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Effects of Voluntary Running and Soy Supplementation on Diet-Induced Metabolic Disturbance and Inflammation in Mice

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ABSTRACT: We investigated the effects of diet (AIN93G or high-fat), physical activity (sedentary or voluntary running), and protein source (casein or soy protein isolate (SPI)) and their interactions on metabolic disturbance and inflammation in mice. After 14 weeks of feeding, the high-fat diet increased body weight gain by 34.5% ($p < 0.01$), whereas running reduced weight gain by 30.5% ($p < 0.01$) compared to their respective AIN93G and sedentary controls; SPI did not affect weight gain. The high-fat diet significantly increased plasma concentrations of insulin, glucose, triglycerides, leptin, and monocyte chemotactic protein-1 (MCP-1); running and SPI significantly reduced these parameters compared to their respective controls. The high-fat diet significantly increased and running significantly reduced plasma plasminogen activator inhibitor-1. A unique finding was that SPI supplementation to the high-fat diet reduced plasma insulin by 11% ($p < 0.05$), MCP-1 by 21% ($p = 0.03$), and tumor necrosis factor-α (TNF-α) by 50% ($p = 0.05$) compared to casein. As adipose tissues produce many adipocytokines, including MCP-1 and TNF-α, that contribute to a state of chronic low grade systemic inflammation and facilitate metabolic disturbance in obesity, further investigations are warranted into the roles of soy protein in reducing the risk of obesity.

KEYWORDS: *soy, physical activity, metabolic disturbance, inflammation, mice*

INTRODUCTION

The worldwide prevalence of overweight and obesity has been considered a global epidemic.[1] Although genetics may play a role in the regulation of body weight homeostasis, sedentary lifestyles and eating habits are important environmental contributors to the dramatic increase in the occurrence of overweight and obesity worldwide.[1]

Obesity occurs when there is an imbalance between energy intake and expenditure which leads to excessive accumulation of fat in various adipose tissues and organs. Hyperglycemia and insulin resistance are the most common underlying abnormalities of obesity. Furthermore, adipose tissues are considered an endocrine organ which produces many adipocytokines that contribute to a state of chronic low grade systemic inflammation and facilitate metabolic disturbance in obesity.[2]

Physical activity, defined as bodily movement due to skeletal muscle contraction, and dietary modification are two key approaches that may reduce the risk of obesity. Moderate exercise, by increasing energy expenditure, reduces body weight, insulin resistance, dyslipidemia, and inflammation and enhances insulin sensitivity and glycemic control.[3] Plant-derived food has been shown to be useful, by decreasing dietary energy density, in reducing the risk of obesity and obesity-related abnormalities. Soybeans provide one of the most abundant plant sources of dietary protein, and soy protein is considered a complete protein in that it contains most of the essential amino acids that are found in animal sources of proteins.[4] The nutritional value of soy protein is approximately equivalent to that of animal protein of high biological value.[4] Soy consumption is considered beneficial to heart health.[5] Clinical investigations demonstrated that a low-caloric soy-based meal replacement formula lowers body weight and fat mass and improves lipid profiles in obese human subjects.[6] Laboratory studies showed that soy protein feeding reduces total and liver adiposity in obese rodents.[7] Furthermore, available evidence indicates that certain soy components, e.g., β-conglycinin[8] and isoflavones,[9] may contribute to such benefits.

Despite the great effort undertaken to study the roles of physical activity and soy protein in weight management and obesity, investigations of their combination have not been documented. To examine the interaction of moderate physical activity with soy consumption in reducing the risk of obesity, the present study investigated the effects of voluntary running in combination with soy protein supplementation on diet-induced metabolic abnormalities and inflammation in mice.

MATERIALS AND METHODS

Chemicals. The soybean cultivar U03-120139, developed specifically for food-grade markets for production of tofu and soymilk, was used in this study. The seeds from this cultivar contain 37.4% protein on a 13% moisture basis, which is greater than the average of 35.3% for soybeans produced in the United States.[10] Soy protein isolate (SPI) was prepared from cleaned seeds (harvest year 2009; Agronomy Research Farm, University of Nebraska—Lincoln) at Food Protein R&D Center, Texas A&M University. The protein isolate (1:20, w/w in water) was heat-inactivated at 98 °C for 30 min, centrifuged, and lyophilized to dry powder before being used for diets. The characterization of the protein isolate is in Table 1.

