Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1740–1748

Journal of Nutritional Biochemistry

The functional effect of soybean extract and isolated isoflavone on myocardial infarction and ventricular dysfunction
The soybean extract on myocardial infarction

Ana C. Miguez[a], Julio C. Francisco[a], Sílvio H. Barberato[a], Rossana Simeoni[a], Dalton Précoma[a], Vivian Ferreira do Amaral[a], Edson Rodrigues[b], Márcia Olandoski[a], Lúcia de Noronha[a], Fernando H. Greca[a], Katherine Athayde Teixeira de Carvalho[c], José Rocha Faria-Neto[a], Luiz C. Guarita-Souza[a,*]

[a]Experimental Laboratory of the Center for Biological and Health Sciences. Pontifical Catholic University of Parana (PUCPR), Brazil
[b]Taubate University, UNITAU, SP, Brazil
[c]Pelé little Prince Institute-Child and Adolescent Health Research, Brazil

Received 30 December 2010; received in revised form 11 May 2011; accepted 25 May 2011

Abstract

Background: Myocardial infarction is a public health problem. Functional food is an alternative treatment for cardiovascular diseases.
Objective: The objective was to analyze the functional and anatomopathological post-myocardial-infarction effects of soybean extract (SE) and isoflavone (IF).
Methods: Myocardial infarction was induced in adult Wistar rats. After 5 days, an echocardiogram was performed to determine heart rate (HR), ejection fraction (EF), systolic volume (LVESV) and diastolic volume (LVEDV). Animals with ventricular dysfunction (EF<45%) were selected for study. The animals were divided into three groups: control (n=14), SE (n=15) and IF (n=12). The IF group received 120 mg/kg/day isolated IF, and the SE group received 12.52 g/day. After 30 days, a new echocardiogram was performed. A histological exam was carried out to determine the collagen. Activity of biochemical markers [arginase, lactate dehydrogenase (LDH) and malate dehydrogenase] was measured.
Results: The animals of the control, IF and SE groups showed a reduction in EF after the infarction (P=.432, P=.017 and P=.320, respectively). An increase of LVESV and LVEDV was observed in all groups (P=.009, P=.001 and P=.140; and P=.003, P=.008 and P=.205, respectively). A reduction of HR was found in the SE group (P=.020). There was a greater activity of LDH in the SE group. A smaller quantity of mature collagen was found in the region proximal to the myocardial infarction in the SE group.
Conclusion: A protective effect in the SE group was observed 30 days after the myocardial infarction.

Keywords: Isoflavone; Soybean; Myocardial infarction; Ventricular dysfunction; Functional foods; Arginase

1. Introduction

Acute myocardial infarction (AMI) is a widespread public health problem resulting in high indices of morbidity and mortality and is relevant with respect to clinical epidemiology [1]. In the United States, 25% of deaths are a consequence of AMI, representing 1,500,000 individuals per year. One in every five patients discharged from the hospital dies within the first year after AMI [2].

Functional food may act as an adjunctive therapy/alternative treatment of these pathologies, and scientific studies are appearing more frequently demonstrating that this hypothesis is, indeed, a reality. Soybean containing isoflavone (IF) and protein is considered a functional food item.

Several articles have defined that a diet including a combination of antioxidant components and foods rich in flavonoids appears to be effective in decreasing the oxidation capacity of low-density lipoprotein (LDL) particles, leading to cardiovascular benefits and reduction in total and LDL cholesterol and triglycerides [3]. Nagata et al. reported that serum levels of cholesterol in Japanese males and females decreased when consuming higher levels of soybean, and Anthony demonstrated a decrease in cholesterol levels as well as prevention of cardiovascular diseases [4,5]. In another similar study, authors suggested that IFs act as phytofibrates and phytoglitazones and can contribute to the lipolytic process. These results are related primarily by alterations in lipid metabolism in the liver [6].

In a study by Lin et al. in a myocardial infarction model by left anterior descending coronary artery ligation, resveratrol, considered to be a functional food, reduced the size of the infarction and increased ventricular function, acting as a strong cardioprotective agent [7].

With respect to the therapeutic effect of soybean, Ma et al. identified a reduction in the toxic effect of Adriamycin in a dilated

* Corresponding author. Rua Rosa Kaint Nadolny, 190, ap 501, CEP: 81200-525, Curitiba (Pr). Brazil. Tel.: +55 41 84177188/32711657, fax: +55 41 32712285.
E-mail address: guaritasouzalc@hotmail.com (L.C. Guarita-Souza).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2011.05.017

myocardiopathy model as well as cardiac contractility recovery [8]. Hagen et al. also reported a cardiovascular benefit after myocardial infarct, as a result of the antioxidant action of soybean [9].

The exact mechanisms involved in soybean action are not yet well established. Based on the fact that soybean not only reduces cholesterol and triglyceride levels but presents a beneficial effect in a dilated myocardiopathy model as well as an antioxidant effect on myocardial infarction models, the aim of this study was to analyze the functional, biochemical and anatomopathological effects of soybean extract (SE) and isolated IF in AMI with ventricular dysfunction.

2. Methods and materials

This project was presented to the Ethics Committee on Animal Research of Pontifical Catholic University of Parana (PUCPR) and subsequently approved (number 201, 4/2/2007). All animals received care in accordance with the ethical principles of the Brazilian College of Animal Experimentation [10].

2.1. Inducing infarction

All Wistar rats (*Rattus norvegicus*) from 3 to 4 months of age with a mean weight of 330.5±28.9 g underwent myocardial infarction and were given general anesthesia of 50 mg/kg of weight of ketamine (Ketalar, Laboratórios Parke & Davis) and 10 mg/kg weight xylazine administered *via* intramuscular. The animals were given paracetamol (1 mg/kg of weight) diluted in water for 7 days as a postoperatory analgesic.

After anesthesia, the animals underwent orotracheal intubation and mechanical ventilation with a volume of 2.5 ml per respiration at a frequency of 68 cycles per min (HARVARD, Inc., respirator model 683, MA, USA).

The myocardial infarction was induced with surgical ligation of the left coronary artery by left thoracotomy with a single incision of 7.0 microfilament thread.

2.2. Experimental groups

Following the AMI and an echocardiographic analysis after 5 days, the animals that presented an EF of less than 45% were included in the study and randomized into three groups: control (C) (n=14), IF (n=12) and SE (n=15).

The functional food was administered through gastrogavage by means of a short nasogastric probe (siliconized PVC no. 08) inserted in the oral cavity of the animal until it reached the stomach, into which the modified diet was delivered in the case of the study groups and water in the case of the C group [11,12].

