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Weight Gain Reduction in Mice Fed *Panax ginseng* Saponin, a Pancreatic Lipase Inhibitor

NAAMA KARU, RAM REIFEN, AND ZOHAR KEREM*

The Institute of Biochemistry, Food Science and Nutrition, and the Otto Warburg Minerva Center for Agricultural Biotechnology, The Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

Roots of the herb *Panax ginseng* are known to contain high levels of bioactive saponins. Here, we isolated saponins from ginseng root powder and studied their inhibitory effect on the absorption of dietary fat in male Balb/c mice. Consumption of ginseng saponins suppressed the expected increase in body weight and plasma triacylglycerols, following a high-fat diet and observed higher intake. Consumption of ginseng saponins had no effect on the concentration of the total plasma cholesterol in both chow and high-fat diets in mice. The mode by which saponins from ginseng inhibit lipid metabolism was assessed as the in vitro inhibition of pancreatic lipase. Ginseng saponin inhibited pancreatic lipase with an apparent IC_{50} value of 500 μg/mL. Our results suggest that the anti-obesity and hypolipidemic effects of Ginseng in high-fat diet-treated mice were attributed to the isolated saponin fraction. These metabolic effects of the ginseng saponins may be mediated by inhibition of pancreatic lipase activity.

KEYWORDS: Obesity; dietary fat; blood lipids; triacylglycerol; cholesterol; pancreatic lipase; ginseng; saponin

INTRODUCTION

Roots of *Panax ginseng* have long been used in Eastern Asia as an herbal remedy to restore and enhance well-being. Currently, ginseng is one of the most popular and top-selling herbs (*1*, *2*). The main constituents contributing to the beneficial biological activities of ginseng are ginsenosides, that is, steroidal saponins (*3*). Ginseng and ginsenosides have been demonstrated to exert therapeutic effects on vitality, immune function, cancer, and cardiovascular diseases, and improve cognitive, physical, and sexual performance (*3*, *4*).

Since the 1950s, numerous studies have suggested that dietary saponins may reduce obesity and hyperlipidemia (*3*, *5*−*7*). In addition to their many other published biological activities (*3*, *4*), ginseng saponins were also shown to beneficially affect lipid metabolism in rodents (*8*). More than two decades ago, ginseng saponins were shown to reduce plasma lipids when injected intramuscularly to rats (*9*). Saponins from ginseng berry and isolated ginsenosides showed anti-hyperglycemic and anti-obesity activities following intraperitoneal injection to db/db and ob/ob mice (*10*). Crude fractions of ginsenosides have also proved to have an antiobesity effect in rats fed a high-fat diet (*8*).

While drug makers are looking forward to the prospect of producing antiobesity medications, obesity is a rising pandemic worldwide affecting 60 million adults in the United States alone. Although most physicians advocate diet and exercise as the best means to fight fat (*11*), many are seeking alternative ways to avoid corpulence and consequences of obesity. Increased consumption of more energy- dense, nutrient-poor foods with high levels of sugar and saturated fats, combined with reduced physical activity, have led to obesity reaching epidemic proportions globally (*12*). Obesity and being overweight are also associated with diet-related chronic diseases, including type 2 diabetes, cardiovascular disease, and several forms of cancer, all resulting in excess morbidity and mortality (*12*, *13*). Antiobesity drugs must tamper with the biochemistry of metabolism or appetite control, systems that are sometimes fatal to disrupt (*11*). This has been the case as increasingly more potent obesity pills were introduced into the market (*13*).

Dietary natural products that are generally recognized as safe (GRAS) may prove to be effective, but vigorous research is needed to establish mechanisms of action and active doses and to prove their safety. *Panax ginseng* is commonly consumed as powder, a beverage (e.g., tea), or a food supplement (*3*), whereupon the relevance of results achieved by injection is limited. The aim of this work was to study the potential of a diet amended with saponins isolated from *Panax ginseng* root in lowering blood lipid levels in a dietary-induced model of hyperlipidemic mice.

MATERIALS AND METHODS

All materials were purchased from Sigma (Sigma, St. Louis, MO) unless otherwise indicated. HPLC grade solvents were purchased from Baker (J.T. Baker, Phillipsburg, NJ). High capacity C_{18} (SPE)

* Author to whom correspondence should be addressed [telephone 972-8-9489278; fax 972-8-9476189; e-mail kerem@agri.huji.ac.il].

