

Changes in Arterial Blood Pressure of a Soluble Cocoa Fiber Product in Spontaneously Hypertensive Rats

D. SÁNCHEZ,^{†,‡} M. QUIÑONES,^{†,‡} L. MOULAY,[§] B. MUGUERZA,[§]
 M. MIGUEL,^{*,†,||} AND A. ALEIXANDRE[†]

[†]Departamento de Farmacología, Facultad de Medicina, Universidad Complutense, Avda. Complutense s/n, 28040 Madrid, Spain, [§]Natraceutical Group, Autovia A-3, Salida 343, Camino de Torrent s/n, Quart de Poblet, 46930 Valencia, Spain, and ^{||}Instituto de Fermentaciones Industriales (CSIC), C/ Juan de la Cierva, 3, 28006, Madrid, Spain. [‡]D.S. and M.Q. contributed equally to this study.

The effect produced by long-term intake of a soluble cocoa fiber product (SCFP) on the development of hypertension of spontaneously hypertensive rats (SHR) was evaluated. Twenty male 3-week-old SHR were divided into two groups of 10 animals that drank either tap water (control) or a solution of SCFP (0.75 g/day SCFP) until the 20th week of life. Five 20-week-old rats of each group were sacrificed. Tap water as drinking fluid was given to all the animals from the 20th to 24th week of life. The 24-week-old rats were also sacrificed. Body weight, liquid and dry food intake, and arterial blood pressure (tail cuff) were recorded weekly. Malondialdehyde (MDA), glucose and angiotensin converting enzyme (ACE) activity in the plasma from the sacrificed rats were also obtained, and we evaluated the relaxation caused by acetylcholine in the aorta from these animals. SCFP attenuated the development of hypertension in SHR; however, the withdrawal of SCFP caused an increase in blood pressure in the rats. Body weight gain was slower in the group treated with SCFP. SCFP increased liquid intake but decreased dry food intake in the rats. SCFP decreased plasma MDA concentrations and slightly decreased plasma ACE activity, but no differences were observed in plasma glucose and in the aorta responses to acetylcholine in both groups of 20-week-old SHR. We have demonstrated the antihypertensive and antioxidant properties of SCFP. The control of body weight and the control of increased angiotensin II may be involved in the antihypertensive effect of this product.

KEYWORDS: Antihypertensive effect; soluble fiber; cocoa; polyphenols; spontaneously hypertensive rats

INTRODUCTION

Cardiovascular diseases have been known to be the first cause of morbidity and mortality in Western countries, with hypertension affecting about 20% of the world's adult population. Lifestyle modifications and diet therapy are two of the most important tools in the prevention and treatment of hypertension (1). A larger number of studies in humans and animals have evidenced the efficacy of dietary fiber in regulating body weight, food intake, glucose homeostasis and insulin sensitivity, and the serum lipid profile. In addition, it has been proposed that fiber could control different cardiovascular risk factors such as hypertension and systemic inflammatory markers (2–4). The epidemiologic evidence linking fiber intake to blood pressure levels was first demonstrated in studies in vegetarian people (5–8). Moreover, some studies also indicated that increased dietary fiber consumption was reported to be a way to reduce blood pressure in patients with hypertension (9–13).

In recent decades, different experimental studies have also investigated the effects of fiber-enriched diets on arterial blood pressure. These studies indicated that a diet high in fiber could decrease arterial blood pressure in hypertensive, obese and diabetic rats (14–16). Nevertheless, the mechanism implicated in the antihypertensive properties of dietary fibers has not been clearly established.

Endothelial dysfunction and lipid peroxidation are important factors in the pathogenesis of atherosclerosis, hypertension, and heart failure (17). Insulin resistance and its concomitant compensatory hyperinsulinemia have been also suggested as major underlying pathogenetic mechanism for the development of hypertension (18). Essential hypertension can nowadays be considered an insulin resistant state, but how exactly insulin resistance influences blood pressure is controversial (19, 20). Early insulin signaling steps are impaired in essential hypertension, and a large body of data suggests that there is a crosstalk at multiple levels between the signaling transduction pathways that mediate insulin and angiotensin II actions (21–23). It has been proposed that the blockade of the renin–angiotensin system may lead to improve the insulin sensitivity from the insulin resistant status (24, 25), and it has also been suggested that a high level of

*Author for correspondence. Mailing address: Instituto de Fermentaciones Industriales (CSIC), C/ Juan de la Cierva, 3, 28006 Madrid, Spain. Tel: + 34 91 5622900. Fax + 34 91 5644853. E-mail: marta.miguel@ifi.csic.es.

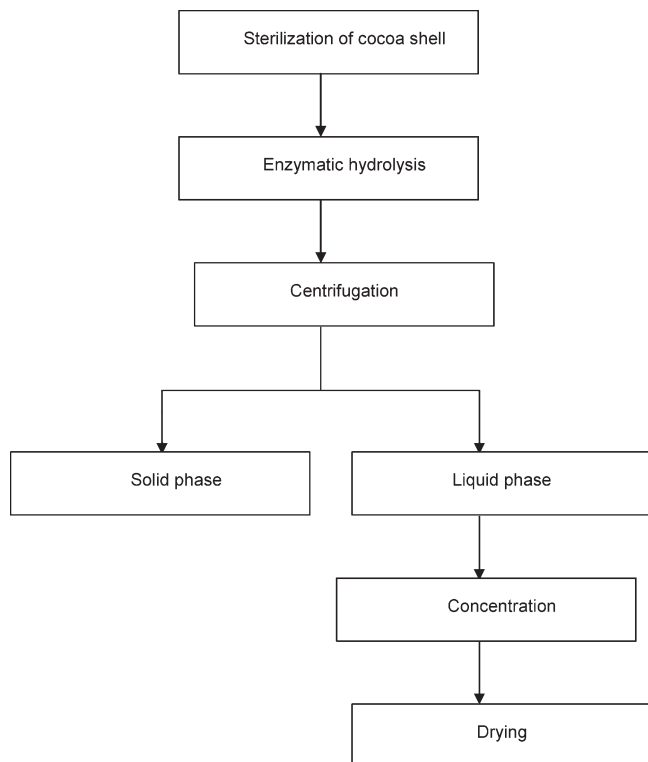


Figure 1. Steps of the process of obtaining SCFP.

insulin in resistance states induces the activation of the tissue renin–angiotensin system in blood vessels and heart and leads to the overproduction of angiotensin II in these tissues (25).

Cocoa husks constitute a byproduct of the cacao industry, with an estimated content of up to 50% total dietary fiber, mostly insoluble fiber (26). A soluble cocoa fiber product (SCFP) is a product rich in soluble fiber obtained from cocoa husks via a patented enzymatic process (27). This new source of soluble fiber was shown to have potential application as a functional food ingredient (28, 29).

The aim of this study was to investigate the possible antihypertensive effect of SCFP, and to elucidate the possible involved mechanisms. We evaluated the blood pressure lowering effect and the possible improvement of lipid peroxidation and endothelial function produced by long-term intake of SCFP in spontaneously hypertensive rats (SHR), a model known for essential hypertension in humans (30). The effect of SCFP on angiotensin converting enzyme activity (ACE), the enzyme that facilitates the conversion of angiotensin I into the vasoconstrictor peptide angiotensin II, and plasma glucose were also determined.

MATERIALS AND METHODS

Soluble Cocoa Fiber Product. This product was supplied by Nutraceutical Group (Valencia, Spain). The SCFP was obtained via an enzymatic patented process (27). Cocoa husks (*Theobroma cacao L.*) as a raw material were used for the extraction of this soluble fiber rich product using a multiactive β -glucanase enzyme. For this enzymatic extraction, time, temperature and pH were the controlled parameters as well as the sterilization of the raw material in this process as was previously described (28). The steps of the process are described in **Figure 1**.