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Table 1. Characterization of Soy Protein Isolate[a]

	g/100 g isolate
Amino Acid	
taurine	0.07
hydroxyproline	0
aspartic acid	9.78
threonine	3.00
serine	3.58
glutamic acid	15.45
proline	4.59
lanthionine	0
glycine	3.57
alanine	3.49
cysteine	1.17
valine	4.48
methionine	1.07
isoleucine	4.27
leucine	6.98
tyrosine	3.01
phenylalanine	4.65
hydroxylysine	0
ornithine	0.06
lysine	5.30
histidine	2.24
arginine	6.61
tryptophan	1.22
total	84.6
crude protein[b]	87.1
moisture	4.76
crude fat	0
crude fiber	0.07
	U/mg isolate
trypsin inhibitors	3.89
	μg/g isolate, aglucon units
Isoflavones	
genistein	982
daidzein	608
glycitein	123
total	1713

[a]Amino acid composition, protein content, and trypsin inhibitors were quantified at the University of Missouri Experiment Station Chemical Laboratories. Isoflavones were analyzed at Nestle Purina Analytical Laboratories, St. Louis, MO. [b]Kjeldahl crude protein = %$N \times 6.25$.

Animals and Diets. Three-week old male C57BL/6 mice (Harlan, Madison, WI) were housed in a pathogen-free room on a 12:12 h light−dark cycle and maintained at 22 ± 1 °C. Four diets were compared: the AIN93G diet,[11] a modified AIN93G diet with 45% calories from corn oil, and those diets supplemented with 20% SPI at the expense of casein (Table 2). The caloric content of the AIN93G and the modified 45% high-fat diets was 4.4 ± 0.1 and 5.2 ± 0.1 kcal/g and that of soy-supplemented diets was 4.2 ± 0.1 and 5.2 ± 0.1 kcal/g, respectively, quantified using bomb calorimetry (model 6200, oxygen bomb calorimeter, Parr Instrument, Moline, IL). All diets were pelleted and stored at −20 °C before being provided to mice.

Experimental Design. This study was approved by the Animal Care and Use Committee of the U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center. The procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals.[12] One hundred and twenty mice were acclimated for 1 week before they were randomly assigned into eight groups of 15 each; they were fed the AIN93G diet, the modified 45% high-fat diet, or the corresponding SPI-supplemented diets with or without access to in-cage activity wheels. Sedentary mice were individually housed in wire-topped plastic boxes, and running mice were individually housed in cages with freely accessible in-cage activity wheels (Lafayette Instrument, Lafayette, IN). Mice had free access to their diets and deionized water, and they were weighed weekly. Running distance (kilometers) was recorded daily for the running mice. The duration of the experiment was 14 weeks. Food intake was recorded 4 days per week for 7 consecutive weeks from the sixth week of the experimental feeding. One week before the end of the experiment, body composition analysis of fat and lean body mass of conscious, immobilized mice was performed using quantitative magnetic resonance (Echo whole-body composition analyzer, model 100, Echo Medical System, Houston, TX). At the termination, mice were fasted for 8 h and then anesthetized with a mixture of ketamine and xylazine. Soleus muscles from both legs were collected for analyses of citrate synthase activity, and plasma was collected for quantification of insulin, glucose, triglycerides, and adipocytokines.

Citrate Synthase Activity Assay. The activity of citrate synthase, which mediates skeletal muscle oxidative capacity, was used as an index of the exercise status of the mice. Maximal citrate synthase activity was determined spectrophotometrically on soleus muscle homogenates using the method of Kennedy et al.[13] Citrate synthase activity was expressed as μmol/min/mg protein at 25 °C.

Quantification of Plasma Insulin, Glucose, Triglycerides, and Adipocytokines. Sandwich enzyme-linked immunosorbent assay kits were used to quantify plasma insulin, leptin, adiponectin, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-α (TNF-α; all were from R&D System, Minneapolis, MN), plasminogen activator inhibitor-1 (PAI-1; Molecular Innovations, MN), glucose, and triglycerides (both were from Cayman Chemical, Ann Arbor, MI) following the manufacturers' protocols. Samples were read within the linear range of the assay, and the accuracy of the analysis was confirmed by the controls provided in each assay kit.

