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Effect of Ginseng Saponin on Liver Glycogen Content

Takako Yokozawa, Kumiko Kanai, Michiko Takefuji, and Hikokichi Oura

Department of Biochemistry, Research Institute for WAKAN-YAKU (Oriental Medicines), Toyama University[1]

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Investigations were carried out to determine whether or not a decreasing action of hepatic glycogen content in rats by ginseng extract is due to its main constituent, saponin. The experimental results suggested that ginseng saponin decreases the glycogen stores, but the degree of its effect is regulated by the nutritional status of rats.

As reported previously, treatment of rats with an extract from the roots of *Panax ginseng* C.A. Meyer produced various reactions on lipid and sugar metabolism.[2,3] Particularly, intraperitoneal administration of ginseng extract showed a dramatic decrease in the liver glycogen content. Since this active preparation from ginseng radix contained mostly saponin,[4] whether or not the decreasing action on hepatic glycogen stores is truly due to the saponin should be investigated. This has become possible by the successful isolation and purification of ginseng saponin.[5]

However, it has been shown that the glycogen content of rat liver is altered over a wide range by varying the antecedent diet.[6] In this work, therefore, special care was taken to consider the nutritional status of rats in elucidating the effect of ginseng saponin on liver glycogen content.

Materials and Methods

Animals and Diet——Male rats of the Wistar strain (80—100 g) were maintained in an air-conditioned room with lighting from 6 a.m. to 6 p.m. The composition of the synthetic diet used is shown in Table I. The dry components of the diet and oil were thoroughly mixed in a mortar, and choline chloride (50% EtOH solution) and an appropriate amount of tap water were added for kneading the diet to a suitable consistency. The diet was prepared late in the afternoon and given freely. The rats were weighed every morning, at 10 a.m. if possible.

Chemicals——Casein, oil mixture, salt mixture, vitamin mixture, and choline chloride used in the synthetic diet were purchased from Tanabe Amino Acid Research Foundation, Osaka. All other chemicals used in this study were of analytical or reagent grade and were used without further purification.

Extraction and Purification of Ginseng Radix——Fraction 4 was prepared from the extract of root of *Panax ginseng* C.A. Meyer produced in Kumsan, Korea, as previously described.[2] This fraction contained 91.7% saponin as detected by the vanillin–H_2SO_4 reaction.[4] Therefore, further purification was carried out. Fraction 4 was dissolved in distilled water and dialyzed against distilled water for 1 week in a cold room to remove a small amount of brown resinoid substance. A white precipitate (fraction 5) was observed in the dialysis bag, and its crystallization from distilled water gave a white powder, mp 182—186° [*Anal.* Found: C, 54.80; H, 8.54; N, O; ash, none]. Chemical examination of fraction 5 gave a positive Liebermann-Burchard reaction, and it was found to contain 69.6% of hexose (glucose equivalent) determined by the Tsugita-Akabori method.[7] Further study indicated that fraction 5 consisted of ginsenoside-Rb_1, -Rb_2, -Rc, -Rc_2, -Rd, -Re, and -Rg_1.[5]

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2) T. Yokozawa, H. Seno, and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **23**, 3095 (1975).
3) T. Yokozawa and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **24**, 987 (1976).
4) S. Hiai, H. Oura, Y. Odaka, and T. Nakajima, *Planta Med.*, **28**, 363 (1975).
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Liver Glycogen——Rats were sacrificed by decapitation. Their liver was quickly excised, a sample of about 300 mg was cut, and frozen in a Dry Ice-acetone bath. The tissue was digested with 3 ml of 1N NaOH in a boiling water bath for 40 min and glycogen was precipitated by the addition of 2 ml of EtOH and purified according to the method of Roe and Dailey.[8] Liver glycogen was determined by the anthrone-H_2SO_4 method, with glucose used as the standard.[9]

TABLE I. Composition of the Synthetic Diet

	Fat diet			Casein diet		
	2%	5%	10%	10%	25%	70%
Casein	25	25	25	10	25	70
Dextrin	50.9	47.9	42.9	65.9	50.9	5.9
Sucrose	15	15	15	15	15	15
Oil mixture[a)]	2	5	10	2	2	2
Salt mixture[b)]	4	4	4	4	4	4
Vitamin mixture[c)]	1	1	1	1	1	1
Cellulose powder	2	2	2	2	2	2
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1

a) Oil mixture contains 80% soybean oil and 20% cod liver oil.
b) Salt mixture contains 29.29% $CaCO_3$, 0.43% $CaHPO_4 \cdot 2H_2O$, 34.31% KH_2PO_4, 25.06% NaCl, 9.98% $MgSO_4 \cdot 7H_2O$, 0.623% $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.156% $CuSO_4 \cdot 5H_2O$, 0.121% $MnSO_4 \cdot H_2O$, 0.02% $ZnCl_2$, 0.0005% KI, 0.0025% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$.
c) Vitamin mixture contains 0.059% thiamine-HCl, 0.059% riboflavin, 0.294% nicotinic acid, 0.235% calcium pantothenate, 0.029% pyridoxine-HCl, 0.006% menadione, 0.001% biotin, 0.002% folic acid, 0.0002% vitamin B_{12}, 1.176% inositol, 0.588% ascorbic acid, 97.551% lactose.

