

# Effect of Shengmai San on Insulin Resistance, Tumor Necrosis Factor-Alpha and Oxidative Stress in Rats Fed on a High-Fat Diet

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(Received: August 17, 2010; Accepted: November 29, 2010)

## ABSTRACT

Shengmai San (SMS) is a well-known traditional Chinese medicine formula prescribed for patients with coronary heart disease (CHD). Insulin resistance is the most likely explanation for the development and progression of this disease. To investigate the effect of SMS on high-fat diet-induced insulin resistance, inflammatory cytokine production and oxidative stress, male Wistar rats were fed with low-fat control diet, high-fat diet and high-fat diet supplemented with 4% SMS for eight weeks. An oral glucose tolerance test was conducted in the seventh week. Rats fed the SMS diet had significantly lower plasma fructosamine concentration ( $p < 0.05$ ) and tended to have lower ( $p < 0.1$ ) AUC values of plasma glucose and insulin concentrations after glucose challenge, compared to those fed the high-fat diet. Moreover, SMS reduced TNF- $\alpha$  and lipid peroxidation levels in the liver and heart. However, SMS had no effect on fasting plasma concentrations of glucose, insulin, triglyceride, non-esterified fatty acids, total cholesterol, HDL-cholesterol and insulin resistance index (homeostasis model assessment; HOMA) in rats. Our results show that SMS may have little or no significant effect on reducing insulin resistance, but display anti-oxidative and anti-inflammatory properties.

Key words: shengmai san, insulin resistance, high-fat diet, TNF- $\alpha$ , rats

## INTRODUCTION

Various clinical studies have shown that chronic elevated plasma glucose and insulin are associated with an increased risk for coronary heart disease (CHD), and insulin resistance is the most likely explanation for the development and progression of this disease<sup>(1)</sup>. With insulin resistance, endothelium may impair vasodilator nitric oxide (NO) release and increase oxidative stress that may damage endothelial function<sup>(1,2)</sup>. In addition, increased circulatory levels of inflammatory cytokine, such as tumor necrosis factor-alpha (TNF- $\alpha$ ), have been shown to be positively associated with insulin resistance, suggesting that the inflammatory factors may potentiate

the cardiovascular risk factor's role of insulin resistance<sup>(3)</sup>. Indeed, TNF- $\alpha$  is over-expressed in patients with heart failure<sup>(4)</sup> and involved in a number of etiologies of cardiac disease possibly due to increased oxidative stress and cardiac myocyte injury<sup>(5)</sup>. Besides a change of lifestyle, the effective administration of dietary supplements or medicinal herbs with anti-inflammatory and/or anti-oxidative activities is considered a promising approach to reduce insulin resistance and inflammation, thus lowering the risk of developing CHD<sup>(6,7)</sup>.

Traditional Chinese medicines (TCM) are widely used in Asia for treating a variety of disorders. For convenience, the traditional decoction process has been improved by concentrating the extracts and by formulating of powdered dosage forms. Shengmai San (SMS), which comprises the medicinal herbs of *Panax ginseng* C. A. Meyer, *Schisandra chinensis* Baill., and *Ophiopogon*

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*japonicus* Ker-Gawl (2 : 1 : 2), is a well-known TCM with long-time clinical use. It is usually prescribed for patients with CHD and has been found to display anti-oxidative effects<sup>(8)</sup>. A recent clinical study has shown that the long-term consumption of SMS as a supplement is safe and can accelerate the recovery of heart function in patients<sup>(9)</sup>. Moreover, studies have also shown that conventional treatment with shengmai injection (added to 5% dextrose or normal saline as intravenous infusions) for 3-4 weeks can improve not only vascular endothelial and heart functions<sup>(10)</sup>, but also insulin resistance in patients with heart disease<sup>(11)</sup>. Although each herbal component in SMS has been shown to exert beneficial effects on reducing the risk of developing CHD, such as reducing insulin resistance<sup>(12)</sup> and increasing anti-oxidative and anti-inflammatory activities<sup>(13-15)</sup>, it is not known whether SMS can ameliorate insulin resistance and inflammatory reaction when administered orally.

