Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 361–367
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats

María A. Manso [a], Marta Miguel [a,b], Jeanne Even [a], Rosario Hernández [c], Amaya Aleixandre [b], Rosina López-Fandiño [a,*]

[a] *Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain*
[b] *Instituto de Farmacología y Toxicología (CSIC), Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain*
[c] *CDEAS, Hospital Central de la Defensa, Plaza del Ejército s/n, 28007 Madrid, Spain*

Received 27 September 2007; received in revised form 29 October 2007; accepted 18 December 2007

Abstract

This paper examines the effects of the long-term consumption of egg white hydrolysed with pepsin (hEW) on the antioxidant status and lipid profile of spontaneously hypertensive rats (SHR). The antioxidant capacity was measured by the oxygen radical absorbance capacity (ORAC) and the oxidative status by the malon-dialdehyde (MDA) assay. The lipid profile was analysed spectrophotometrically. The radical-scavenging capacity of the plasma was increased and the MDA concentration in the aorta was decreased in the SHR treated with 0.5 g/kg/day of hEW. Our findings indicate that hEW played an important role in antioxidative defence of SHR and exerted a beneficial effect on the lipid profile, lowering triglycerides and total cholesterol without changing HDL levels. Therefore, hEW may be useful to prevent or reverse abnormalities associated with the metabolic syndrome and its complications, such as hypertension, oxidative stress and hyperlipidemia.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Antioxidant status; Lipid profile; Bioactive peptides; Egg white; Spontaneously hypertensive rats

1. Introduction

Free radicals generated *in vivo*, including reactive oxygen species (ROS), are responsible for the oxidative damage of lipids, proteins, DNA and small molecules. As a result, they have been implicated in a number of multifactor degenerative diseases and aging processes, such as diabetes, cancer and cardiovascular diseases, including the initiation and maintenance of hypertension (Ames, Shigenaga, & Hagen, 1993; Schnackenberg, Welch, & Wilcox, 1998). Recent evidence also indicates oxidative stress as the main mechanism responsible for cardiovascular complications observed in patients with metabolic syndrome, e.g. initiation or progression of the atherosclerotic process and alteration in lipid metabolism (Palmieri, Grattagliano, Portincasa, & Palasciano, 2006).

Some substances, termed antioxidants, might be able to prevent, or at least attenuate, the organic impairment originated by excessive oxidative stress. Since endogenous antioxidants may not be sufficient to prevent damage, diet-derived or supplemented antioxidants could be important to maintain health. Some food proteins and peptides were found to have scavenging activity against free radicals (Okada & Okada, 1998; Suetsuna, Ukeda, & Ochi, 2000) or to exert antioxidant activity against peroxidation of lipids or fatty acids (Hernández-Ledesma, Dávalos, Bartolomé, & Amigo, 2005; Kim, Je, & Kim, 2007; Kim et al., 2001; Nagai, Inoue, Suzuki, & Nagashima, 2006; Saiga, Tanabe, & Nishimura, 2003; Tsuge, Eikawa, Nomura, Yamamoto,

* Corresponding author. Tel.: +34 91 5622900; fax: +34 91 5644853.
E-mail address: rosina@ifi.csic.es (R. López-Fandiño).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.049

& Sugisawa, 1991; Virtanen, Pihlanto, Akkanen, & Korhonen, 2007; Zhu, Zhou, & Qian, 2006). However, neither the structure-activity relationship, nor the antioxidant mechanism of peptides is fully understood (Pihlanto, 2006). On the other hand, very few studies have demonstrated the physiological activity of antioxidant food peptides and their ability to modify the lipid profile and markers of oxidative stress *in vivo*.

In previous work, we reported that the enzymatic hydrolysis of egg white with pepsin resulted in the production of peptides with free radical-scavenging capacity and lipid peroxidation inhibition ability (Dávalos, Miguel, Bartolomé, & López-Fandiño, 2004). Some of these peptides also possessed *in vitro* angiotensin converting enzyme (ACE) inhibitory activity (Miguel, Recio, Gómez-Ruiz, Ramos, & López-Fandiño, 2004) and vasodilator properties (Miguel, Álvarez, López-Fandiño, Alonso, & Salaices, 2007). The short-term administration of egg white hydrolysed with pepsin (hEW) decreased arterial blood pressure in spontaneously hypertensive rats (SHR) and the long-term intake of hEW attenuated the development of hypertension in these animals (Miguel, López-Fandiño, Ramos, & Aleixandre, 2005, 2006). Furthermore, the reductions in arterial blood pressure caused by the short- and long-term treatments with hEW in SHR correlated with an altered ACE activity in plasma and various tissues (Miguel, Manso, Alvarez, Aleixandre, & López-Fandiño, 2007).

