

# Effects of a soybean protein diet on ovariectomised female albino rats subjected to myocardial infarction

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## ABSTRACT

**Introduction:** Cardiovascular disease is the leading cause of death among menopausal women in developed countries, mostly due to the loss of endogenous oestrogen protection. Soybean protein (SP) is rich in isoflavone phytoestrogens. This study aimed to determine the effect of SP on ovariectomised rats subjected to myocardial infarction and its possible cardio-protection.

**Methods:** The study was conducted on 30 adult female albino rats, which were divided into three groups: Group I comprised the sham-operated rats; Group II, the ovariectomised (OVX) rats fed a standard diet; and Group III, OVX rats fed a standard diet supplemented with SP (OVX plus SP). The rats were anaesthetised, and electrocardiograms were conducted. The rats were then sacrificed, after which their hearts and livers were removed, weighed and subjected to histopathological examination. Blood was collected to determine the lipid profile, and the levels of total triiodothyronine, tetraiodothyronine (T4), thyroid-stimulating hormone (TSH), creatinine phosphokinase (CPK), lactate dehydrogenase, superoxide dismutase (SOD) and malonaldehyde (MDA).

**Results:** The biochemical studies showed a significant increase in plasma CPK (Group II), MDA and triacylglycerol (Groups II and III) levels compared to Group I. The plasma SOD showed a significant decrease in Group II compared to Group I. Total cholesterol, low and very low density lipoprotein cholesterol levels showed a significant increase in Group II, and a significant decrease compared to Group I. Significant increases in T4 and TSH were found in Group III compared to Group II.

**Conclusion:** SP intake can be valuable in

protecting the heart against an attack of acute myocardial infarction.

**Keywords:** isoflavone phytoestrogens, lipid profile, myocardial infarction, ovariectomy, soybean protein

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## INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of hospitalisation and death among menopausal women in developed countries.<sup>(1)</sup> The incidence of CVD among postmenopausal women has been directly related to the loss of endogenous oestrogen protection.<sup>(2)</sup> Oestrogen replacement therapy, or oestrogen and progestin hormone replacement therapy (HRT) has favourable effects on blood lipids and lipoprotein concentrations, antioxidant protection, endothelial function and vascular reactivity.<sup>(3)</sup> Several studies have raised concerns about the early adverse effect of HRT on cardiovascular risk.<sup>(4)</sup> The mechanism of the increased cardiovascular risk is not known; a possible explanation could be an increased risk of venous thromboembolism reported with oestrogen use.<sup>(5)</sup> Many foods contain phytoestrogen, but soybeans are particularly rich in isoflavones, one of the common classes of phytoestrogens.<sup>(6)</sup> Phytoestrogens are a diverse group of non-steroidal, plant-derived compounds that are structurally similar to oestrogenic steroids and have an affinity for oestrogen receptors.<sup>(7)</sup>

Epidemiological evidence suggests that populations that consume soy products as a staple have a lower incidence of cardiovascular disease than those in which soy protein consumption is minimal,<sup>(8)</sup> and this has led to the suggestion that isoflavones may be beneficial for cardiovascular health. Therefore, this study was performed to determine the effect of a soybean protein diet on the lipid profile and hormones of ovariectomised rats, as well as to investigate the possible cardio-protective effect of a soybean protein diet in ovariectomised rats that were subjected to myocardial infarction.

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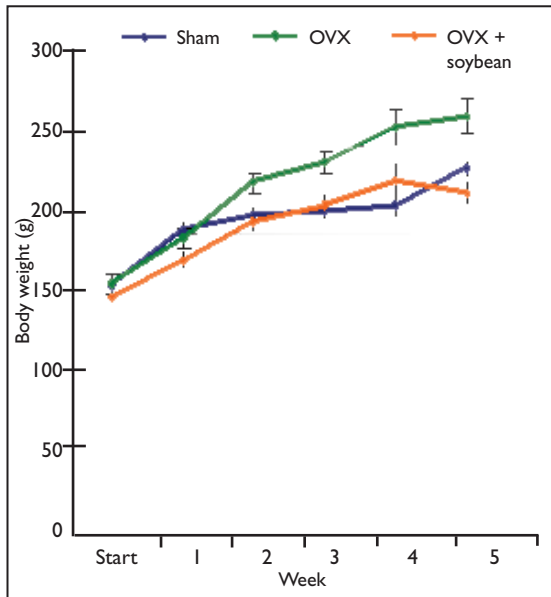


Fig. 1 Graph shows the mean body weight  $\pm$  standard error of the three groups of rats during the five-week study period.

