

The Short-Term Effects of Soft Pellets on Lipogenesis and Insulin Sensitivity in Rats

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ABSTRACT: The purpose of the present study was to investigate the short-term effects of a 12-day, soft pellet (SP) diet with a 3-h restricted feeding schedule on caloric intake, body weight, lipid metabolism, and insulin sensitivity. Glucose and insulin levels were measured pre-, mid-, and post-feeding. The SP rats exhibited postprandial hyperglycemia compared to rats fed control pellets (CP). The insulin response of SP rats during a meal was significantly higher than that of CP rats. There were no significant differences in the hepatic triacylglycerol contents and lipogenesis gene mRNA levels of SP and CP rats. However, the hepatocytes of SP rats were slightly hypertrophic. In addition, histological analysis revealed that the pancreases of SP rats had more islet areas than those of CP rats. This study demonstrated that feeding an SP-only diet for 12 days induces glucose intolerance, suggesting that the consumption of absorbable food, like a soft diet, may trigger glucose metabolism insufficiency and lead to life-threatening diseases.

Keywords: soft pellets, short-term feeding, lipogenesis, insulin sensitivity

INTRODUCTION

Individuals with insulin resistance are predisposed to develop type 2 diabetes mellitus (T2DM) (1). T2DM is associated with a number of comorbidities, including dyslipidemia and inflammation, which are collectively referred to as metabolic syndrome (2). In the insulin resistant state, the effect of insulin to maintain blood glucose homeostasis is compromised, leading to hypersecretion of insulin from pancreatic beta cells (3,4).

Food texture is thought to affect feeding behavior and energy metabolism (5). Eating behavior has long been of interest as a factor contributing to the development of obesity (6-9). Fujise et al. reported that meal size and eating speed increased in rats fed a soft pellet (SP) diet compared to rats fed a hard pellet diet (10). In previous studies, long-term feeding of an SP diet induced a larger increase in body weight and body fat content than long-term feeding of a hard pellet diet (11,12). These findings indicate that there is a close relationship between eating habits, food texture differences, and obesity or obesity-related disease. Similarly, we recently reported that rats fed an SP diet for 14 weeks displayed signs of glucose in-

tolerance and *de novo* lipogenesis (13). These findings suggest that long-term intake of an SP diet may induce obesity or insulin resistance. However, it is not yet known whether short-term intake of an SP diet affects lipid metabolism and insulin sensitivity. Therefore, in the present study we investigate the effects of short-term (i.e., 12 days) feeding of an SP diet on lipid metabolism and insulin sensitivity in rats.

MATERIALS AND METHODS

Animals and diet

Twenty 7-week-old, male Wistar rats (Charles River Japan, Inc., Shiga, Japan) were used for this study. Rats were individually housed in plastic cages kept at a constant room temperature with a 12 h light/12 h dark cycle (light: 08:00~20:00). The rats were randomly divided into two groups (n=10 per group) and were fed either control pellets (CP) or SPs for 12 days. The CP group was fed a standard laboratory chow containing 51% carbohydrate, 25% protein, and 4.6% fat (CLEA Japan, Inc., Tokyo, Japan). The SP group was fed a mix-

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ture of standard laboratory chow and water (1 g of standard laboratory chow with 1.4 mL of water). Because we planned to measure glucose and insulin concentrations pre-, mid-, and post-feeding, the rats were given access to food between 09:00 and 12:00 (i.e., a 3-h restricted feeding schedule). All rats were allowed *ad libitum* access to water throughout the study. Body weight and food intake were measured daily throughout the study. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. The study protocol was approved by the Ethics Review Committee for Animal Experimentation in the Faculty of Medicine, University of Miyazaki.

Plasma samples preparation and analysis

Blood samples were obtained from the tail vein of 9-week-old CP and SP rats (n=6 per group) immediately pre-meal (t=0 min), mid-meal (t=60 min into the meal), and post-meal (t=30 min postprandial). Blood was collected into tubes containing a protease inhibitor cocktail (Roche, Basel, Switzerland), immediately centrifuged (2,000 g, 4°C, 10 min), and then stored at -80°C until analysis. Plasma insulin levels were measured with ELISA kits (Insulin; Morinaga Institute of Biological Science, Inc., Yokohama, Japan) at the three points described above. Blood glucose measurements were performed with a hand-held glucometer (Bayer, Osaka, Japan) at the three points mentioned above.