High-fat diets have been associated with insulin resistance, a risk factor for both Type II diabetes and heart disease in humans<sup>(16)</sup>. The symptoms of insulin hypersecretion and glucose intolerance after glucose challenge have also been demonstrated in rats fed the high-fat diets<sup>(17)</sup>. The aim of this study is to investigate the effect of SMS supplementation on insulin resistance, TNF- $\alpha$  level and oxidative stress in the liver and heart of rats fed a high-fat diet. The dosage of 4% SMS in the diet was chosen because it had been shown to significantly reduce hepatic lipid contents and lipid peroxidation<sup>(18)</sup>.

## MATERIALS AND METHODS

### I. Shengmai San

Shengmai San (SMS) concentrated powder was obtained from Sheng Chang Pharmaceutical (Taipei, Taiwan). SMS comprises the medicinal herbs of *Panax ginseng* C. A. Meyer, *Schisandra chinensis* Baill. and *Ophiopogon japonicus* Ker-Gawl (2 : 1 : 2). The yield from water extraction was 16% (w/w). In the preparation used in this study, the SMS powder contained 11.9  $\mu$ g/g of ginsenoside Rf, 13.7  $\mu$ g/g of ginsenoside Rg<sub>2</sub>, 6.5  $\mu$ g/g of ginsenoside Rg<sub>3</sub>, < 0.2  $\mu$ g/g of ginsenoside Rh<sub>1</sub>, 8.6  $\mu$ g/g of gomisin A, 20.5  $\mu$ g/g of schizandrin and < 0.2  $\mu$ g/g of methyl-ophiopanaxone B, respectively, determined by LC/MS<sup>(18)</sup>.

### II. Animals and Treatment

Twenty-four 9-week-old male Wistar rats (BioLASCO Taiwan Co., Ltd., Ilan, Taiwan) were randomly divided into three groups: (a) the control group (CTR), rats in this group were fed a standard laboratory rat diet; (b) the high-fat diet group (HF), rats in this group were fed a high-fat diet; (c) the SMS group (SMS), rats in this group were fed a high-fat diet containing 4% SMS (w/w). The

high-fat diet contained 85% standard laboratory rat diet and 15% lard (w/w). The cholesterol content in the high-fat diet was 0.015%. The rats were housed in individual cages in a room kept at a temperature of 23  $\pm$  1°C and relative humidity of 60  $\pm$  5% with a 12-h light and dark cycle. Food and drinking water were available *ad libitum* for eight weeks. Food intake was measured every two days and body weight was measured once a week. After seven weeks of feeding, an oral glucose tolerance test (OGTT) was conducted. At the end of the 8-week experimental period, the rats were fasted for 12 h prior to being sacrificed (at 10:00 AM). The rats were killed by exsanguination via the abdominal aorta, while under carbon dioxide (70%/30%, CO<sub>2</sub>/O<sub>2</sub>) anesthesia. Heparin was used as the anticoagulant. Plasma was separated from the blood by centrifugation (1,750  $\times$ g) at 4°C for 20 min. The liver, heart and adipose tissues (epididymal and abdominal) from each animal were excised, weighed and immediately stored frozen at -80°C.

All animal experiments were performed based on the animal ethics guidelines of the Institutional Animal Ethics Committee in the Taiwan National Health Research Institute (NHRI).

### III. Oral Glucose Tolerance Test (OGTT)

After the seventh week of the feeding period, the rats were deprived of food for 12 h, and then a 2 g/kg dose of glucose solution was administered intragastrically. Serial blood samples were collected from the tail vein of each rat immediately before dosing (0 min) and at 30, 60, 120 and 180 min after dosing. Plasma was separated from the blood samples and analyzed for glucose and insulin concentrations according to the methods described below. The area under the plasma glucose or insulin concentration *versus* time curve (AUC) was calculated using the trapezoidal method.

### IV. Insulin Resistance Measurement

The homeostasis model assessment (HOMA) was expressed as an index of insulin resistance<sup>(19)</sup>. It was calculated by the formula HOMA = fasting glucose [mmol/L]  $\times$  fasting insulin [ $\mu$ U/mL] / 22.5.