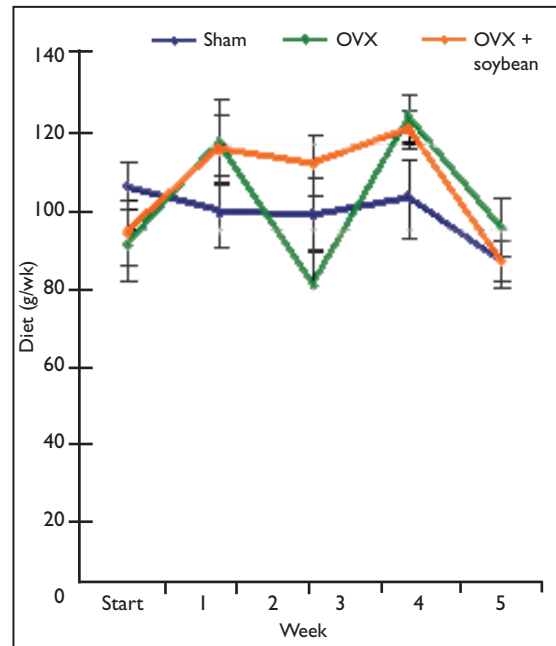


Fig. 2 Graph shows the mean dietary intake  $\pm$  standard error of the three groups of rats during the five-week study period.

## METHODS

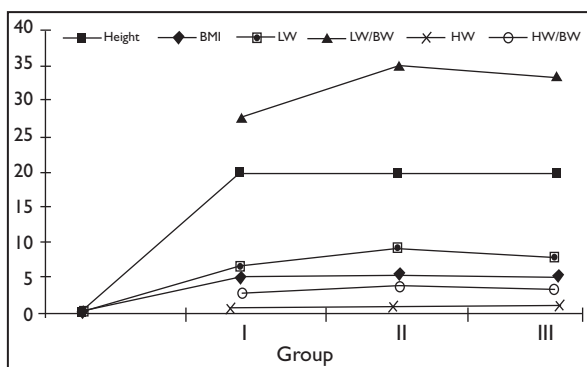
This study was approved by the high society of scientific ethics committee of General Organisation for Teaching Hospitals and Institutes, Cairo, Egypt. A total of 30 adult female albino rats weighing 150–200 g at the start of the study were housed individually in suspended wire-mesh cages. All the rats were initially fed standard rat chow for ten days before the start of the experiment. The standard rat chow diet (AIN-93 M diet formulated for the maintenance of adult rodents) was prepared according to the nutritional requirements of the National Research Council.<sup>(9,10)</sup> DL-methionine was added to the soy diets. The methods used to produce soy protein resulted in a preparation that retained the isoflavones. The isoflavone concentrations were 0.6–1.0 mg/g protein in isolated soy protein.<sup>(11)</sup>

The rats were divided into three groups, with ovariectomy performed in Groups II and III. Group I consisted of the sham-operated rats ( $n = 10$ ) and served as a control group. These rats were fed a standard rat chow diet. Group II comprised ovariectomised (OVX) rats ( $n = 10$ , 1 died at the end of the study) that were also fed a standard rat chow diet. Group III comprised OVX rats ( $n = 10$ ) that were fed a soybean protein-enriched diet. Water and food were provided ad libitum. The experiment lasted for 35 days. The food intake and body weight of the animals were measured once a week. On the 33rd day, isoproterenol was injected subcutaneously at a dose of 150 mg/kg/day for two successive days into the rats in Group II and III in order

to induce myocardial infarction.<sup>(12)</sup>

Ovariectomy was conducted according to the technique described by Farris and Griffith.<sup>(13)</sup> The animals were anaesthetised using ether, and a single longitudinal skin incision was made in the ventral midline of the abdomen above the symphysis pubis. The skin was retracted laterally, and the abdominal wall and peritoneum were incised from the level of the urinary bladder to the lower poles of the kidneys. The ovary was exposed on each side, and haemostasis was insured by ligation of the upper horns of the uterus with a chromic catgut thread (3/0). Each ovary, together with its surrounding fat and oviduct, was removed. The abdominal muscles were then closed with 3–4 interrupted stitches using chromic catgut thread (3/0). The skin was closed with 3–4 interrupted stitches using a silk thread. Aseptic conditions were maintained during the operation, and antibiotic powder was sprinkled within the wound. The sham-operated rats were treated in a similar way, but only the ovaries and oviduct were manipulated.

