Research Article

Angiotensin-converting enzyme activity in plasma and tissues of spontaneously hypertensive rats after the short- and long-term intake of hydrolysed egg white

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This paper evaluates the effects of the short- (1 g/kg) and long-term (0.5 and 1 g/kg/day) oral intake of egg white hydrolysed with pepsin (hEW) and the long-term oral intake (1 g/kg/day) of egg white (EW) on local angiotensin-converting enzyme (ACE) activities in plasma and other tissues of spontaneously hypertensive rats (SHR), as compared to the effect of the ACE inhibitor prototype captopril. The rats treated with hEW were classed in a different group than the control rats and the rats treated with EW by cluster analysis, taking into account their tissue ACE activities and their systolic blood pressure (SBP). Principal component analysis (PCA) showed that SBP in SHR was negatively related with ACE activity in plasma and positively related with ACE activity in aorta and kidney. ACE activity in plasma significantly increased after the long-term treatment with hEW (0.5 g/kg/day). ACE activity in aorta and kidney was significantly inhibited 4 h after the short-term administration of hEW. The long-term treatment with hEW caused local effects on ACE activity in aorta, kidney and lungs that followed a pattern similar, but less pronounced, than that caused by captopril.

Keywords: Angiotensin-converting enzyme / Bioactive peptides / Egg white / Hypertension / Spontaneously hypertensive rats

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1 Introduction

High blood pressure is the main risk factor for stroke, coronary heart disease and renal vascular disease. The rennin–angiotensin system plays an important role in blood pressure regulation and fluid homeostasis [1]. Within the enzyme cascade of this system, angiotensin-converting enzyme (ACE), a peptidyl-dipeptide hydrolase (EC 3.4.15.1), generates angiotensin II from its inactive precursor, angiotensin I, by cleaving a dipeptide from its C-terminal end [2]. Angiotensin II induces vasoconstriction, aldosterone release, production of reactive oxygen species and other physiological actions that act to raise the blood pressure. ACE also catalyses the degradation of bradykinin, a blood pressure lowering peptide in the kallikrein–kinin system [3]. ACE is widely distributed, not only in the cardiovascular system, but also in various noncardiovascular tissues. It has been localized in endothelial cells throughout the body, in the epithelial cells of various organs and in male germinal cells [4].

The control of hypertension through the diet is a focal point of public health and mass media attention. Apart from their basic nutritional function, food proteins and among them egg proteins, are capable of modulating specific biological functions, such as blood pressure [5]. Many food protein-derived sequences exerting ACE inhibitory activity *in vitro* have been described, some of which have also been tested *in vivo*, proving their antihypertensive effect [6]. We recently demonstrated that the short-term administration of egg white hydrolysed with pepsin (hEW) decreased the

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Abbreviations: ACE, angiotensin-converting enzyme; **ANOVA,** analysis of variance; **EW,** egg white; **hEW,** egg white hydrolysed with pepsin; **PCA,** principal component analysis; **SBP,** systolic blood pressure, **SHR,** spontaneously hypertensive rats

arterial blood pressure in spontaneously hypertensive rats (SHR) and that the long-term intake of hEW attenuated the development of hypertension in these animals, while the effect of nonhydrolysed egg white (EW) was significantly less pronounced [7, 8]. Since hEW inhibited ACE in an *in vitro* assay [9], its antihypertensive effects could be attributed to the *in vivo* blockage of this enzyme. Furthermore, some of the peptide sequences contained in hEW, that acted as ACE inhibitors *in vitro*, were identified [9] and their antihypertensive activity in SHR was subsequently demonstrated [7].

Only a better understanding of the mechanism and the factors determining the antihypertensive activity of food peptides will allow a rational development of peptide-based functional foods for blood pressure control. However, very few studies have been conducted to confirm the *in vivo* inhibition of ACE of food protein-derived sequences with *in vitro* ACE inhibitory activity [10] and, in some cases, evidence has been provided for the existence of an additional mode of action [11]. The aim of this study was to investigate the effect of short- and long-term treatments with hEW on ACE activities in different tissues of SHR, a rat model of hypertension showing an alteration of local ACE activity [12]. The effects of hEW were compared with those of captopril, an antihypertensive drug that is considered a classical ACE-inhibitor.

2 Materials and methods

2.1 Products

Crude EW was obtained from fresh hen eggs. The hEW was prepared by pepsin hydrolysis as previously described [9]. Captopril (Sigma, St. Louis, MO, USA) was also used in this study. All products were dissolved in distilled water for administration to the rats.

2.2 Treatment protocols and blood pressure measurements

The short-term experiments were performed with a set of 17–20-week-old male SHR (Charles River Laboratories España S.A., Barcelona, Spain), weighing 314 ± 3 g. The animals were housed at a temperature of 23°C with 12/12 h light/dark cycles, and consumed tap water and a standard diet for rats (A04 Panlab, Barcelona, Spain) *ad libitum* during the experimental period. The SHR were randomly divided into three groups of five animals each and were given different products by gastric intubation (1 mL *per* rat) between 9 and 10 a.m.: hEW (1 g/kg), tap water (negative control) and captopril (100 mg/kg, positive control). These short-term experiments were similar to those described by Miguel *et al.* [7], but with higher doses of the antihypertensive products. Before administration and also 4–6 h postadministration, the systolic blood pressure (SBP) of the conscious rats was measured indirectly by the tail-cuff method [13]. The rats of the three different groups were weighed and killed by decapitation just after the postadministration measurement of the arterial blood pressure.