Statistical Analyses. Three-way (2 × 2 × 2) analysis of variance (ANOVA) was used to test the effects of diet (AIN93G or high-fat), physical activity (sedentary or running) and protein source (casein or SPI) and their interactions. Tukey contrasts were used for post hoc comparisons among the groups. All data are presented as means ± standard error of the mean (SEM). Differences with a p value of 0.05 or less were considered significant. All statistical analyses were performed using SAS software (version 9.3, SAS Institute, Inc., Cary, NC).

RESULTS

Mice assigned to the four running groups ran approximately 4−8 km/d for the duration of the experiment (Figure 1), which is similar to previously reported daily running distances for the C57BL/6 strain.[14] Over the 14 week study, there were no significant differences in daily running distance among the groups. The citrate synthase activity of running mice was significantly higher than that of their sedentary controls (1.50 ± 0.03 vs 0.97 ± 0.03 μmol/min/mg protein, $p < 0.01$). Neither dietary fat nor SPI supplementation affected citrate synthase activity (data not shown). The caloric intakes were 17.4 ± 0.3 and 14.6 ± 0.3 kcal/d for the high-fat diet-fed and the AIN93G-fed mice ($p < 0.01$), 16.4 ± 0.3 and 15.7 ± 0.3 kcal/d for the running and sedentary mice ($p = 0.07$), and 16.0 ± 0.3 and 16.1 ± 0.3 kcal/d for mice fed SPI-based and casein-based diets ($p = 0.79$), respectively.

Consumption of the high-fat diet increased body weight gain by 34.5% ($p < 0.01$) and voluntary running reduced weight gain by 30.5% ($p < 0.01$) compared to their respective AIN93G-fed and sedentary controls; SPI supplementation did not affect weight gain ($p = 0.35$; Figure 2a). Voluntary running had a greater effect on reducing weight gain in mice fed the high-fat diet compared to those fed the AIN93G diet ($p < 0.01$; Figure 2a); weight gain of running mice was on average 6.1 g less than

Table 2. Composition of Experimental Diets[a]

	AIN93G		AIN93G/SPI		high-fat		high-fat/SPI	
ingredient	g	kcal	g	kcal	g	kcal	g	kcal
corn starch	390	1558	395	1580	28.5	114	31.3	125
casein	200	800			200	800		
soy protein isolate			200	800			200	800
dextrin	132	528	132	528	200	800	200	800
sucrose	100	400	100	400	100	400	100	400
corn oil	70	630	70	630	200	1802	202	1814
cellulose	50		50		50		50	
mineral mix[a]	35	30	35	30	35	30	35	30
AIN93G vitamin mix	10	39	10	39	10	39	10	39
L-cystine	4.4	18	2.6	10	4.4	18	2.6	10
L-methionine	0.3	1	2.8	11	0.3	1	2.8	11
choline bitartrate	2.5		2.5		2.5		2.5	
sodium carbonate	6.22				6.22			
t-butylhydroquinone	0.01		0.01		0.04		0.04	
total	1000	4004	1000	4029	837	4004	836	4029

[a]Mineral mix contained calcium carbonate, anhydrous, 40.04% Ca, 357 g/kg; potassium phosphate, monobasic, 22.76% P, 28.73% K, 196 g/kg; potassium citrate, tri-potassium, monohydrate, 27.3 g/kg; potassium sulfate, 44.87% K, 46.6 g/kg; potassium chloride, 52.45% K, 47.55% Cl, 30g/kg; magnesium oxide, 60.32% Mg, 24 g/kg; ferrous sulfate, 7-hydrate, 20.09% Fe, 4.98 g/kg; zinc carbonate, 52.14% Zn, 1.65g/kg; sodium meta-silicate, 9-hydrate, 9.88% Si, 1.45 g/kg; manganese carbonate, 47.79% Mn, 0.63 g/kg; cupric carbonate 57.47% Cu, 0.3 g/kg; chromium potassium sulfate, 12-hydrate, 10.42% Cr, 0.275 g/kg; boric acid, 17.5% B, 81.5 mg/kg; sodium fluoride, 45.24% F, 63.5 mg/kg; nickel carbonate, 45% Ni, 31.8 mg/kg; lithium chloride, 16.38% Li, 17.4 mg/kg, sodium selenate, anhydrous, 41.79% Se, 10.25 mg/kg; potassium iodate, 59.3% I, 10 mg/kg; ammonium paramolybdate, 4-hydrate, 54.34% Mo, 7.95 mg/kg; ammonium vanadate, 43.55% V, 6.6 mg/kg; powdered sucrose 341.236 g/kg. The sodium content of soy protein isolate was 13.5 g/kg (Thermo 6500 ICP spectrometer, Thermo Fisher Scientific Inc. Waltham, MA). Sodium was omitted from the mineral mix; it was supplied to the casein-based diets at 2.7 g/kg as sodium carbonate (6.22 g/kg). SPI: soy protein isolate.