At the end of the experimental period, all the rats were subjected to overnight fasting, weighed and anaesthetised with intraperitoneal thiopental sodium (40 mg/kg BW). The height (from the tip of the nose to the anus) was measured and an electrocardiogram (ECG) was generated for each rat. Then the abdominal aorta was exposed and the blood samples were collected in two centrifuge tubes; a plain tube to obtain serum and a Na<sub>2</sub>EDTA tube to obtain plasma. Both tubes were



**Fig. 3** Graph shows the changes in the height, body mass index (BMI), liver weight (LW), LW/body weight (BW), heart weight (HW) and HW/BW in the three groups of rats.

centrifuged to allow for separation of the serum and plasma, and were stored at  $-20^{\circ}\text{C}$  until they were used for the determination of biochemical measurements.

ECG recording was done by placing needle electrodes under the skin of the four limbs of each rat near the paws, and connected through an ECG coupler to a two-channel oscillograph (Cardimax FX 121, Fukuda Denshi Co Ltd, Tokyo, Japan). The electrocardiographic tracing was recorded using standard limbs. From lead II-ECG tracing with a paper speed of 25 mm/s, the heart rate (HR), P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The HR was calculated using the formula:

$$\text{HR} = [7500/\text{distance (mm) between six successive peaks of R waves}]$$

The hearts were subjected to histopathological examination after they were removed, washed with sterilised saline, dried between filter papers and weighed. They were then kept in 10% formalin for histological examination, dehydrated, cleared in zylol and embedded in parablax. Paraffin sections were cut serially at 6  $\mu\text{m}$  thickness and stained using haematoxylin and eosin, as described by Drury and Wallington.<sup>(14)</sup> The serum total cholesterol (TC) and serum high density lipoprotein cholesterol (HDL-C) levels were determined using colourimetric enzymatic kits (SGM Italia, Rome, Italy), according to the methods described by Allain et al<sup>(15)</sup> and Lopes-Virella et al, respectively.<sup>(16)</sup> The serum low density lipoprotein cholesterol (LDL-C) and serum triacylglycerol (TG) levels were determined using colourimetric enzymatic kits, according to the methods described by Fruchart et al<sup>(17)</sup> and Bucolo and David, respectively.<sup>(18)</sup> The serum very low density lipoprotein cholesterol (VLDL-C) level was calculated using the equation:  $\text{VLDL-C} = \text{TC} - (\text{HDL-C} + \text{LDL-C})$ .

The serum tT3 and tT4 levels were determined using

AccuBind ELISA Microwells (Monobind Inc, Costa Mesa, CA, USA), according to the method described by Gharib et al<sup>(19)</sup> and Chopra et al, respectively.<sup>(20)</sup> The serum thyroid-stimulating hormone (TSH) (radioimmunoassay) level was determined using a rat TSH kit (Amersham Life Science, Amersham, IL, USA), while the plasma creatine phosphokinase (CPK) and plasma lactate dehydrogenase (LDH) levels were determined using kinetic endpoint kits (SGM Italia, Rome, Italy), according to the method described by Morin et al<sup>(21)</sup> and Kreutzer et al,<sup>(22)</sup> respectively. The superoxide dismutase (SOD) level was determined according to the method described by Concetti et al,<sup>(23)</sup> and the plasma malondialdehyde (MDA) level, according to the method described by Draper and Hadley.<sup>(24)</sup>

All statistical data and significance tests were performed using the Statistical Package for the Social Sciences version 11 (SPSS Inc, Chicago, IL, USA).<sup>(25)</sup> Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between the means of the different groups. Further analysis was conducted by the least significance difference multiple-range test to determine the inter-group differences. A probability of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