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cartridges of 10 g/60 mL were purchased from Alltech (Deerfield, IL). TLC silica gel plates were obtained from Merck (Darmstadt, Germany). Isolated standards of ginseng saponins were purchased from Extrasynthese (Lyon, France). Root powder of *Panax ginseng* was purchased from Fitness R Us (Qiryat Shmona, Israel). Assay kit for cholesterol was purchased from Raichem (San Diego, CA).

Animal Care. Male Balb/c mice, 8 weeks of age (Harlan, Indianapolis, IN), were housed individually in plastic cages under a 12-h light:dark cycle (0700−1900) in a temperature-controlled room (22 ± 1 °C). Mice were allowed free access to feed and water. Ethical approval was obtained for the study, and all of the procedures were conducted in full compliance with the strict guidelines of the Hebrew University Policy on Animal Care and Use.

Diets. Standard chow diet was supplied by Koffolk Ltd. (Tel Aviv, Israel; Cat. No. 19520) (ingredients of the diet, 210 g/kg total protein, 40 g/kg total fat, 535 g/kg carbohydrate, 45 g/kg cellulose, 70 g/kg ash, 0.4 g/kg choline chloride; energy content, 16.53 MJ/kg (3950 kcal/kg)). High-fat diet was purchased from Harlan-Teklad (Madison, WI; Cat. No. TD 96125) (ingredients of the diet, 198 g/kg total protein, 212 g/kg total fat, 492.2 g/kg carbohydrate, 36.7 g/kg cellulose, 3 g/kg choline bitartrate; energy content, 18.83 MJ/kg (4500 kcal/kg)). The diets were stored at −20 °C to avoid oxidation of lipids.

After 1 week of feeding chow diet, the mice were divided into four diet groups: a control group of 9 mice fed chow diet, a group of 5 mice fed chow diet containing 3% (w/w) GSE, a group of 9 mice fed high-fat diet (HFD), and a group of 9 mice fed HFD containing 3% GSE.

Sampling. The body weight of each mouse was measured twice a week, and the total amount of feed consumed was recorded daily. Individual feces were collected twice a week and stored at −80 °C until analysis. Mice were anesthetized with diethyl ether and killed by cervical dislocation on the morning of day 22 of the experiment. Urine was collected directly and stored at −80 °C until analysis. Blood was drawn from the portal vein into heparinized tubes, centrifuged at 2000*g* for 15 min, and the plasma was frozen at −80 °C. The liver was dissected and weighed, and sections from the middle jejunum were collected and frozen at −80 °C for later analyses.

Preparation of Ginseng Saponin Extract. Ginseng saponins were extracted on the basis of the method of Pietta et al. (*14*) with modifications. Ginseng root powder was extracted with 80% methanol in a solvent-to-meal ratio of 20:1 mL/g, by ultrasonication (Transsonic 420, Elma, Germany) for 1 h at 40 °C. Following filtration, the solid residue was similarly re-extracted. The filtrates were combined, concentrated under reduced pressure to a volume of 50 mL, and 150 mL of distilled water was added. Extracted ginseng saponins were separated by SPE (*14*). SPE cartridge was preconditioned by passing through 25 mL of methanol followed by 25 mL of water. The saponin crude extract was sonicated for 2 min, and 50 mL was loaded. The cartridge was washed with 50 mL of water and 25 mL of 40% methanol, followed by elution with 50 mL of 100% methanol. The cartridge was washed with 50 mL of acetone. Eluate volume was decreased under reduced pressure and consequently freeze-dried. The dried ginseng saponin-rich extract (GSE) was weighed, and a sample of 10 mg was reconstituted in 1 mL of 50% methanol for further analyses. The yield of the process was 15% (w/w) out of the original ginseng root powder.

Saponin Analysis. Thin-layer chromatography (TLC) was performed on a silica gel 60 plate developed with methanol/water/acetic acid, 12:2:1 (v/v/v). The dried plate was sprayed with acetic acid/sulfuric acid/*p*-anisaldehyde (100:2:1; v/v/v), dried, and heated to 110 °C to develop purple bands, indicating the presence of saponins (*15*). Analyses by TLC and HPLC were conducted to determine the concentration of saponins in GSE and facilitate the analysis of saponins from ginseng in jejunum, feces, and urine.