### V. Determination of Plasma Lipids, Fructosamine, Glucose, Insulin, Total Antioxidant Status and Aminotransferase Activity

Plasma concentrations of glucose, total cholesterol and triglyceride were determined by the enzymatic methods provided by the kits purchased from Audit Diagnostics (Cork, Ireland). The concentrations of non-esterified fatty acid (NEFA) and insulin in plasma were determined by the enzymatic methods provided by the kits purchased from Waco Pure Chemical Industries, Ltd. (Osaka, Japan) and Mercodia (Uppsala, Sweden),

respectively. High density lipoprotein (HDL) cholesterol was measured after the removal of very low density lipoprotein (VLDL) and LDL cholesterol by heparin-manganese precipitation (Randox Laboratories, Crumlin, U.K.). Plasma total antioxidant status (TAS) and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the commercial kits from Randox Laboratories (Antrim, U.K.). Fructosamine was determined by a colorimetric method using the kit purchased from Hospitex Diagnostic (Firenze, Italy).

#### VI. Determination of Liver Lipids

Lipids were extracted from the liver by the method of Folch *et al.*<sup>(20)</sup> and solubilized in Triton X-100 according to the method of Carlson and Goldfarb<sup>(21)</sup>. The hepatic total cholesterol, triglyceride and NEFA contents were assayed enzymatically by the same method, as described above.

#### VII. Determination of Hexokinase, Glucose-6-Phosphatase and Glutathione Peroxidase (GSH-Px) Activities

Tissues were homogenized in ice-cold 1.15% KCl to obtain a final 10% (w/v) solution. The homogenates were immediately centrifuged at 10,000 × g for 15 min at 4°C. The resulting supernatant was used for the assay of enzyme activity. The protein content was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Hepatic hexokinase and glucose-6-phosphatase were assayed according to the method of Nagayama *et al.*<sup>(22)</sup>. The enzyme activities were expressed as the rate of NADPH and inorganic phosphate formation, respectively. GSH-Px activity was determined spectrophotometrically according to the method of Mohandas *et al.*<sup>(23)</sup> and expressed as nanomoles of NADPH decrease per minute per milligram of protein.

#### VIII. Determination of Lipid Peroxidation, Glutathione and Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) Levels in the Liver and Heart

GSH was determined by a LC/MS method reported previously<sup>(24)</sup>. Lipid peroxidation, as measured by thiobarbituric acid reactive substances (TBARS), in plasma and tissues were assessed by the methods of Yagi<sup>(25)</sup> and Uchiyama and Mihara<sup>(26)</sup>, respectively. The calibration curve of a 1,1,3,3-tetramethoxypropane (Sigma) was used to determine the concentrations of TBARS in samples. Fluorescence was measured at excitation and emission wavelengths of 515 nm and 553 nm, respectively. The concentrations of TNF- $\alpha$  in plasma and tissues (liver and heart) were determined by immunoassay, according to the method provided by R&D Systems, Inc. (Minneapolis, MN, USA).

#### IX. Statistical Evaluation

Statistical differences among the groups were calculated using one-way ANOVA (SAS Institute, Cary, NC, USA) and were considered to be significant at  $p < 0.05$ , as determined by Duncan's new multiple-range test.

## RESULTS

As shown in Table 1, after 8 weeks of feeding, there was no difference in the body weight and the relative liver and heart weights among the animals in the three groups. Lower daily food intake and higher adipose tissue weight were observed in rats of the HF and SMS groups, compared to rats of the CTR group. There was no difference in adipose tissue weight and daily food intake between the rats of the HF and SMS groups.

Figures 1A and 1C show the plasma concentrations

**Table 1.** Body weight, tissue weight and food intake of rats of the CTR, HF and SMS groups after eight weeks of feeding regimen<sup>a</sup>

Diet <sup>b</sup>	CTR	HF	SMS
Initial body weight (g)	363.6 ± 17.9	364.3 ± 17.1	365.4 ± 17.0
Final body weight (g)	490.6 ± 35.5	523.7 ± 30.0	512.2 ± 22.2
Food intake <sup>c</sup> (g/day)	31.2 ± 2.3	26.5 ± 1.3*	27.3 ± 1.7*
Liver weight (g/100g b.w.)	2.9 ± 0.2	2.8 ± 0.1	2.9 ± 0.2
Heart (g/100g b.w.)	0.28 ± 0.02	0.28 ± 0.02	0.30 ± 0.02
Adipose tissue (g /100g b.w.)	2.8 ± 0.5	4.6 ± 1.2*	5.2 ± 0.9*

<sup>a</sup> Values are mean ± SD of eight rats in each group.