SCFP was characterized physicochemically in duplicate samples. Fat and protein content were determined by Soxhlet extraction and Kjeldhal respectively (31). Theobromine and caffeine were determined by high performance liquid chromatography (HPLC) (31). Total, soluble and insoluble dietary fiber contents were determined by enzymatic–gravimetric method (31). Total polyphenol content was determined in SCFP as previously described (32). Duplicate samples of 1 g were extracted with

100 mL of acetone:water (70:30; v:v) under reflux at 60 °C for two hours, and the acetone was removed under vacuum at 45 °C. The polyphenol content of the product was determined by means of the Folin–Ciocalteu method (33). Polyphenols were expressed as catechin using a calibration curve.

To determine the concentration of flavan-3-ol compounds in SCFP, a modified protocol of extraction was applied as described elsewhere (34). The product was milled using a MS 50 laboratory mill. Approximately 0.5 g was dissolved in 5 mL of distilled water at 100 °C and agitated in a vortex for one minute. A sample of 20 mL of the extraction solution (methanol:HCl; 99.77:0.22 v:v) was added, and the extract was agitated in a vortex for two minutes. The homogenate was centrifuged at 1600g, 4 °C for 15 min in a Digicen20-R centrifuge (Oltoalresa, Spain). This procedure was repeated twice, and the supernatants were pooled and concentrated under reduced pressure (35 °C) to remove the methanol. The remaining aqueous extract was made up to 50 mL with distilled water. One aliquot of 5 mL was diluted 4 times, and finally the sample extract was passed through a 0.45 μ m PTFE filter before 20 μ L was injected. Chromatographic separation was performed on an Agilent 1100 HPLC system equipped with a diode array detector (DAD), quaternary pump, column heater and manual injector. Separation was carried out on a reverse-phase Zorbax Eclipse XDB-C₁₈ (150 \times 2.1 mm, 5 μ m) column at 35 °C. The mobile phase consisted of water–formic acid (99.9:0.1; v:v) (solvent A) and acetonitrile (solvent B) applied at a flow rate of 0.6 mL/min. The gradient was as follows: 0–20 min, 6–10% B linear; 20–25 min, 10–13% B linear; 25–30 min, 13–15% B linear; 30–40 min, 15–10% B linear; 40–45 min, 10–6% B linear followed by 10 min of re-equilibration of the column before a new injection. Flavan-3-ols, (+)-catechin, (–)-epicatechin, procyanidin B2 and B1 were quantified at 280 nm using the corresponding commercial standards. The results are expressed on wet basis as mean values \pm standard deviation of the mean (SD).

General Protocol in Rats. After being weaned at 3 weeks, male SHR (Charles River Laboratories España S.A.) were housed in groups of five rats at a temperature of 23 °C with 12 h light/dark cycles. They were in turn randomly divided with *ad libitum* intake into two groups of 10 animals, and during the experimental period (until the rats were 24 weeks old), the SHR of both groups were fed on a solid standard diet (A04 Panlab, Barcelona, Spain).

Until the rats were 20 weeks old, the drinking fluids in these groups were tap water (control) or a solution of SCFP prepared to give 0.75 g/day of this fiber to the animals. After overnight fasting, five 20-week-old rats of each group were sacrificed by decapitation. Blood samples from the sacrificed animals were collected into tubes containing lithium heparin as anticoagulant. These samples were centrifuged at 2500g for 20 min at 4 °C to obtain the plasma, which was divided into aliquots and kept frozen at –80 °C until analysis of malondialdehyde (MDA), plasma glucose and ACE activity. In addition, the thoraxes of the rats were opened and the aorta was quickly extracted to evaluate the endothelial function in accordance with the studies published by Furchgott in 1980 and in 1999 (35, 36). We describe below the procedures to evaluate plasma malondialdehyde, plasma glucose and plasma ACE activity. The *in vitro* experiments in aorta rings are also described below. The drinking fluid was always tap water in both groups of animals from the 20th to 24th week of life.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured weekly in the rats from the sixth to 24th week of life, by the tail cuff method (37). The original technique for measuring arterial blood pressure using the tail cuff method provides only SBP values, but the equipment used in this study, LE 5001 (Letica, Hospitalet, Barcelona, Spain), has a high sensitivity pulse transducer coupled with an accurate microprocessor program, and allows us to distinguish between SBP and DBP. Before the measurements, the rats were kept at 38 °C for 10–15 min to make the pulsations of the tail artery detectable. Arterial blood pressure measurements were performed at the same time of day (between 9 a.m. and 13 p.m.) in order to avoid the influence of the circadian cycle, and the values of SBP and DBP were obtained by estimating the average reading of five measurements. Moreover, the person who measured the arterial blood pressure in the animals from both groups did not know the drinking fluid that had been administered to each of these groups.

The rat weight in both groups was recorded weekly up to the 24th week of life. Daily intake of drinking fluids and freely accessible feed was also

estimated weekly in the animals from the different groups throughout the experimental period.

At the end of the experimental period, the 24-week-old rats were sacrificed by decapitation after overnight fasting, and the same determinations and procedures described above were done in these animals.

All the above-mentioned experiments were performed as authorized for scientific research (European Directive 86/609/CEE and Royal Decree 223/1988 of the Spanish Ministry of Agriculture, Fisheries and Food).

Malondialdehyde Determination. Plasma malondialdehyde (MDA) levels were measured by a thiobarbituric acid assay (38). Plasma were mixed with 20% trichloroacetic acid in 0.6 M HCl (1:1, v/v), and the tubes were kept in ice for 20 min to precipitate plasma components and avoid possible interferences. Samples were centrifuged at 1500g for 15 min before adding thiobarbituric acid (120 mM in Tris 260 mM, pH 7) to the supernatant in a proportion of 1:5 (v/v); then, the mixture was boiled at 97 °C for 30 min. Spectrophotometric measurements at 535 nm were made at 20 °C. The plasma MDA values were expressed as nmol MDA.

Plasma Glucose Determination. Plasma glucose was assayed by using the glucoseoxidase enzymatic test with commercial kits (Roche Diagnostics S.L., Spain). The different concentrations were determined spectrophotometrically with using a Hitachi 911 autoanalyser (wavelength: 700 nm).

Determination of ACE Activity. ACE activity in plasma was measured by a fluorimetric method (39). Briefly, triplicate plasma (3 μ L) were incubated for 15 min at 37 °C with 40 μ L of assay buffer containing the ACE substrate 5 mM Hip-His-Leu (Sigma). The reaction was stopped by addition of 190 μ L of 0.35 N HCl. The generated product, His-Leu, was measured fluorimetrically following 10 min incubation with 100 μ L of 2% *o*-phthalaldehyde in methanol. Fluorescence measurements were carried out at 37 °C in a Fluostar Optima plate reader (BMG Labtech, GmbH, Offenburg, Germany) with 350 nm excitation and 520 nm emission filters. The fluorescent plate reader was controlled by the Fluostar Optima software. Black 96-well polystyrene microplates (Biogen Cientifica, Madrid, Spain) were used. A calibration curve with ACE from rabbit lung (Sigma, St. Louis, MO) was included in each plate.