HPLC Assay of Ginsenosides. The HPLC system (Thermo Separation Products, Riviera Beach, FL) consisted of an auto-sampler (AS3000), an injector (100 μL), a column oven (32 °C), a pump (P3000), and a diode-array detector (UV6000). A reverse-phase C-18 column (250 × 4.6 mm, “Inertsil ODS-3V”, GL Sciences Inc., Tokyo, Japan) was employed. Gradient elution was performed using water (A) and acetonitrile (B). Initial conditions were 80% A, linearly changed to 75% A at 20 min and 69% A at 25 min. Over the next 30 min, the percentage of mobile-phase B increased linearly to 42%, followed by a gradient to 98% B at 60 min (*16*−*18*). Flow rate was 1.0 mL/min, and UV absorption was measured at wavelengths of 203 and 254 nm. Retention time of all ginsenosides was confirmed by authentic standards.

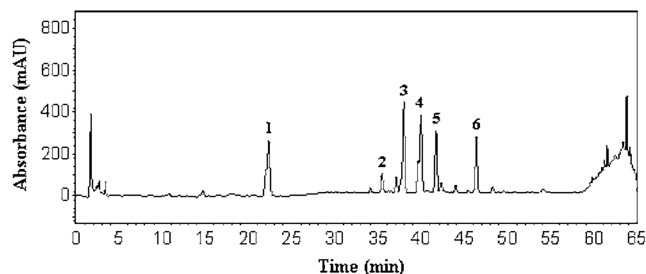

Figure 1. HPLC chromatogram at 203 nm, of ginseng saponin fraction eluted from a C-18 SPE column with 100% methanol (GSE). The weight of dry material injected was 100 μg. (1) Ginsenosides Rg_1 and Re; (2) ginsenoside Rf; (3) ginsenoside Rb_1; (4) ginsenoside Rc; (5) ginsenoside Rb_2; (6) ginsenoside Rd.

Plasma Lipid Analysis. Plasma concentration of total cholesterol was determined enzymatically using a commercial assay kit.

Free glycerol and total triacylglycerol in the plasma were determined enzymatically using Sigma reagents, and the true plasma triacylglycerol value was calculated.

Extraction of Saponins from Jejunum and Feces. Thawed jejunum sections, obtained from each of 5 animals in each diet group, were pooled, weighed, and cut to small pieces. To extract sapogenins, the pooled sections were homogenized in 100 mL of diethyl ether, centrifuged, and the supernatant was collected. Solvent was evaporated until dryness under a stream of nitrogen and reconstituted in 80% ethanol in water for further analyses. To extract saponins, residues of the ether extract were homogenized in 100 mL of 80% ethanol in water, and the supernatant was again collected (*19*, *30*).

Fecal saponin extraction was carried out using a similar procedure, with one sample of 200 mg of dry ground feces for each diet group.

Extraction of Saponins from Urine. Thawed urine (750 μL) collected from one mouse was loaded on a SPE C-18 cartridge (5 mL, packed with 200 mg of ODS) preconditioned by passing through 5 mL of methanol followed by 5 mL of water. The cartridge was washed with 10 mL of water and 4 mL of methanol 20%, followed by elution with 5 mL of methanol 70%. The eluate was evaporated to dryness under reduced pressure and reconstituted in 200 μL of acetonitrile 50%. A 20 μL aliquot was injected into the HPLC (*18*).

Pancreatic Lipase Activity Assay. Porcine pancreatic lipase activity was determined by the rate of oleic acid released from triolein, as described previously (*21*, *22*). The assay was conducted with five replicates for each treatment.

Statistical Analysis. Results are expressed as the mean ± SEM. Individual comparisons of means were made with Student’s *t*-test (two-tailed). If required, the data were log transformed before analysis of variance. Two-way ANOVA was used to identify significant effects of diet and treatment. Post tests for differences within diet groups were made with Bonferroni adjustments of the *P* values. In all instances, differences were considered significant at $P < 0.05$. Analyses were performed using Prism GraphPad statistical software (version 4.0 for windows).