Hepatic triacylglycerol content

Nine-week-old CP and SP rats (n=3 per group) were fasted overnight and then anesthetized with an intraperitoneal injection of a 50 mg/kg dose of pentobarbital. To measure hepatic triacylglycerol content, the lipids from 25 mg of liver tissue were extracted in 1 mL of chloroform-methanol [2:1 (v/v)] mixture, as previously described (14), and the triacylglycerol content was quantified with a triglyceride E kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Histological analysis

The liver and pancreas were removed from the anesthetized rats (n=3 per group), rinsed with saline and fixed in a buffer solution of 3.7% formalin. Portions of each fixed tissue specimen were processed for paraffin embedding. Three-micrometer tissue sections were cut from the paraffin-embedded specimens, spread onto a slide, and baked at 60°C for 1 h. The slides were stained with hematoxylin and eosin (H&E) examined under an optical microscope (Nikon, Tokyo, Japan). The islets of Langerhans were measured with ImageJ software (<http://rsb.info.nih.gov/ij/download.html>).

Table 1. PCR amplification primer sequences

Gene		Primer sequence (5'→3')
SREBP-1c	Forward	GCGGAGCCATGGATTGCAC
	Reverse	CTCTTCCTTGATACCAGGCC
ACC	Forward	GAGGTTGGAGGCCAAAGGACAT
	Reverse	TACAGCATCGCCAGCTTAAGG
FAS	Forward	AGGCGTCGAACTTGGACAGAT
	Reverse	AGTTCTGGGCCAACCTCATTG
36B4	Forward	AGGTCCTCCTTGGTGAACACAAA
	Reverse	TCATTGTGGGAGCAGACAATGTG

Quantitative real-time PCR

The liver was removed from overnight-fasted, anesthetized 9-week-old rats (n=5 or 6 per group), and total RNA was rapidly extracted with TRIzol[®] reagent (Invitrogen Corp., Carlsbad, CA, USA). First-strand cDNA was synthesized from 1 µg of total RNA using a commercially available Superscript[®] III First-Strand Synthesis System kit (Invitrogen Corp.), and the resulting cDNA was used for quantitative PCR analysis. Quantitative PCR was conducted on a LightCycler[®] system (Roche Diagnostics GmbH, Mannheim, Germany) using the SYBR[®] Premix Ex Taq[™] system (Takara Bio Inc., Shiga, Japan) with sterol regulatory element-binding protein (SREBP)-1c, acetyl CoA carboxylase (ACC), and fatty acid synthase (FAS) primers. The gene specific primers used are described in Table 1. The relative abundance of all reaction products was normalized to the level of ribosomal protein 36B4 mRNA.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) soft version 12.0 for windows (SPSS Inc., Chicago, IL, USA). All data are presented as the mean±SEM. Statistical significance was evaluated by Student's *t*-tests. The trapezoidal method was used for all area under the curve (AUC) calculations. *P*-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

In the present study, we investigated lipogenesis and insulin sensitivity in rats fed an SP diet for 12 days. The caloric intake, body weight, and energy efficiency rate were not significantly different between groups (Fig. 1).

Blood glucose and insulin responses to feeding are shown in Fig. 2. At 30 min post-feeding, the blood glucose level was significantly greater in the SP group than the CP group (Fig. 2A). However, there was no difference between the blood glucose AUC values of the two groups (Fig. 2B). Furthermore, at the 60 min (i.e., mid-meal) time point, SP rats had significantly greater insulin levels than CP rats (Fig. 2C). The insulin response