<sup>b</sup> CTR: control group; HF: high-fat diet group; SMS: high-fat diet with SMS group.

<sup>c</sup> Food intake was measured every two days over a period of 8 weeks.

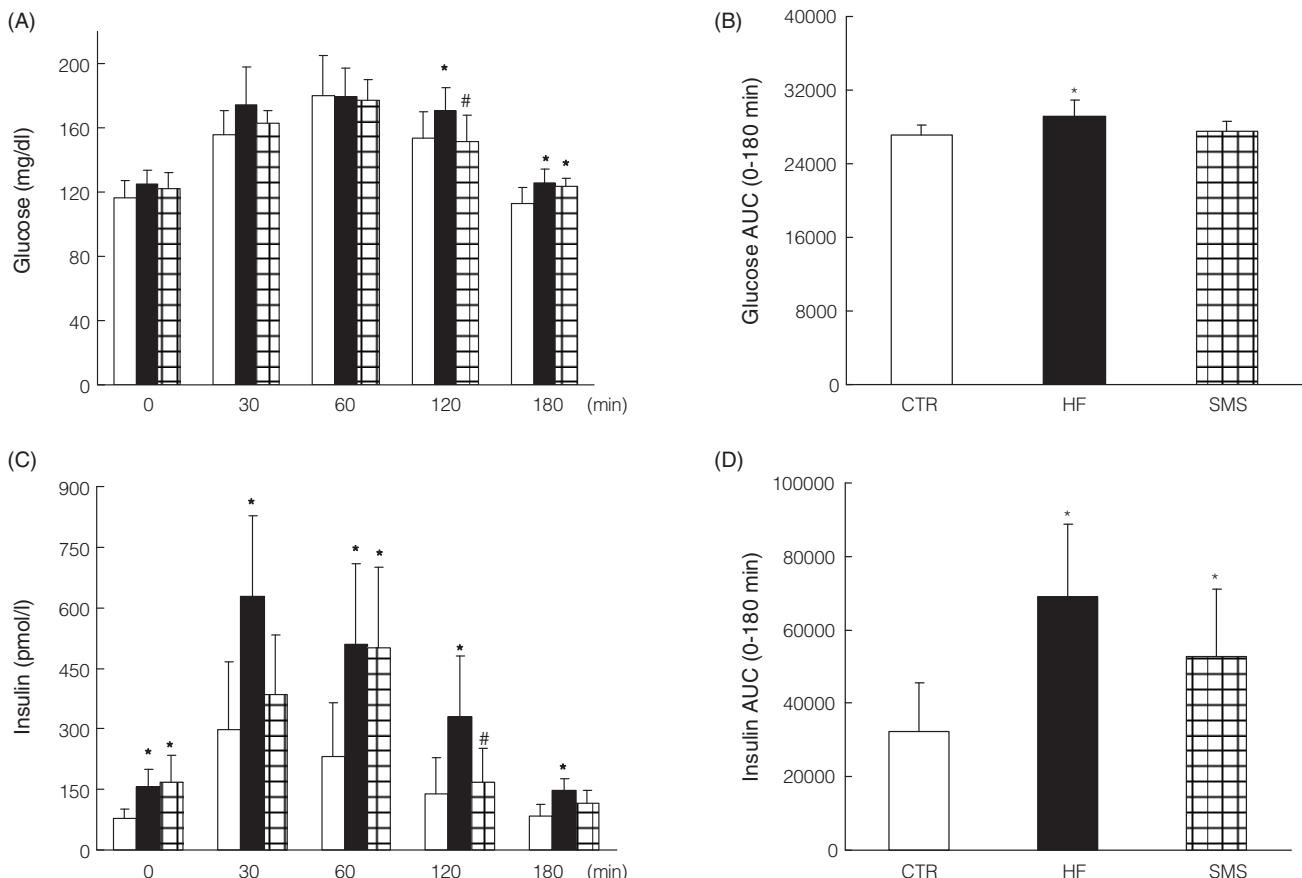
\*Significantly different from the CTR group at  $p < 0.05$ .