RESULTS

Saponins in GSE, Jejunum, Feces, and Urine. The concentration and profile of ginsenosides in GSE prepared and used here showed the prevalence of saponins in the extract, as determined initially by TLC (*15*). To further establish the composition of the saponin powder used here, the powder was dissolved and chromatographed by RP-HPLC (*16*−*18*). The resultant chromatogram showed a profile typical to ginsenosides found in *Panax ginseng* root (**Figure 1**). Spiking with commercially available individual ginsenosides revealed the fol-

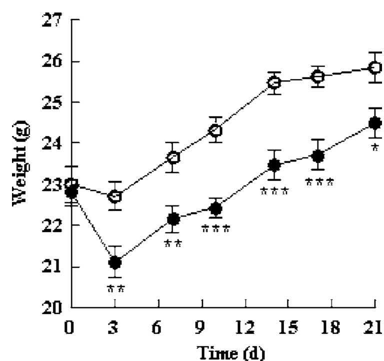

Figure 2. Effect of GSE on body weight gain in mice fed HFD. Values are means ± SEM, $n = 9$; (○) HFD; (●) HFD+GSE. Significance as determined by Student's *t*-test: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

lowing saponin composition (w/w): 24% Rb_1, 22% Rb_2, 26% Rc, 20.5% Rd, 3.5% Re, 2% Rf, 2% Rg_1.

Similarly, TLC and HPLC of jejunum, feces, and urine extracts showed the presence of ginseng saponins. An identical ginsenoside profile was observed in the jejunum extract of mice that consumed GSE, whereas the feces and urine extracts revealed a different profile with a higher concentration of Rd and lower concentration of Rb_1. The calculated amounts of total ginsenosides in the feces and urine samples were 110 mg/1 g of dry feces and 0.9 mg/1 mL of urine. These values suggest that up to 95% of a daily GSE intake was secreted in feces, and only about 1% of the daily intake was absorbed and secreted in the urine.

Physiological Data. Consumption of 3% GSE-amended diet for 3 weeks reduced the body-weight gain of mice fed HFD (**Figure 2**). Calculated energy consumption (mean ± SEM, in kJ/mouse/wk (to convert to kcal/mouse/wk, multiply by 0.239)) was dramatically higher in mice consuming GSE: 431 ± 22 in the HFD group; 629 ± 109 in the amended HFD group; 537 ± 38 in the chow diet group; and 718 ± 68 in the amended chow diet group. Changes in total feed intake and body weight of mice during the experiment are presented in **Table 1**. Feed efficiency ratio (FER), calculated from total feed intake and weight gain, demonstrated a low ratio for mice fed amended HFD, while amending GSE to chow diet reduced FER value only slightly. Two-way ANOVA of the FER values revealed a strong interaction between the diet and the GSE treatment, accounting for the main source of variation. Liver weight, expressed as a normalized weight per 100 g of mouse body weight, showed close results in mice fed either HFD- or HFD-amended diets. On the other hand, consumption of chow diet amended with 3% GSE increased liver weight relative to liver of mice fed control chow diet (**Table 1**).

Plasma Lipids. Determination of plasma lipids showed that amending either chow or high-fat diets with 3% GSE did not affect plasma total cholesterol concentrations of mice. Unlike cholesterol, plasma triacylglycerol concentration was reduced when HFD amended with GSE was consumed (**Table 1**). Two-way ANOVA of plasma triacylglycerols values revealed an interaction between the diet and the GSE treatment. Nevertheless, diet was the main source of variation ($P < 0.0001$).

Pancreatic Lipase Activity. The latter results suggested that the effect of dietary saponins is better expressed when the diet contains high amounts of both fat and saponins. We have thus tested the inhibition of lipid metabolism by pancreatic lipase in vitro. A dose-dependent inhibition of pancreatic lipase activity was demonstrated: A reduction of 20−80% of the control was measured with GSE concentrations of 200−800 μg/mL (100 μg/mL GSE is approximately 100 μmol/L ginsenosides), resulting in an apparent IC_{50} of 492 μg/mL (**Figure 3**).