The C group animals received 2 ml of mineral water at room temperature during 25 days, 7 days per week. The study substances were administered twice a day. IF was given in quantities of 120 mg/kg/day [8], and the animals in the SE group were given 12.52 g/kg/day of SE for the same period. In all three groups, each rat received a mean of 60.75 kcal/day from their food sources. All measurements were performed on a precision scale (BEL MARK U210A) with a maximum capacity of 210 g and precision of 100 mg.

In addition, all animals had free access to water and traditional animal chow Nuvilab CR1, Nutrival brand (registered in Ministry of Agriculture number from PR58033-00103). According to the data from Sliva, each adult animal consumed on average 25–30 g of food per day. Since the food contained 2900 kcal/1000 g of metabolizable energy, each rat consumed 72.5–87 kcal/day, a mean of 80 kcal/day [13]. The animals were given IF (Novasoy) 152–400 in a concentration of 40% (genistein, daidzein, glycitein: 1.3:1.0:0.3), lot 0507111. ADM of Brasil Ltda. supplied the IF for this study.

The mortality rates for the groups after the end of treatment were 50% in the C group, 60% in the IF group and 54% for the SE group.

2.3. Functional assessment

Left ventricular function was assessed 5 days and 30 days after myocardial infarction by bidimensional transthoracic echocardiography (5500 Sonos model Hewlett Packard Company, USA), frame rate 21–64 Hz, with S12 (5–12 mHz) sector and 15L6 (7–15 mHz) linear transducers, specifically designed for ultrasound studies in small animals. Under anesthesia, the transducer was positioned in the left anterolateral portion of the thorax, and the heart was visualized by using a two-dimensional mode with an axial view of the left ventricle, including the mitral and aortic valves and the apex in the same image. Digital conversion of the image was obtained by identifying the interventricular septum and the left ventricular posterior wall. Left ventricular end-systolic (LVESV) and end-diastolic volumes (LVEDV) and ejection fraction (EF) were calculated using the following formula: ventricular volume (V) was $8\times(S)2/(3\times3.1415926\times C)$, where V=volume, S=area and C=weight. The formula for calculating the left ventricular EF was as follows: EF=LEDV−LESV/LEDV. All measurements were blind and performed three times by the same technician, and mean values were recorded [14].

2.4. Euthanasia

The animals were euthanized 30 days after infarction and echocardiography following the methodology described by the Federal Counsel of Veterinary Medicine. Rats were euthanized approximately 24 h after the last feeding by means of intraperitoneal injection of 0.5 g of Thiopentax (sodium thiopental; intravenous sterile powder form) [15].

2.5. Biochemical analysis

After euthanasia, the animal's hearts were frozen in liquid nitrogen. The cardiac muscle was homogenized in the Potter/Elvehjem device in a proportion of 1 g of tissue to 5 ml of buffer solution Tris-HCl 20 mM (pH 7.4) and sonicated for 30 s. After centrifugation at 14,000g for 10 min, the supernatant of the homogenized tissue was utilized for identification of enzymatic activity and total protein. All steps of the homogenized tissue preparation were completed at temperatures between zero and 4°C.

Activity of enzymes malate dehydrogenase (MDH: E.C. 1.1.1.37) and lactate dehydrogenase (LDH: E.C. 1.1.1.27) was verified in a buffer solution of Tris-HCl 50 mM (pH 7.4) containing (a) $MgCl_2$ 20 mM, oxaloacetate 0.4 mM and NADH+H^+ 150 mM for MDH and (b) sodium pyruvate 1 mM, KCl 100 mM and NADH+H^+ 150 μM for LDH [16,17]. In both cases, enzymatic activity was identified accompanying a reduction of absorbance of the reaction system at wavelengths of 340 nm. Arginase (ARG: E.C. 3.5.3.1) activity was measured by the modified Iyamu method [18]. The reaction system was composed of a glycine buffer of 50 mM (pH 9.5) containing L-arginine 100 mM and $MnCl_2$ 1 mM. The reaction was initiated by the addition of 20 μl of homogenized tissue to 230 μl of system reaction. The system remained in incubation at 37°C for 2 h, and the reaction was interrupted by the addition of 250 μl of hydrochloric acid 0.75 M. After centrifugation at 14,000g for 10 min, 50-μl portions of supernatant were transferred to microtubes containing chromogenic reagent [50 μl water and 200 μl of ninhydrin 6% (p/v) dissolved in 2-methoxyethanol], heated for 25 min at 100°C, cooled, transferred to microplates of 96 wells and read in wavelengths of 505 nm.

The spectrophotometric reading of the microplates was performed using the spectroflourometer Fluostar manufactured by BMG. Enzyme activity was expressed in international units (U), defined as the enzyme quantity that catalyzes the conversion of 1 μmol of substrate in product in 1 min at 37°C.

Protein was measured by the Bradford method utilizing bovine serum albumin as the standard [19]. The protein content of the samples was used to calculate the specific enzyme activity expressed in mU/mg of protein (international milliunits/mg of protein).

2.6. Histopathology

The hearts were removed from all animals for morphological evaluation at the Anatomic Pathology Laboratory of PUCPR. Sirius red method was utilized for the anatomopathological analysis, promoting red coloration in collagen, reticular fibers, cartilage and basal membranes; blue in DNA and RNA by Harris hematoxylin; and pink or yellow in the cytoplasm of epithelial and muscular cells by the picric acid. The rat hearts were cut longitudinally and subsequently fixed in a 10% formaldehyde solution. After this procedure, they were embedded in paraffin, cut into widths of 4 μm, and stained with picrosirius red for the histomorphometric analysis. An optical microscope magnified at 200 times, a binocular microscope (Olympus model BX50) and a Dell computer with Image Pro Plus software were utilized for the analysis. Using the digital image of the field by the software and a standardized reference color, the area of the sample presenting the referred color was calculated. Imaging analysis was performed in triplicate [20].

For all sites (site 0: region of myocardial infarction, site 1: transition region of infarct and whole myocardium and site 2: free wall of the left ventricle) and in each field (1, 2 and 3), the total area of mature collagen and total area of immature collagen were calculated using all readings taken. The value for the area of mature and immature collagen in the comparative analysis of the groups was the mean area for all the fields. Using these results, mature and immature collagen percentages were calculated in relationship to the sum of the area of the two types of collagen, as well as total collagen (mature and immature) for the three groups, the sum of percentages being 100%.

2.7. Statistical analysis

The paired-sample Student's t test was used to compare quantitative variables for pre and post time periods for each group. The variance analysis model with one factor was used for preanalysis of results comparing the two groups. The covariance analysis was used for comparison of the groups postevaluation and the differences between pre and post results, using the preanalysis measurement as the covariable. Variables with asymmetric distributions were analyzed by application of the nonparametric Kruskal–Wallis test. The Shapiro–Wilk test was utilized for symmetrical variables. P values of <.05 indicated statistical significance. The data were organized on an Excel spreadsheet and analyzed using the statistical program Statistica v.8.0 [21].