The body weight and dietary intake of the rats during the study period are shown in Figs. 1 and 2. Despite the rats' matched body weight at the start of the study, Fig. 1 shows that rats in Group II gained significantly more body weight than those in Group I and III. In terms of the body mass index (BMI), a significant increase was observed in Group II rats compared to those in Group I and III (Fig. 3). A significant increase in the weight of the livers and hearts was also observed in Group II rats compared to Group I and III rats. A significant increase in the liver weight/body weight and heart weight/body weight ratio was observed in Group II and III rats compared to Group I rats (Fig. 3). In terms of the ECG parameters, significant prolongation of the PR interval was observed in Group II rats compared to Group I and Group III rats ( $61.1 \pm 3.5$  ms vs.  $51 \pm 2.8$  ms and  $52 \pm 2.0$  ms). In addition, the Q wave voltage was significantly increased in Group II rats compared to Group I rats ( $55.6 \pm 3.7$   $\mu\text{V}$  vs.  $45 \pm 3.3$   $\mu\text{V}$ ). The ST segment showed significant upward deflection in Group II rats compared to Group I rats ( $155.6 \pm 12.4$   $\mu\text{V}$  vs.  $42.5 \pm 3.8$   $\mu\text{V}$ ). In Group III, the upward deflection of the ST segment was still present compared to the Group I rats ( $67.5 \pm 5.3$   $\mu\text{V}$  vs.  $42.5$

**Table I. Changes to the plasma creatine phosphokinase (CPK), plasma superoxide dismutase (SOD), plasma malondialdehyde (MDA), lipid profile and hormone levels in the three study groups.**

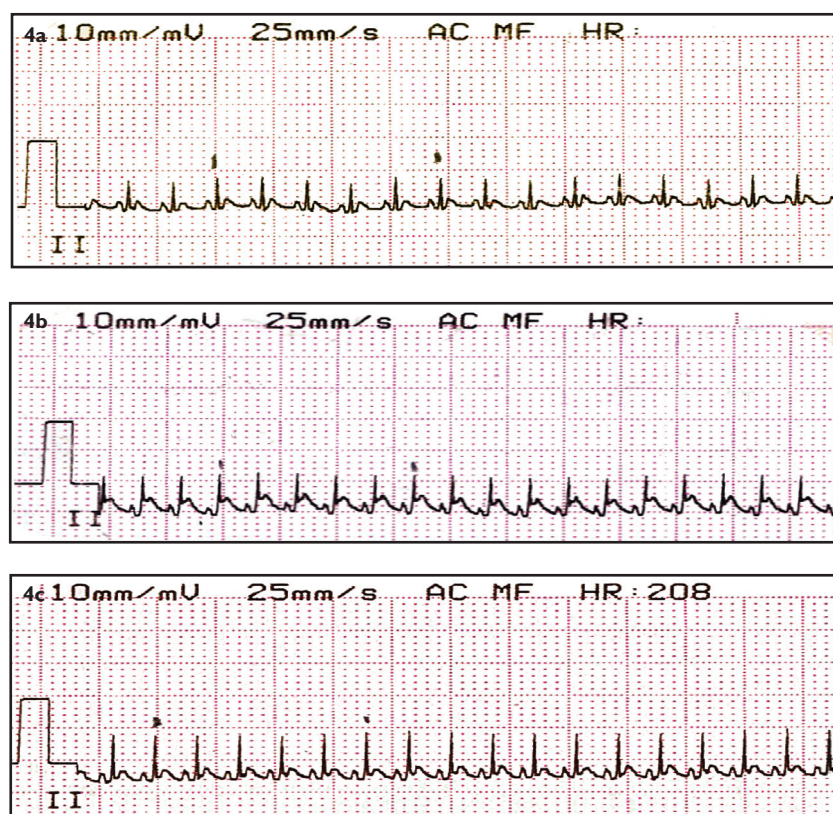
Group	Plasma CPK (U/L)	Plasma SOD (μg/mL)	Plasma MDA (μmol/L)	TC (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	TG (mg/dL)	T4 (μg/dL)	TSH (μIU/mL)
I (n = 10)	699.5 ± 24.4	40.5 ± 1.0	69.11 ± 1.3	86.74 ± 3.7	39.6 ± 2.6	17.6 ± 0.8	90.39 ± 4.1	6.5 ± 0.5	0.43 ± 0.03
II (n = 9)	1010.4 ± 73.1	36.6 ± 1.4	119.3 ± 4.2	133.02 ± 3.8	75.2 ± 2.8	25.5 ± 0.7	129.55 ± 7.4	5.5 ± 0.4	0.37 ± 0.02
III (n = 10)	824.76 ± 39.3	42.4 ± 0.8	80.2 ± 1.8	109.50 ± 6.3	55.9 ± 4.3	21.6 ± 1.2	115.5 ± 5.2	7.6 ± 0.5	0.24 ± 0.02
p-value	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05	< 0.001