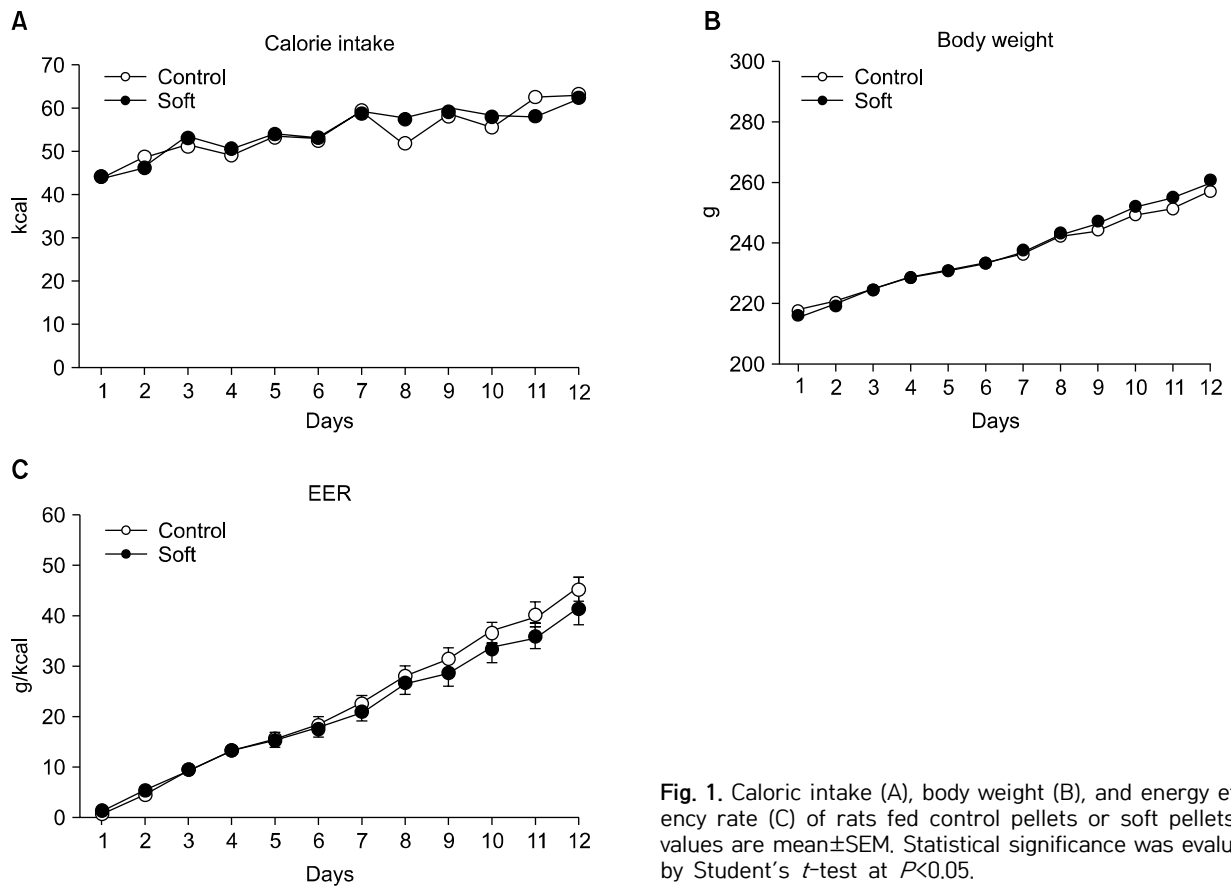


Fig. 1. Caloric intake (A), body weight (B), and energy efficiency rate (C) of rats fed control pellets or soft pellets. All values are mean±SEM. Statistical significance was evaluated by Student's *t*-test at $P<0.05$.