3. Results

3.1. Weight analysis

The intragroup analysis for the period between AMI and 30 days after revealed an increase in weight for the three groups studied. The values expressed, shown in Fig. 1, were 327.78 g to 399.33 g (P=<.001), 321.16 g to 350.87 g (P=.001) and 342.96 g to 377.20 g (P=<.001) for the IF, SE and C groups, respectively. No significant difference was found in the pre mass evaluation when comparing the three groups (P=.116). However, a significant difference was found between the three groups for the postevaluation (P=<.001). The overall gain in the IF group was roughly 71.55 g (22.30%) vs. 29.71 g (9.65%) in the SE group vs. 34.24 g (10.10%) in the C group.

Upon comparison between groups after 30 days, a significant difference was identified between the C group and IF (P=.030), C and ES (P=.007), and ES and IF (P<.001), as shown in Tables 1 and 2.

3.2. Food Intake

Table 3 expresses the amount of food eaten by each group during the experiment.

3.3. Biochemical markers

The mean argininolytic activity of the IF, SE and C is presented in Fig. 2. The mean ARG activity in the IF and SE groups was lower than that of group C, although not significantly.

The differences between the mean values of LDH activity in cardiac tissue of the rats in groups IF, SE and C are presented in Fig. 3. The specific activity of LDH in the group treated with SE was more expressive, with a significant difference.

There was a significant difference for LDH in the two groups compared: C×SE and IF×SE. Accordingly, the groups were compared two by two, and the P values are presented in Table 4.

The differences between mean values of MDH activity of group C and the two treated groups were significant and are shown in Fig. 4.

For the variable MDH, there was a statistical difference between the two groups compared: C×ES and IF and SE. The groups were then compared two by two, and the P values are presented in Table 5.

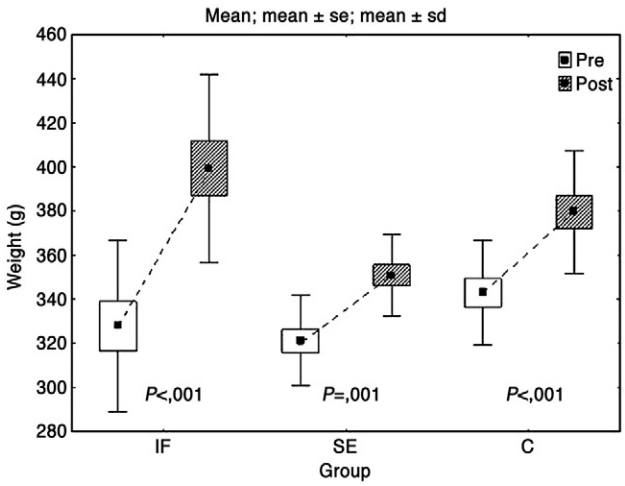

Fig. 1. Comparative analysis of weight for the three groups.

Table 1
P values for weight expression for the three groups

Groups compared	P value
SE×C	.007
SE×IF	<.001
C×IF	.030

3.4. Histopathology

Mature and immature collagen is identified in Figs. 5 and 6. As observed, the SE group presented less mature collagen compared to the other groups.

The data regarding the collagen analysis are expressed in Table 6. Site 0 refers to the region of the myocardial infarct, site 1 refers to the transition region between infarct and whole myocardium, and site 2 refers to the free wall of the left ventricle.

For the mature collagen variable area in site 1, a significant difference was found between the three groups. The groups were then compared two by two, and the P values are presented in Table 7.

For total collagen in site 1, a significant difference was found between the three groups. The groups were compared two by two, and the P values are presented in Table 8.

3.5. Echocardiographic analysis

Descriptive statistics for each variable of each group are presented in the following figures. Pre- and postevaluations and P values for statistics related to pre- and posttreatment within each group, as well as between the groups, are also presented.

The values for heart rate were 232.67 bpm to 237.67 bpm (P=.624), 269.31 bpm to 219.85 bpm (P=0.020) and 270.93 bpm to 256.36 bpm (P=.520) for the intragroup analysis of IF, SE and C, respectively. There was no statistical difference observed in pretreatment cardiac frequency between the three groups (P=.080). Similarly, there was no significant difference seen in the postevaluation between the three groups (P=.071), as shown in Fig. 7.

There was a decrease in left ventricular EF identified for the three groups. Values expressed in the intragroup analysis were 39.42% to 32.91% (P=.017), 36.77% to 34.17% (P=.0320) and 40.38% to 38.33% (P=.432) for the IF, SE and C groups, respectively. No significant difference for the pretreatment evaluation of EF was observed upon comparison of the three groups (P=.401). Similarly, no difference was observed between groups in the postevaluation (P=.382), as shown in Fig. 8. The pre and post difference presented a P value of .422.

Upon intragroup analysis of the LVESV, we observed an increase for the three groups. The values expressed in Fig. 9 were from 0.24 ml to 0.45 ml (P=.001), 0.36 ml to 0.41 ml (P=.140) and 0.31 ml to 0.43 ml (P=.009) for the IF, SE and C groups, respectively. A significant difference in LVESV was observed when comparing the three groups among themselves (P=.012). No statistical difference was found among the three groups in the postevaluation (P=.253). The pre and post difference presented a P value of .021.

The intragroup analysis of the LVEDV identified an increase for the three groups. The values expressed in Fig. 10 were from 0.44 ml to 0.61 ml (P=.008), 0.57 ml to 0.63 ml (P=.205) and 0.53 ml to

Table 2
Percentage of weight values for three groups

IF×SE×C	IF×SE	IF×C	SE×C
(P)<.001	<.001	.001	.885

Table 3
Quantity of chow and calories intake for the three groups

Group (calories)	Chow intake (g/rat/day)
IF (81.2 kcal)	28.0
SE (49.3 kcal)	17.0
C (87.0 kcal)	30.0

0.67 ml (P=.014) for the IF, SA and C groups, respectively. Upon comparison of the LVEDV, there was a significant difference between the groups IF and SE (P=.048). In the postevaluation, there was no significant difference between the groups (P=.639). The difference before and after presented a P value of .382.

Results of the LVEDV index analysis of the three groups for both pre- and posttreatment are expressed in Fig. 11.

Results of the LVEDV index analysis of the three groups for both pre- and posttreatment are expressed in Table 9.

For LVEDV index analysis of the three groups, for both pre- and posttreatment, a significant difference was found between the three groups. The groups were compared two by two, and the P values are presented in Table 10.