The results are expressed as mean ± standard error.

The p-value is the significance by one-way ANOVA among the three study groups.

TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol;

TG: triacylglycerol; TSH: thyroid stimulating hormone



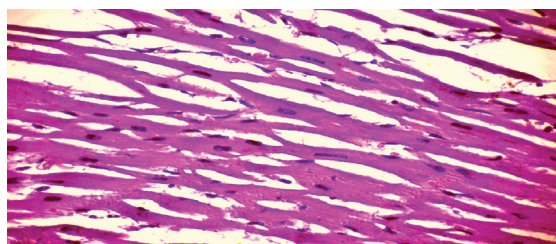
**Fig. 4** Electrocardiographic records (lead II) of (a) Group I; (b) Group II; and (c) Group III rats.

± 3.8 μv), although it was significantly decreased compared to Group II rats (Figs. 4a–c).

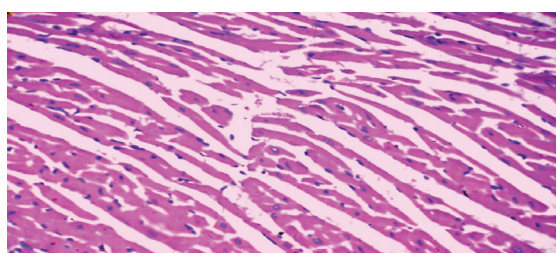
Table I shows a significant increase in plasma CPK in Group II compared to Group I rats. Feeding a soybean protein-enriched diet to OVX rats resulted in a significant reduction in the CPK level compared to OVX rats that were on a standard rat chow diet. However, the plasma LDH levels showed no statistically significant difference among the three groups (Table I). A significant decrease in the plasma SOD was observed in Group II rats compared to both Groups I and III. The plasma MDA level showed significant elevation in Group II compared to Group I rats. Feeding a soybean protein-enriched

diet to OVX rats resulted in a significant reduction in the plasma MDA level compared to OVX rats that were on a standard rat chow diet, although the level was still significantly higher than that recorded in Group I rats.

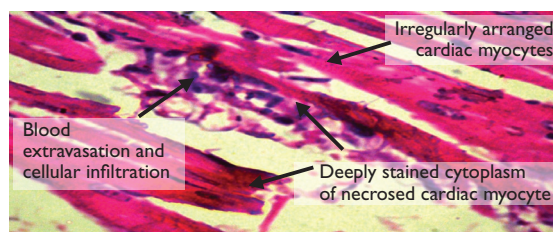
Table I shows a significant increase in serum TC, LDL-C, VLDL-C and TG in Group II compared to Group I rats. Feeding a soybean protein-enriched diet to OVX rats resulted in a significant reduction in the TC, LDL-C, VLDL-C and TG levels compared to OVX rats that were on a standard rat chow diet, although the levels were still higher than in Group I. In terms of serum HDL-C, no statistically significant difference was recorded among the three groups. For serum T4, a significant increase



**Fig. 5** Photomicrograph of the heart isolated from Group I rats shows regularly arranged cardiac myocytes with branching and anastomosis, with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibres (Haematoxylin & eosin, × 250).



**Fig. 7** Photomicrograph of the heart isolated from Group III rats shows regularly arranged cardiac myocytes with pale acidophilic staining and vesicular nuclei (Haematoxylin & eosin, × 250).



**Fig. 6** Photomicrograph of the heart isolated from Group II rats shows irregularly arranged cardiac myocytes with areas of degeneration and necrosis that are deeply stained, with blood extravasation and cellular infiltration (Haematoxylin & eosin, × 560).