of glucose and insulin in rats of the CTR, HF and SMS groups. Figures 1B and 1D show the area under the plasma concentrations of glucose and insulin *versus* time curve (AUC) values of rats in the three groups. After glucose challenge, the plasma glucose concentration of rats in the HF group was significantly higher than that of the CTR group at 120 and 180 min. For rats in the SMS group, the plasma glucose concentration was significantly higher than that of the CTR group at 180 min after the challenge. The plasma insulin concentration of rats in the HF group was significantly higher than that of the CTR group at all time points. There was no significant difference in plasma glucose and insulin concentration between the rats of the HF and SMS groups, except at 120 min when plasma glucose and insulin concentration was lower in the SMS group than that of the HF group (Figure 1A and 1C). The plasma glucose AUC value of rats in the HF group was significantly higher than that of the CTR group. No significant difference in the plasma glucose AUC values between the rats of the HF and SMS groups was observed (Figure 1B). The plasma insulin AUC values of rats in the HF and SMS groups were both significantly higher than that of the CTR group. However, there was no significant difference between

the plasma insulin AUC values of the rats in the HF and SMS groups (Figure 1D). These results indicated that rats fed the high-fat diet had developed insulin resistance and glucose intolerance. Rats in the SMS group tended to have lower plasma glucose (-5.7%,  $p = 0.064$ ) and insulin (-23.7%,  $p = 0.068$ ) AUC values, compared to that of the HF group, suggesting that SMS may have a mild effect on reducing insulin resistance and glucose intolerance.

Table 2 shows the plasma parameters of rats in the CTR, HF and SMS groups. Rats in the HF and SMS groups had significantly higher glucose, insulin, fructosamine and HOMA levels than those of the CTR group. Plasma fructosamine level of rats in the SMS group was significantly lower than that of the HF groups. However, there were no significant differences in glucose, insulin and HOMA levels between the rats in the HF and SMS groups. No significant change in the concentrations of plasma lipids (total cholesterol, HDL-cholesterol, triglyceride and NEFA), total antioxidant status (TAS) and lipid peroxide (TBARS), aminotransaminases (AST and ALT; data not shown) were observed among the rats in the three groups. Plasma TNF- $\alpha$  was not detected in rats of all three groups.

Table 3 shows the activities of hexokinase and glucose-6-phosphatase and the concentration (mg/g of



**Figure 1.** Plasma concentrations of glucose and insulin in rats of the CTR, HF and SMS groups after a 2 g/kg glucose-loading dose. Results are mean  $\pm$  SD of eight rats in each group. \*HF vs. CTR group,  $p < 0.05$ . #HF vs. SMS group,  $p < 0.05$ . AUC: Area under the plasma concentration of glucose or insulin *versus* time curve.

liver) of triglyceride, cholesterol and NEFA in the liver of rats in the CTR, HF and SMS groups. Rats of the HF and SMS groups had significantly higher triglyceride level and glucose-6-phosphatase activity than that of the CTR group. There was no significant difference in the triglyceride level and glucose-6-phosphatase activity between the rats in the HF and SMS groups. There was also no significant difference in the cholesterol and NEFA levels and hexokinase activity among the rats in the three groups.

The activities of GSH-Px and concentrations of

GSH, TBARS and TNF- $\alpha$  in the liver and heart are shown in Table 4. In the liver, rats of the HF group had significantly higher TBARS, GSH and TNF- $\alpha$  concentrations and GSH-Px activity than that of the CTR group. Significantly reduced TBARS and TNF- $\alpha$  concentrations and GSH-Px activity were observed in the rats of the SMS group, compared to that of the HF group. In the heart, there was no difference in TBARS, GSH and TNF- $\alpha$  concentrations and GSH-Px activity between the rats of the HF and CTR groups. However, significantly lower