4. Discussion

The Food and Drug Administration suggested the use of SE in 1999, recognizing that the components of soybean could bring some type of cardioprotective benefit [22]. However, IF supplementation in tablet form or in foods had not been recommended by the American Heart Association in 2006 [23]. This may have been due to the lack of scientific information regarding the action of IF and the constituents of soybeans.

The presumed protective effects of IF and phytochemical polyphenol compounds of soybean in cardiovascular disease have been the focus of several studies [24]. The cardiovascular effects of IF, *in vitro* and *in vivo*, involve the combination of vasodilator system activation and inhibition of constrictor mechanisms. The molecular mechanisms of IF action include signaling pathways such as ERKe/2, PI3-kinases/Akt and eNOS activation [25]. The benefits of this class of compounds in treatment of osteoporosis, menopause and breast cancer have also been studied [26–28].

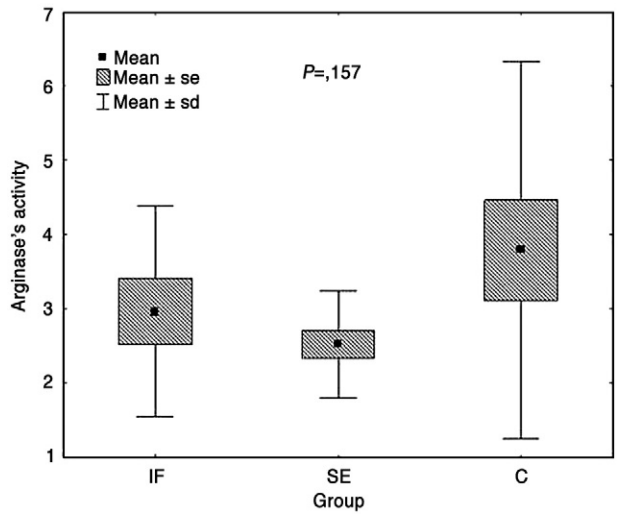

Fig. 2. Expression of ARG's activity for the three groups.

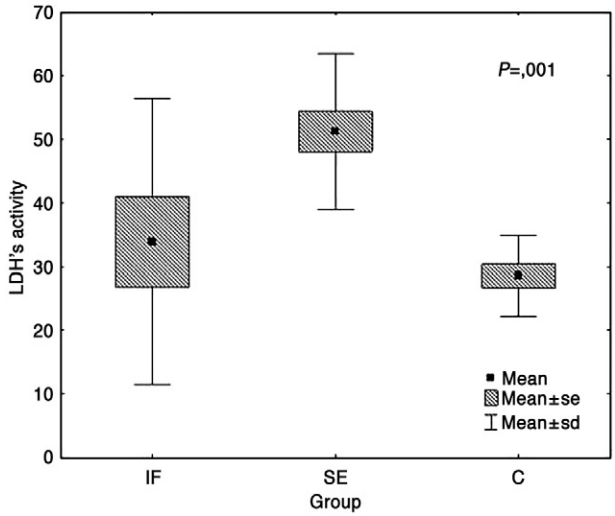

Fig. 3. Expression of LDH's activity for the three groups.

The effects of soybean on the mechanisms for cholesterol reduction have been described; however, therapeutic effects in myocardial infarction have not yet been reported. With respect to soybean effects on myocardiopathy, Ma et al. suggested that these functional foods present benefits. The mechanisms that justified these findings involved the fact that IF kept myofibrils organized, thus decreasing the toxic effect of Adriamycin in the dilated myocardiopathy model [8]. It was suggested that the anti-inflammatory effect of IF decreased the toxic effect of Adriamycin and, consequently, an improvement in cardiac function was identified.

Contrary to what Ma et al. reported, the present study identified that, in the IF group, EF decreased and LVESV and LVEDV increased as in the C group, suggesting alterations characteristic of late evolution of the myocardial infarct and cardiac failure. However, in the SE group, stabilization in EF was observed as well as for final diastolic and systolic volumes of the left ventricle, suggesting a myocardial protective effect. This is confirmed by the fact that the difference in LVESV pre- and posttreatment in the SE group showed a statistical significance of P=.0021. Another important factor to point out is that the LVEDV index, suggesting that the SE group presented worse hemodynamic values in the pretreatment period compared to the other groups (P=.045), presented a greater benefit in relationship to the other groups (P=.007).

We observed that the SE group presented a significant reduction in cardiac frequency between the pre- and post-myocardial-infarction period. In contrast, we observed an increase in CF in the IF group. This parameter, however, cannot be analyzed independently, only in relationship to other parameters. When analyzing the data for the SE group, the relationship to EF stabilization and the antiremodeling effect suggest a myocardial protective effect in this group.

Table 4
P values for expression of LDH's activity in the three groups

Groups compared	P value
C×IF	.388
C×SE	<.001
IF×SE	.006

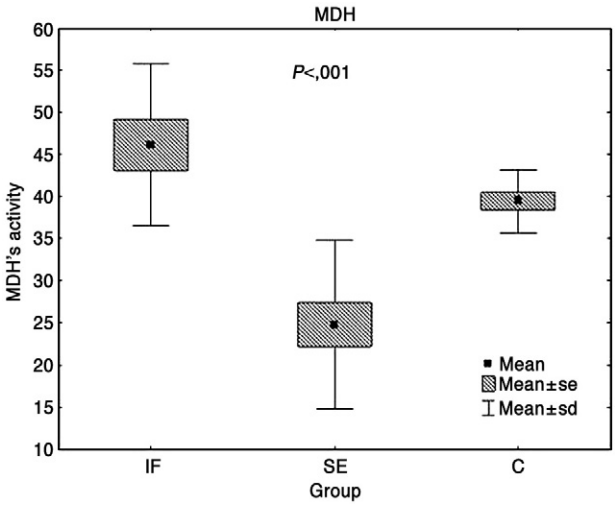

Fig. 4. Expression of MDH's activity for the three groups.

These data imply that isolated IF does not present post-myocardial-infarction benefits. It is true, however, that the base physiopathology of the models of dilated myocardiopathy induced by Adriamycin and myocardial infarction is different.

With respect to the IF group, the weight gain from water retention associated to the drop in EF of the left ventricle with ventricular remodeling suggests signs of the development of cardiac failure. Calories were ingested by gavage.

In the functional analysis of SE, we observed post-myocardial-infarct cardiac function stabilization. This was based on the fact that EF, which guarantees cardiac inotropism, did not present a significant reduction, nor did the ventricular diastolic and systolic volumes show a significant increase. An increase in final systolic and diastolic volumes of the left ventricle was observed in all groups, however, with statistical significance in the IF and C groups, suggesting the cardioprotective effect of SE. Corroborating with our data, Lin et al. reported an improvement in cardiac function and a decrease in histological and hemodynamic parameters using the functional food, polyphenol, in a model similar to that used in this study [7].