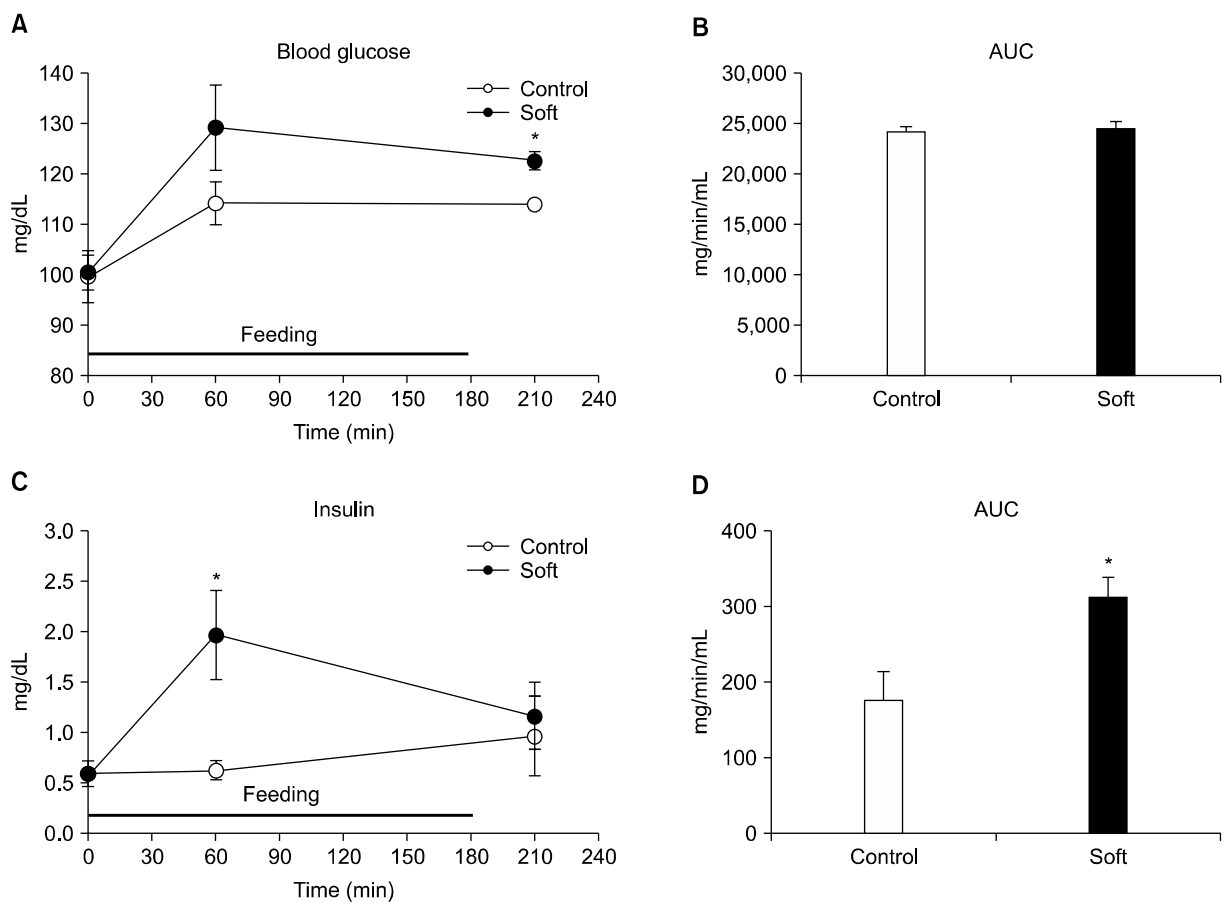


Fig. 2. Blood glucose (A and B) and insulin (C and D) levels in rats fed control pellets or soft pellets. All values are mean±SEM. Statistical significance was evaluated by Student's *t*-test at $P<0.05$. AUC, area under the curve.

AUC of the SP group was also significantly greater than the insulin response AUC of the CP group (Fig. 2D). Our data are in agreement with the work of Nojima et al., which showed that consumption of a soft diet can have a blood glucose increasing effect (15). Previous studies have also indicated that the texture of absorbable food can trigger hyperglycemia and hyperinsulinemia (13). In other words, even short-term consumption of an SP diet may promote glucose metabolism insufficiency in rats.

Insulin producing pancreatic β -cells maintains blood glucose levels within a narrow range by secreting insulin in response to glucose (16). In rats, glucose infusion induces β -cell replication and increased β -cell size and mass (17,18). In the early stages of diabetes, pancreatic β -cells, which are closely regulated by glucose flux and hormonal effects (19), are hyperplastic and produce a lot of insulin (20). H&E staining was used to assess the islet morphology of pancreas sections collected from CP and SP rats (Fig. 3A). The surface area of pancreatic islets from the SP group was 90.45% greater than the surface area of pancreatic islets from the CP group (Fig. 3B). Recent studies suggest that both CP and SP diets increase β -cell mass and worsen insulin sensitivity (13). The present data suggest that the short-term consumption of an absorbable SP diet induces sustained hypergly-

cemia. This is followed by β -cell hyperplasia, which causes an increase in plasma insulin levels.

We examined the histology, triacylglycerol content, and lipogenesis-related gene (i.e., SREBP-1c, ACC, and FAS) expression of livers collected from rats in the CP and SP groups. Photomicrographs of H&E-stained liver tissue sections revealed the histological structure of the liver (Fig. 4A). Triacylglycerol levels were increased in the SP group, but not significantly (Fig. 4B). There were no significant differences in hepatic SREBP-1c, ACC, and FAS mRNA expression between the CP and SP groups. However, the ACC and FAS mRNA levels of the SP group were greater than the ACC and FAS mRNA levels of the CP group (Fig. 4C). SREBP-1c expression is stimulated by insulin. The targets (e.g., ACC and FAS) of SREBP-1c are involved in the lipogenic pathway. Activation of the lipogenic pathway increases liver triglyceride concentrations (21,22). In the present study, we found that triacylglycerol levels, ACC mRNA expression, and FAS mRNA expression tended to be greater in the SP rats compared to the CP rats; however, these differences were not significant. Our previous studies indicated that rats fed an SP diet for 14 weeks had signs of liver de novo lipogenesis (13), indicating that a longer period of SP diet consumption is necessary for the induction of hepatic lipogenesis.