**Table 2.** Plasma parameters of rats of the CTR, HF and SMS groups after eight weeks of feeding regimen<sup>a</sup>

Diet <sup>b</sup>	CTR	HF	SMS
Glucose (mg/dL)	127.1 $\pm$ 5.5	150.6 $\pm$ 7.3*	148.4 $\pm$ 16.7*
Insulin (pmol/L)	112.2 $\pm$ 48.3	690.2 $\pm$ 340.1*	679.7 $\pm$ 400.1*
Fructosamine ( $\mu$ mol/L)	216.2 $\pm$ 45.8	347.8 $\pm$ 40.4*	277.6 $\pm$ 30.2*,#
Total cholesterol (mg/dL)	70.9 $\pm$ 9.5	81.7 $\pm$ 14.3	80.8 $\pm$ 10.8
Triglyceride (mg/dL)	61.8 $\pm$ 18.4	69.8 $\pm$ 13.7	67.3 $\pm$ 19.8
HDL-cholesterol(mg/dL)	25.4 $\pm$ 2.9	28.5 $\pm$ 4.9	27.0 $\pm$ 2.6
NEFA (mEq/mL)	0.38 $\pm$ 0.08	0.41 $\pm$ 0.14	0.38 $\pm$ 0.07
TBARS (nmol/mL)	1.5 $\pm$ 0.9	2.0 $\pm$ 0.9	1.7 $\pm$ 0.8
TAS (mmol/dL)	0.97 $\pm$ 0.09	0.88 $\pm$ 0.23	0.90 $\pm$ 0.18
HOMA-IR index	3.5 $\pm$ 1.2	37.3 $\pm$ 10.3*	36.1 $\pm$ 14.2*

<sup>a</sup> Values are mean  $\pm$  SD of eight rats in each group.

<sup>b</sup> CTR: control group; HF: high-fat diet group; SMS: high-fat diet with SMS group.

\*Significantly different from the CTR group at  $p < 0.05$ .

#Significantly different from the HF group at  $p < 0.05$ .

**Table 3.** Hepatic lipid concentrations and enzyme activities of rats of the CTR, HF and SMS groups after eight weeks of feeding regimen<sup>a</sup>

Diet <sup>b</sup>	CTR	HF	SMS
Lipids			
Cholesterol (mg/g)	2.4 $\pm$ 0.3	2.8 $\pm$ 0.7	2.7 $\pm$ 0.7
Triglyceride (mg/g)	10.1 $\pm$ 2.7	23.8 $\pm$ 10.8*	20.3 $\pm$ 8.6*
NEFA (mEq/g)	4.7 $\pm$ 1.1	5.2 $\pm$ 2.4	3.9 $\pm$ 1.8
Enzyme activities <sup>c</sup>			
Hexokinase	46.9 $\pm$ 6.0	42.4 $\pm$ 8.7	45.8 $\pm$ 6.5
Glucose-6-phosphatase	288.7 $\pm$ 69.3	444.7 $\pm$ 63.1*	429.2 $\pm$ 23.7*
Glucose-6-phosphatase/ Hexokinase	6.2 $\pm$ 1.5	10.7 $\pm$ 2.4*	9.5 $\pm$ 1.2*

<sup>a</sup> Values are mean  $\pm$  SD of eight rats in each group.

<sup>b</sup> CTR: control group; HF: high-fat diet group; SMS: high-fat diet with SMS group.

<sup>c</sup> Enzyme activities are expressed as nmol/min/mg protein.

\*Significantly different from the CTR group at  $p < 0.05$ .

**Table 4.** TBARS, GSH and TNF- $\alpha$  concentrations and GSH-Px activity in the liver and heart of rats of the CTR, HF and SMS groups after eight weeks of feeding regimen<sup>a</sup>

Diet <sup>b</sup>	Liver			Heart		
	CTR	HF	SMS	CTR	HF	SMS
TBARS (pmol/mg protein)	215.6 $\pm$ 22.8	255.2 $\pm$ 30.9*	196.3 $\pm$ 25.7 <sup>#</sup>	382.4 $\pm$ 120.8	356.4 $\pm$ 41.2	296.4 $\pm$ 30.6 <sup>#</sup>
GSH (nmol/mg protein)	23.3 $\pm$ 8.6	41.9 $\pm$ 5.9*	45.4 $\pm$ 8.5*	14.0 $\pm$ 3.1	15.5 $\pm$ 1.5	13.9 $\pm$ 1.5
GSH-Px (nmol/min/mg protein)	735.9 $\pm$ 90.6	871.2 $\pm$ 45.5*	770.4 $\pm$ 61.5 <sup>#</sup>	193.1 $\pm$ 16.8	195.2 $\pm$ 18.3	174.9 $\pm$ 15.7 <sup>#</sup>
TNF- $\alpha$ (ng/mg protein)	159.9 $\pm$ 23.2	207.3 $\pm$ 21.6*	180.9 $\pm$ 11.5 <sup>#</sup>	417.9 $\pm$ 53.2	428.5 $\pm$ 47.9	302.1 $\pm$ 53.4 <sup>#</sup>

<sup>a</sup> Values are mean  $\pm$  SD of eight rats in each group.