Corroborating with the data in this study, although using another experimental model and another type of functional food, Lin et al. administered resveratrol in rats after myocardial infarction. In the echocardiographic analysis after treatment, they reported an important reduction of infarction as well as an increase in left ventricular EF, a regression of left ventricular dilatation and a reduction in left ventricular final diastolic pressure. In the histological analyses, positive results were also observed, demonstrating that resveratrol is an important cardioprotective agent [7].

One relevant factor is that IF, when separated from the soy protein, appears to lose part of its functional effect, particularly with respect to cardiovascular disease, which implies that its intake from the leguminous source is indispensable. This effect was also observed in studies on primates and even more relevant, when IF was removed from the soy protein, and previously restored to the protein matrix, this food item lost all functional effect. When soy protein is associated with IF, the lipoprotein profile decreases considerably. We can see from these results that IF should be consumed in its natural form (naturally inserted in the food itself) since humans are physiologically programmed to retrieve the components from foods that will cause the functional effect, and this component will therefore have greater bioavailability [29–31].

Table 5
P values of expression of MDH's activity in the three groups

Groups compared	*P* value
C×IF	.072
C×SE	<.001
IF×SE	<.001

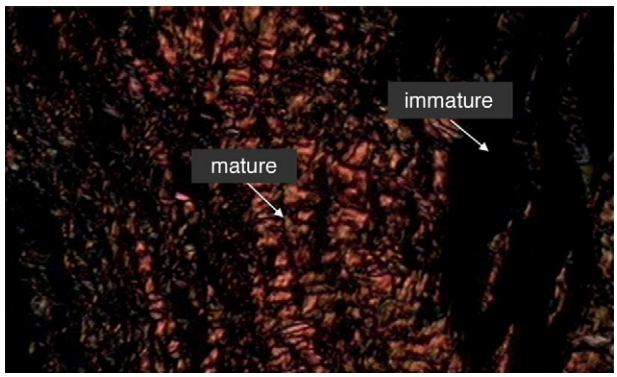

Fig. 5. Expression of mature and immature collagen of the IF group. Rat n° 07-region proximal to AMI.

In a study with rats pretreated with betaine, following the AMI provoked by isoprenaline, the authors reported that this compound presented a cardioprotective effect, keeping plasma LDH levels close to those of the C group [32]. The identification of specific LDH activity in cardiac tissue reflects an anaerobic adenosine triphosphate (ATP) generating potential in the tissue. Pretreatment of rats with crocin, a carotenoid found in saffron, followed by myocardial infarction induced by isoproterenol revealed that this compound had a cardioprotective effect and was able to maintain cardiac levels of LDH close to those of the C group. In the present study, posttreatment with IF was not effective in elevating LDH levels in relationship to the C group; however, in the SE group, a cardioprotective effect was observed, maintaining elevated LDH levels in cardiac tissue. In our study, there was a greater expression of LDH in the SE group, suggesting that ischemic tissue can obtain a better potential for ATP generation *via* anaerobic metabolism, important for cardiac muscle, since it increases tissue capacity to overcome the O_2 deficit caused by the cardiac lesion.

With respect to MDH expression, utilizing rats pretreated with betaine for 30 days and infarction induced with isoprenaline, Ganesan

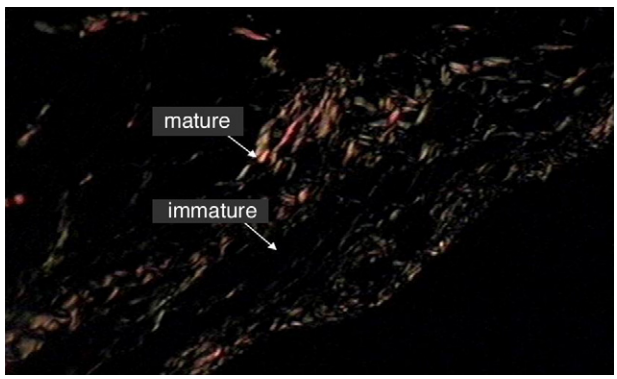

Fig. 6. Expression of mature and immature collagen in the SE group. Rat n° 13-region proximal to AMI.

Table 6
Analysis of the two collagen types and total collagen for the three groups

Site	Variable	Group	*n*	Mean	Median	Minimum	Maximum	Standard deviation	*P* value
Site 0	Mature col area	C	14	5496	5221	3481	8706	1650	
		SE	9	5670	5742	1745	7462	1766	
		IF	9	5390	5164	4061	7044	969	.925 *
	Immature col area	C	14	2806	2618	1554	4551	762	
		SE	9	3077	2909	2192	4150	743	
		IF	9	3484	3096	2126	6326	1420	.288 *
	Total col area	C	14	8301.8	8193.2	5391.9	10715	1758.7	
		SE	9	8747.6	8159.4	3959.0	11613	2368.8	
		IF	9	8873.9	8849.1	6187.1	10775	1390.4	.740 *
	Mature col %	C	14	65.52	66.68	49.41	81.26	8.64	
		SE	9	63.61	64.66	44.08	72.37	8.11	
		IF	9	61.54	65.63	41.29	74.33	10.89	.600 *
	Immature col%	C	14	34.48	33.32	18.74	50.59	8.64	
		SE	9	36.39	35.34	27.63	55.92	8.11	
		IF	9	38.46	34.37	25.67	58.71	10.89	.600 *
Site 1	Mature col area	C	14	3497	3650	1156	5901	1548	
		SE	9	1741	1574	580	2941	815	
		IF	9	2866	2932	922	4747	1292	.014 *
	Immature col area	C	14	1941	1755	805	3756	856	
		SE	9	1415	1243	356	2366	725	
		IF	9	2229	2408	493	4486	1293	.209 *
	Total col area	C	14	5438.1	5424.6	2634.1	9407	1903.8	
		SE	9	3156.0	3046.0	936.3	5281	1378.5	
		IF	9	5094.6	5417.9	1503.4	9232	2483.0	.030 *
	Mature col %	C	14	63.03	68.13	25.85	80.49	14.61	
		SE	9	55.79	55.69	32.50	72.75	10.98	
		IF	9	57.86	56.28	49.00	67.51	7.44	.341 *
	Immature col %	C	14	36.97	31.87	19.51	74.15	14.61	
		SE	9	44.21	44.31	27.25	67.50	10.98	
		IF	9	42.14	43.72	32.49	51.00	7.44	.341 *
Site 2	Mature col area	C	14	1484	1140	459	3425	1044	
		SE	9	1246	676	470	5169	1510	
		IF	9	2309	2283	269	4802	1615	.418 **
	Immature col area	C	14	940	759	241	2776	704	
		SE	9	620	516	233	1620	411	
		IF	9	1835	1629	67	5034	1637	.153 **
	Total col area	C	14	2423.8	2205.6	699.9	4390	1471.3	
		SE	9	1865.1	1214.2	702.8	6789	1905.2	
		IF	9	4143.9	3912.5	335.7	9836	3218.2	.242 **
	Mature col %	C	14	59.99	62.49	35.24	80.32	13.78	
		SE	9	61.78	62.86	49.59	76.14	8.63	
		IF	9	59.78	57.48	48.81	79.98	11.03	.921 *
	Immature col %	C	14	40.01	37.51	19.68	64.76	13.78	
		SE	9	38.22	37.14	23.86	50.41	8.63	
		IF	9	40.22	42.52	20.02	51.19	11.03	.921 *