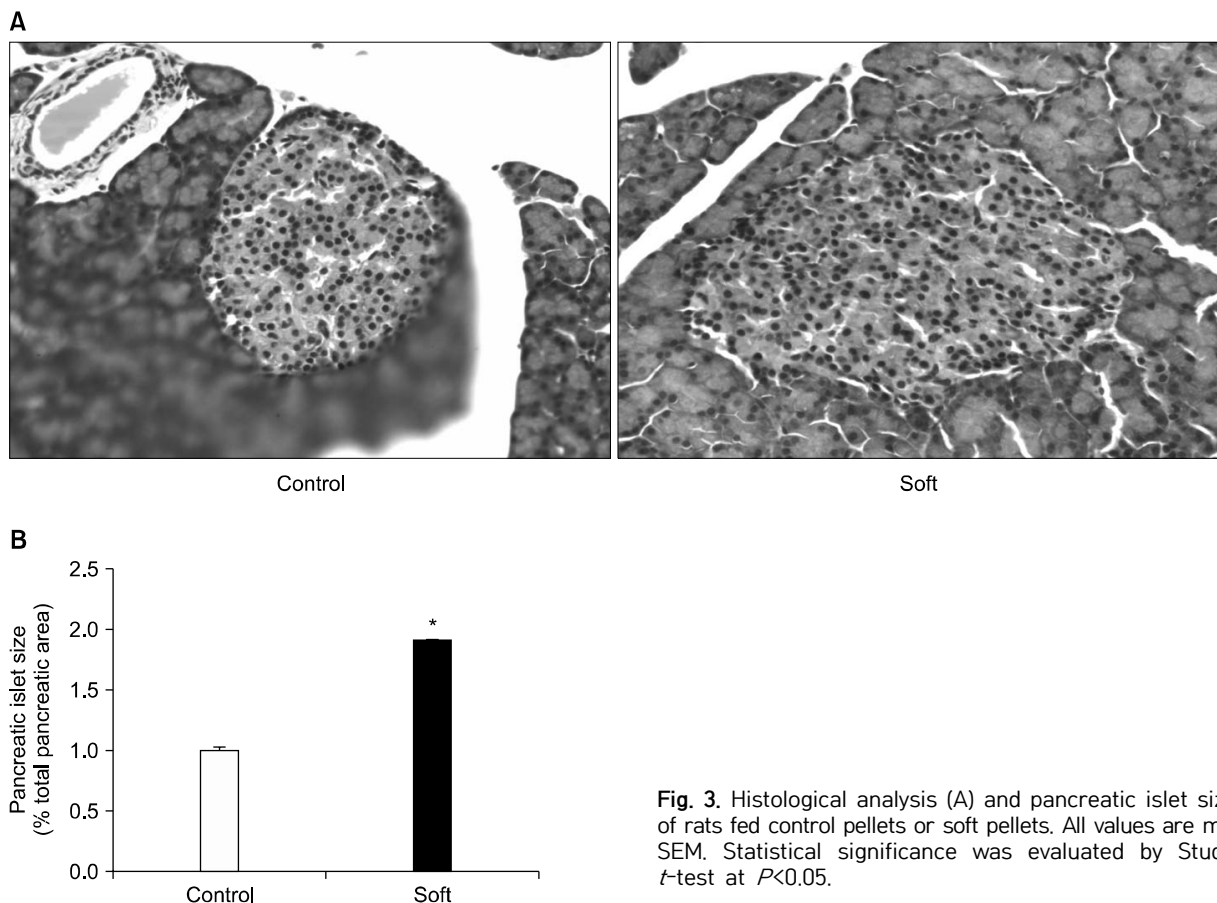


Fig. 3. Histological analysis (A) and pancreatic islet size (B) of rats fed control pellets or soft pellets. All values are mean \pm SEM. Statistical significance was evaluated by Student's *t*-test at $P < 0.05$.