Experiments in Aorta Rings. Excess fat and connective tissue were removed from the aorta, and the tissue was cut into rings (approximately 4 mm in length). The aortic rings were mounted between two steel hooks in isolated tissue chambers containing Krebs–Henseleit solution with the following composition (mmol/L): NaCl, 118.2; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; and glucose, 10.0. The medium was maintained at 37 °C, and continuously bubbled with a 95% O₂–5% CO₂ mixture, which gave a pH of 7.4. An optimal resting tension of 2 g was applied to all aortic segments. This tension was adjusted every 15 min during a 60–90 min equilibration period before adding drugs. Isometric tension was recorded by using an isometric force displacement transducer connected to an acquisition system (Protos 5, Panlab, Spain). After the equilibration period, the rings were first contracted by 80 mmol/L KCl to assess their functionality, and when the contraction had reached the steady state (about 15 min after the administration) the preparations were washed until the basal tension was recovered. Then the rings were exposed to 10⁻⁶ mol/L methoxamine, and dose–response curves to acetylcholine (10⁻⁹–10⁻⁵ mol/L) were performed in the methoxamine-precontracted rings. Relaxant responses to ACh were expressed as a percentage of the precontraction induced by methoxamine.

Statistical Analysis. The results are expressed as mean values \pm SEM for 4–6 rats, and were analyzed by one- or two-way analysis of variance (ANOVA), using the GraphPad Prism software. We considered, on the one hand, the treatment period (from weaning until the 20th week of life) and, on the other hand, the follow-up period (from the 21st to the 24th week of life). Differences between the groups were assessed by the Bonferroni test. Differences between the means were considered to be significant when $P < 0.05$.

RESULTS

The physicochemical characteristics of SCFP are shown in **Table 1**. The characterization of SCFP revealed an undetectable amount of insoluble fiber and a main soluble dietary fiber fraction (**Table 1**). The polyphenolic content and the procyanidine profile of the SCFP are presented in **Table 2**. The monomeric flavanols

Table 1. Physicochemical Composition of the SCFP (g/kg Dry Matter^a)

components	composition (g/kg dry matter)
soluble dietary fiber	412.0 \pm 13.19
moisture	23.0 \pm 1.2
fat	53.0 \pm 0.56
protein	179.0 \pm 1.2
ash	190.0 \pm 0.5
theobromine	26.80 \pm 0.02
caffeine	2.00 \pm 0.004
polyphenols	22.4 \pm 1.4

^aThe values are expressed as the mean \pm SD ($n = 2$).

Table 2. Total Polyphenols and Procyanidins Profile of the Soluble Cocoa Fiber Product (mg/g Dry Matter^a)

components	composition (g/kg dry matter)
total polyphenols content ^b	22.4 \pm 1.4
total procyanidins content ^c	0.415 \pm 0.030
procyanidin B1	0.120 \pm 0.006
catechin	0.114 \pm 0.006
procyanidin B2	0.051 \pm 0.023
epicatechin	0.130 \pm 0.010

^aThe values are expressed as the mean \pm SD ($n = 3$). ^bMeasured by Folin–Ciocalteu's method. ^cMeasured by HPLC-DAD.

epicatechin and catechin together with appreciable amounts of the dimeric procyanidins B1 were the main phenolics.

Body weight of the SHR increased progressively from weaning, this increase being more accentuated until the 11th week of life (**Figure 2**). The group treated with SCFP gained weight steadily, but at week 17 of life the animals of this group reached the same body weight as the control group. Liquid intake and dry food increased progressively in the SHR of both groups from the beginning of the experimental period. Liquid intake was significantly higher in the SHR that drank the SCFP solution than in the control group, and this difference in liquid intake between both groups was maintained through the follow-up period (**Figure 3A**). The consumption of dry food was significantly lower in the rats treated with SCFP than in the control group. However during the follow-up period no significant differences in dry food intake were observed between both groups of animals (**Figure 3B**).

The SHR that drank tap water showed a gradual increase in SBP and DBP from weaning that reached maximal values about 17 weeks of life. From this age, the SBP and DBP values of these rats remained constantly high, and no differences were found between weeks 17 and 25. From the seventh to the 20th week of life, the values of SBP were lower (about 10–15 mm Hg), in the SHR that drank the solution of SCFP than in the SHR that drank tap water. A significant decrease in DBP was observed in the SCFP group only from the start of the study until the animals were 14 weeks old. From the 20th week of life an increase in SBP and DBP was observed in the SHR that had been treated with SCFP. In fact, these rats achieved SBP and DBP values similar to those of the control rats during the follow-up period. **Figures 4 and 5** show the evolution of SBP and DBP respectively of the SHR taking the different drinking fluids.

There were statistical differences in plasma MDA concentrations between both groups of SHR. The values were lower in the SHR that drank the solution of SCFP than in the SHR that drank tap water. Moreover, at the end of the follow-up period (when the rats had 24 weeks of life), the levels of this metabolite were also lower in the rats that had drunk SCFP than in the rats of the control group (**Figure 6**). No differences were observed in plasma

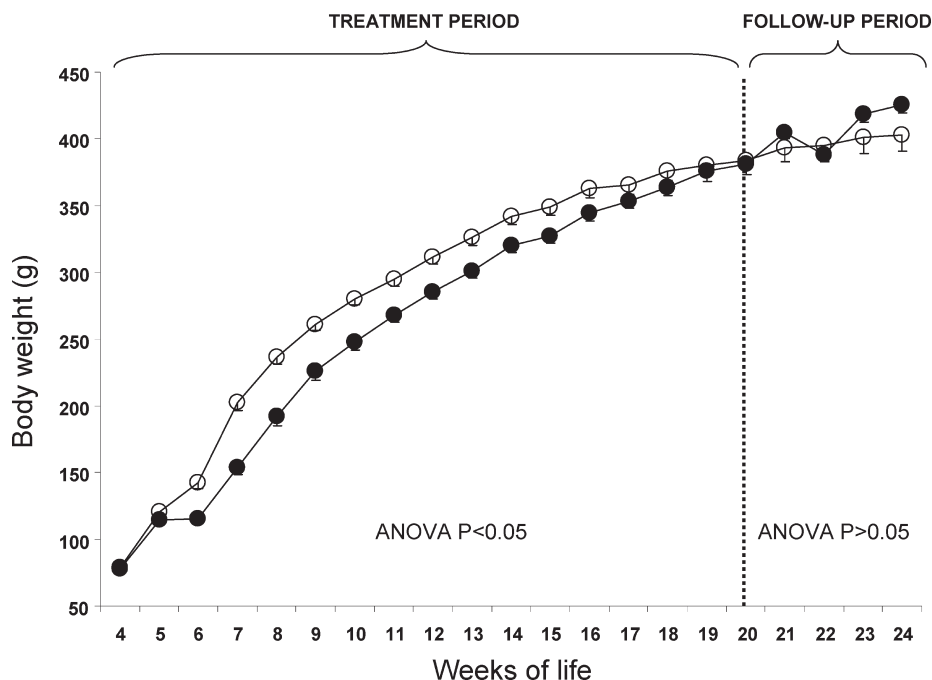


Figure 2. Body weight of spontaneously hypertensive rats. The animals drank water (○) or a solution of SCFP (0.75 g/day of fiber) (●) from weaning until the 20th week of life (treatment period indicated by a solid bar). All rats drank tap water from the 20th week of life until the 24th week of life (follow-up period indicated by an open bar). Data are mean values \pm SEM (10 animals in the treatment period and 5 animals in the follow-up period). The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by two-way ANOVA.

glucose levels in both groups of rats (104.2 ± 2.4 for the control group and 112.6 ± 1.3 for the SCFP treated group at the end of the treatment period; 108 ± 6.3 for the control group and 101.4 ± 2.3 for the group that had been treated with SCFP at the end of the follow-up period). The values of plasma ACE activity were slightly lower in the group of animals that received SCFP than in the control group. The differences in plasma ACE activity between both groups of animals were more accentuated at the end of the follow-up period, but statistical differences were not attained in the 24-week-old animals either (Figure 7).