## DISCUSSION

The results of the present study revealed a significant increase in the body weight of ovariectomised rats that were fed a standard rat chow diet compared to sham-operated rats, although their food consumption was comparable throughout the study. BMI, which is a clinical index of body adiposity, was also significantly increased, indicating increased body fat either due to increased lipogenesis or decreased lipolysis, or both. The euthyroid state of these rats excludes the possibility of hypothyroidism as a cause of the increased body weight and hyperlipidaemia. Ovariectomy-induced obesity has been attributed to metabolic changes as a result of ovarian hormone deficiency, which leads to increased fat synthesis and deposition in the adipocytes. When adipocytes reach their capacity of fat storage, fat becomes mobilised to be deposited in the viscera as the skeletal muscles, heart and liver (ectopic fat syndrome).<sup>(26)</sup>

In the present study, ovariectomised rats showed significant increases in both their absolute and relative liver weights, as well as in their total plasma cholesterol levels, particularly in the LDL fraction. This indicates either enhanced cholesterol synthesis or decreased excretion by the liver. These findings are in accordance with the findings of some other studies that have reported increased liver weight following ovariectomy.<sup>(27-29)</sup> The increase in liver weight has been reported to be due to triglyceride infiltration as early as three weeks after ovariectomy,<sup>(29)</sup> as well as due to the increased deposition of TC and LDL-C, leading to adiposis hepatica.<sup>(27)</sup>

Oestrogen has been reported to increase both the hepatic output of cholesterol in bile and the expression of 3-hydroxy-3-methyl glutaryl co-enzyme A (HMG-CoA) reductase, a rate limiting enzyme in cholesterol synthesis.<sup>(30,31)</sup> The higher the basal expression of hepatic HMG-CoA reductase, the greater the “cholesterol buffering capacity” and the greater the resistance to dietary cholesterol, while deficiencies in hormones that increase the hepatic HMG-CoA reductase gene expression lead to elevations in serum cholesterol levels.<sup>(32)</sup> The significant

was observed in Group III compared to Group II rats. However, no significant difference was observed among the three groups in terms of the T3 level, while the serum TSH level showed a significant decrease in Group III compared to both Group I and II rats.

Histological examination of the hearts isolated from Group I rats revealed regularly arranged branching and anastomosing chain of cardiac muscle fibres with pale acidophilic cytoplasm and apparent myofibrils. The myofibrils revealed a single vesicular nucleus that was present in the centre (Fig. 5). Histological examination of the hearts isolated from Group II rats revealed some areas with deeply acidophilic degenerated cardiac muscle fibres. The necrotic fibres revealed small pyknotic nuclei. Cardiac muscle fibres in some areas appeared in irregular arrangement with the destruction of their fibres. Mononuclear cellular infiltration was observed between the cardiac fibres. Some blood vessels were ruptured and extravasated blood cells were observed in the interstitial tissue. Some homogenous pale areas of damaged myofibrils were seen in between the cardiac fibres (Fig. 6). In contrast, the histological examination of the hearts isolated from Group III rats revealed that most of the areas were in regularly arranged branching and anastomosing fibres. The cytoplasm appeared to be pale acidophilic. The nuclei were single, central and vesicular; very few fibres were destructed with little connective tissue in between (Fig. 7).

increase in plasma LDL-C reflects a decreased uptake by the LDL receptors, which are known to be induced by oestrogen at both the protein and mRNA levels.<sup>(31,33)</sup>

After the induction of myocardial infarction by isoproterenol, the OVX rats showed a significant increase in Q wave voltage as well as a highly significant increase in ST voltage compared to the sham-operated control rats. All these changes indicate myocardial injury and infarction. The significant prolongation of the PR interval in these rats in comparison to the controls may be due to atrioventricular nodal damage, thus delaying atrioventricular impulse conduction. Cardiac muscle infarction was further confirmed by the significant elevation in serum CPK levels as well as the microscopic picture, which revealed patchy degeneration of cardiac muscle fibres, mononuclear cellular infiltration and thick-walled irregular blood vessels engorged with blood. Myocardial infarction induced by isoproterenol was attributed to the inotropic activity of isoproterenol, leading to an increased intracellular calcium level, isoproterenol-induced lipid peroxide generation as well as increased procoagulant activity.<sup>(34)</sup> These facts, together with the observation that these rats had increased lipid peroxidation as evidenced by the significantly higher level of plasma MDA and decreased level of the plasma antioxidant SOD, may provide clues to explain the extensive cardiac muscle damage induced by isoproterenol compared to soybean-fed rats. Thus, after ovariectomy, several changes occur that contribute to an increased cardiovascular risk. These changes include increased BMI, hyperlipidaemia, increased lipid peroxidation and decreased plasma antioxidants, all of which increase the susceptibility of the cardiac muscle to myocardial infarction. These unfavourable changes can be attributed to the loss of the physiological actions of ovarian hormones.

