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Dietary β-conglycinin prevents fatty liver induced by a high-fat diet by a decrease in peroxisome proliferator-activated receptor γ2 protein☆,☆☆

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Abstract

Diets high in sucrose/fructose or fat can result in hepatic steatosis (fatty liver). Mice fed a high-fat diet, especially that of saturated-fat-rich oil, develop fatty liver with an increase in peroxisome proliferator-activated receptor (PPAR) γ2 protein in liver. The fatty liver induced by a high-fat diet is improved by knockdown of liver PPARγ2. In this study, we investigated whether β-conglycinin (a major protein of soy protein) could reduce PPARγ2 protein and prevent high-fat-diet-induced fatty liver in ddY mice. Mice were fed a high-starch diet (70 energy% [en%] starch) plus 20% (wt/wt) sucrose in their drinking water or a high-safflower-oil diet (60 en%) or a high-butter diet (60 en%) for 11 weeks, by which fatty liver is developed. As a control, mice were fed a high-starch diet with drinking water. Either β-conglycinin or casein (control) was given as dietary protein. β-Conglycinin supplementation completely prevented fatty liver induced by each type of diet, along with a reduction in adipose tissue weight. β-Conglycinin decreased sterol regulatory element-binding protein (SREBP)-1c and carbohydrate response element-binding protein (ChREBP) messenger RNAs (mRNAs) in sucrose-supplemented mice, whereas it decreased PPARγ2 mRNA (and its target genes CD36 and FSP27), but did not decrease SREBP-1c and ChREBP mRNAs, in mice fed a high-fat diet. β-Conglycinin decreased PPARγ2 protein and liver triglyceride (TG) concentration in a dose-dependent manner in mice fed a high-butter diet; a significant decrease in liver TG concentration was observed at a concentration of 15 en%. In conclusion, β-conglycinin effectively prevents fatty liver induced by a high-fat diet through a decrease in liver PPARγ2 protein.

Keywords: Soy protein; Steatosis; Sterol regulatory element-binding protein; Peroxisome proliferator-activated receptor gamma; Butter

1. Introduction

The prevalence of obesity in Western societies, which is associated with an increased consumption of fat or carbohydrate, has increased dramatically. Among the consequences of obesity are the emerging epidemics of hepatic steatosis and nonalcoholic fatty liver disease (NAFLD) [1]. Nonalcoholic steatohepatitis (NASH), an advanced form in NAFLD, is characterized by macrovesicular steatosis and parenchymal inflammation [2]. The prevalence of NAFLD ranges from 10% to 24% of the general population, whereas NASH affects about 3% of the lean population and almost half of morbidly obese people [3].

The accumulated hepatic lipids in patients with NAFLD include plasma nonesterified fatty acids (NEFAs) from adipose tissue, fatty acids produced in the liver via *de novo* lipogenesis (DNL) and dietary fatty acids, which enter the liver via spillover of NEFA derived from the lipolysis of chylomicron and via hepatic uptake of chylomicron remnants. Analysis of multiple stable isotopes in patients with NAFLD has revealed that of the triglyceride (TG) in the liver, 59% is derived from NEFAs, 26% is from DNL and 15% is from dietary fatty acids [4]. Roughly one fourth of fatty acids in the liver is produced from *de novo* 2-carbon precursors derived from glucose, fructose and amino acids. Dietary fat also contributes significantly to liver TG storage pools. Transcription factors, including sterol regulatory element-binding protein (SREBP)-1c, carbohydrate response element-binding protein (ChREBP), peroxisome proliferator-activated receptor (PPAR)α and PPARγ, that regulate liver TG concentrations contribute to fatty liver induced by dietary manipulations [5–8].