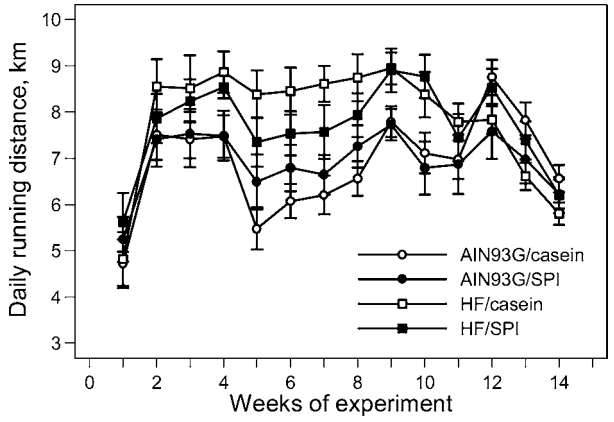

Figure 1. Average daily running distance of mice for the duration of the experiment. Values are means ± SEM (n = 15 per group). SPI: soy protein isolate, HF: high-fat diet.

sedentary mice in high-fat diet-fed groups (12.6 ± 0.4 vs 18.7 ± 0.4 g, $p < 0.01$) and 3.7 g less on average in AIN93G-fed groups (9.8 ± 0.4 vs 13.5 ± 0.4 g, $p < 0.01$). Compared to the AIN93G-fed controls, the high-fat diet increased the percent fat mass by 39.5% ($p < 0.01$; Figure 2b), which was largely due to a net increase in body fat weight (9.3 ± 0.3 vs 5.9 ± 0.3 g, $p < 0.01$). Correspondingly, it lowered the percent lean mass by 8.7% ($p < 0.01$) compared to the AIN93-fed controls (Figure 2c). Voluntary running reduced the percent fat mass by 21.1% ($p < 0.01$; Figure 2b) and increased the percent lean mass by 6.8% ($p < 0.01$; Figure 2c) compared to their sedentary controls. The SPI did not affect the percent fat mass ($p = 0.23$; Figure 2b) or the percent lean mass ($p = 0.74$; Figure 2c) compared to casein.

Consumption of the high-fat diet increased plasma concentrations of insulin by 21% ($p < 0.01$), whereas voluntary running and SPI lowered insulin by 10% ($p < 0.01$) and 8.6% ($p < 0.01$) compared to their respective sedentary and casein-based controls (Figure 3a). The SPI had a greater effect on insulin normalization in the high-fat diet-fed mice than in the AIN93G-fed mice ($p < 0.05$, Figure 3a). Compared to casein-based diets, SPI feeding significantly reduced plasma insulin in the high-fat diet-fed mice (0.45 ± 0.01 vs 0.40 ± 0.01 ng/mL, $p < 0.01$) but not in the AIN93G-fed mice (0.36 ± 0.01 vs 0.34 ± 0.01 ng/mL, $p = 0.44$). The high-fat diet increased plasma glucose concentration by 10% ($p < 0.01$), whereas running and SPI reduced glucose by 6% ($p < 0.05$) and 9% ($p = 0.01$) compared to their respective controls (Figure 3b). The high-fat diet increased plasma levels of triglycerides by 18% ($p < 0.01$), whereas running and SPI reduced triglycerides by 8% ($p < 0.01$) and 13% ($p < 0.01$) compared to their respective controls (Figure 3c).

Feeding mice the high-fat diet resulted in a 2.2-fold increase in plasma levels of leptin compared to the AIN93G diet ($p < 0.01$), whereas voluntary running and SPI lowered leptin by 36.8% ($p < 0.01$) and 15.7% ($p = 0.04$) compared to their respective controls (Figure 4a). Plasma concentrations of adiponectin were 14.1% lower in the high-fat diet-fed mice than in the AIN93G-fed controls ($p < 0.01$), and it was 26.4% higher in running mice than in their sedentary controls ($p < 0.01$); there was no significant difference in plasma adiponectin between groups fed SPI-based or casein-based diet ($p = 0.18$; Figure 4b).