DISCUSSION

The presented results demonstrate that dietary supplementation of ginseng saponins to male Balb/c mice inhibits increase in body weight and decreases the hypertriacylglycerolemia that follows consumption of a high-fat diet. The ginseng saponin extract was prepared from commercially available ginseng root powder. Using TLC and RP-LC, we confirmed the composition of the isolated saponin preparation to be identical to that of the genuine *Panax ginseng* saponins, where saponins comprise 95.4% of the powder.

The amended diets were designed to contain a high concentration of saponin preparation, considering 3% saponins in rodents' diet as the safety limit (*21*, *23*). To test the potency of ginseng saponins as a dietary supplement, we used oral administration rather than intraperitoneal injection. Indeed, the inclusion of GSE at 3% in the diet did not exert any observable toxic effect on the studied animals. Moreover, consumption of HFD amended with 3% GSE for 21 days suppressed body weight gain in mice, although during that period their cumulative and average energy consumption were much higher than that of mice consuming HFD alone. The high doses of GSE used here support previous results demonstrating significant metabolic effects of saponins in mice and rats (*6*, *21*). However, to our knowledge, no previous reports have shown the antiobesity effect of ginseng saponins following oral administration to mice. The apparent discrepancy between studies reporting inhibition of lipid metabolism and others that showed no beneficial effects may reflect the use of insufficient dosage.

The hypolipidemic effect of saponins from several plant sources has been previously demonstrated; for example, soyasaponins fed to female golden Syrian hamsters lowered plasma cholesterol and triacylglycerol concentrations (*6*). In our study, consumption of GSE lowered plasma triacylglycerol concentrations in mice fed HFD. This effect was not observed in mice fed chow diet, suggesting that the effect of dietary ginseng saponins is induced only when the fat concentration in the diet is elevated. This may be supported by the two-way ANOVA, demonstrating an interaction between the fat in the diet and the GSE consumption.

The suggested mechanism by which saponins exert their hypolipidemic effect is commonly attributed to disruption in cholesterol metabolism (*24*, *25*). This was supported by the observation of a decrease in plasma triacylglycerol and LDL concentrations in 8 humans who ingested 6 g of *Panax ginseng* extract containing 10% saponins per day for 8 weeks (*26*). In our study, however, consumption of GSE failed to induce any decrease in cholesterol concentration. These results are consistent with those observed in diabetic patients who consumed 100 mg of non-purified ginseng per day, for 8 weeks, resulting in lowering of plasma triacylglycerol concentrations, while plasma total cholesterol concentrations remained unaffected (*27*).

The lack of hypocholesterolemic activity may support a mechanism that is based on triacylglycerol metabolism in the gastrointestinal tract. Inhibition of hydrolysis of dietary fats may decrease intestinal absorption of fat and reduce blood chylomicron, leading to a reduction in hyperlipidemia and obesity. To further test the hypothesis that the decrease in plasma triacylglycerol concentration observed here was due to GSE administration, we assessed the potential of GSE to inhibit pancreatic lipase activity in vitro. Indeed, GSE inhibited lipase activity, with an apparent IC_{50} value of about 500 μg/mL.

Table 1. Effects of GSE Consumption on Physiological Data and Liver Weight in Mice Fed High-Fat and Chow Diets for 3 weeks[a]

	chow diet			high-fat diet (HFD)			DxGSE[b]
	control	+3% GSE	*P*	control	+3% GSE	*P*	*P*
n	9	5		9	9		
initial weight, g	22.8 ± 0.5	22.5 ± 0.7	>0.05	23.0 ± 0.4	22.8 ± 0.3	>0.05	0.678
final weight, g	25.7 ± 0.6	25.4 ± 0.3	>0.05	25.8 ± 0.4	24.5 ± 0.4	>0.05	0.278
total weight gain, g/3 wk	2.9 ± 0.3	3.0 ± 0.6	>0.05	2.8 ± 0.2	1.7 ± 0.3	<0.05	0.093
total feed intake, g/3 wk	97.5 ± 2.3	134.3 ± 6.4	<0.001	68.7 ± 1.2	103.3 ± 5.8	<0.001	0.403
FER	2.9 ± 0.3	2.3 ± 0.5	>0.05	4.4 ± 0.4	2.1 ± 0.4	<0.01	0.008
liver weight, g/100 g BW	5.4 ± 0.1	6.3 ± 0.4	<0.01	5.1 ± 0.1	5.5 ± 0.2	>0.05	0.155
plasma triacylglycerols,[c] mmol/L	0.93 ± 0.06	0.82 ± 0.02	>0.05	1.92 ± 0.05	1.40 ± 0.09	<0.001	0.007
plasma total cholesterol,[d] mmol/L	2.60 ± 0.06	2.15 ± 0.29	>0.05	4.12 ± 0.20	3.82 ± 0.29	>0.05	0.772