The C57BL/6J inbred mouse strain has been used for studies of obesity and diabetes because of its susceptibility to these diseases in response to a high-fat (HF) diet [9,10]. C57BL/6J mice also develop fatty liver in response to an HF diet [11–13]. However, these mice are resistant to sucrose/fructose-induced fatty liver because they possess adenine −468 bp from the putative 5′ end of the SREBP-1c gene [14]. The ddY mice possess guanine −468 bp in the SREBP-1c promoter and develop hepatic steatosis when fed either sucrose supplementation or

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Table 1
Dietary composition of experimental diets

β-Conglycinin (20 en%)	St		Suc		Safflower oil		Butter	
	−	+	−	+	−	+	−	+
	g/100 g							
Safflower oil	3.9	3.9	3.9	3.9	32.6	32.6		
Butter							32.6	32.6
Casein	18.0		18.0		25.5		25.5	
β-Conglycinin		18.9		18.9		26.8		26.8
α-Starch	68.3	67.4	68.3	67.4	27.7	26.4	27.7	26.4
Vitamin mix (AIN-93)	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5
Mineral mix (AIN-93)	3.5	3.5	3.5	3.5	5.1	5.1	5.1	5.1
Cellulose powder	5.0	5.0	5.0	5.0	7.3	7.3	7.3	7.3
L-Cystine	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4

St, high-starch diet; Suc, high-starch diet with 20% (wt/wt) sucrose drink. β-Conglycinin contains approximately 5% carbohydrate per protein as glucose.

an HF diet. In our previous study, fish oil supplementation could prevent sucrose-induced fatty liver but did not prevent HF-diet-induced fatty liver in ddY mice [15]. This was due to the difference in mechanisms of dietary-induced fatty liver formation. Fish oil decreases liver SREBP-1c activity, which counteracts the increase of SREBP-1c messenger RNA (mRNA) in response to sucrose overconsumption. Fish oil does not decrease liver PPARγ mRNA that is increased in response to HF diets.

In the present study, we sought to find a dietary compound to prevent HF-diet-induced fatty liver. Soy protein could prevent fat accumulation [16]. We therefore investigated β-conglycinin, a major component of soy protein, to determine if this protein might effectively prevent HF-diet-induced fatty liver and the methods by which it might do so.

2. Materials and methods

2.1. Animals

Six-week-old male ddY mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and fed a normal laboratory diet (CE2; Clea, Tokyo, Japan) for 1 week to stabilize metabolic conditions. Mice were exposed to a 12-h light/12-h dark cycle, and the room was maintained at a constant temperature of 22°C. Four mice were housed per plastic cage, each of which was equipped with plastic partitions to separate individual mice. Mice were cared for in accordance with the National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals*. All animal procedures were reviewed and approved by the National Institute of Health and Nutrition (No. 0606).

2.2. Diet

At 7 weeks of age, ddY mice were assigned to one of several groups (n=5–9 in each group). To examine the effects of β-conglycinin on fatty liver induced by sucrose, high-safflower-oil, and high-butter diets, four groups were created: control mice were fed a high-starch diet, sucrose-supplemented mice were fed a high-starch diet plus 20% sucrose (wt/wt) in their drinking water, high-safflower-oil-fed mice were given 60 energy% (en%) safflower oil and high-butter-fed mice were given 60 en% butter. To examine the effects of β-conglycinin, all casein in the diet of each group was replaced by β-conglycinin. Detailed compositions of the experimental diets are listed in Table 1. To examine the dose-dependency of β-conglycinin in mice fed a high-butter-oil diet, 0, 5, 10, 15 and 20 en% of β-conglycinin were given in replacement of casein. Duration of the dietary manipulations was 11 weeks in all experiments. Fatty acid compositions of dietary oils were measured by gas–liquid chromatography. Safflower oil (high-oleic type) contained 45% (wt/wt) oleic acid (18:1n-9) and 46% linoleic acid (18:2n-6), and butter (salt-free type) contained 71% saturated fatty acid including 12% myristic acid (14:0), 33% palmitic acid (16:0) and stearic acid 11% (18:0). Diet preparations were similar to those of our previous studies [15]. Butter was purchased from Snow Brand Milk Corp. (Hokkaido, Japan). Safflower oil and β-conglycinin were kindly provided by Benibana Food (Tokyo, Japan) and Fuji Oil Co. (Osaka, Japan), respectively. β-Conglycinin was prepared by treatment of soybean protein extract with phytase [17]. Consumption of food was measured daily, and intake of sucrose water was measured weekly. Food intake per day was estimated by subtracting the food weight of that day from the initial food weight of the previous day. Average energy intakes during total experimental periods in each group of mice were calculated with these data.