* One-factor variance analysis, $P<.05$.
** Nonparametric Kruskal–Wallis test, $P<.05$.

et al. concluded that this compound had a cardioprotective effect on mitochondrial function [32]. In this case, the mean mitochondrial activity of MDH in the group injected with isoprenaline and pretreated with betaine for 30 days was nearly two times greater than the mean activity of the nontreated group. The authors also reported that the mean concentration of ATP in the group pretreated with betaine was twice that of the nontreated group. Accordingly, in this study, the posttreatment with IF and SE was unable to raise the potential aerobic generation of ATP in cardiac muscle of infarcted rats due to a lower expression of MDH for the SE group, demonstrating that this tissue was adapting for anaerobiosis (LDH) rather than aerobiosis (MDH).

The protective effect of potential aerobic generation of ATP observed by Ganesan et al. in pretreatment with betaine was related to an increase in antioxidant potential in the myocardium, considering that levels of the enzymes catalase, superoxide dismutase and glutathione peroxidase in the group treated with betaine were about 25% greater in comparison to those in the C group (standard diet and injected with saline solution) and 120% greater in comparison to those in the group with infarction induced by isoprenaline (no betaine diet). In this case, the authors also observed that lipid peroxide levels were nearly 200% higher in the group of rats infarcted with isoprenaline without betaine pretreatment when compared to those in the group pretreated with betaine [32].

Table 7
P values for the expression of mature collagen found in the three groups

Groups compared	*P* value
SE×C	.004
SE×IF	.269
C×IF	.079

Table 8
P values for the expression of total collagen found in the three groups

Groups compared	*P* value
SE×C	.011
SE×IF	.685
C×IF	.045

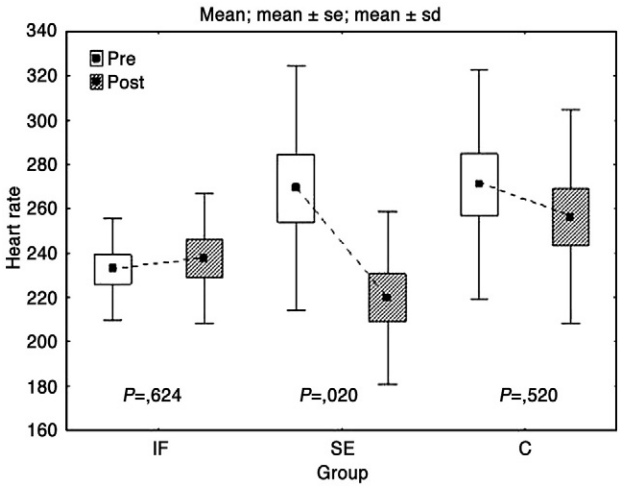

Fig. 7. Comparative analysis of cardiac frequency for the three groups.

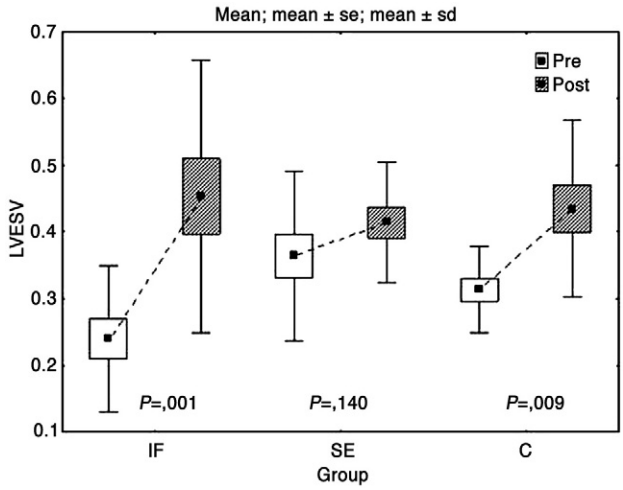

Fig. 9. Comparative analysis of final systolic volume for the three groups.

As the data suggest, cardiac tissue damage does not only occur during the ischemic phase of myocardial infarct but also during reperfusion. The lack of oxygen impedes the mitochondrial electron transporter system, saturating it with electrons. The reintroduction of oxygen in ischemic tissue reduces oxygen inappropriately and leads to the production of large quantities of reactive oxygen species, greater than the antioxidant defense potential in this tissues [34,35]. In this case, the protective effect of betaine pretreatment in myocardial infarction recovery could be related to an increase in aerobic potential of ATP generation associated with an increase in antioxidant defense [33].

In the present study, the effects of IF treatment increased the aerobic potential of ATP generation (MDH) when compared to the C group (P=.072). This increase most likely confers some protective advantage associated with the increase in antioxidant defense potential, as observed by Ganesan et al. [32].

On the other hand, treatment with SE reduced MDH levels compared to those in the C group (P<.001). The increase of anaerobic potential of ATP generation was accompanied by the maintenance of final diastolic and systolic volumes at levels near those observed prior to myocardial infarction. Accordingly, the reductive effect of SE on MDH levels and the increase of LDH apparently provided a protective advantage in postinfarcted rats since the differences between final diastolic and systolic volumes in the IF group and C groups for both pre- and postinfarction were enhanced.

Collagen was utilized as a parameter in the anatomic pathology analysis quantifying the presence of fibrosis in the myocardial infarcted area and the transition zone between infarction and whole myocardium. There was also a marked protective effect of SE in myocardial recovery when we analyzed the collagen content in the transition region between normal and infarcted tissue. A small quantity of mature collagen in this region, compared to that in the C group and IF group, indicates that the lesioned area was smaller, possibly explained by the extensive modular effect of soy extract on the metabolism of these animals. It is presumed that this effect can occur as a consequence of vasodilatation produced by a greater presence of available nitric oxide, causing better irrigation of affected myocardial cells around the infarcted area and thus inhibiting apoptosis.