For the long-term experiments, we have used the plasma and tissue samples from the animals used in the long-term study published by Miguel *et al.* [8]. In summary, male SHR, after being weaned at 3 wk, were randomly divided into five groups of 16 animals each. Until the rats were 20 wk old (treatment period), the drinking fluids in these groups were as follows: hEW (0.5 g/kg/day), hEW (1 g/kg/day), EW (1 g/kg/day), tap water and captopril (100 mg/kg/day). From the 20th to the 25th week of life, the drinking fluid was always tap water for all the rats (follow-up period). SBP and diastolic blood pressure (DBP) were measured by the tail-cuff method, as described above. The values of SBP and DBP, body weight of SHR and solid and liquid diet are described in detail in Miguel *et al.* [8]. Eight rats of each group were weighed and killed by decapitation, just after the measurement of arterial blood pressure, at the end of the treatment period (20-wk-old rats) and at the end of the follow-up period (25-wk old rats).

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).

2.3 Plasma and tissue preparations

Blood samples were collected into tubes containing lithium heparin as an anticoagulant and centrifuged at 3500 g for 20 min to obtain the plasma. Brain, heart, aorta, lungs, liver, kidney and spleen were immediately excised and weighed, and the percent wet organ weight to body weight ratio was calculated for each organ. Aorta, lung and kidney were homogenized at 4°C in a Potter with PBS (0.01 M PBS, 0.15 M NaCl, pH 7.4), the homogenates were centrifuged at 5000 g for 15 min at 4°C and the supernatant was recovered. The plasma and the supernatants of the centrifuged samples were kept frozen at −80°C until used for ACE activity assays. The protein content was determined by the bicinchoninic acid assay (Pierce, Rockford, Ill, USA), using BSA as the standard.

2.4 Determination of ACE activity

ACE activity was measured by a fluorimetric method adapted from Friedland and Silverstein [14]. Briefly, triplicate plasma (3 µL) and supernatant (30–80 µg protein) aliquots from homogenized tissues were incubated for 15–90 min at 37°C with 40 µL of assay buffer containing the ACE substrate 5 mM Hip-His-Leu (Sigma). The reaction was stopped by the addition of 190 µL of 0.35 N HCl. The generated product, His-Leu, was measured fluorimetrically following 10 min incubation with 100 µL of 2% *o*-phthal-

dialdehyde in methanol. Fluorescence measurements were carried out at 37°C in a Fluostar Optima plate reader (BMG Labtech, 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 Científica, Madrid, Spain) were used. A calibration curve with ACE from rabbit lung (Sigma) was included in each plate.

2.5 Statistical analysis

Cluster analysis was applied to the data of ACE activity and SBP of all the rats with the aim to discover natural groupings of the samples of the study. The squared Euclidean distance was taken as a measure of proximity between two samples and Ward's method was used as a linkage rule. Principal component analysis (PCA) (from standardized variables) was used to examine the relationship among the analysed variables. When required for the statistical analysis, data of SBP of the animals of the long-term study were taken from Miguel *et al.* [8].

Significant differences were evaluated by one-way analysis of variance (ANOVA) followed by posthoc analysis by Bonferroni's test and Student *t*-test. $P < 0.05$ was used as the threshold for statistically significant differences. The results were expressed as mean values ± SEM for 4 – 8 rats. The STATISTICA program for Windows (release 7.1; Statsoft, Tulsa, OK) was used for data processing.

3 Results

3.1 ACE activity in plasma and tissues

In order to obtain a preliminary view of the distribution of the rats by virtue of their profile concerning ACE activity, cluster analysis was applied to the data of the ACE activity in plasma and other tissues and the SBP of all the rats after the different treatments. Figure 1 shows the dendrogram obtained. Two large sample groups can be distinguished. The first one consists of the SHR treated with captopril, with rats from the short- and the long-term treatments being well separated into two different subgroups. The second group includes the rest of the rats of the study and it is divided into two subgroups, one including all the SHR treated with hEW, at a small distance to the captopril group, and the second including the SHR treated with EW, the control rats SHR, and the SHR rats after the follow-up period. We could also observe that, in general terms, the rats after the follow-up period presented a shorter distance to the SHR treated with hEW than the control SHR.