Food enrichment with soy protein isolate (SPI) for five weeks improved many, if not all, the cardiovascular risk factors that were present in the standard chow OVX rats. In comparison to the OVX and control rats, the body weight and BMI, which have been reported to be major cardiovascular risk factors,<sup>(35)</sup> were significantly decreased with an SPI diet, although the average food intake of soybean protein-fed rats was not significantly different from the other two groups of rats. This comparable food intake excludes the possibility of hypophagia as a cause of decreased body weight and BMI, and suggests enhanced thermogenesis as the underlying mechanism of less weight gain in these rats. These results are consistent with the findings of Aoyama et al,<sup>(36)</sup> who reported that soy protein and its peptide

reduced the body fat, fat-pad weight and perirenal fat, which suggests an increase in energy production. Soybean protein was found to increase the expression of the brown fat uncoupling protein-1 in rats,<sup>(37)</sup> leading to increased food utilisation and energy production.

Thyroid gland activity was found to be significantly increased in Group III rats, as evidenced by the increased T4 level and decreased TSH level. This finding is in contrast with those of other studies that reported insignificant changes in thyroid function with SPI,<sup>(38,39)</sup> and decreased thyroid function with SPI feeding.<sup>(40,41)</sup> The increased activity of the thyroid gland enhances lipolysis and decreases depot fat via the induction of hormone sensitive lipase.<sup>(33)</sup> Thyroid hormone also enhances TC and LDL-C excretion by increasing hepatic LDL-receptors.<sup>(33)</sup> In addition, it has been hypothesised that a diet containing alcohol-washed SPI significantly increases the hepatic thyroid receptor (TR $\beta$ 1) protein content in both male and female rats compared to a casein-based diet.<sup>(42)</sup> This thyroid receptor was found to be a mediator of the thyroid hormone effect on cholesterol 7  $\alpha$ -hydroxylase, a rate-limiting enzyme responsible for the conversion of cholesterol to bile acids.<sup>(43)</sup> It also regulates the expression of other hepatic genes involved in cholesterol catabolism, including those for the LDL-receptor and lecithin,<sup>(44)</sup> as well as cholesterol acyltransferase.<sup>(45)</sup> In addition, the genes for lipogenic enzymes, including malic enzyme,<sup>(46)</sup> fatty acid synthase<sup>(47)</sup> and acetyl-CoA carboxylase,<sup>(48)</sup> have been shown to contain a thyroid hormone response element in their promoters and to be regulated by thyroid hormones. Hence, the hypocholesterolaemic and lipid-lowering actions of soybean protein may be mediated by increased hepatic TR $\beta$ 1 through the regulation of cholesterol catabolism and lipogenesis. These effects of soy protein on gene expression and the activities of hepatic lipogenic enzymes have been observed in both normal and obese rats.<sup>(49,50)</sup> Thus, it can be concluded that soybean protein-induced hypocholesterolaemia is mediated at least in part by enhanced thyroid hormone activity.