TABLE II. Effect of Fraction 5 on Liver Glycogen Content in Rats Fed on Diets containing Various Fat Levels

Fat level (%)	Treatment	Glycogen content (% of wet weight)	Statistical significance
2	control	4.34±0.56	
	fraction 5	2.50±0.36	$p<0.01$
5	control	3.59±0.14	
	fraction 5	2.65±0.19	$p<0.01$
10	control	2.70±0.36	
	fraction 5	2.63±0.20	N.S.

Rats were fed freely. After 7 days, rats were administered intraperitoneally with 10 mg of fraction 5 per rat. Control animals were treated with an equal volume of saline. Rats were killed by decapitation 8 hr after the administration. Groups of 5 to 6 rats were used in each experiment. The data presented are mean±S.E.

Results and Discussion

It is considered that dietary composition is of prime importance in determining the effect of ginseng saponin. In the first experiment, rats were fed a synthetic diet containing 2, 5, or 10% oil mixture for 7 days, and they were administered ginseng saponin intraperitoneally. Exactly 8 hr after the administration, they were killed and their liver was rapidly excised and assayed. As shown in Table II, the administration of ginseng saponin produced a significant decrease of hepatic glycogen content in rats fed a 2% fat diet. The data in Table II further indicate that the effect of ginseng saponin was completely abolished when the fat in the diet was raised from 2 to 10%.

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Further, it seemed of interest to examine whether or not a quantitative difference in dietary protein could modify the effect of ginseng saponin. For this, rats were fed a synthetic diet containing 10, 25, or 70% casein. The amount of casein was raised at the expense of dextrin, and the diet contained exactly 2% oil mixture. Under these dietary conditions, ginseng saponin decreased hepatic glycogen stores in rats fed a 25% casein diet (see Table III). A decrease of hepatic glycogen content by ginseng saponin was also observed in rats fed a 10% casein diet, but there was no statistically significant difference between the control and fraction 5-treated group. Table III shows, in addition, that the effect of ginseng saponin was completely abolished by a high-casein diet.

Table III. Effect of Fraction 5 on Liver Glycogen Content in Rats Fed on Diets containing Various Protein Levels

Protein level (%)	Treatment	Glycogen content (% of wet weight)	Statistical significance
10	control	7.29±0.52	
	fraction 5	5.72±0.47	N.S.
25	control	3.23±0.20	
	fraction 5	1.76±0.09	$p<0.001$
70	control	2.09±0.18	
	fraction 5	1.83±0.28	N.S.

Details are described in the legend to Table II.

From these results it is apparent that ginseng saponin decreases the glycogen content, but its effect is regulated by the dietary condition.

Rats are nocturnal and eat mainly at night. Consequently, food intake during the night increases. From these facts, it is considered that food intake, especially the fasting-and-eating cycle, is significant in controlling the effect of ginseng saponin. Therefore, we determined the effect of ginseng saponin at two periods of the day; fraction 5 was administered intraperitoneally at 10 p.m. the period of increasing glycogen content, in one experiment (A in Fig. 1) and at 6 a.m. the period of decreasing glycogen content, in another experiment (B in Fig. 1). Control rats were treated with an equal volume of saline. In both cases, animals were killed 8 hr later by decapitation. As shown in Fig. 1, ginseng saponin effectively suppressed the circadian increase and accelerated the circadian decrease, but the amount of the two cases was different.

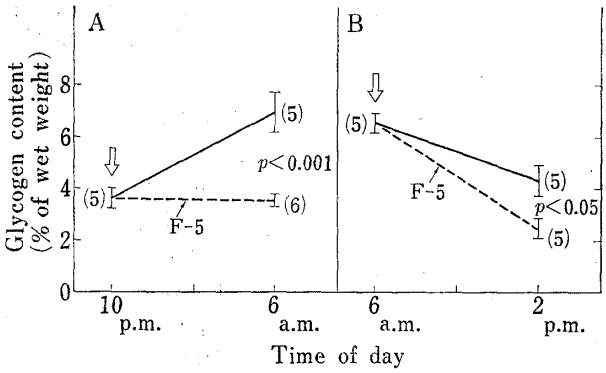

Fig. 1. Effect of Fraction 5 on Liver Glycogen Content in Rats Fed a 25% Casein Diet

Rats were fed freely for 7 days. In experiment A, rats were administered intraperitoneally with 10 mg of fraction 5 per rat at 10 p.m. and were killed at 6 a.m. In experiment B, rats were administered intraperitoneally with 10 mg of fraction 5 per rat at 6 a.m. and were killed at 2 p.m. Control animals were treated with an equal volume of saline. Vertical bars show standard error. Numbers in parentheses are number of animals used.

Thus, it may be considered that the stimulating action of ginseng saponin is influenced by the nutritional status of rats. However, the underlying mechanism that control these changes is beyond the scope of this experiment. Further studies will be needed for the elucidation of this mechanism.

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