of ginsenoside-Rb_2. A significant increase was maintained until 12 h after the treatment. The phospholipid content was also increased up to 16 h after the treatment. However, there was no significant change in the level

TABLE II. Effect of Ginsenoside-Rb_2 on the Enzyme Activities in the Adipose Tissue

Time after treatment (h)	Lipoprotein lipase (neq/h/mg protein)	Hormone-sensitive lipase (neq/h/mg protein)
Control	306.9 (100)	12.4 (100)
4	755.5 (246)	8.6 (69)
8	483.1 (157)	8.5 (69)
12	669.1 (218)	10.0 (81)
16	805.6 (262)	11.2 (90)
24	593.4 (193)	11.4 (92)

Values are means of 6 rats. Figures in parentheses are percentages of the control value.

TABLE III. Effect of Ginsenoside-Rb_2 on the Lipid Constituents in the Adipose Tissue

Time after treatment (h)	Triglyceride (mg/tissue)	Phospholipid (mg/tissue)	Non-esterified fatty acid (mg/tissue)
Control	175.9±11.2 (100)	17.8±1.6 (100)	2.63±0.23 (100)
4	286.0±41.7[a] (163)	28.4±3.6[a] (160)	3.12±0.83 (119)
8	298.4±30.1[a] (170)	27.0±2.5[a] (152)	2.39±0.30 (91)
10	289.2±30.6[a] (164)	26.3±1.6[a] (148)	1.90±0.26 (81)
12	305.0±52.7[a] (173)	24.6±1.4[a] (138)	2.41±0.29 (92)
16	246.3±30.3 (140)	25.2±2.4[a] (142)	2.36±0.19 (90)
24	294.1±37.9[a] (167)	23.9±3.7 (134)	2.50±0.18 (95)

Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$.

of non-esterified fatty acid.

Time Course of the Effect of Ginsenoside-Rb_2 on Lipid Constituents in the Serum

As shown in Table IV, a slight decrease (25%) of the triglyceride level was seen 24 h after the treatment. A significant decrease was observed 24 h after the treatment in the level of total cholesterol; the mean values in the control and ginsenoside-Rb_2 treated groups were 116.7 and 95.3 mg/dl, respectively. However, non-esterified fatty acid, 3-hydroxybutyrate, and acetoacetate in the serum showed no appreciable changes.

Discussion

It is generally accepted that the activity of acetyl-CoA carboxylase regulates lipogenesis.[12,13] In the present experiment using ginsenoside-Rb_2, an increase in the activity of this enzyme, a rate-limiting enzyme of fatty acid biosynthesis, was observed (Table I). In addition, the treatment with ginsenoside-Rb_2 altered the $NADPH/NADP^+$ ratio *in vivo* and this ratio is known to be involved in the control mechanism of fatty acid biosynthesis[11] (Table I). It seems clear that ginsenoside-Rb_2 does have a stimulating effect on the lipogenic pathway.

TABLE IV. Effect of Ginsenoside-Rb_2 on the Lipid Constituents in the Serum

Time after treatment (h)	Triglyceride (mg/dl)	Non-esterified fatty acid (μeq/l)	T. cholesterol (mg/dl)	3-Hydroxybutyrate (μmol/ml)	Acetoacetate (μmol/ml)
Control	65.1±6.9 (100)	662.4±22.6 (100)	116.7± 7.0 (100)	0.14±0.04 (100)	N.D.
4	70.4±6.1 (108)	637.3±36.1 (96)	113.3± 4.7 (97)	0.12±0.02 (86)	N.D.
8	67.3±2.9 (103)	537.9±68.2 (81)	112.1± 4.6 (96)	0.07±0.03 (50)	N.D.
10	57.0±6.4 (88)	583.0±66.0 (88)	118.7± 4.1 (102)	—	—
12	54.6±4.4 (84)	661.7±37.8 (100)	126.7±11.1 (109)	0.11±0.04 (79)	N.D.
16	52.6±3.7 (81)	523.6±97.4 (79)	107.7± 3.3 (92)	0.13±0.05 (93)	N.D.
24	49.1±5.1 (75)	599.4±38.2 (90)	95.3± 3.4[a] (82)	0.13±0.05 (93)	N.D.

N.D., not detectable. Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value.
a) Significantly different from the control value, $p<0.05$.