In the hypertensive state, angiotensin II mediates many of its cellular actions by stimulating the formation of intracellular ROS, thereby participating in the increase of oxidative stress (Schiffrin & Touyz, 2004). In fact, the blockage of angiotensin II production by ACE inhibitors has been shown to decrease lipid peroxidation and increase the antioxidant defences in animals and humans (de Cavanagh, Inserra, Ferder, & Fraga, 2000; Kedziova-Kornatowska, Luciak, & Paszkowski, 2000). Therefore, because of their radical-scavenging and ACE-inhibitory properties, the peptides contained in hEW could be potentially used as natural antioxidants. The aim of the present study was to evaluate the effect of the long-term consumption of hEW on the *in vivo* antioxidant status in plasma and several tissues of SHR, using biomarkers of oxidative stress, and to investigate the possible hypolipidemic properties of the hydrolysate.

2. Materials and methods

2.1. Protocol in animals

Hydrolysed egg white (hEW) was prepared by pepsin hydrolysis, as previously described (Miguel et al., 2004). Briefly, lyophilised crude egg white (EW), obtained in the laboratory from chicken fresh shell eggs, was dissolved in Milli-Q water at a concentration of 100 mg/ml and the pH was set to 2.0 by the addition of 1 M HCl. Pepsin (E.C 3.4.23.1 type A, 10,000 U/mg was from pork stomach, Sigma Chemicals Co., St. Louis, MO, USA). Dissolved EW was hydrolysed with pepsin (E/S 1/100, wt/wt) at 37 °C for 3 h. Pepsin was inactivated by increasing the pH to 7.0 with 1 M NaOH and the hydrolysate was centrifuged at 4500*g* for 15 min.

A set of male SHR (Charles River Laboratories España S.A., Barcelona, Spain), after being weaned at 3 weeks, was randomly divided into five groups of 16 animals each and housed at a temperature of 23 °C with 12/12 h light/dark cycles. The animals consumed a standard diet for rats (A04 Panlab, Barcelona, Spain) *ad libitum* during the experimental period. Until the rats were 20 weeks 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, Sigma Chemicals). From the 20th to the 25th week of life, the drinking fluid was always tap water for all the rats (follow-up period). The values of SBP and DBP, body weight of SHR and solid and liquid diet intake during this long-term study are described in detail in Miguel et al. (2006). 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-week-old rats) and at the end of the follow-up period (25-week-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).

2.2. Plasma and tissue preparations

Blood samples were collected into tubes containing lithium heparin as anticoagulant and centrifuged at 3500*g* for 20 min to obtain the plasma. Brain, aorta, and liver 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 antioxidant capacity and oxidative status assays. The protein content was determined by the bicinchoninic acid assay (Pierce, Rockford, Ill, USA), using bovine serum albumin as standard.

2.3. Oxygen radical absorbance capacity

The oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay was based on that proposed by Ou, Hampsch-Woodill, and Prior, (2001) and modified as previously described (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). The final assay mixture (200 μl) contained 70 nM fluorescein (Sigma) as oxidable substrate, 12 mM 2,2′-azobis (2-methylpropionamidine) dihydrochloride AAPH (Aldrich, Milwaukee, WI, USA) as oxygen radical generator, and either 1–8 μM of the antioxidant trolox [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid] as standard, or plasma samples. Samples and standards were dissolved in 75 mM PBS (pH 7.4). A fluorescein stock solu-

tion (1.17 mM) was made in the same buffer and stored in dark conditions at 4 °C for a maximum of 4 weeks. AAPH and trolox solutions in 75 mM PBS (pH 7.4) were prepared daily. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with a 485-P excitation and a 520-P emission filter was used. The fluorescent plate reader was controlled by the Fluostar Galaxy software version (4.11-0). Black 96-well microplates (96F untreated microwell, Nunc™, Denmark) were used. Fluorescence measurement was carried out at 37 °C and was recorded every minute for 80 min. All reaction mixtures were prepared in duplicate and at least three independent runs were performed for each sample. ORAC-FL values were calculated and expressed as nmol of trolox equivalent/ml of plasma as described by Dávalos, Gómez-Cordovés, et al. (2004).