No differences were observed in the responses to KCl and methoxamine of the aorta rings from the SCFP group and the corresponding responses of the aorta rings from the control group (data not shown). Figure 8 shows the endothelium-dependent vasodilator responses to acetylcholine in the arteries from both groups. The responses to acetylcholine were similar in both groups when the rats were 20 weeks old, but at the end of the follow-up period, the relaxation caused by this drug in the aorta rings from the rats that had been treated with SCFP was more accentuated than the correspondent relaxation in the aorta rings from the control rats.

DISCUSSION

The effects of dietary fiber on arterial blood pressure have been studied to a much lesser extent than other healthy effects of fiber. The physicochemical data of SCFP showed that the described process using cocoa husks resulted in a product rich in soluble dietary fiber with cocoa polyphenols. In the present study, SCFP treatment clearly decreased SBP and DBP in the SHR. These results are in agreement with other experimental studies, even when the antihypertensive properties of dietary fiber are still debatable. A decrease in systolic blood pressure could also be observed when spontaneously hypertensive rats (14) and obese diabetic Zucker rats (15) received a diet supplemented with soluble fiber. Another study describes reduced systolic blood

pressure levels in Goto Kakizaki type 2 diabetic rats following long-term consumption of cereal dietary fiber (16). In addition, it has been shown that a diet containing fiber-rich grain, fruit and vegetables significantly decreases the need for antihypertensive treatments, and improved blood pressure levels in hypertensive patients (9). Small reductions in blood pressure levels were also described in humans after intake of high-fiber diets containing particularly β -glucan and psyllium (10, 11).

Several mechanisms have been hypothesized to explain the potential effect of dietary fiber intake on blood pressure. Blood pressure is linked to the degree of insulin resistance, and the amount of dietary fiber is inversely related to insulin resistance (19). In this context, effectiveness of both soluble and insoluble fibers in reducing insulin resistance and insulin levels in both diabetic and healthy persons would contribute to treating or preventing hypertension (40–42). Exactly how insulin resistance influences blood pressure is controversial (19, 20), but the results obtained in our laboratory when Zucker fatty rats were fed with a 5% soluble cocoa fiber-enriched diet seems to support this correlation (29). SCFP decreased plasma glucose and insulin resistance in the Zucker fatty rats, and a slight decrease of SBP and DBP was also observed in these animals (29). The SHR has been reported to be also insulin-resistant compared to the parent strain (43), and the study of DeLano and Schmid-Schönbein (44) also points out a possible very important overlap of an insulin resistance mechanism with hypertension in these animals. Nevertheless, in the present study, a blood pressure lowering effect was observed when 0.75 g/day SCFP, a mean dose of 3.67 ± 0.54 g/kg/day of SCFP, was administered to SHR, but long-term administration of this fiber did not affect plasma glucose in this strain. Therefore, the effect of SCFP on blood pressure was mainly observed in the hypertensive rat strain that shows only a slight degree of insulin resistance, and the effect of SCFP on glucose metabolism was mainly observed in the insulin resistant Zucker fatty rats that had normal arterial blood pressure values.

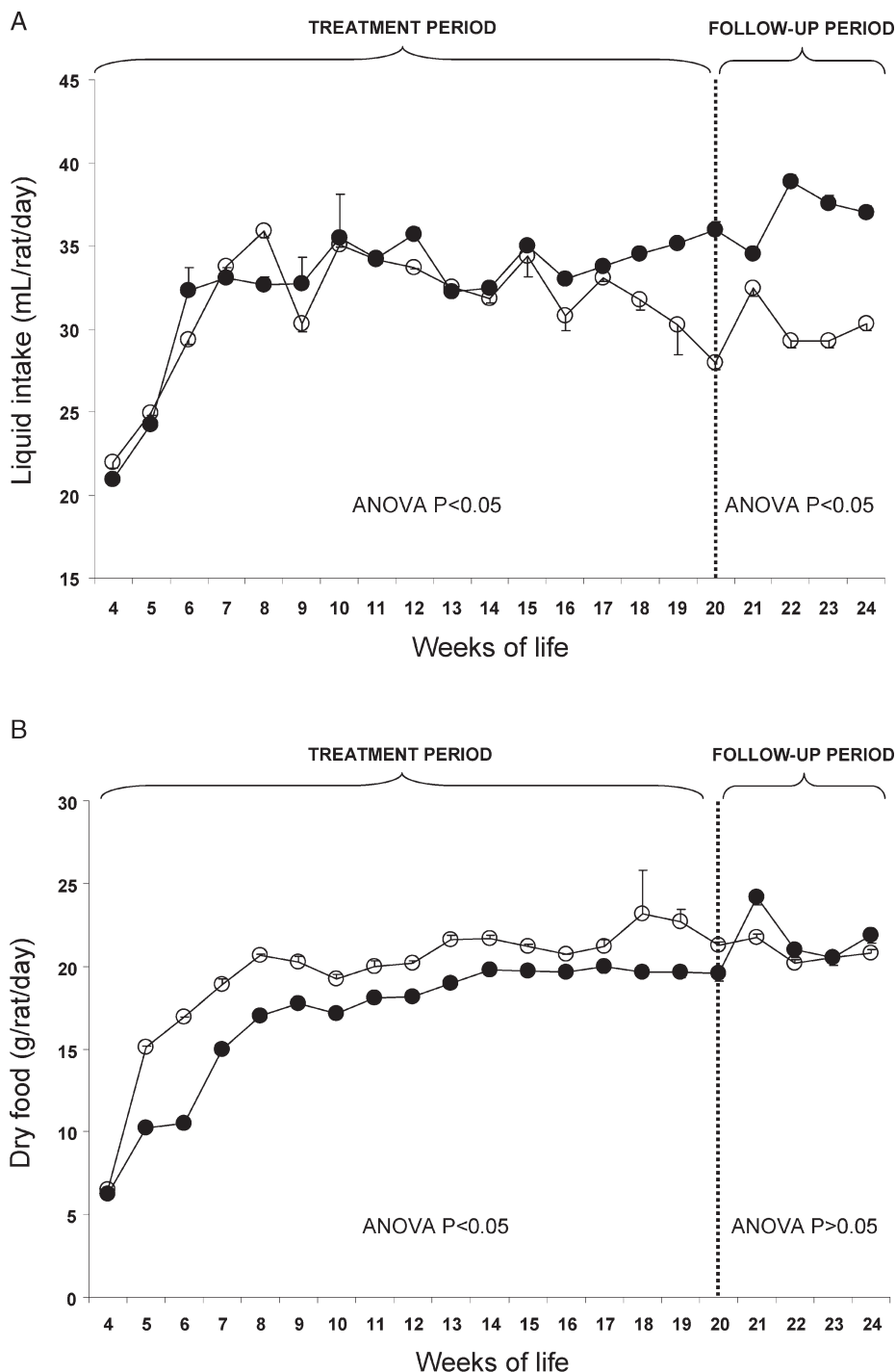


Figure 3. Liquid (A) and solid (B) intake of spontaneously hypertensive rats. The animals drank water (○) or a solution of SCFP (0.75 g/day of fiber) (●) from weaning until the 20th week of life (treatment period indicated by a solid bar). All rats drank tap water from the 20th week of life until the 24th week of life (follow-up period indicated by an open bar). Data are mean values \pm SEM (10 animals in the treatment period and 5 animals in the follow-up period). The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by two-way ANOVA.