<sup>b</sup> CTR: control group; HF: high-fat diet group; SMS: high-fat diet with SMS group.

\*Significantly different from the CTR group at  $p < 0.05$ .

#Significantly different from the HF group at  $p < 0.05$ .

TBARS and TNF- $\alpha$  concentrations and GSH-Px activity were observed in rats of the SMS group, compared to that of the CTR and HF groups.

## DISCUSSION

In our previous study, SMS was shown to reduce hepatic lipids and lipid peroxidation in rats fed on a high-cholesterol diet<sup>(18)</sup>. However, it is not known whether SMS can reduce insulin resistance, which is the most likely explanation for the development and progression of CHD. In this study, we evaluated this possibility using another animal model of insulin resistance which is induced by a high-fat diet. Our results demonstrated that the beneficial effect of SMS on glucose control was mild, but SMS exerted anti-oxidative and anti-inflammatory properties in rats that were fed the high-fat diet.

Liver plays an important role in maintaining plasma glucose concentration through the regulation of metabolic pathways of gluconeogenesis and glycolysis<sup>(27)</sup>. Studies indicate that fat accumulation in the liver can reduce the insulin sensitivity of the liver cells and defect in insulin suppression of hepatic glucose production<sup>(28,29)</sup>. In the rats that were fed the high-fat diet, fat accumulation in liver may lead to insulin resistance. This impairment of insulin action in rats has been partly attributed to the inflammatory response elevated by high-fat diet, such as an overproduction of TNF- $\alpha$ <sup>(30)</sup>. Similar to the results of previous studies, our results showed that rats fed the HF diet had increased TNF- $\alpha$  level (Table 4) and up-regulated glucose-6-phosphatase activity (Table 3) in the liver. These results were accompanied with higher fasting plasma glucose and insulin concentration and hyperinsulinemia in response to glucose challenge. Moreover, a higher HOMA value was also found in rats that were fed the HF diet, indicating that insulin resistance had been developed in those animals. Although the rats that were fed the SMS diet had no effect on fasting

plasma glucose and insulin concentration as well as insulin resistance (HOMA), it had reduced plasma fructosamine level, a glycated plasma protein which reflects average blood glucose concentration and monitoring glucose control over the previous 1 - 3 weeks. After the glucose challenge (OGTT), the rats of the SMS group had lower plasma concentrations of insulin (at 30 and 120 min) and glucose (at 120 min) than the rats of the HF group. Lower AUC values of plasma glucose ( $p = 0.064$ ) and insulin ( $p = 0.068$ ) were also observed in SMS group than those in the HF group, despite the fact that these differences were not statistically significant (Figure 1B and 1D). Furthermore, the TNF- $\alpha$  level in the liver of rats in the SMS group was lower than that of the rats in the HF group (Table 4). These results suggested that the beneficial effect of SMS on glucose control was mild and might be partially attributed to a lower hepatic TNF- $\alpha$  level. However, in this study, the extent of hepatic TNF- $\alpha$  level lowering (-12.7%) by SMS might not reach the threshold to affect insulin resistance for no significant change on HOMA value was observed.

In addition to insulin resistance, TNF- $\alpha$  is also a signal to trigger oxidative stress in the cells. TNF- $\alpha$  is up-regulated in patients with non-alcoholic fatty liver disease (NAFLD)<sup>(31)</sup> and cardiac disease<sup>(4)</sup>, which may lead to increased ROS production and lipid peroxidation in these tissues. Previous studies indicated that SMS has protective effects on the prevention of chemical-induced oxidative damage (e.g. adriamycin, carbon tetrachloride, hydrogen peroxide). Under severe oxidative stress, reduced lipid peroxidation and increased enzymatic (i.e. GSH-Px) and/or non-enzymatic (i.e. GSH) antioxidants are responsible for the therapeutic effect of SMS<sup>(32,33)</sup>. In the present study, higher hepatic TBARS level was observed in the rats of the HF group than that of the CTR group, indicating that a high-fat diet resulted in an increase of lipid peroxidation in the liver. In addition, GSH level and GSH-Px activity, which act as antioxidants and are known to be up-regulated under oxidative