2.4. Measurement of malon-dialdehyde production

Plasma, brain, aorta and liver malon-dialdehyde (MDA) levels were measured by the thiobarbituric acid-reactive substances (TBARS) assay (Rodríguez-Martínez & Ruíz-Torres, 1992). Plasma and homogenates of tissues 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 1500*g* for 15 min before adding TBA (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/L and, for the different tissues, as nmol MDA/g tissue protein.

2.5. Biochemical measurements

The lipid profile (plasma triglycerides, total and HDL-cholesterol) was analysed using enzymatic and colorimetric methods with commercial kits (Roche Diagnostics S.L., Spain). The concentrations were determined spectrophotometrically using a Hitachi 911 autoanalyser.

2.6. Statistical analysis

The results were expressed as mean values ± SEM for 4–8 rats. Significant differences were evaluated by one-way analysis of variance (ANOVA), followed by *post-hoc* analysis by Bonferroni's test and by Student *t*-test. In some cases we have used a non-parametric test. $P < 0.05$ was used as the threshold for statistically significant differences. The Graph Pad 4 Prism software was used for data processing.

3. Results and discussion

3.1. Antioxidant activity of hydrolysed egg white

In order to estimate the antioxidant effect of the intake of hEW in SHR, the radical-scavenging activity of the plasma was evaluated by the ORAC test. The production of free radicals was also assessed by an indirect measurement of tissue damage products using the TBARS test assay, based on the measurement of the MDA formed as a consequence of lipid peroxidation.

The results (Fig. 1) indicated that the long-term intake of hEW (0.5 g/kg/day) and captopril promoted the ability of plasma to neutralize an excessive formation of ROS. At the end of the treatment period (week 20), a significant increase was found in the ORAC-FL values of the plasma of the rats treated with hEW (0.5 mg/kg/day) and captopril, as revealed by the one-way ANOVA of the data ($P < 0.05$). The effect caused by captopril was more important than that of hEW, with average values of around 18 nmol trolox eq/ml plasma. As revealed by the Student *t*-test, in all cases, the ORAC-FL value reverted to basal figures, of around 12 nmol trolox eq/ml plasma, at the end of the follow-up period, that is 5 weeks after the treatments were removed.

Despite these differences in the radical-scavenging activity, the MDA concentration in the plasma did not change during the treatment period with the different products (Fig. 2A). However, the formation of MDA significantly increased from the 20th to the 25th week of life in all the experimental groups, probably reflecting a higher level of lipid peroxidation at aging due to an increased oxidative stress (Finkel & Holbrook, 2000; Sawada, Sester, & Carlson, 1992). No significant differences were found in the MDA concentrations in the brain during the treatment and follow-up periods (data not shown). In the aorta, the treatment with 0.5 g/kg/day of hEW significantly decreased MDA concentration, an effect that disappeared after the follow-up period (Fig. 2B). Long-term treatment with 1.0 g/kg/day of hEW significantly increased the MDA concentration in the liver of SHR ($P < 0.05$), as revealed by the

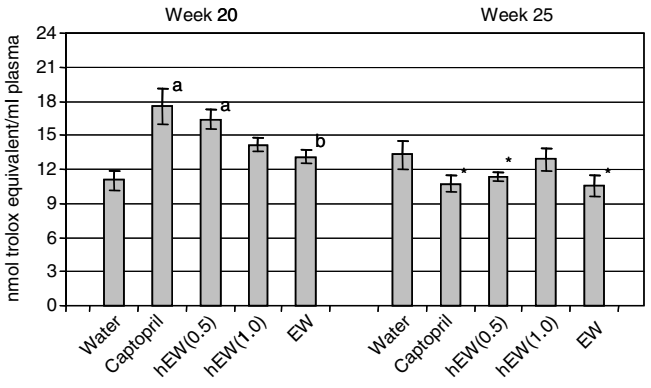

Fig. 1. Antioxidant capacity in plasma of SHR obtained by the ORAC test (nmol trolox eq/ml) after long-term treatments with $hEW_{(0.5)}$ (0.5 g/kg/day), $hEW_{(1.0)}$ (1 g/kg/day), EW (1 g/kg/day) and captopril (100 mg/kg/day). The animals drank different fluids from weaning until the 20th week of life (treatment period) and tap water from the 20th to the 25th week of life (follow-up period). Data are mean values ± SEM for 6–8 rats. $^{a}P < 0.05$ vs. water; $^{b}P < 0.05$ vs. captopril; $^{c}P < 0.05$ vs. EW, as estimated by one-way ANOVA. $^{*}P < 0.05$ vs. the same group at week 20, as estimated by Student *t*-test.