Increased body weight is a strong risk factor for hypertension (45), and the intake of dietary fiber has been shown to efficiently reduce body weight (46,47) and weight gain (48,49). In fact, a significant reduction in body weight was also observed when 10% of soluble cocoa fiber-enriched diet was given to hypercholesterolemic rats (28). In another study carried out in our laboratory, Zucker fatty rats fed a 5% of soluble cocoa fiber-enriched diet showed less weight gain than the Zucker fatty rats fed the standard diet (29). Nevertheless, since in this study growth

patterns were similar in the SHR that consumed SCFP and in the SHR control rats, we might also assume that the decrease in body weight could only be a mechanism implicated in antihypertensive effect of fiber when fiber decreases arterial blood pressure in overweight conditions.

The possible contribution of cocoa polyphenols in the lowering blood pressure effect of SCFP cannot be ruled out. As a consequence of their high procyanidin contents, cocoa has high antioxidant potential (50–52). In addition, an increase in plasma

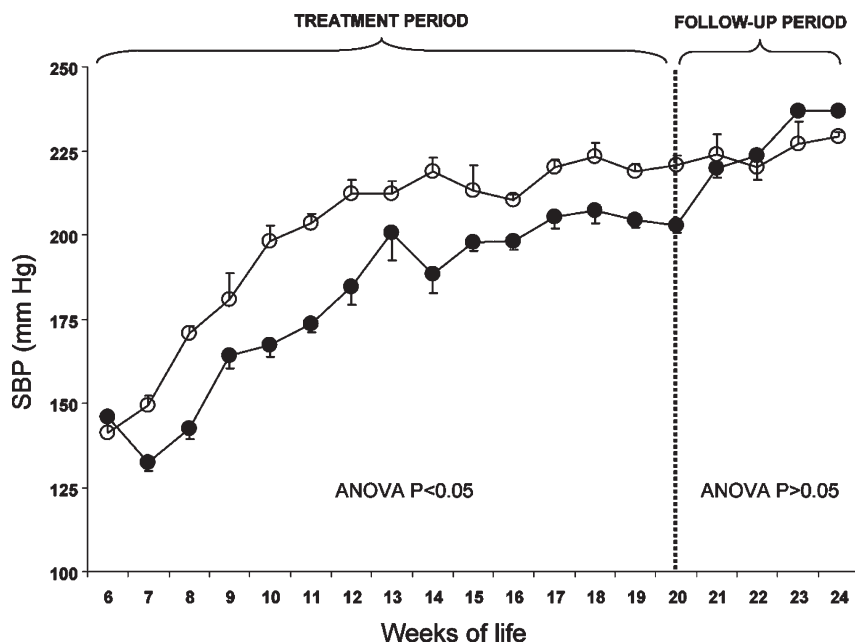


Figure 4. Systolic blood pressure (SBP) of spontaneously hypertensive rats. The animals drank water (○) or a solution of SCFP (0.75 g/day of fiber) (●) from weaning until the 20th week of life (treatment period indicated by a solid bar). All rats drank tap water from the 20th week of life until the 24th week of life (follow-up period indicated by an open bar). Data are mean values \pm SEM (10 animals in the treatment period and 5 animals in the follow-up period). The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by two-way ANOVA.

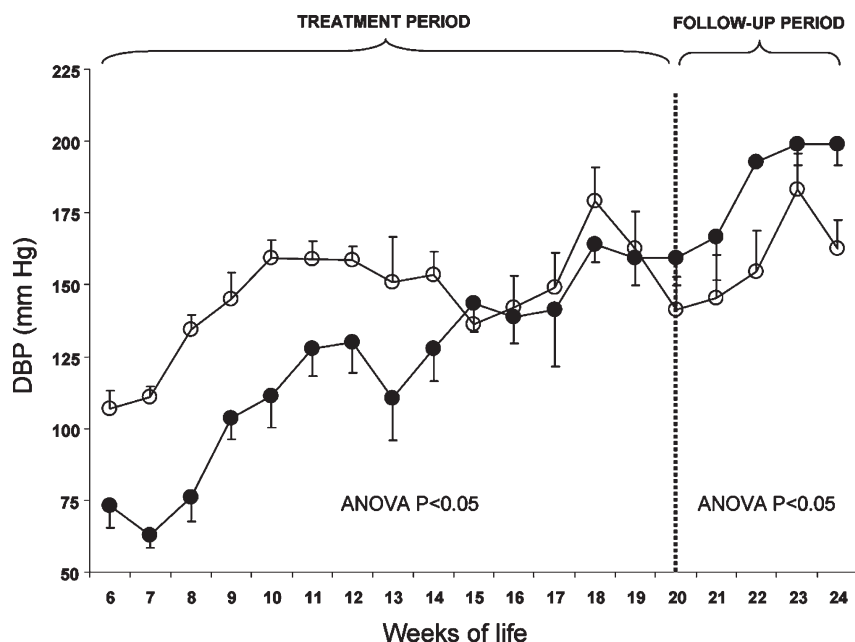


Figure 5. Diastolic blood pressure (DBP) of spontaneously hypertensive rats. The animals drank water (○) or a solution of SCFP (0.75 g/day of fiber) (●) from weaning until the 20th week of life (treatment period indicated by a solid bar). All rats drank tap water from the 20th week of life until the 24th week of life (follow-up period indicated by an open bar). Data are mean values \pm SEM (10 animals in the treatment period and 5 animals in the follow-up period). The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by two-way ANOVA.

total antioxidant capacity after the intake of cocoa polyphenols has been reported (53–56). These compounds have also shown antihypertensive properties in different studies (57–62). In spite of the relatively modest content of flavan-3-ols in SCFP (0.415 mg/g up to 2-mers), we have to bear in mind that amounts as small as 30 mg/day (up to 5-mers) of these compounds reduced blood pressure in humans (61). Therefore, 38 g/day of SCFP would be sufficient to provide the amount of flavan-3-ols (up to 2-

mers) reported to exhibit a reduction in blood pressure (61). In addition, the SCFP revealed a high antioxidant capacity (ORAC values of $569 \pm 76 \mu\text{mol TE/g}$ dry matter) (28), which could in part explain the important decrease in the blood pressure observed in the present study. It is nowadays assumed that plasma MDA reflects free radical production, increase in oxidative stress and lipid peroxidation. This in turn conducts to atherogenesis and alteration of arterial tone (63–66). In this study, we have shown

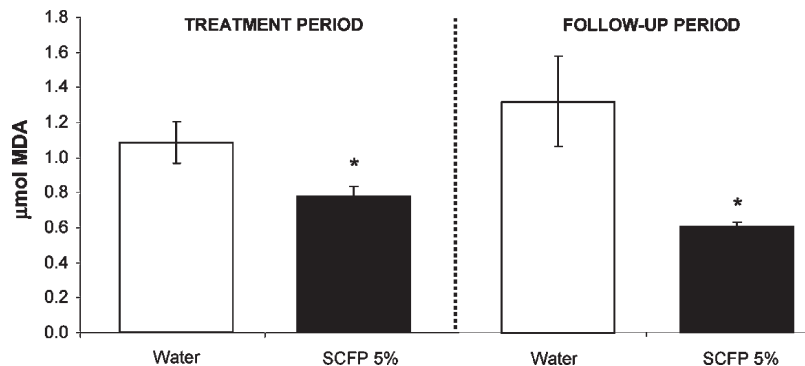


Figure 6. Histograms of plasma malondialdehyde (MDA) of spontaneously hypertensive rats. The animals drank water (□) or a solution of SCFP (0.75 g/day of fiber) (■) from weaning until the 20th week of life. All rats drank tap water from the 20th week of life until the 24th week of life. Data are mean values \pm SEM for 5 animals. The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by one-way ANOVA.