The reductive effect of SE on aerobic potential ATP generation can act together with the reduction in free radical formation in the lesion site during reperfusion and consequently reduce the area of the lesion. LDH generation as a result of SE increases the anaerobic potential of ATP generation and collaborates in the maintenance of energy demand without increasing the risk of free radical formation.

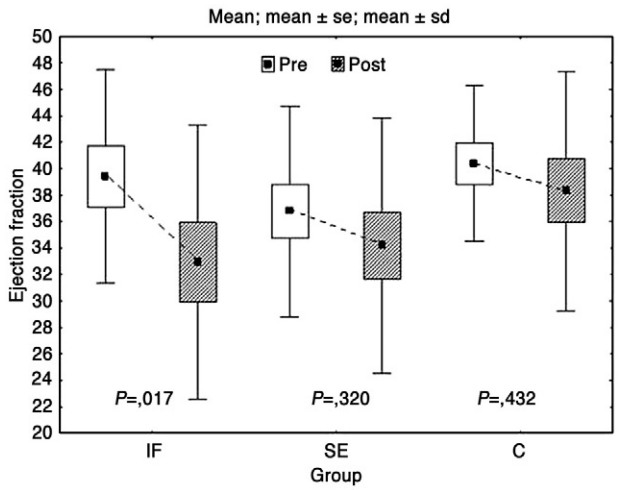

Fig. 8. Comparative analysis of EF for the three groups.

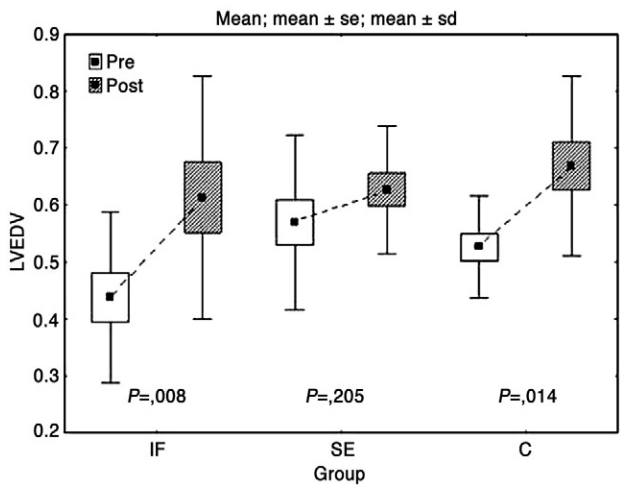

Fig. 10. Comparative analysis of final diastolic volume for the three groups.

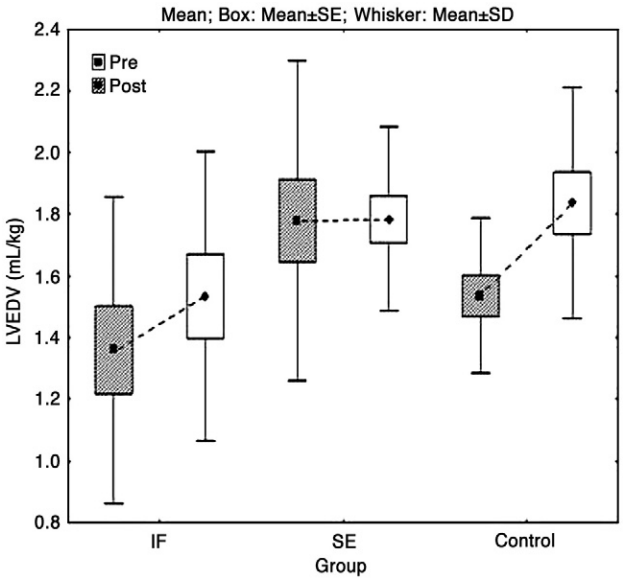

Fig. 11. Comparative analysis of LVEDV index for the three groups.

The mechanisms behind the beneficial effects of SE are not yet clear; however, it is believed that vegetable flavonoids positively affect the production of NO, a potent vasodilator, through *in vivo* and *in vitro* inducing of NOS [36]. The occasional cardioprotective benefits of SE could be related to the L-arginine content present in soy protein and the precursor of NO synthesis [37]. The presence of a smaller quantity of mature collagen in the SE group also indirectly suggests a greater quantity of NO in the tissue and, consequently, greater vasodilatation of reperfusion in cardiac muscle.

Furthermore, in the SE group, the fact that a greater LDH and lower MDH were identified indicates that the cardiac muscle created compensatory mechanisms that assist in tissue recovery. Consequently, the energy metabolism profile was induced. Ischemic tissue in the SE group showed a greater capacity to generate ATP through anaerobic metabolism (increased LDH activity).

Despite an increased stress on the animals, we opted for the gavage system of feeding [12,13] since studies have shown that IF, in order to be absorbed and metabolized more efficiently, requires intestinal metabolism. This occasionally does not occur when animals are given free access to food. In addition, correct doses of daily functional food intake are not accurately identified [38].

Many factors can influence the effects of flavonoids in individuals, such as cultural differences and lifestyle, as well as the pattern of food intake. It is not well established to what point the effects of these compounds are influenced by the environment in which the individual is placed. More studies should be performed in order to elucidate the real benefits and adverse effects of soybean, its derivatives and particularly concentrated forms of IF. These studies will allow for a more thorough understanding of adequate dietetic recommendations for each of these bioactive components and will create a worldwide consensus as well as specific goals for each country.

Table 9
LVEDV index

	Group	Valid *N*	Mean	S.D.
LVEDV/weight pre	IF	12	1.36	0.50
	SE	15	1.78	0.52
	C	14	1.54	0.25
LVEDV/weight post	IF	12	1.53	0.47
	SE	15	1.78	0.30
	C	14	1.84	0.38

Post- and pretreatment for the three groups.

Table 10
P values of LVEDV index analysis in the three groups

	IF×SE×C	IF×SE	IF×C	SE×C
Pre (*P*)	.045	.013	.191	.200
Post (*P*)	.007	<.1001	.082	.030

Acknowledgments

We would like to thank the Graduate Program in Health Sciences (PUCPR, Brazil) for the research support and methodological expertise.

We also thank the University of Taubate in Sao Paulo, Brazil, for the high-quality scientific partnership allowing for important methodological enrichment.