stress, was increased in the liver of rats that were fed the HF diet. This result might be explained by an adaptive mechanism in response to the oxidative stress in liver<sup>(34)</sup>. However, in the heart, there was no difference in TBARS and GSH levels and GSH-Px activity between the rats of the HF and CTR groups. These results indicated that the liver was more sensitive to oxidative stress induced by high-fat diet than the heart. It is interesting to note that SMS reduced TBARS level and GSH-Px activity in both liver and heart, suggesting that SMS might reduce oxidative stress in these tissues. Moreover, reduced TBARS level in liver and heart by SMS was also accompanied with a lower TNF- $\alpha$  level in these tissues. Since TNF- $\alpha$  plays a central role in initiating and sustaining the inflammatory response and increase oxidative stress in tissues<sup>(5)</sup>, it is proposed that reduced TNF- $\alpha$  levels in the liver and heart by SMS might result in a lower level of oxidative stress in these tissues. Our results supported previous findings showing that SMS had beneficial effects on reducing tissue injury caused by oxidative damage<sup>(8,33)</sup>.

In contrast to our previous result that SMS reduced hepatic triglyceride and cholesterol levels in rats that were fed with cholesterol-enriched diet (0.5% cholesterol and 0.1% cholic acid)<sup>(18)</sup>, in the present study, however, administration of SMS had no effect on liver lipids in rats that were fed the high-fat diet. The possible explanation for this discrepancy may have been due to the differences in the amount of the cholesterol provided by the experimental diets. It has been shown that rats that were fed a high cholesterol diet increase the hepatic cholesterol content, and that may result in the increased triglyceride biosynthesis and reduced fatty acid oxidation<sup>(35)</sup>. When rats were fed the high-cholesterol diet, increased excretion of fecal bile acid, the primary route of endogenous degradation of cholesterol, by SMS is suggested to be responsible for its hepatic lipids-lowering effect<sup>(18)</sup>. However, in the present study, when rats were fed the high-fat diet (containing only 0.015% cholesterol) without additional cholesterol supplementation, SMS failed to change the level of hepatic lipids. These results indicated that SMS may only reduce high-cholesterol diet-induced lipogenesis, but has no effect on high-fat diet-induced fat accumulation in liver.

Recent clinical studies have shown that conventional treatment with shengmai injection for 3 - 4 weeks can improve not only vascular endothelial and heart functions<sup>(10)</sup>, but also insulin resistance in patients with heart disease<sup>(11)</sup>. However, when administered orally with shengmai capsule, long-term application (6 months) was required to accelerate the recovery of heart function<sup>(36)</sup>. These results imply that certain active components in SMS can improve insulin resistance and heart functions. However, these active components may have limited systemic exposure when administered orally. Indeed, studies indicated that saponins in *Panax ginseng* may have low oral bioavailability due to poor

membrane permeability and active biliary excretion<sup>(37)</sup>, even though *Panax ginseng* has been reported to have beneficial effects on reducing insulin resistance<sup>(12)</sup> and inflammation<sup>(13)</sup>. Schizandrin, the most abundant (20.5  $\mu$ g/g) active component in SMS, is the main effective orally-absorbed ingredient of *Fructus Schisandrae* and its oral absorption can be enhanced by some ingredients in SMS<sup>(38)</sup>. Schizandrin has been demonstrated to have anti-oxidative and anti-inflammatory activities<sup>(39,40)</sup> and is believed to be the major myocardial protective component in SMS<sup>(41)</sup>. Therefore, in this study, lower TNF- $\alpha$  and TBARS levels in liver and heart caused by SMS might be partially related to schizandrin. However, in addition to schizandrin, other constituents in SMS should also be considered as SMS is a composite formula comprising of three herbal components. Therefore, the synergism effect among herbal components may have occurred, as seen in other TCM<sup>(42)</sup>.

In summary, our results show that SMS supplementation may have little or no effect on reducing insulin resistance, but display anti-oxidative and anti-inflammatory properties in rats.

## ACKNOWLEDGMENTS

This study was financially supported by grant aid from the China Medical University (CMU98-N1-11) and the National Health Research Institutes of Taiwan.

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