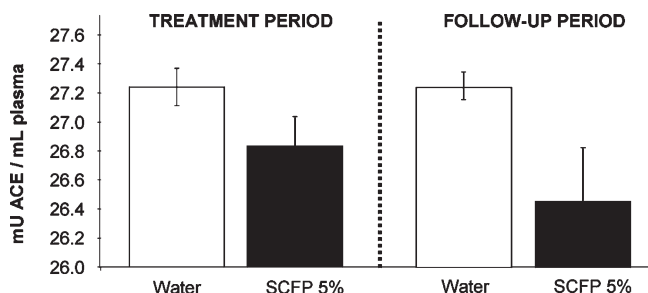


Figure 7. Histograms of the plasma angiotensin converting enzyme activity (ACE) of spontaneously hypertensive rats. The animals drank water (□) or a solution of SCFP (0.75 g/day of fiber) (■) from weaning until the 20th week of life. All rats drank tap water from the 20th week of life until the 24th week of life. Data are mean values \pm SEM for 5 animals. The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by one-way ANOVA.

that SCFP markedly decreases MDA in the plasma of SHR, and we can therefore assume that the antioxidant polyphenols present in this fiber could decrease the processes mentioned above that are finally responsible of the increased arterial blood pressure in these rats. This finding confirms recent results obtained from a study in hypercholesterolemic rats where an important reduction in MDA was obtained when the rats were fed 10% soluble cocoa fiber-enriched diet (28).

Endothelial dysfunction is also associated with the increased production of free radicals. The consequent decrease in nitric oxide (NO) and vasorelaxing endothelial factors conveys vasoconstriction and hypertension. Some researchers have shown that fiber supplements improve also endothelial dysfunction (67). Since SHR show endothelial dysfunction (68–70), we also hypothesized that the administration of SCFP to these animals could normalize the vasodilator processes that depend on endothelial NO production. Moreover, we have already linked the antihypertensive properties of SCFP with the polyphenol content of this fiber. In addition, different polyphenols have been demonstrated to increase NO levels and to improve endothelial dysfunction (71–73). In this study, the endothelial-dependent responses to acetylcholine in the SHR that had been treated with SCFP only improved paradoxically after the withdrawal of this treatment. It is true that the mechanisms implicated in the biological properties of soluble fibers could have a delay to be started, but we cannot forget that the withdrawal of SCFP was

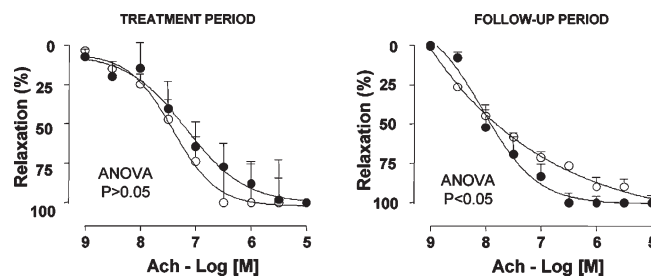


Figure 8. Acetylcholine relaxation in aortic rings from spontaneously hypertensive rats. The animals drank water (○) or a solution of SCFP (0.75 g/day of fiber) (●) from weaning until the 20th week of life. All rats drank tap water from the 20th week of life until the 24th week of life. Data are mean values \pm SEM (10 animals in the treatment period and 5 animals in the follow-up period). The figures indicate significant differences between the groups of rats in the established periods. Different letters represent statistical differences ($p < 0.05$). P estimated by two-way ANOVA.

accompanied with the return to increased SBP and DBP levels in SHR.

We can state that there is a clear link between the vasopressor peptide angiotensin II and insulin activity. This study also shows that SCFP slightly decreases ACE activity in SHR. This mechanism has not been actually involved in the antihypertensive effect of other fibers, but it has been recently shown that SCFP presents a high content of nitrogen compounds and a possible effect of cocoa peptides with ACE-inhibitory activity could not be ruled out (74). Moreover, the inhibition of ACE has been reported to be associated with the polyphenol content of cocoa (75, 76). Therefore, this effect of polyphenols could be involved in the decrease of the blood pressure. Our results suggest that at least in the case of SCFP the inhibition of this enzyme may contribute to the fiber antihypertensive effect.

In conclusion, the beneficial properties of SCFP in lowering blood pressure have been demonstrated. We have shown that the antioxidant properties of SCFP and the polyphenol content of this fiber could be in part responsible for its antihypertensive effect. The fiber may also benefit hypertension through the control of body weight, and it may also control increased angiotensin II in the hypertensive condition by inhibiting ACE. Nevertheless, it remains still difficult to explain the exact mechanisms involved in the antihypertensive effect of SCFP, and more studies are recommended to elucidate them. Therefore, further clinical trials should also be carried out before suggesting SCFP as a functional

ingredient to prevent and/or attenuate the development of hypertension.

ACKNOWLEDGMENT

We thank Manuel Bas Caro, Technician in Pharmacology, for his excellent care of the rats and control of the diets in the different groups of animals and Olga Vilanova Technician from Natra-ceutical Group for the characterization of the SCFP. M. Miguel is the recipient of a Ramón y Cajal contract from MICINN.