Consumption of the high-fat diet resulted in a 42% increase in plasma concentrations of MCP-1 ($p < 0.01$) compared to the AIN93G diet, whereas running and SPI lowered MCP-1 by 16.8% ($p < 0.01$) and 13.5% ($p = 0.02$) compared to their respective controls (Figure 5a). Compared to casein, SPI significantly reduced MCP-1 in the high-fat diet-fed mice (73.47 ± 3.21 vs 58.12 ± 3.21 pg/mL; $p < 0.01$) but not in the

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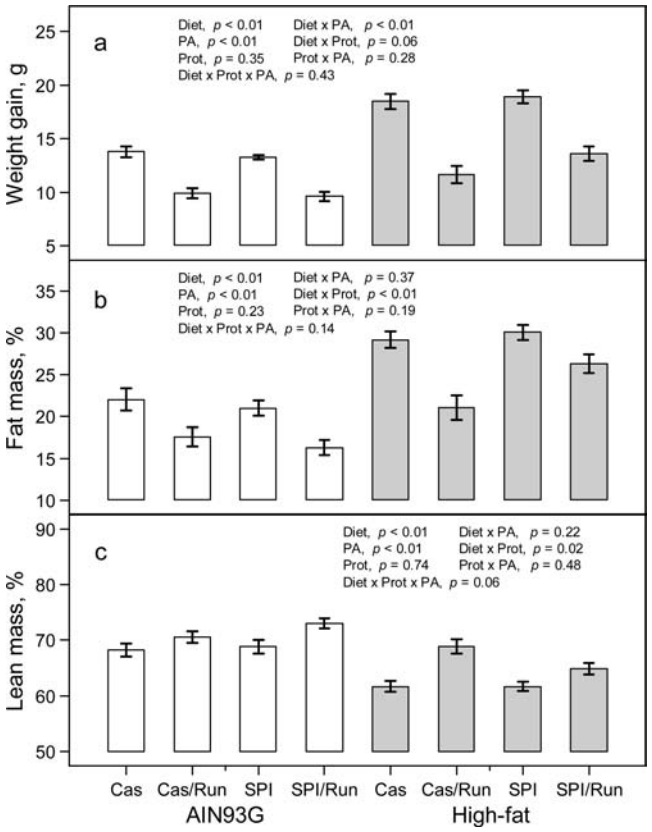

Figure 2. Body weight gain (a), fat mass:body mass ratio (b), and lean mass:body mass ratio (c) of mice after 14 weeks of experimental feeding and voluntary running. Data were analyzed by three-way ANOVA and Tukey contrasts. Values are means ± SEM (n = 15 per group). Cas: casein. Run: running. SPI: soy protein isolate. Diet: AIN93G vs high-fat. PA: physical activity (sedentary vs running). Prot: protein source (casein vs SPI).

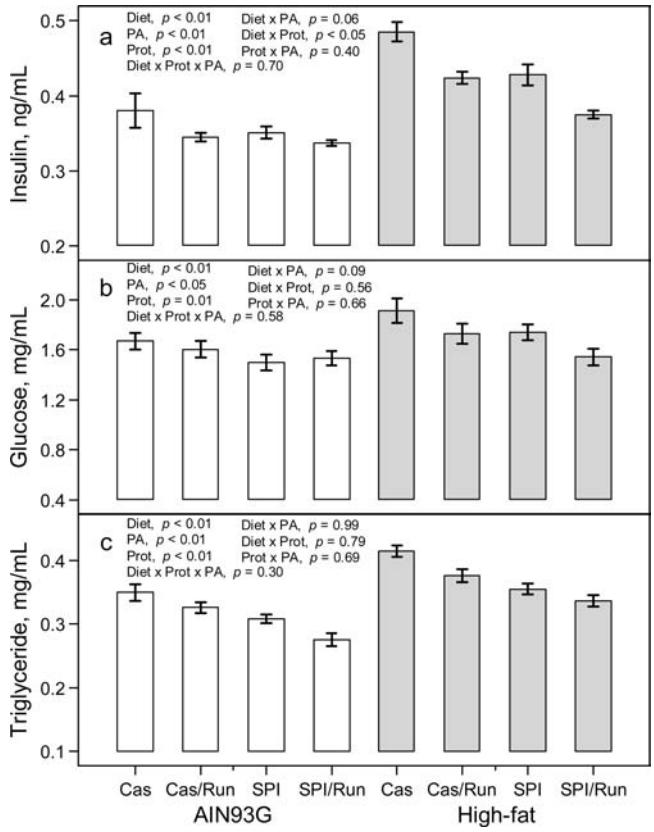

Figure 3. Plasma concentrations of insulin (a), glucose (b), and triglycerides (c) of mice after 14 weeks of experimental feeding and voluntary running. Data were analyzed by three-way ANOVA and Tukey contrasts. Values are means ± SEM (n = 10 per group). Cas: casein. Run: running. SPI: soy protein isolate. Diet: AIN93G vs high-fat. PA: physical activity (sedentary vs running). Prot: protein source (casein vs SPI).