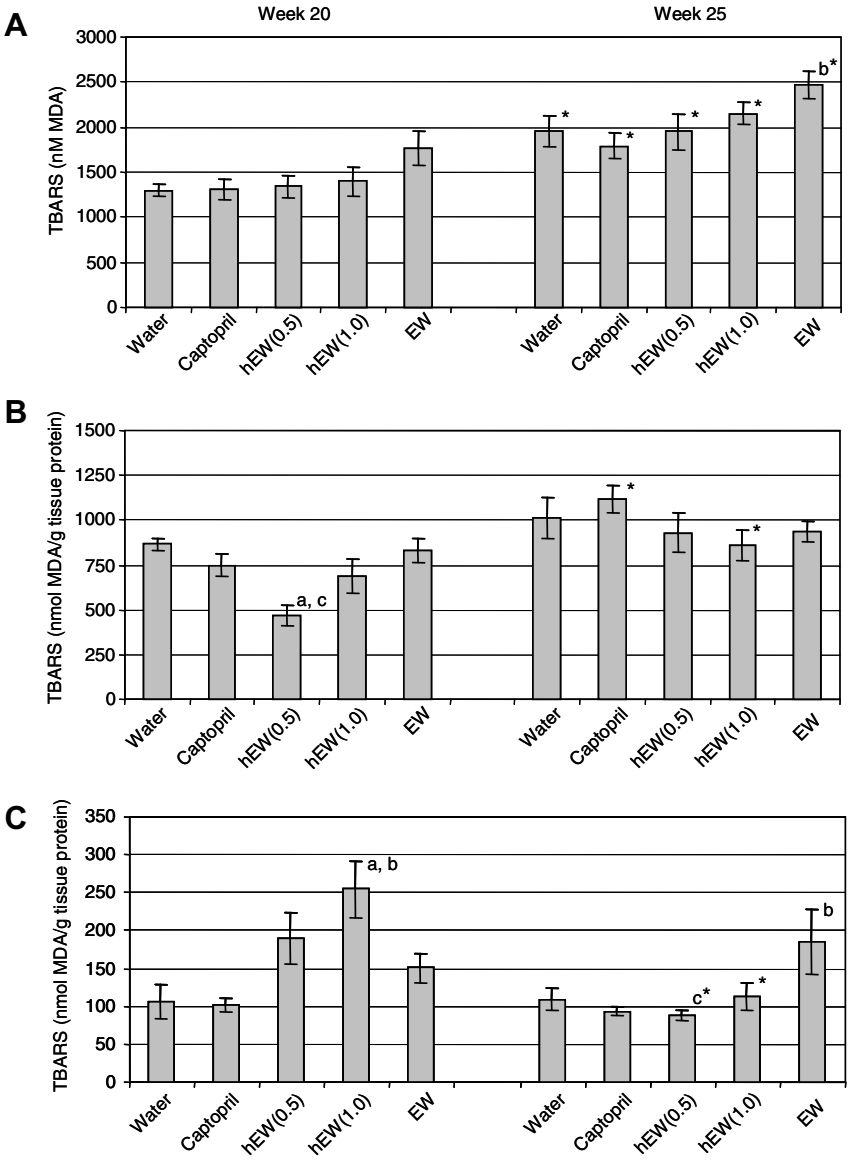

Fig. 2. Oxidative status (TBARS) in (A) plasma, (B) aorta and (C) liver of SHR after long-term treatments with $hEW_{(0.5)}$ (0.5 g/kg/day), $hEW_{(1.0)}$ (1 g/kg/day), EW (1 g/kg/day) and captopril (100 mg/kg/day). The animals drank different fluids from weaning until the 20th week of life (treatment period) and tap water from the 20th to the 25th week of life (follow-up period). Data are mean values ± SEM for 4–8 rats. [a] $P < 0.05$ vs. water; [b] $P < 0.05$ vs. captopril; [c] $P < 0.05$ vs. EW as estimated by one-way ANOVA. [*] $P < 0.05$ vs. the same group at week 20, as estimated by Student *t*-test.

one-way ANOVA of the data (Fig. 2C). The concentration of MDA in the liver reverted to basal values, of around 100 nmol/g of protein, at the end of the follow-up period, as shown by the Student *t*-test. The reason for the enhanced MDA concentration in the liver, brought about by the administration of hEW, is unknown and needs further investigation. Unlike the case of hEW, the long-term administration of captopril did not modify the MDA levels in the aorta or liver of SHR.