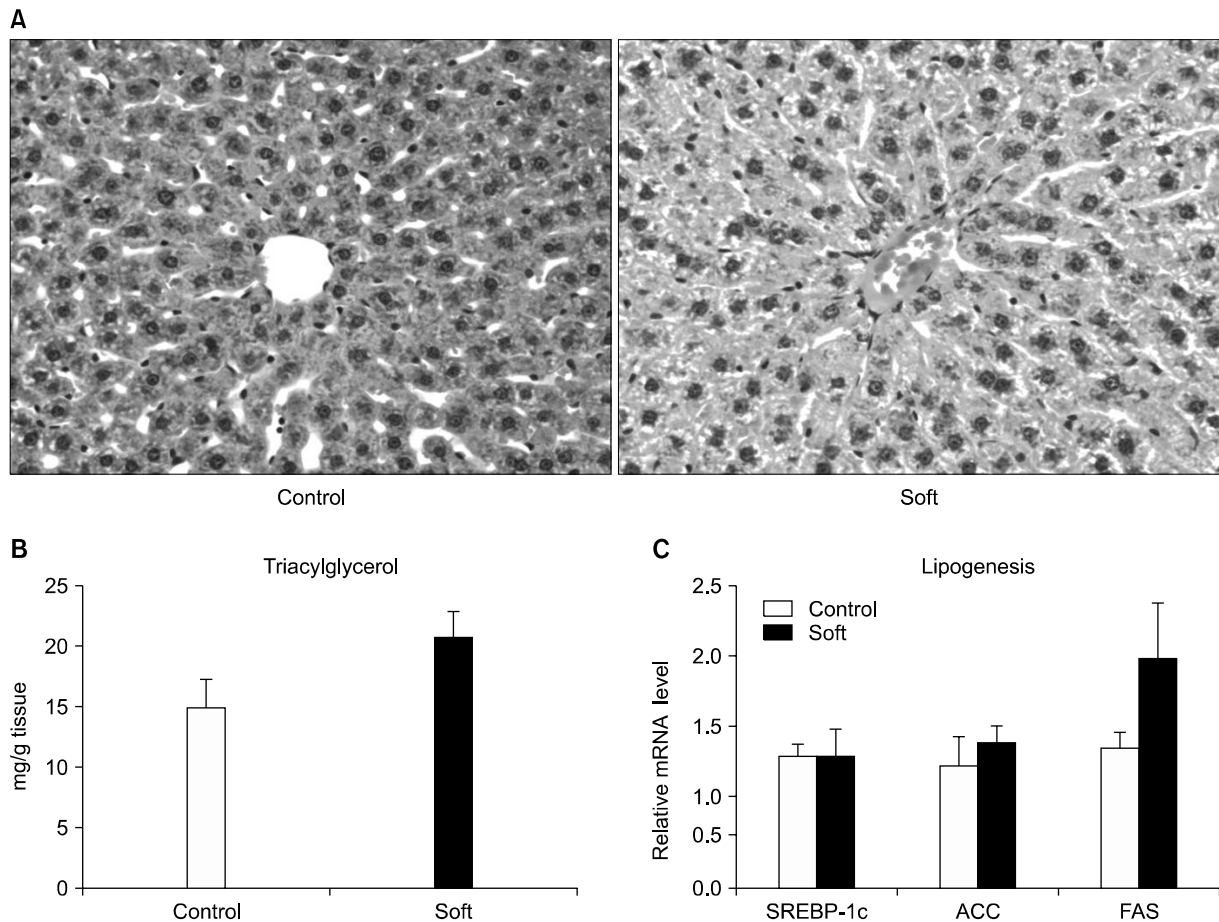


Fig. 4. Photomicrographs of H&E-stained liver sections (A), hepatic triacylglycerol content (B), and lipogenesis-related mRNA levels (C). All values are mean \pm SEM. Statistical significance was evaluated by Student's *t*-test at $P<0.05$.

In conclusion, our current data demonstrate that rats fed an SP diet, even for a short period (i.e., 12 days), develop hyperinsulinemia with postprandial hyperglycemia, but not *de novo* lipogenesis. Our previous study revealed that rats fed an SP diet for 14 weeks developed insulin resistance and lipogenesis. However, the data obtained from the present study suggest that the SP diet itself could disturb glucose homeostasis, indicating that continuous consumption of an SP diet would lead to insulin resistance and lipogenesis. The results of our present study, as well as those of our previous study, have provided a new insight into the development and prevention of metabolic syndrome. Food texture might be an important factor when selecting foods to improve the control of human lifestyle-related diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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