AIN93G-fed mice (46.41 ± 3.21 vs 45.71 ± 3.21 pg/mL; p = 0.99). The high-fat diet increased plasma PAI-1 levels by 30% (p < 0.01), whereas voluntary running reduced PAI-1 by 13% (p < 0.01) compared to their respective AIN93G and sedentary controls; SPI did not affect plasma PAI-1 compared to casein (p = 0.36; Figure 5b). Plasma concentrations of TNF-α were not detectable in groups fed the AIN93G-based diets. In the high-fat diet-fed mice, voluntary running, SPI, or their combination resulted in an approximately 50% reduction in plasma TNF-α compared to the controls, but only the difference between SPI and the controls was statistically significant at p = 0.05 (Figure 5c).

DISCUSSION

Consumption of the high-fat diet significantly increased body weight gain in mice, which was largely attributed to an increase in body fat mass, compared to the AIN93G-fed controls. Voluntary running on average of 4−8 km/d significantly reduced the weight gain and fat mass compared to the sedentary controls. This reduction was not due to changes in caloric intake. In fact, the intake of running mice was approximately 4% greater than that of their sedentary counterparts. Dietary supplementation with SPI affected neither compared to casein.

The high-fat diet-induced increase in body fat mass was accompanied with increases in plasma insulin, glucose, triglycerides, and leptin and a reduction in plasma adiponectin. Insulin secretion occurs in direct response to caloric intake; leptin regulates body weight through its effects on food intake and energy expenditure,[15] and circulating leptin concentrations are directly proportional to the total amount of visceral adiposity in the body.[16] Adiponectin modulates glucose regulation and fatty acid catabolism,[17] and its plasma levels are reduced in overweight and obese individuals and elevated in those with normal body weight.[18] Voluntary running in the present study significantly reduced fat mass which was accompanied with reductions in plasma insulin, glucose, triglycerides, and leptin and at the same time with a significant increase in plasma adiponectin. Replenishment of adiponectin completely resolved insulin resistance in obese and diabetic mice, which was associated with increased expression of molecules involved in both fatty-acid combustion and energy dissipation in muscles.[19] Thus, the reduction in fat mass by voluntary running is likely secondary to insulin normalization and metabolic homeostasis.

Voluntary running reduced plasma concentrations of MCP-1, PAI-1, and TNF-α. In agreement with our findings, others also showed that long-term exercise reduces diet-induced inflammation in mice.[20] Overexpression of inflammatory cytokines such as MCP-1,[21] PAI-1,[22] and TNF-α[23] contributes to obesity. Blood levels of these cytokines are elevated in obese humans[21,24] and laboratory rodents.[25,26] Weight loss decreases serum concentrations of inflammatory cytokines such as MCP-

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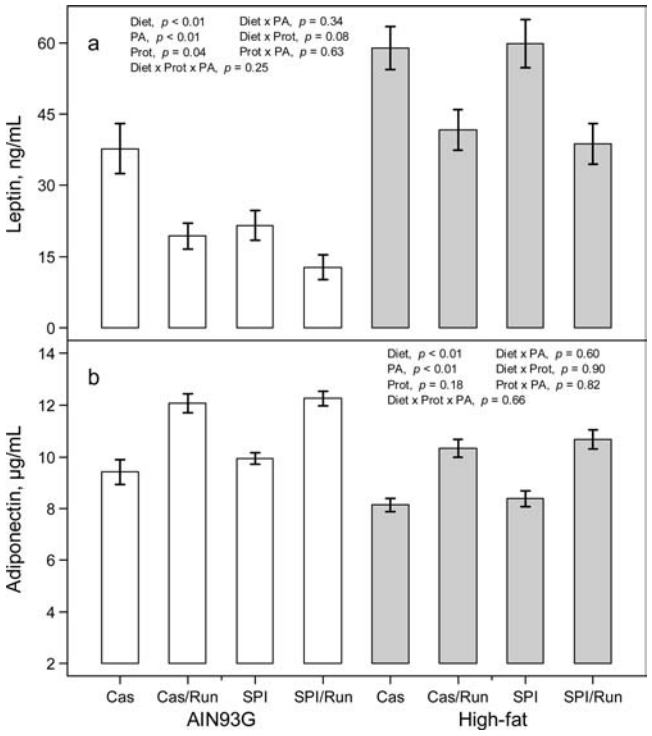