LITERATURE CITED

- (1) Hermansen, K. Diet, blood pressure and hypertension. *Br. J. Nutr.* **2000**, *83*, 113S–119S.
- (2) Davy, B. M.; Melby, C. L. The effect of fiber-rich carbohydrates on features of Syndrome X. *J. Am. Diet. Assoc.* **2003**, *103*, 86–96.
- (3) Delzenne, N. M.; Cani, P. H. Some studies have reported a satiety effect of soluble fibers. *Curr. Opin. Clin. Nutr. Metab.* **2005**, *8*, 636–640.
- (4) Ven, B. J.; Mann, J. I. Cereal grains, legumes and diabetes. *Eur. J. Clin. Nutr.* **2004**, *58*, 1443–1461.
- (5) Sacks, F. M.; Rosner, B.; Kass, E. H. Blood pressure in vegetarians. *Am. J. Epidemiol.* **1974**, *100*, 390–398.
- (6) Armstrong, B.; Van Merwyk, A. J.; Coates, H. Blood pressure in Seventh-Day Adventist vegetarians. *Am. J. Epidemiol.* **1977**, *105*, 444–449.
- (7) Rouse, I. L.; Beilin, L. J. Vegetarian diet and blood pressure. *J. Hypertens.* **1984**, *2*, 231–240.
- (8) Sacks, F. M.; Kass, E. H. Low blood pressure in vegetarians: effects of specific foods and nutrients. *Am. J. Clin. Nutr.* **1988**, *48*, 795–800.
- (9) Burke, V.; Hodgson, J. M.; Beilin, L. J.; Giangiulio, N.; Rogers, P.; Puddey, I. B. Dietary protein and soluble fiber reduce ambulatory blood pressure in treated hypertensives. *Hypertension* **2001**, *38*, 821–826.
- (10) He, J.; Streiffer, R. H.; Muntner, P.; Krousel-Wood, M. A.; Whelton, P. K. Effect of dietary fiber intake on blood pressure: a randomized, double-blind, placebo-controlled trial. *J. Hypertens.* **2004**, *22*, 73–80.
- (11) Whelton, S. P.; Hyre, A. D.; Pedersen, B.; Yi, Y.; Whelton, P. K.; He, J. Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *J. Hypertens.* **2005**, *23*, 475–481.
- (12) Berkow, S. E.; Barnard, N. D. Blood pressure regulation and vegetarian diets. *Nutr. Rev.* **2005**, *63*, 1–8.
- (13) Streppe, M. T.; Arends, L. R.; Van 't Veer, P.; Grobbee, D. E.; Geleijnse, J. M. Dietary fiber and blood pressure: a meta-analysis of randomized placebo-controlled trials. *Arch. Intern. Med.* **2005**, *165*, 150–156.
- (14) Obata, K.; Ikeda, K.; Yamasaki, M.; Yamori, Y. Dietary fiber, psyllium, attenuates salt-accelerated hypertension in stroke-prone spontaneously hypertensive rats. *J. Hypertens.* **1998**, *16*, 1959–1964.
- (15) Galisteo, M.; Sanchez, M.; Vera, R.; Gonzalez, M.; Anguera, A.; Duarte, J.; Zarzuelo, A. A diet supplemented with husks of *Plantago ovata* reduces the development of endothelial dysfunction, hypertension, and obesity by affecting adiponectin and TNF- α in obese Zucker rats. *J. Nutr.* **2005**, *135*, 2399–2404.
- (16) Li, J.; Wang, J.; Kaneko, T.; Qin, L. Q.; Sato, A. Effects of fiber intake on the blood pressure, lipids, and heart rate in Goto Kakizaki rats. *Nutrition* **2004**, *20*, 1003–1007.
- (17) Ross, R. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **1999**, *340*, 115–126.
- (18) Ferrannini, E.; Buzzigoli, G.; Bonadonna, R.; Giorico, M. A.; Oleggini, M.; Graziadei, L.; Pedrinelli, R.; Brandi, L.; Bevilacqua, S. Insulin resistance in essential hypertension. *N. Engl. J. Med.* **1987**, *317*, 350–357.
- (19) Ferri, C.; Bellini, C.; Desideri, G.; Valenti, M.; De Mattia, G.; Santucci, A.; Hollenberg, N. K.; Williams, G. H. Relationship between insulin resistance and nonmodulating hypertension: linkage of metabolic abnormalities and cardiovascular risk. *Diabetes* **1999**, *48*, 1623–1630.
- (20) Osei, K. Insulin resistance and systemic hypertension. *Am. J. Cardiol.* **1999**, *84*, J33–J36.
- (21) Folli, F.; Kahn, C. R.; Hansen, H.; Bouchie, J. L.; Feener, E. P. Angiotensin II inhibits signalling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J. Clin. Invest.* **1997**, *100*, 2158–2169.
- (22) Andreozzi, F.; Laratta, E.; Sciacqua, A.; Perticone, F.; Sesti, G. Angiotensin II impairs the inulin signalin pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser 616 in human umbilical vein endothelial cells. *Circ. Res.* **2004**, *94*, 1211–1220.
- (23) Muscogiuri, G.; Chavez, A. O.; Gastaldelli, A.; Perego, L.; Tripathy, D.; Saad, M. J.; Velloso, L.; Folli, F. The crosstalk between insulin and rennin-angiotensin-Aldosterone signalling systems and its effect on glucose metabolism and diabetes prevention. *Curr. Vasc. Pharmacol.* **2008**, *6*, 301–312.
- (24) Dahlöf, B.; Devereux, R. B.; Kjeldsen, S. E.; Julius, S.; Beevers, G.; de Faire, U.; Fyhrquist, F.; Ibsen, H.; Kristiansson, K.; et al. LIFE Study Group. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* **2002**, *359*, 995–1003.
- (25) Kamide, K.; Tuck, M. K. Renin-angiotensin system and vasculature-focusing on insulin action. *Int. Congr. Ser.* **2004**, *1262*, 462–465.
- (26) Martín-Cabrejas, M. A.; Valiente, C.; Esteban, R. M.; Mollá, E.; Waldron, K. Cocoa hull: a potential source of dietary fibre. *J. Sci. Food Agric.* **1994**, *66*, 307–311.
- (27) Moulay L.; Sniderman Z.; Ibarra A.; Marti Bartual V. Method of preparing a highly-soluble cocoa extract. 2005. WO/2006/117416.
- (28) Ramos, S.; Moulay, L.; Granado-Serrano, A. B.; Vilanova, O.; Muguerza, B.; Goya, L.; Bravo, L. Hypolipidemic effect in cholesterol-fed rats of a soluble fiber-rich product obtained from cocoa husks. *J. Agric. Food Chem.* **2008**, *56*, 6985–6993.
- (29) Quiñones, M.; Sánchez, D.; Muguerza, B.; Moulay, L.; Hernández, R.; Miguel, M.; Aleixandre, A. Effect of a soluble cocoa fibre-enriched diet in Zucker fatty rats. *Methods Find. Exp. Clin. Pharmacol.* **2007**, *29*, 105.
- (30) FitzGerald, R. J.; Murray, B. A.; Walsh, D. J. Hypotensive peptides from milk proteins. *J. Nutr.* **2004**, *134*, 980S–988S.
- (31) AOAC. *Official methods of analysis*, 17th ed.; AOAC International: Arlington, VA, 2000.
- (32) Tomas-Barberan, F. A.; Cienfuegos-Jovellanos, E.; Marin, A.; et al. A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. *J. Agric. Food Chem.* **2007**, *55*, 3926–35.
- (33) Singleton, V. L.; Rossi, J. A. Colorimetric of total Phenolics with phosphomolybdc-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–58.
- (34) Cienfuegos-Jovellanos, E.; Quiñones, M.; Muguerza, B.; Moulay, L.; Miguel, M.; Aleixandre, A. Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed preserving the original flavonoids of the cocoa beans. *J. Agric. Food Chem.* **2009**, *57*, 6156–6162.
- (35) Furchgott, R. F.; Zawadzki, J. V. The obligatory role of the endothelium in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **1980**, *288*, 373–376.
- (36) Furchgott, R. F. Endothelium-derived relaxing factor: discovery, early studies, and identification as nitric oxide. *Biosci. Rep.* **1999**, *19*, 233–251.
- (37) Buñag, R. D. Validation in awake rats of a tail-cuff method for measuring systolic pressure. *J. Appl. Physiol.* **1973**, *34*, 279–282.
- (38) Manso, M. A.; Miguel, M.; Even, J.; Hernandez, R.; Aleixandre, M. A.; López-Fandiño, R. Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. *Food Chem.* **2008**, *109*, 361–367.
- (39) Miguel, M.; Manso, M. A.; Aleixandre, M. A.; López-Fandiño, R. Angiotensin converting enzyme activity in plasma and tissues of spontaneously hypertensive rats after short- and long-term intake of an egg white hydrolysate. *Mol. Nutr. Food Res.* **2007**, *51*, 555–563.
- (40) McKeown, N. M.; Meigs, J. B.; Liu, S.; Wilson, P. W.; Jacques, P. F. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *Am. J. Clin. Nutr.* **2002**, *76*, 390–398.