Other mechanisms by which soybean protein may exert its hypocholesterolaemic effect include stimulation of the faecal excretion of cholesterol and bile acids. This effect has been explained by the characteristic amino acid composition of soybean protein, which has a low content of methionine and arginine, and a high content of lysine.<sup>(51,52)</sup> In addition, some authors have proposed that soybean protein-associated compounds such as saponins, trypsin inhibitors and phytic acid contribute to the soybean protein hypocholesterolaemic effect by

increasing bile excretion, releasing cholecystokinin which stimulates gall bladder contraction and decreasing the Zn/Cu ratio in blood, which has a direct relationship with the plasma cholesterol level.<sup>(53)</sup> A possible mechanism that is currently receiving a great deal of interest could be the phytoestrogens that are present in different proportions in all soy products. Isoflavones (the main phytoestrogens in soybean that are structurally similar to endogenous oestrogen and bind with intranuclear oestrogen receptors) have been found to exert an oestrogen-like effect on plasma lipids (i.e. decrease TC, particularly in the LDL fraction, VLDL and triglycerides, as well as elevate HDL-C).<sup>(54,55)</sup>

In the present study, SPI feeding appears to have preserved the myocardial structure and function. This is evidenced by the finding that soybean-fed OVX rats had a significantly lower prolonged PR interval, less Q wave voltage and less ST-segment elevation, which may reflect less extensive myocardial infarction compared to OVX rats that were fed a standard rat chow diet. The observation that lipid peroxidation decreased significantly and the plasma SOD level was restored to the sham-operated control value suggests that the hearts of these rats were better protected, and thus were able to better tolerate the damaging effect of isoproterenol. This was further confirmed by the histological examination of their hearts, which revealed less fibre destruction and a cardiac structure similar to the sham-operated rats. This may explain the significantly decreased absolute and relative heart weights of these rats compared to OVX rats due to less inflammatory changes and cellular infiltration.

There are many reports in the literature that explain the cardioprotective effects of soy isoflavones. These include potent antioxidant activity,<sup>(56)</sup> interference with the arterial LDL uptake, the oxidation and inflammation pathway being implicated in the initiation and progression of atherosclerosis,<sup>(57)</sup> interactions with oestrogen receptors,<sup>(58)</sup> both oestrogen receptors  $\alpha$  and  $\beta$ ,<sup>(59)</sup> stimulation of the transcriptional activity of the oestrogen receptor,<sup>(60)</sup> maintenance of nitric oxide production during ischaemic reperfusion,<sup>(61)</sup> preservation of the mitochondrial structure and function,<sup>(61)</sup> inhibition of  $Ca^{++}$  accumulation during ischaemic reperfusion which was reported to depress the recovery of myocardial function,<sup>(62-64)</sup> inhibition of smooth muscle migration and intimal proliferation in response to vascular injury in rats,<sup>(65,66)</sup> and improvement of flow-mediated arterial dilation and platelet function.<sup>(67,68)</sup>

Soy isoflavones have strong biological properties in animals, such as causing arterial vasodilation, lowering serum cholesterol levels and inhibiting

atherosclerosis in postmenopausal monkeys.<sup>(69)</sup> This has led to the intriguing idea that the presence and amount of isoflavones explains the variable results of soy studies. The three major isoflavones found in soybeans are genistin, daidzin and glycitin. The amount of these isoflavones in soy protein preparations varies widely and depends on the processing techniques used during production.<sup>(70)</sup> These compounds have oestrogenic activity.<sup>(71,72)</sup> Isoflavone concentrations range from 0.6 to 1.0 mg/g protein in isolated soy protein.<sup>(11)</sup>

SPI, the soybean product used in the present study, is a soy product from which oil, carbohydrates and water are extracted so as to obtain the protein content in the product exceeding 90%, which means that its isoflavone content is essentially less than that of the native soybean seeds. Thus, it is possible that the isoflavone content of the SPI was enough to exert a substantial cardioprotective effect or that the protein part of the SPI also had a cardioprotective effect, possibly by decreasing the body iron stores that were found to increase after menopause.<sup>(73,74)</sup> Iron is considered to be a cardiovascular risk factor because it catalyses the reactions that produce the hydroxyl radical, which is highly reactive and can result in structural damage to proteins, lipids and DNA, thus increasing the predisposition to heart disease.<sup>(75)</sup>

In conclusion, the data obtained in the present study suggests that soybean protein intake can be of value in protecting the heart against an attack of acute myocardial infarction. The lines of protection include decreased BMI, plasma lipids and lipid peroxidation, as well as increased plasma antioxidants. Menopausal women, particularly those who have metabolic syndrome and are at increased risk of ischaemic heart disease, may benefit from regular dietary intake of soybean protein as an alternative to traditional hormone replacement therapy. There is a need to better define the optimum dose of soy phytoestrogen in future studies.

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