Our observations also suggest that a striking change of triglyceride content in the adipose tissue occurs after the intraperitoneal administration of ginsenoside-Rb_2 (Table III). Rat epididymal adipose tissue is known to contain at least two distinct lipases, hormone-sensitive lipase[14] and lipoprotein lipase.[15] Adipose tissue triglyceride is hydrolyzed by hormone-sensitive lipase, which is thought to be activated by catecholamine and a variety of hormones. Lipoprotein lipase regulates the rate of uptake of plasma triglyceride by adipose tissue, because it catalyzes the hydrolysis of circulating chylomicrons and lipoprotein triglyceride that occurs during the uptake of fatty acid components.[16,17] A time course study on the effect of ginsenoside-Rb_2 on the two enzyme activities in adipose tissue showed that lipoprotein lipase activity was increased during the experimental period, whereas the activity of hormone-sensitive lipase was decreased (Table II). The above results appear to be consistent with the increase of triglyceride fatty acid in the adipose tissue in response to ginsenoside-Rb_2. No significant change occurred in the concentration of serum lipids (Table IV), however. Thus, intraperitoneal administration of the ginsenoside to rats stimulated the lipolytic and lipogenic activities with little concomitant increase in the levels of triglyceride, total cholesterol, non-esterified fatty acid, *etc.*, in the serum.

On the other hand, the effect of an extract of Ginseng on lipid metabolism is considered to be strongly affected by the nutritional status of the body. Yamamoto *et al.* have reported that in rats fed on a high cholesterol diet, the concentrations of total cholesterol and triglyceride in the serum were decreased and the excretion of sterol was increased by the administration of *Panax ginseng*.[18,19] Moreover, long-term administration of ginseng powder in hyperlipidemic patients improved the atherogenic index.[20] Recent data from our laboratory have also shown that ginsenoside-Rb_2 reduced the total blood cholesterol in rats fed a high-cholesterol diet. Furthermore, an elevation of high density lipoprotein (HDL)-cholesterol, which is proposed to play a major role in cholesterol transport in the circulation,[21] was noted. Therefore, attention should be paid to the factor of nutritional status in future studies.

The experimental results described above suggest that ginsenoside-Rb_2 has a stimulating effect on lipogenesis in the liver and adipose tissue. On the basis of the present evidence, in

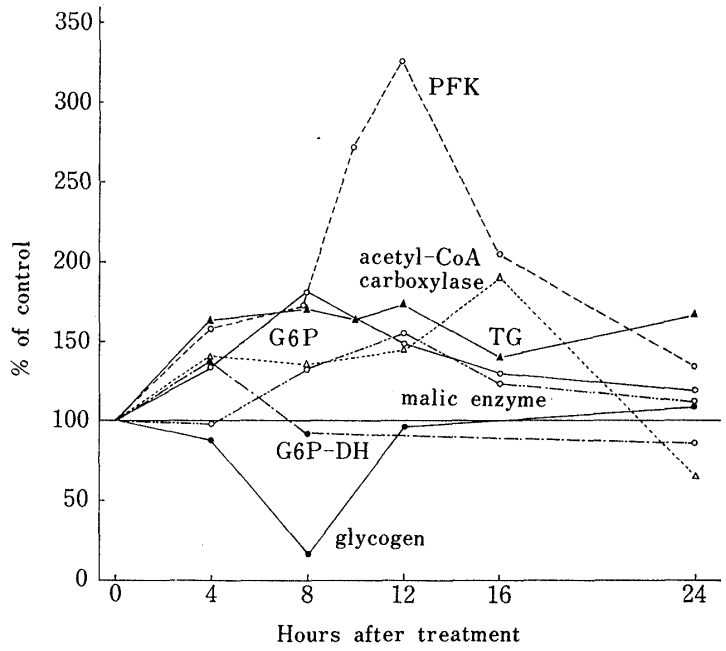

Fig. 1. Summary of Data on the Effects of Ginsenoside-Rb_2 in Rats

TG, triglyceride; G6P-DH, glucose-6-phosphate dehydrogenase; G6P, glucose-6-phosphate; PFK, phosphofructokinase.

conjunction with the preceding results on sugar metabolite concentrations and the activities of various enzymes concerned with sugar metabolism,[1] we can summarize the biochemical effects of a single injection of ginsenoside-Rb_2 in rats as shown in Fig. 1. The first phenomenon observed was a stimulation of glucose-6-phosphate dehydrogenase activity beginning 4 h after the administration of ginsenoside-Rb_2. Almost simultaneously a slight increase of acetyl-CoA carboxylase was observed. A decrease in the hepatic glycogen content reached a maximum at 8 h after the treatment. At this time, a drastic increase in the glucose-6-phosphate level took place, leading to increased activities of phosphofructokinase, malic enzyme, and acetyl-CoA carboxylase. Finally, adipose tissue triglyceride was increased. The effects of ginsenoside-Rb_2 fell almost to the control level at 24 h after the treatment, with the exception of the triglyceride content in adipose tissue. Accordingly, it may be concluded that ginsenoside-Rb_2 turns the metabolic flow toward the anaerobic glycolytic and lipogenic pathways. Previously, we showed that a semi-purified saponin (fractions 4 and 5) from the roots of *Panax ginseng* C. A. MEYER stimulates various metabolic reactions related to lipid and sugar metabolism.[22-26] Our present results indicate that most of the biochemical actions of the semi-purified saponin might be due to ginsenoside-Rb_2.

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