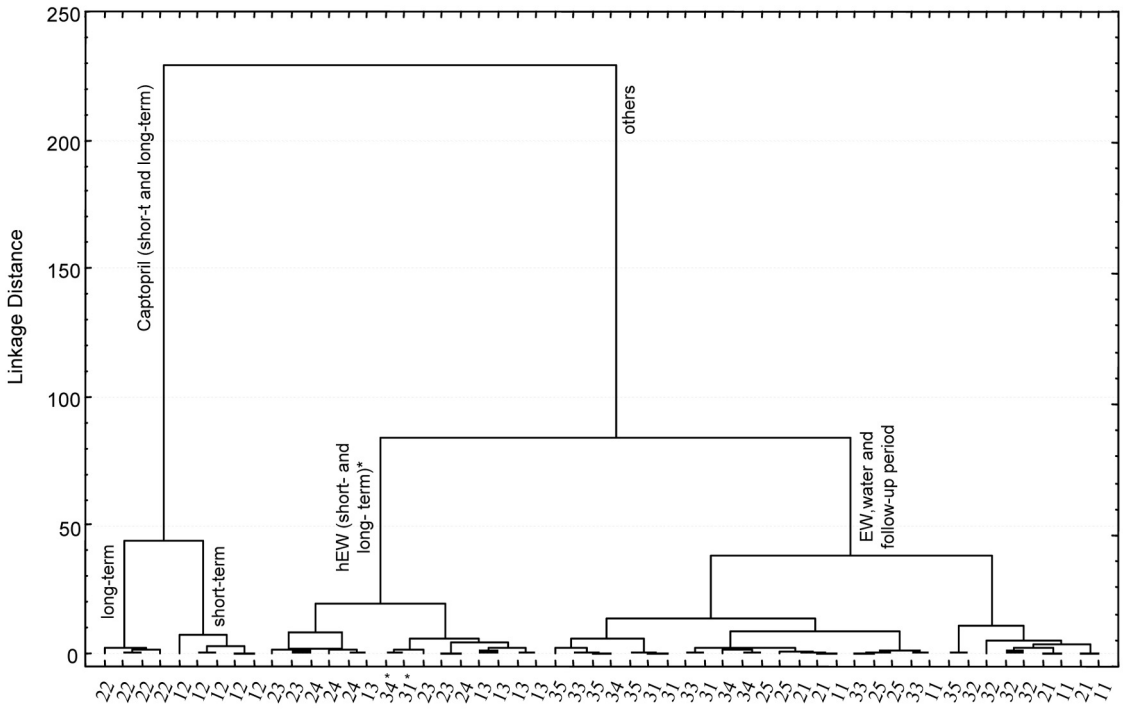

Figure 1. Dendrogram obtained by the cluster analysis with the data from the determinations of ACE activity in plasma and other tissues and the SBP of all the rats after the different treatments. The squared Euclidean distance was taken as a measure of proximity between two samples and Ward's method was used as a linkage rule. Samples are represented by AB: A = 1, Short-term treatment; 2, Long-term treatment; 3, Follow-up period. B = 1, Control (water); 2, captopril; 3, hEW (1 g/kg or 1 g/kg/day); 4, hEW (0.5 g/kg/day); 5, EW (1 g/kg/day). * Samples that are an exception to this group.

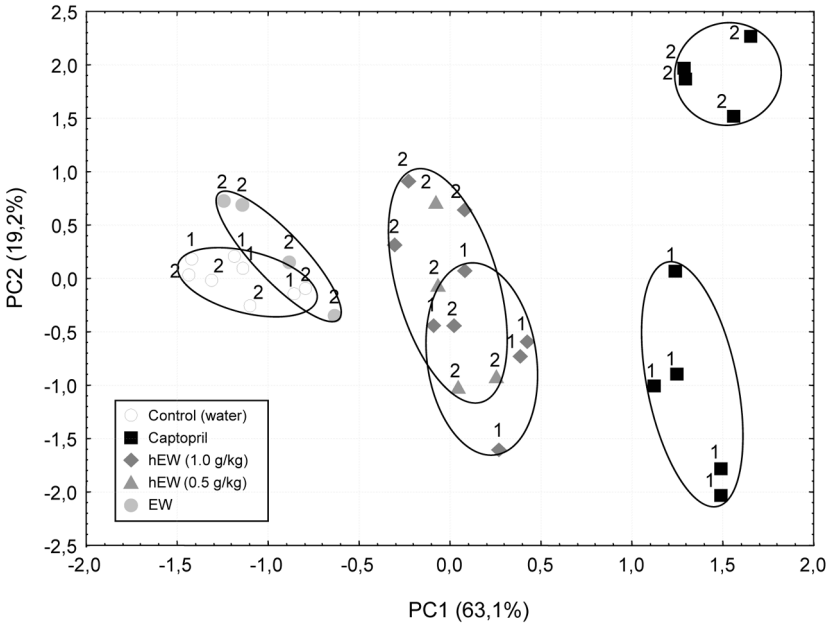

Figure 2. Representation of the different rats plotted on the plane defined by the two first principal components obtained by PCA of the data from the determinations of ACE activity in plasma and other tissues and the SBP after the short- (1) and long-term (2) treatments.