[a] Values are means ± SEM; *P* values calculated by two-way ANOVA with Bonferroni correction. [b] Abbreviations: D, diet; GSE, ginseng saponin extract; FER, feed efficiency ratio [total weight gain (g/3 wk)/total feed intake (g/3 wk)] × 10^2; BW, body weight. [c] To convert to mg/dL, multiply by 88.57. [d] To convert to mg/dL, multiply by 38.67.

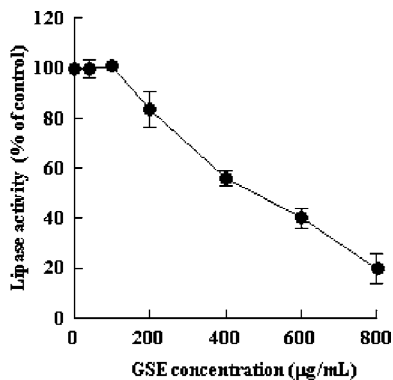

Figure 3. Inhibition of porcine pancreatic lipase by GSE in vitro. Values are means ± SEM, $n = 5$. 100 μg/mL GSE is approximately 100 μmol/L ginsenosides.

Inhibition potency of the same range was demonstrated in previous works focusing on saponins from medicinal plants less common than ginseng (*7*, *21*, *28*). In our study, mice consumed a dose of 150 mg/d GSE, suggesting that the actual concentration in the intestine was higher than the observed IC_{50}.

Pancreatic lipase attains its full catalytic activity only in the presence of oil in water (o/w) emulsions (*29*). Saponins act as emulsifiers, stabilizing the o/w interface (*25*), but, while aggregating with dietary fat droplets to form micelles, saponins might reduce the interface contact of lipase (by co-lipase) with the substrate. Human studies using the currently available drug inhibiting pancreatic lipase, Orlistat (XENICAL), reduced obesity and hyperlipidemia through inhibition of fat absorption (*30*). Unlike Orlistat, GSE activity on lipase is more likely mediated through disturbing the formation of an ideal substrate rather than the direct inhibition of the enzyme. Such mechanism of action should not affect the expression and metabolism of pancreatic lipase or other enzymes in the gastro-intestinal tract.

Another possible mechanism that may underlie the reduced weight gain and plasma triacylglycerol concentration induced by consumption of GSE is related to the capability of saponins to form micelles with bile acids (*25*). Reduced availability of bile acids for the correct arrangement of micelles with lipolytic products (of triacylglycerol hydrolysis by lipases) may decrease the amount of lipids dispersed in micelles for absorption by cells lining the gut (*29*).

Both mechanisms suggested here involve the duodenum and proximal jejunum (emulsification, lipolysis, solubilization, and lipid absorption). Our results suggest that the principal part of the administered GSE is secreted in feces. Orally ingested ginsenosides were shown in a previous report to pass through the stomach and small intestine without decomposition (*31*). Together, this evidence supports a mechanism based on small-intestinal lumen processes, rather than a mechanism based on absorption. Such inhibition of lipid metabolism by saponins might avoid induction of adaptation reaction in the body.

Consumption of a few grams of ginseng a day has been proven safe for humans, as reviewed by Coon and Ernst (*4*). Ingestion of isolated ginseng saponins along with a fatty meal may result in a high enough concentration to reduce weight gain and hyperlipidemia in healthy or overweight patients who wish to preserve their life-style habits. Our results suggest that ginseng saponins may be good candidates for a natural dietary supplement that reduces weight through delaying intestinal absorption of dietary fat by inhibiting pancreatic lipase activity.

ABBREVIATIONS USED

FER, feed efficiency ratio; GRAS, generally recognized as safe; GSE, ginseng saponin extract; HFD, high-fat diet.

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