- (41) King, D. E.; Mainous, A. G.; Egan, B. M.; Woolson, R. F.; Geesey, M. E. Fiber and C-reactive protein in diabetes, hypertension, and obesity. *Diabetes Care* **2005**, *28*, 1487–1489.
- (42) Qi, L.; Rimm, E.; Liu, S.; Rifai, N.; Hu, F. B. Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. *Diabetes Care* **2005**, *28*, 1022–1028.
- (43) Schiffrin, E. L. Efectos cardiovasculares de los receptores activadores del proliferador de peroxisomas (PPAR) en hipertensión. *Bol. Con. Argent. Hipertens.* **2003**, *2*, 14–18.
- (44) DeLano, F. A.; Schmid-Schönbein, G. W. Proteinase activity and receptor cleavage: mechanism for insulin resistance in the spontaneously hypertensive rat. *Hypertension* **2008**, *52*, 415–423.
- (45) Neter, J. E.; Stam, B. E.; Kok, F. J.; Grobbee, D. E.; Geleijnse, J. M. Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension* **2003**, *42*, 878–884.
- (46) Solum, T. T.; Rytting, K. R.; Solum, E.; Larsen, S. The influence of a high-fibre diet on body weight, serum lipids and blood pressure in slightly overweight persons. A randomized, double-blind, placebo-controlled investigation with diet and fibre tablets (DumoVital). *Int. J. Obes.* **1987**, 1167–1171.
- (47) Rigaud, D.; Rytting, K. R.; Angel, L. A.; Apfelbaum, M. Overweight treated with energy restriction and a dietary fibre supplement: a 6-month randomized, double-blind, placebo-controlled trial. *Int. J. Obes.* **1990**, *14*, 763–769.
- (48) Liu, S.; Willett, W. C.; Manson, J. E.; Hu, F. B.; Rosner, B.; Colditz, G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am. J. Clin. Nutr.* **2003**, *78*, 920–927.
- (49) Koh-Banerjee, P.; Franz, M.; Sampson, L.; Liu, S.; Jacobs, D. R.; Spiegelman, D.; Willett, W.; Rimm, E. Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. *Am. J. Clin. Nutr.* **2004**, *80*, 1237–1245.
- (50) Hannum, S. M.; Schmitz, H. H.; Keen, C. L. Chocolate: A Heart-healthy Food? Show Me the Science!. *Nutr. Today* **2002**, *37*, 103–109.
- (51) Kris-Etherton, P. M.; Keen, C. L. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr. Opin. Lipidol.* **2002**, *13*, 41–49.
- (52) Steinberg, F. M.; Bearden, M. M.; Keen, C. L. Cocoa and chocolate flavonoids: Implications for cardiovascular health. *J. Am. Diet. Assoc.* **2003**, *103*, 215–223.
- (53) Serafini, M.; Bugianesi, R.; Maiani, G.; Valtuena, S.; De Santis, S.; Crozier, A. Plasma antioxidants from chocolate. *Nature* **1993**, *424*, 1013.
- (54) Rein, D.; Lotito, S.; Holt, R. R.; Keen, C. L.; Schmitz, H. H.; Fraga, C. G. Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status. *J. Nutr.* **2000**, *130*, 2109S–2114S.
- (55) Wan, Y.; Vinson, J. A.; Etherton, T. D.; Proch, J.; Lazarus, S. A.; Kris-Etherton, P. M. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr.* **2001**, *74*, 596–602.
- (56) Hermann, F.; Spieker, L.; Ruschitzka, F.; Sudano, I.; Hermann, M.; Binggeli, C.; Luscher, T. F.; Riesen, W.; Noll, G.; Corti, R. Dark chocolate improves endothelial and platelet function. *Heart* **2006**, *92*, 119–120.
- (57) Fraga, C. G. Cocoa, diabetes, and hypertension: should we eat more chocolate? *Am. J. Clin. Nutr.* **2005**, *81*, 541–542.
- (58) Grassi, D.; Necozione, S.; Lippi, C.; Croce, G.; Valeri, L.; Pasqualetti, P.; Desideri, G.; Blumberg, J. B.; Ferri, C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* **2005**, *46*, 398–405.
- (59) Grassi, D.; Lippi, C.; Necozione, S.; Desideri, G.; Ferri, C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am. J. Clin. Nutr.* **2005**, *81*, 611–614.
- (60) Sarr, M.; Chataigneau, M.; Martins, S.; Schott, C.; El Bedoui, J.; Oak, M. H.; Muller, B.; Chataigneau, T.; Schini-Kerth, V. Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. *Cardiovasc. Res.* **2006**, *71*, 794–802.
- (61) Taubert, D.; Roesen, R.; Lehmann, C.; Jung, N.; Schömig, E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *JAMA* **2007**, *298*, 49–60.
- (62) Taubert, D.; Roesen, R.; Schömig, E. Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Arch. Intern. Med.* **2007**, *167*, 626–634.
- (63) Holvoet, P. Endothelial dysfunction, oxidation of low-density lipoprotein, and cardiovascular disease. *Ther. Apher. Dial.* **1999**, *3*, 287–293.
- (64) Mizuno, T.; Matsui, H.; Imamura, A.; Numaguchi, Y.; Sakai, K.; Murohara, T.; Okumura, K. Insulin resistance increases circulating malondialdehyde-modified LDL and impairs endothelial function in healthy young men. *Int. J. Cardiol.* **2004**, *97*, 455–461.
- (65) Ahmed, F. N.; Naqvi, F. N.; Shafiq, F. Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. *Ann. N.Y. Acad. Sci.* **2006**, *1084*, 481–489.
- (66) Yang, R. L.; Shi, Y. H.; Hao, G.; Li, W.; Le, G. W. Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. *J. Clin. Biochem. Nutr.* **2008**, *43*, 154–158.
- (67) Ascherio, A.; Rimm, E. B.; Giovannucci, E. L.; Colditz, G. A.; Rosner, B.; Willett, W. C.; Sacks, F.; Stampfer, M. J. A prospective study of nutritional factors and hypertension among US men. *Circulation* **1992**, *86*, 1475–1484.
- (68) Watt, P. A.; Thurston, H. Endothelium-dependent relaxation in resistance vessels from the spontaneously hypertensive rats. *J. Hypertens.* **1989**, *7*, 661–666.
- (69) Küng, C. F.; Lüscher, T. F. Different mechanisms of endothelial dysfunction with aging and hypertension in rat aorta. *Hypertension* **1995**, *25*, 194–200.
- (70) Lang, M. G.; Noll, G.; Lüscher, T. F. Effect of aging and hypertension on contractility of resistance arteries: modulation by endothelial factors. *Am. J. Physiol.* **1995**, *269*, H837–H844.
- (71) Stoclet, J. C.; Chataigneau, T.; Ndiaye, M.; Oak, M. H.; El Bedoui, J.; Chataigneau, M.; Schini-Kerth, V. B. Vascular protection by dietary polyphenols. *Eur. J. Pharmacol.* **2004**, *500*, 299–313.
- (72) Perez-Vizcaino, F.; Duarte, J.; Andriantsitohaina, R. Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. *Free Radical Res.* **2006**, *40*, 1054–1065.
- (73) Grassi, D.; Aggio, A.; Onori, L.; Croce, G.; Tiberti, S.; Ferri, C.; Ferri, L.; Desideri, G. Tea, flavonoids, and nitric oxide-mediated vascular reactivity. *J. Nutr.* **2008**, *138*, 1554S–1560S.
- (74) Ottaviani, J.; Actis-Goretti, L.; Villordo, J.; Fraga, C. Procyanidin structure defines the extent and specificity of angiotensin I converting enzyme inhibition. *J. Agric. Food Chem.* **2006**, *88*, 359–365.
- (75) Actis-Goretti, L.; Ottaviani, J.; Fraga, C. Inhibition of angiotensin converting enzyme extent and specificity of inhibition. *J. Agric. Food Chem.* **2006**, *54*, 229–234.
- (76) Muguerza, B.; Monzó, H.; Alepuz, N.; Bataller, E.; Genoves, S.; Enrique, M.; Martorell, P.; Ramón, D. Obtención de extractos de cacao ricos en péptidos bioactivos con actividad inhibidora de las enzimas ECA y PEP. PCT/ES2008/000540. Filling date: 01/08/08.

Received for review May 19, 2009. Revised manuscript received November 25, 2009. Accepted December 14, 2009. This study was supported by Natraceutical Group (36/2007 UCM Project).