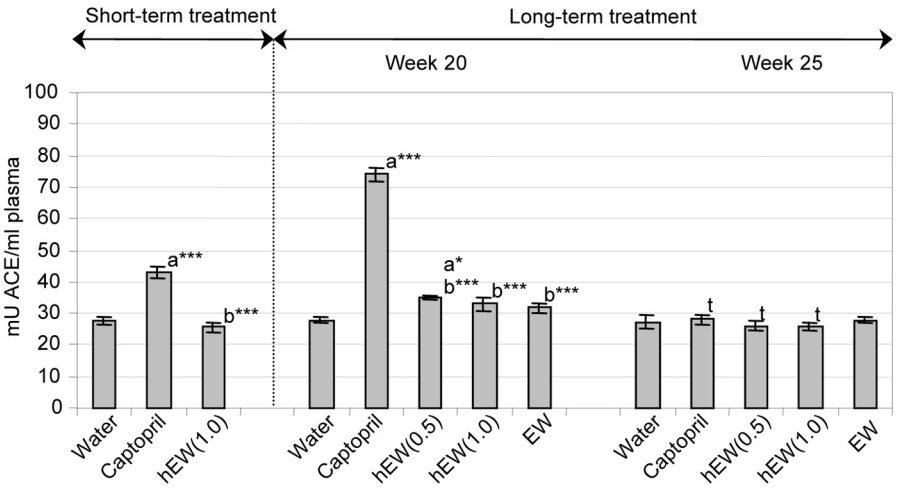

Figure 3. ACE activity (mU/mL) in plasma of SHR after short-term treatments with $hEW_{(1.0)}$ (1 g/kg) and captopril (100 mg/kg), and long-term treatments with $hEW_{(0.5)}$ (0.5 g/kg/day), $hEW_{(1.0)}$ (1 g/kg/day), EW (1 g/kg/day) and captopril (100 mg/kg/day). Data are mean values ± SEM for 4–5 rats in the case of the short-term treatment and 6–8 rats in the case of the long-term treatment. $^{a}P < 0.05$ *versus* water; $^{b}P < 0.05$ *versus* captopril; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, as estimated by one-way ANOVA. $^{t}P < 0.05$ *versus* the same group at week 20, as estimated by a Student *t*-test.

PCA was applied to establish relationships among the analysed variables. Two principal components were obtained which explained 82.3% of the total variance of the data. The first principal component, which explains 63.1% of the total variance, was strongly related with ACE activities in kidney (−0.89), SBP (−0.85), aorta (−0.81) and plasma (0.73). The second principal component, which explains 19.2% of the total variance, was mainly related with the variable ACE activity in lungs (0.63). As shown in Fig. 2, the SHR treated with captopril and hEW appeared as clearly separated groups according to their lower values of SBP, ACE activity in aorta and kidney and higher ACE activity in plasma, as compared to the SHR taking water and EW. Short- and long-term treatments with captopril separated according to the values of ACE activity in the lungs. The correlation matrix just taking into account the data for the short-term treatment showed a positive correlation between SBP and ACE activity in aorta (84%), while, for the long-term treatment, there was a negative correlation between SBP and ACE activity in plasma (89%).

Short-term treatment with hEW did not produce a significant effect on the ACE activity in plasma of SHR, while

the long-term treatment with 0.5 g/kg/day of hEW significantly increased the plasma ACE activity ($P < 0.05$), as revealed by the one-way ANOVA of the data (Fig. 3). ACE activity in plasma of SHR significantly increased with respect to the control ($P < 0.001$), both after the short- and the long-term treatment with captopril. In all groups, plasma ACE activity had reverted to basal values as shown by the Student-*t* test, of around 28 mU/mL, at the end of the follow-up period.

Figure 4 shows the effect of the different treatments on tissue ACE activities. The one-way ANOVA showed significant differences in the short-term treatment between the