Figure 4. Plasma concentrations of leptin (a) and adiponectin (b) of mice after 14 weeks of experimental feeding and voluntary running. Data were analyzed by three-way ANOVA and Tukey contrasts. Values are means ± SEM (n = 10 per group). Cas: casein. Run: running. SPI: soy protein isolate. Diet: AIN93G vs high-fat. PA: physical activity (sedentary vs running). Prot: protein source (casein vs SPI).

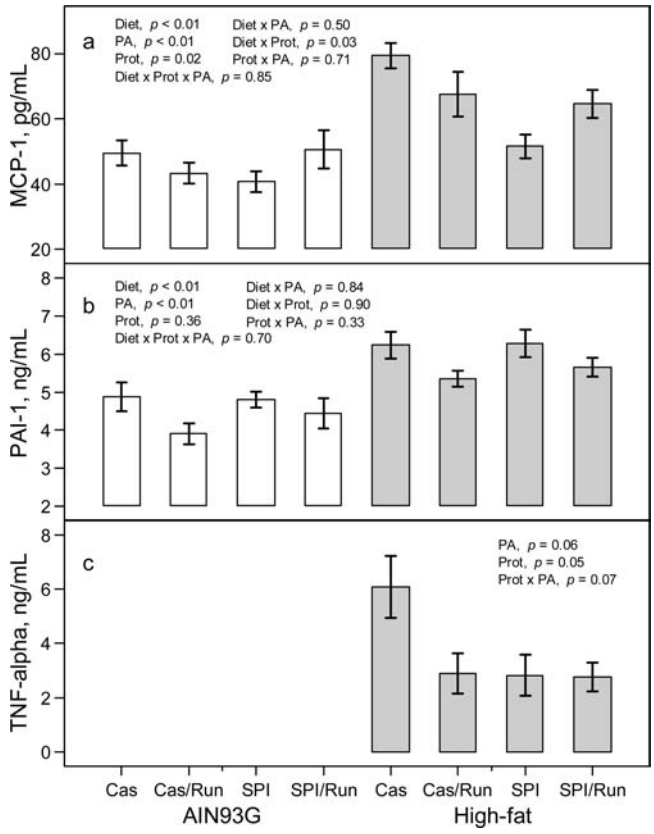

Figure 5. Plasma concentrations of monocyte chemotactic protein-1 (MCP-1 (a)), plasminogen activator inhibitor-1 (PAI-1 (b)), and tumor necrosis factor-α (TNF-α (c)) of mice after 14 weeks of experimental feeding and voluntary running. Data were analyzed by three-way ANOVA and Tukey contrasts. Values are means ± SEM (n = 10 (a, b) or 15 (c) per group). Cas: casein. Run: running. SPI: soy protein isolate. Diet: AIN93G vs high-fat. PA: physical activity (sedentary vs running). Prot: protein source (casein vs SPI).

1,[27] PAI-1 deficiency prevents fat accumulation in genetically obese and diabetic mice,[26] and exercise reduces adipose TNF-α in high-fat diet-fed mice.[28] Taken together with existing knowledge, our results indicate that the reduction in weight gain and fat mass by voluntary running is likely mediated, at least in part, by attenuating diet-induced inflammation. Furthermore, the production of adipocytokines is inducible and actions of adipocytokines are interrelated. For example, insulin up-regulates the expression of MCP-1;[25,29] TNF-α up-regulates MCP-1 expression,[29] contributes to insulin resistance,[30] and is a potent inducer of PAI-1;[31] adiponectin down-regulates MCP-1 expression[32] and TNF-α-mediated inflammatory responses.[33] Thus, the observed changes in plasma concentrations of adipocytokines by voluntary running are likely through a concomitant down-regulation of diet-induced inflammation rather than an action on any individual adipocytokines.