2.3. Quantitative reverse transcriptase polymerase chain reaction

Mice were killed, and livers were isolated for RNA preparation in the morning from 3-h fasted animals to avoid acute effects of food intake. RNA was extracted with TRIzol Reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA isolated from liver was reverse transcribed with ReverTra Ace (Toyobo Co., Ltd., Osaka, Japan) with random hexamers. The resulting complementary DNA was polymerase chain reaction (PCR) amplified in the 96-well format with SYBR Green PCR Master Mix and a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Expression levels of test genes were normalized to those of an endogenous control, acidic ribosomal phosphoprotein P0 (36B4). The primers used for quantitative

Table 2
Body and tissue weights and total energy intake of mice after 11 weeks on experimental diets

	β-Conglycinin	St	Suc	Safflower oil	Butter	Two-way ANOVA P value		
						Diet	β-Conglycinin	Diet×β-conglycinin
n	−	8	8	8	8			
	+	8	8	8	8			
Weight (g)								
BW at start	−	28.3±0.8	28.3±0.8	28.4±1.2	28.6±0.9			
	+	28.4±1.4	28.4±1.4	28.6±1.4	28.5±1.4	.998	.946	.999
BW	−	45.3±1.9^{a,b}	53.4±1.0^{d}	53.7±2.1^{d,e}	59.8±2.2^{f}			
	+	36.6±1.7^{c}	41.0±1.2^{a,c}	47.7±2.2^{b,e}	46.1±1.7^{b}	<.001	<.001	.162
Liver	−	1.66±0.18^{a,b}	1.98±0.07^{b,c}	2.11±0.08^{b}	2.56±0.25^{d}			
	+	1.54±0.06^{a}	1.62±0.08^{a}	1.83±0.06^{a,b,c}	1.97±0.08^{b,c}	<.001	<.001	.308
Epididymal WAT	−	1.51±0.27^{a,b}	2.33±0.17^{b,d}	2.77±0.24^{d}	2.67±0.17^{d}			
	+	0.74±0.11^{c}	1.23±0.08^{a,c}	2.15±0.31^{b,d}	1.80±0.29^{a,b}	<.001	<.001	.716
Retroperitoneal WAT	−	0.38±0.07^{a,b}	0.52±0.07^{a,d}	0.60±0.07^{d}	0.58±0.07^{a,d}			
	+	0.16±0.03^{c}	0.27±0.03^{b,c}	0.51±0.05^{a,b}	0.41±0.07^{a,b}	<.001	<.001	.588
Mesenteric WAT	−	0.69±0.11^{a}	0.96±0.10^{c,d}	0.99±0.12^{c,e}	1.14±0.10^{de}			
	+	0.30±0.05^{b}	0.48±0.05^{a}	0.77±0.13^{a,c}	0.67±0.10^{a}	<.001	<.001	.553
Subcutaneous WAT	−	0.81±0.18^{a}	1.25±0.14^{c,d}	1.35±0.15^{c}	1.91±0.23^{e}			
	+	0.34±0.07^{b}	0.48±0.04^{a,b}	1.08±0.13^{a,c}	0.90±0.14^{a,d}	<.001	<.001	.085
Total energy intake (MJ/mouse)	−	5.51±0.03^{a}	6.73±0.12^{b}	7.64±0.09^{c}	7.59±0.13^{d}			
	+	5.52±0.03^{a}	6.93±0.11^{b}	7.45±0.08^{c}	7.71±0.11^{d}	<.001	.616	.184

Values are mean±S.E.M. Means without a common letter^{a,b,c,d,e} differ (P<.05). BW, body weight; St, high-starch diet; Suc, high-starch diet with 20% (wt/wt) sucrose drink; WAT, white adipose tissue.