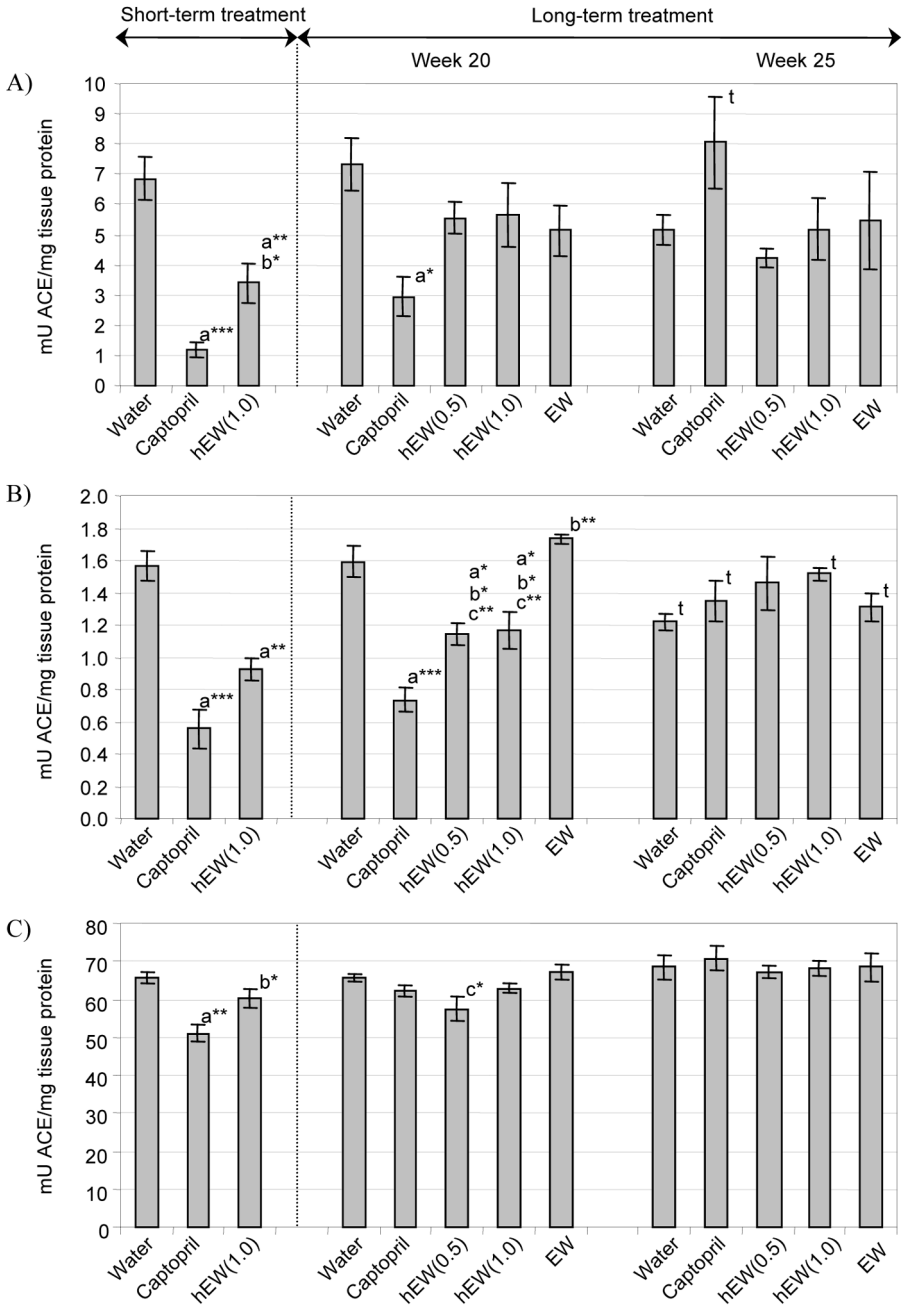

Figure 4. ACE activity (mU/mg protein) in A) aorta, B) kidney and C) lung of SHR after short-term treatments with $hEW_{(1.0)}$ (1 g/kg) and captopril (100 mg/kg), and long-term treatments with $hEW_{(0.5)}$ (0.5 g/kg/day), $hEW_{(1.0)}$ (1 g/kg/day), EW (1 g/kg/day) and captopril (100 mg/kg/day). Data are mean values ± SEM for 4–5 rats in the case of the short-term treatment and 4 rats in the case of the long-term treatment. $^{a}P < 0.05$ *versus* water; $^{b}P < 0.05$ *versus* captopril; $^{c}P < 0.05$ *versus* EW; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, as estimated by one-way ANOVA. $^{t}P < 0.05$ *versus* the same group at week 20, as estimated by a Student *t*-test.

Table 1. Percent organ weight to body weight ratios at the 20th week of life of SHRs administered different long-term treatments. Each value represents the mean ± SEM for 6–8 animals

	Water (control)	hEW_1 (0.5 g/kg/day)	hEW_2 (1 g/kg/day)	EW (1 g/kg/day)	Captopril (100 mg/kg/day)
Brain (%)	0.52 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.55 ± 0.01
Heart (%)	0.41 ± 0.02	0.38 ± 0.01[b)]	0.4 ± 0.01[b)]	0.39 ± 0.004	0.32 ± 0.01[a),c)]
Lung (%)	0.48 ± 0.05	0.47 ± 0.03	0.49 ± 0.03	0.49 ± 0.03	0.44 ± 0.02
Liver (%)	3.26 ± 0.02	3.24 ± 0.02[b)]	3.15 ± 0.03[b)]	3.29 ± 0.04	3.39 ± 0.04
Kidney (%)	0.63 ± 0.01	0.65 ± 0.01	0.64 ± 0.01	0.65 ± 0.01	0.68 ± 0.01[a)]
Spleen (%)	0.19 ± 0.003	0.2 ± 0.004	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.005

a) $P < 0.05$ *versus* water.
b) $P < 0.05$ *versus* captopril.
c) $P < 0.05$ *versus* EW.

Table 2. Percent organ weight to body weight ratios at the 25th week of life of SHRs administered different long-term treatments. Each value represents the mean ± SEM for 6–8 animals

	Water (control)	hEW_1 (0.5 g/kg/day)	hEW_2 (1 g/kg/day)	EW (1 g/kg/day)	Captopril (100 mg/kg/day)
Brain (%)	0.51 ± 0.01	0.50 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.01
Heart (%)	0.39 ± 0.01	0.37 ± 0.01[b)]	0.38 ± 0.01[b)]	0.36 ± 0.01	0.33 ± 0.005[a)]
Lung (%)	0.49 ± 0.04	0.57 ± 0.06	0.53 ± 0.05	0.42 ± 0.01	0.43 ± 0.02
Liver (%)	3.13 ± 0.07	3.07 ± 0.04	3.01 ± 0.03	3.02 ± 0.04	3.21 ± 0.03
Kidney (%)	0.62 ± 0.01	0.6 ± 0.01	0.62 ± 0.01	0.6 ± 0.01	0.62 ± 0.01
Spleen (%)	0.19 ± 0.005	0.19 ± 0.003	0.19 ± 0.01	0.19 ± 0.01	0.17 ± 0.004

a) $P < 0.05$ *versus* water.
b) $P < 0.05$ *versus* captopril.

control group and the group that was administered hEW ($P < 0.01$), with average reductions in ACE activity in the aorta and in the kidney of 50.4 and 40.7%, respectively (Figs. 4A and B). The short-term treatment with captopril also produced a significant ACE activity lowering effect in the aorta and in the kidney ($P < 0.001$), with average decreases in the local ACE activities of 82.5 and 64.4%, respectively. In the case of the long-term treatments, when compared to the negative control, the ACE activity in the kidney was significantly lower with both doses of hEW ($P < 0.05$) and with captopril ($P < 0.001$) but not with the administration of EW ($P > 0.05$) (Fig. 2B). Concerning ACE activities in the aorta, significant differences with respect to the negative control were only observed in the group treated with captopril ($P < 0.05$). No significant differences were observed in kidney and aorta ACE activities among the experimental groups after the follow-up period. In addition, the Student *t*-test confirmed the increase in ACE activity in both tissues at week 25.