That SPI supplementation did not affect body weight gain regardless of the type of diet demonstrated that the quality and nutritional value of SPI are comparable to that of casein in animal growth and weight maintenance. Compared to casein, however, SPI showed a significant reduction in plasma concentrations of insulin, glucose, triglycerides, and leptin, but it did not affect plasma levels of adiponectin. It has been well documented that soy protein consumption reduces blood cholesterols and triglycerides, which leads to the health claim authorized by the U.S. Food and Drug Administration on soy protein and heart health.[5] Our results demonstrated that soy protein consumption improves metabolic disturbance, but it is not through an action of weight reduction nor it is through a mechanism of up-regulation of adiponectin.

A unique finding from the present study was that SPI significantly reduced plasma concentrations of insulin, MCP-1, and TNF-α in the high-fat diet-fed mice, indicating that soy protein consumption may down-regulate the diet-induced inflammation. Available studies on soy and adipocytokines are limited. Short-term intervention with soy milk[34] or soy nuts[35] results in a moderate reduction in TNF-α in postmenopausal women with metabolic syndrome. Nagasawa et al.[36] reported that soy protein in comparison with casein increases adipose mRNA expression of adiponectin and at the same time it decreases expression of leptin and PAI-1 in Wistar rats. Frigolet et al.[37] showed that soy protein reduces serum levels of leptin but it does not affect adiponectin. Considering that adipose tissues produce many adipocytokines, including MCP-1 and TNF-α, that contribute to a state of chronic low grade systemic inflammation and facilitate metabolic disturbance in obesity,[2] further investigations are warranted to understand the roles of soy protein in regulation of inflammation in obesity.

It has been showed that soy β-conglycinin, a major storage protein of soybeans, reduces plasma and liver cholesterol concentrations in rats fed a hypercholesterolemic diet[8] and inhibits atherosclerosis in atherosclerosis-susceptible mice.[38] Furthermore, soy isoflavones, either in a purified form[9] or as a component of a soy protein preparation,[7] improve metabolic abnormality in diet-induced obesity[9] and in genetically obese rats.[7] The present study was not designed to study the specific

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effects of soy β-conglycinin and isoflavone components per se; however, it does not exclude the possibility that SPI-contained β-conglycinin and isoflavones may contribute to the observed soy effects.

Our results do not agree with the report that soy feeding reduced body weight of laboratory animals.[39] In the study by Lee et al.,[39] however, the lipids were extracted from whole soybean powder at 50 °C at 300 bar pressure using supercritical CO_2. That temperature and pressure may not have been sufficient to inactivate trypsin inhibitors.[40] The nutritional value of a soy preparation is determined by its contents of sulfur-containing amino acids and trypsin inhibitors. The former is a growth limiting factor of soy protein,[41] and the latter retards growth if they are not inactivated.[42] The SPI used in the present study was heat-treated before being added to the diet, and the content of sulfur-containing amino acids of each soy diet was adjusted according to National Research Council nutrient requirements of laboratory animals.[43] Our results are consistent with the existing knowledge that heated soy protein isolates are comparable to casein in animal growth and produce no deleterious effects.[42]

We chose the voluntary running model as the means of physical activity because the mice themselves determined the frequency, duration, and intensity of running in a self-controlled, physically capable manner. This is an advantage over the forced running model using a treadmill in which the reinforcement-associated stress is a potential confounding factor that may affect the treatment effect. A limitation of the voluntary running model is the variation in daily activity. We observed consistent within-group reduction in body weight gain and fat mass in running mice with daily activity ranging from 4 to 8 km/d for the duration of the experiment, indicating that the variation in activity was acceptable for the study designed.

The present study demonstrated that both voluntary running and SPI consumption improved diet-induced metabolic disturbance and inflammation in mice. A major difference between running and SPI was that voluntary running-mediated improvement was through an action of weight reduction, which was largely due to a reduction in fat mass, whereas SPI was not through such an action. A unique finding was that SPI supplementation significantly reduced plasma concentrations of insulin and adipocytokines MCP-1 and TNF-α in the high-fat diet-fed mice. As adipose tissues produce many adipocytokines, including MCP-1 and TNF-α, that contribute to a state of chronic low grade systemic inflammation and facilitate metabolic disturbance in obesity, further investigations are warranted into the roles of soy protein as a dietary modification in reducing the risk of obesity, particularly to those with restricted capability to be physically active.

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Notes

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The authors declare no competing financial interest.

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