References

[1] Prunier F, Gaertner R, Louedec L, Michel JB, Mercadier JJ, Escoubet B. Doppler echocardiographic estimation of left ventricular end-diastolic pressure after MI in rats. Am J Physiol Heart Circ Physiol 2002;283(1):H346–52.
[2] Murray CJL, Lopez AD. The global burden of disease: a comprehensive assessment of mortality and disability from disease, injuries and risk factors in 1990 and projected to 2020. USA: Harvard School of Health; 1996.
[3] Lapointe A, Couillard C, Lemieux S. Effects of dietary factors on oxidation of low-density lipoprotein particles. J Nutr Biochem 2006;12:381–7.
[4] Nagata C, Takatsuka N, Kurisu Y, Shimizu H. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J Nutr 1998;128(2):209–13.
[5] Anthony M. Soy and cardiovascular disease: cholesterol lowering and beyond. J Nutr 2000;130:662S–3S.
[6] Shukla A, Brandsch C, Bettzieche A, Hirche F, Stangl GI, Eder K. Isoflavone-poor soy protein alters the lipid metabolism of rats by SREBP-mediated down-regulation of hepatic genes. J Nutr Biochem 2007;18(5):313–21 [Epub 2006 Sep 7].
[7] Lin JF, Lin SM, Chih CL, Nien MW, Su HH, Hu BR, et al. Resveratrol reduces infarct size and improves ventricular function after myocardial ischemia in rats. Life Sci 2008;83(9-10):313–7 [Epub 2008 Jun 27].
[8] Ma SF, Guan SD, Zhu Y. Effect of soybean isoflavones on heart function of rats with Adriamycin-induced heart failure. Zhong Xi Yi Jie He Xue Bao 2004;2(4):278–80.
[9] Hagen MK, Lehenbauer-Lüdke A, Paludo A, Schenkel P, Gonçalves L, Fernandes T, et al. Diet with isolated soy protein reduces oxidative stress and preserves ventricular function in rats with myocardial infarction. Nutr Metab Cardiovasc Dis 2009;19(2):91–7.
[10] www.cobea.org.br . Acesso em 17/07/2009.
[11] Budin P, Crouzat E. La pratique des accouchements. Paris: Octave Doin; 1891.
[12] Budin P, Demelin L. Manuel pratique d'accouchements et d'atla itement. Paris: Octave Doin; 1904.
[13] Sliva S. Imunomodulação da nutrição enteral experimental associada à sepse. Curitiba: Dissertação de mestrado-PPGCS/PUCPR; 2006.
[14] Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989;2(5):358–67.
[15] CFMV-Conselho Federal de Medicina Veterinária. Resolução 714, 20 de junho de 2002.
[16] Childress JJ, Somero GN. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar Biol 1979;52:273–83.
[17] Thuesen EV, McCullough KD, Childress JJ. Metabolic enzyme activities in swimming muscle of medusae: is the scaling of glycolytic activity related to oxygen availability? J Mar Biol Assoc UK 2005;85:603–11.
[18] Iyamu EW, Asakura T, Woods GM. A colorimetric microplate assay method for high-throughput analysis of arginase activity in vitro. Anal Biochem 2008;383:332–4.
[19] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[20] Junqueira LCU, Bignoas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979;11:447–55.
[21] Pagano M, Gauvreau K. Princípios de bioestatística. São Paulo: Pioneira Thonson Learning; 2004.
[22] FDA-Food and Drug Administration. US. Food labeling: health claims: soy protein and coronary heart disease. htpp://www.fda.gov, 1999.

[23] ACC/AHA (American College of Cardiology/American Heart Association-Task Force Report). Update: guidelines for the management of patients with acute myocardial infarction. Guideline. J Am Coll Cardiol 1999;34:890–911.
[24] Rimbach G, Boesch-Saadatmandi C, Frank J, Fuchs D, Wenzel U, Daniel H, et al. Dietary isoflavones in the prevention of cardiovascular disease — a molecular perspective. Food Chem Toxicol 2008;46:1308–19.
[25] Martin D, Song J, Mark C, Eyster K. Understanding the cardiovascular actions of soy isoflavones: potential novel targets for antihypertensive drug development. Cardiovasc Hemat Disord-Drug Targets 2008;8:297–312.
[26] Alekel DL, Van Loan MD, Koehler KJ, Hanson LN, Stewart JW, Hanson KB, et al. The soy isoflavones for reducing bone loss (SIRBL) study: a 3-y randomized controlled trial in postmenopausal women. Am J Clin Nutr 2010;91:218–30.
[27] Taku K, Melby MK, Takebayashi J, Mizuno S, Ishimi Y, Omori T, et al. Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. Asia Pac J Clin Nutr 2010;19:33–42.
[28] Butler LM, Wu AH, Wang R, Koh WP, Yuan JM, Yu MC. A vegetable-fruit-soy dietary pattern protects against breast cancer among postmenopausal Singapore Chinese women. Am J Clin Nutr 2010;91:1013–9.
[29] Crouse JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amount of isoflavones on plasma concentration of lipids and lipoproteins. Arch Intern Med 1998;159:2070–6.
[30] Greaves KA, Parks JS, Williams JK, Wagner JD. Intact dietary soy protein, but not adding an isoflavone-rich soy extract to casein, improves plasma lipids in ovariectomized cynomolgus monkeys. J Nutr 1999;129:1585–92.
[31] Clarkson TB, Anthony MS, Morgan TM. Inhibition of postmenopausal atherosclerosis progression: a comparison of the effects of conjugated equine estrogens and soy phytoestrogens. J Clin Endocr Metab 2001;186:41–7.
[32] Ganesan B, Rajesh R, Anandan R, Dhandapani N. Biochemical studies on the protective effect of betaine on mitochondrial function in experimentally induced myocardial infarction in rats. J Health Sci 2007;53:671–81.
[33] Goyal SN, Arora S, Sharma AK, Joshi S, Ray R, Bhatia J, et al. Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultrastructural alterations in isoproterenol-induced cardiotoxicity in rats. Phytomedicine 2010;17:227–32.
[34] Lorenzen JM, Ückert S, Scheller F, Haller H, Kuczyk MA. Effects of arginase inhibitors on the contractile and relaxant responses of isolated human penile erectile tissue. World J Urol 2009;27(6):805–10.
[35] Cadenas S, Aragonés J, Landázuri MO. Mitochondrial reprogramming through cardiac oxygen sensors in ischaemic heart disease. Cardiovasc Res 2010;88:219–28.
[36] Correa F, Martínez-Abundis E, Hernández-Reséndiz S, García N, Buelna-Chontal M, Arreguín F, et al. Pharmacological strategies to contend against myocardial reperfusion damage: diverse chemicals for multiple targets. Curr Med Chem 2010;17:2261–73.
[37] Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, Knabe DA, et al. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino Acids 2010:1–11.
[38] Schmitt CA, Dirsch VM. Modulation of endothelial nitric oxide by plant-derived products. Nitric Oxide-Biol Ch 2009;21:77–91.