The highest ACE activities were found in lung homogenates, with values ranging between 64–68 mU/μg of protein for the control, that were not significantly altered by the treatment with hEW (Fig. 4C). Short-term administration of captopril decreased ACE activity in the lungs ($P < 0.01$), but no such difference was observed after the equivalent long-term treatment. After the follow-up period, there were no differences in lung ACE activities among the experimental groups.

3.2 Organ weights

No significant differences in percent wet organ weight to body weight ratios were observed among the experimental groups after the short-term treatments. Brain, lungs, liver and spleen weights were not significantly different among the different groups after the long-term treatment (Table 1). The long-term intake of captopril significantly increased the renal wet weight to the total body weight ratio. However, the kidney weight decreased to normal values during the follow-up period (Table 2). In addition, this drug significantly decreased the heart to body weight ratio with respect to the other experimental groups (Table 1), an effect that was maintained during the follow-up period (Table 2).

4 Discussion

The results obtained in the present work relate the reductions in arterial blood pressure caused by short- and long-term treatments with hEW in SHR with an altered ACE activity in plasma and various tissues. This points, at least partially, at a systemic and local ACE-inhibitory mechan-

ism of the antihypertensive effect, which is similar, but less pronounced than that caused by the ACE inhibitor captopril.

Although oral administration of ACE inhibitors has been widely used to lower the blood pressure in animal models and humans, it remains unclear which organs these drugs target to achieve their antihypertensive effects. It has been demonstrated that a variety of tissues, including blood vessels, heart and kidney contain all the essential components of the rennin–angiotensin system. In our study, we extracted plasma, aorta, kidney and lungs to measure the ACE activity. In SHR, ACE activity in vascular tissues increases along with blood pressure during the chronic phase of hypertension and thus, it may play a more important role in the regulation of blood pressure than ACE levels in other tissues or plasma [15]. In addition, local ACE in kidney leads to the production of Ang II and aldosterone, causing a reduction of the urinary excretion of water and sodium, which plays an important role in long-term stabilization of hypertension [16]. Inhibition of ACE activity in the heart may play an important role in suppressing the progression of cardiac hypertrophy [15].

In our work, we found an increase in ACE activity in plasma of SHR after the short-term treatment with captopril and after the long-term treatments with hEW and captopril. ACE activities in plasma of SHR have been reported to decrease in the long-term administration of ACE-inhibitory peptides contained in egg yolk [17] and soya protein hydrolysates [18]. However, an elevation in plasma ACE concentration has been documented in humans and rats during the treatment with ACE inhibitors [10, 19, 20]. Costerousse *et al.* [21] found that the increase in circulating ACE levels during the treatment of rats with ACE inhibitors was associated to a generalized increase in ACE gene transcription and ACE synthesis in somatic cells. This may be due to an adaptative response to the inhibition of the enzyme although it seems to be independent of Ang II suppression. The physiological and pharmacological consequences of ACE induction during inhibition of the enzyme are not known, but it does not seem to reduce the therapeutic effect of these drugs [21]. In addition, inhibition of plasma ACE activity may not necessarily be a blood pressure-lowering mechanism for ACE inhibitors [15].

The ACE activity in kidney was significantly reduced by hEW as well as by captopril. However, due to the particularly high variability obtained for the analyses in aorta homogenates, we failed to provide significant differences in ACE inhibitory activity in the aorta after the long-term treatment with hEW. The short-term oral administration of milk fermented by a starter containing *Lactobacillus helveticus* and *Saccaharomyces cerevisiae* produced a significant decrease in ACE activity in the aorta of SHR [22] that was related to the local presence of the potent *in vitro* ACE inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) [23]. Additionally, the *in vitro* ACE inhibitory peptide His-His-Leu (HHL), derived from a fermented soybean paste, significantly decreased ACE activity in the aorta of SHR [24]. Matsui *et al.* [25] investigated the distribution of an antihypertensive dipeptide from a sardine muscle hydrolyzate, Val-Tyr (VY) in SHR and the results indicated that it preferably accumulated in the tissues rather than in plasma, and that aorta and kidney could be the target sites for the antihypertensive action of this peptide. The present results also suggest that at least the kidney could be a target site for hEW action.

Unlike captopril, which increased the kidney weight significantly, kidney weight in the hEW groups was not significantly different from that of the negative control group, despite liquid consumption was enhanced in all groups with respect to the control, as explained in Miguel *et al.* [8]. ACE-inhibitors produce renal hypertrophy, because they induce thirst, increasing water intake and urine output in rats [26]. It has been reported that diets rich in protein may also increase water consumption, renal blood flow and kidney weight [27, 28]. Similarly, we did not observe a significant reduction in the heart weight to body weight ratios in the SHR treated with hEW although captopril reduced the heart weight to body weight ratio, in agreement with the results obtained by Swislocki *et al.* [29]. This is because ACE inhibitors, such as captopril, are very effective in preventing or reversing the growth of cardiovascular tissues and cardiac fibrosis that result from the high Ang II levels typical of SHR [30, 31].

The present results indicate that the inhibition of ACE activity *in vivo* plays a role in the antihypertensive effect produced by hEW in SHR. Nevertheless tissue ACE inhibitory effects produced by hEW were not as marked as those produced by the strong ACE inhibitor captopril although both exerted a potent blood-pressure lowering effect. Unlike captopril, hEW did not produce significant effects on kidney and heart weights either. These observations suggest that other mechanisms may also be involved in the antihypertensive activity of hEW. Peptides derived from the hydrolysis of ovalbumin (ovokinin, FRADHPFL, and ovokinin 2–7, RADHPF) lower blood pressure in SHR through different vasodilator mechanisms [32, 33]. In fact, hEW and some other of its peptide sequences, such as IVF, RADHPFL and YAEERYPIL induce vasodilatation in intact aortic rings, which suggests that they could reduce the vascular resistance [34, 35]. In addition, it is noteworthy that hEW and some of its peptides have shown antioxidant effects *in vitro* [36] and strong experimental evidence indicates that oxidative stress and associated oxidative damage are mediators in cardiovascular pathologies.

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5 References

[1] Johnston, C. I., Burrell, L. M., Perich, R., Jandeleit, K., Jackson, B., The tissue renin-angiotensin system and its functional role, *Clin. Exp. Pharmacol. Physiol. Suppl.* 1992, *19*, 1 – 5.

[2] Erdös, E. G., Conversion of angiotensin I to angiotensin II, *Am. J. Med*. 1976, *60*, 749 – 759.

[3] Bhoola, K. D., Figueroa, C. D., Worthy, K., Bioregulation of kinins – kallikreins, kininogens, and kininases, *Pharmacol. Rev.* 1992, *44*, 1 – 80.

[4] Danser, A. H. J., Local renin-angiotensin systems, *Mol. Cel. Biochem*. 1996, *157*, 211 – 216.

[5] Kovacs-Nolan, J., Phillips, M., Mine, Y., Advances in the value of eggs and egg components for human health, *J. Agric. Food Chem.* 2005, *53*, 8421 – 8431.

[6] Li, G. H., Le, G. W., Shi, Y. H., Shrestha, S., Angotensin-I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects, *Nutr. Res.* 2004, *24*, 469 – 486.

[7] Miguel, M., López-Fandiño, R., Ramos, M., Aleixandre, A., Short-term effect of egg-white hydrolysate products on the arterial blood pressure of hypertensive rats, *Br. J. Nutr.* 2005, *94*, 731 – 937.

[8] Miguel, M., López-Fandiño, R., Ramos, M., Aleixandre, A., Long-term intake of egg white hydrolysate attenuates the development of hypertension in spontaneously hypertensive rats, *Life Sci.* 2006, *78*, 2960 – 2966.

[9] Miguel, M., Recio, I., Gómez-Ruiz, J. A., Ramos, M., López-Fandiño, R., Angiotensin I-converting enzyme inhibitory activity of peptides derived from egg white proteins by enzymatic hydrolysis, *J. Food Protect.* 2004, *67*, 1914 – 1920.

[10] Wu, J., Ding, X., Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptide derived from soy protein on spontaneously hypertensive rats, *J. Agric. Food Chem.* 2001, *49*, 501 – 506.

[11] Maes, W., Van Camp, J., Vermeirssen, V., Hemeryck, M., *et al*., Influence of the lactakinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells, *Regul. Pept.* 2004, *118*, 105 – 109.

[12] Nakata, K., Nishimura, K., Takada, T., Ikuse, T., *et al*., Effects of an angiotensin-converting enzyme (ACE) inhibitor, SA446, on tissue ACE activity in normotensive, spontaneously hypertensive, and renal hypertensive rats, *J. Cardiovasc. Pharmacol.* 1987, *9*, 305 – 310.

[13] Buñag, R. D., Validation in awake rats of a tail-cuff method for measuring systolic pressure, *J. Appl. Physiol.* 1973, *34*, 279 – 282.

[14] Friedland, J., Silverstein, E., Sensitive fluorimetric assay for angiotensin- converting enzyme, *Am. J. Clin. Pathol*. 1976, *66*, 416 – 424.

[15] Takai, S., Jin, D., Sakaguchi, M., Miyazaki, M., Significant target organs for hypertension and cardiac hypertrophy by angiotensin-converting enzyme inhibitors, *Hypertens. Res.* 2004, *27*, 213 – 219.

[16] Sharifi, A. M., Darabi, R., Akbarloo, N., Larijani, B., Khoshbaten, A., Investigation of circulatory and tissue ACE activity during development of lead-induced hypertension, *Toxicol. Lett*. 2004, *153*, 233 – 238.

[17] Yoshi, H., Tachi, N., Ohba, R., Sakamura, O., *et al*., Antihypertensive effect of ACE inhibitory oligopeptides from chichen egg yolks, *Comp. Biochem. Physiol. Part C*., 2001, *128*, 27 – 33.

[18] Yang, H. Y., Yang, S. C., Chen, J. R., Tzeng, Y. H., Han, B. C., Soybean protein hydrolysate prevents the development of hypertension in spontaneously hypertensive rats, *Br. J. Nutr.* 2004, *92*, 507 – 512.

[19] Fyhrquist, F., Forslund, T., Tikkanen, I., Gronhagen-Riska, C., Induction of angiotensin I-converting enzyme in rat lung with Captopril (SQ 14225), *Eur. J. Pharmacol*. 1980, *67*, 473 – 475.

[20] Boomsma, F., Debruyn, J. H. B., Derkx, F. H. M., Schalekamp, M. A. D. H., Opposite effects of captopril on angiotensin I-converting enzyme activity and concentration: Relation between enzyme inhibition and long-term blood pressure response, *Clin. Sci.* 1981, *60*, 491 – 498.

[21] Costerousse, O., Allegrini, J., Clozel, J. P., Ménard, J., Alhenc-Geñas, F., Angiotensin I-converting enzyme inhibition but not angiotensin II supression alters angiotensin I-converting enzyme gene expression in vessels and epithelia, *J. Pharmacol. Exp. Ther.* 1998, *284*, 1180 – 1187.

[22] Takano, T., Milk derived peptides and hypertension reduction, *Int. Dairy J.* 1998, *8*, 375 – 81.

[23] Masuda, O., Nakamura, Y., Takano, T., Antihypertensive peptides are present in aorta after oral administration of sour milk containing these peptides to spontaneously hypertensive rats, *J. Nutr.* 1996, *126*, 3063 – 3068.

[24] Shin, Z. I., Yu, R., Park, S. A., Chung, D. K., *et al*., His-His-Leu, an angiotensin I converting enzyme inhibitory peptide derived from Korean soybean paste, exerts antihypertensive activity *in vivo*, *J. Agric. Food. Chem*. 2001, *49*, 3004 – 3009.

[25] Matsui, T., Imamura, M., Oka, H., Osajima, K., *et al*., Tissue distribution of antihypertensive dipeptide, Val-Tyr, after its single oral administration to spontaneously hypertensive rats, *J. Pept. Sci*. 2004, *10*, 535 – 545.

[26] Barney, C. C., Katovich, M. J., Fregly, M. J., The effect of acute administration of an angiotensin converting enzyme inhibitor, captopril (SQ 14,225), on experimentally induced thirst in rats, *J. Pharmacol. Exp. Ther.* 1980, *212*, 53 – 57.

[27] Chan, K. C., Lou, P. P., Hargrove, J. L., High casein-lactalbumin diet accelerates blood coagulation in rats, *J. Nutr.* 1993, *123*, 1010 – 1016.

[28] Nevala, R., Vaskonen, T., Vehniäinen, J., Korpela, R., Vapaatalo, H., Soy based diet attenuates the development of hypertension when compared to casein based diet in spontaneously hypertensive rat, *Life Sci*. 2000, *66*, 115 – 124.

[29] Swislocki, A. L., La Pier, T. L., Khuu, D. T., Fann, K. Y., *et al.*, Metabolic, hemodynamic, and cardiac effects of captopril in young, spontaneously hypertensive rats, *Am. J. Hypertens*. 1999, *12*, 581 – 589.

[30] Regan, C. P., Bishop, S. P., Berecek, K. H., Early, short-term treatment with captopril permanently attenuates cardiovascular changes in spontaneously hypertensive rats, *Clin. Exp. Hypertens*. 1997, *19*, 1161 – 1177.

[31] Berecek, K. H., Reaves, P., Raizada, M., Effects of early perturbation of the rennin-angiotensin system on cardiovascular remodelling in spontaneously hypertensive rats, *Vascul. Pharmacol*. 2005, *42*, 93 – 98.

[32] Fujita, H., Usui, H., Kurahashi, K., Yoshikawa, M., Isolation and characterization of ovokinin, a bradykinin B_1 agonist peptide derived from ovoalbumin, *Peptides* 1995, *16*, 785 – 790.

[33] Matoba, N., Usui, H., Fujita, H., Yoshikawa, M., A novel antihypertensive peptide derived from ovalbumin induces nitric oxide-mediated vasorelaxation in an isolated SHR mesenteric artery, *FEBS Lett.* 1999, *452*, 181 – 184.

[34] Miguel, M., Alonso, M. J., Salaices, M., Aleixandre, A., López-Fandiño, R., Antihypertensive, ACE-inhibitory and vasodilator properties of an egg white hydrolysate. Effect of a simulated intestinal digestion, *Food Chem*. 2007, in press, doi: 10.1016/j.foodchem.2006.11.016.

[35] Miguel, M., Alvárez, Y., López-Fandiño, R., Alonso, M. J., Salaices, M., Vasodilator effects of peptides derived from egg proteins, *Regul. Pept*. 2007, *140*, 131 – 135.

[36] Dávalos, A., Miguel, M., Bartolomé, B., López-Fandiño, R., Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis, *J. Food Protect*, 2004, *67*, 1939 – 1944.