It has already been documented that captopril, a potent blocker of the rennin–angiotensin system, possesses free radical-scavenging effects (Bagchi, Prasad, & Das, 1989; Bain, Le Guen, Lunec, & Barnett, 1991). However, it is controversial whether this activity relies on the presence of a –SH group in the molecule, then being independent of ACE-inhibition, or whether both –SH- and non –SH-containing ACE-inhibitors exert free radical-scavenging actions (Pasini et al., 2007; Suzuki, Sato, Shimada, Takashima, & Arakawa, 1993). Thus, it has been reported that captopril, administered to SHR at the same dose as that used in our study (100 mg/kg) for 15 days, induces a small, but significant effect in the TBARS levels in the plasma (Bolterman, Manriquez, Ortiz Ruiz, Juncos, & Romero, 2005). The present results support the free radical-scavenging activity of captopril, although, in our experiments, captopril did not decrease the MDA levels in SHR. On the other hand, hEW, a complex mixture of antihypertensive peptides comprising ACE-inhibitors (Miguel et al., 2004) and vasorelaxing peptides devoid of ACE-inhibitory activity (Miguel, Manso, Aleixandre, Alonso, Salaices,

& López-Fandiño, 2007), also increased the ability of plasma to scavenge free radicals and inhibited TBARS development in the aorta of SHR. Although we could not demostrate a dose-dependent inhibition of lipid oxidation, it should be noted that this effect disappeared when the treatment with 0.5 g/kg/day of hEW was withdrawn.

Spontaneously hypertensive rats, that constitute an accepted model for human essential hypertension, are also considered a model for oxidative stress (Griendling & Ushio-Fukai, 2000). In SHR, impaired endothelium-dependent relaxations have been described, implying a decrease in bioactive nitric oxide and an imbalance in ROS, that may finally lead to target organ damage (Kojsova et al., 2006). It has been postulated that the normal or modestly elevated levels of angiotensin II in SHR may activate oxidative pathways that promote hypertension (Bolterman et al., 2005) and, in fact, antioxidants have been reported to lower blood pressure in these animals (Akpaffiong & Taylor, 1998). We had previously found that the development of hypertension was attenuated in the SHR groups treated with captopril and both doses of hEW (Miguel et al., 2006). Furthermore, the reduction of the systolic blood pressure in these animals was positively related with a decreased ACE activity in the aorta and kidney (Miguel et al., 2007). Our results with the SHR group, that had taken 0.5 g/kg/day of hEW, suggest a relationship between the observed inhibition of the rennin–angiotensin system and the increase in antioxidant systems and reduction in MDA levels, which agrees with the findings of other researchers in rats (Bolterman et al., 2005; Liu, You, Song, Wu, & Liu, 2007; Mervaala et al., 2001). Nevertheless, we could not detect such a correlation after the administration of captopril. In this respect, it should be stressed that the putative mechanisms underlying the blood pressure lowering effects of antioxidant or ACE-inhibitory peptides have not been fully elucidated. For example, it has been demonstrated that the ACE-inhibitory dipeptide, Met-Tyr, inhibits ROS formation through the stimulation of the expression of the antioxidant defence proteins HO-1 and ferritin in endothelial cells, protecting them against oxidative stress. However, neither captopril nor the ACE-inhibitor peptide Met-Phe possess these enzyme-inducing effects, which shows that the antioxidant properties of Met-Tyr are independent of its ACE-inhibitory properties (Erdmann, Grosser, Schipporeit, & Schröder, 2006).

3.2. Effects on the lipid levels

Triglycerides, total cholesterol and high density lipoprotein (HDL)-cholesterol in plasma were analysed (Fig. 3). Significant changes in the blood lipid profile were found after consumption of hEW. The long-term intake of hEW for 20 weeks had a lowering effect on triglycerides and total cholesterol, while no changes were observed in HDL-cholesterol. No differences were observed in those variables within the experimental groups after the follow-up period.

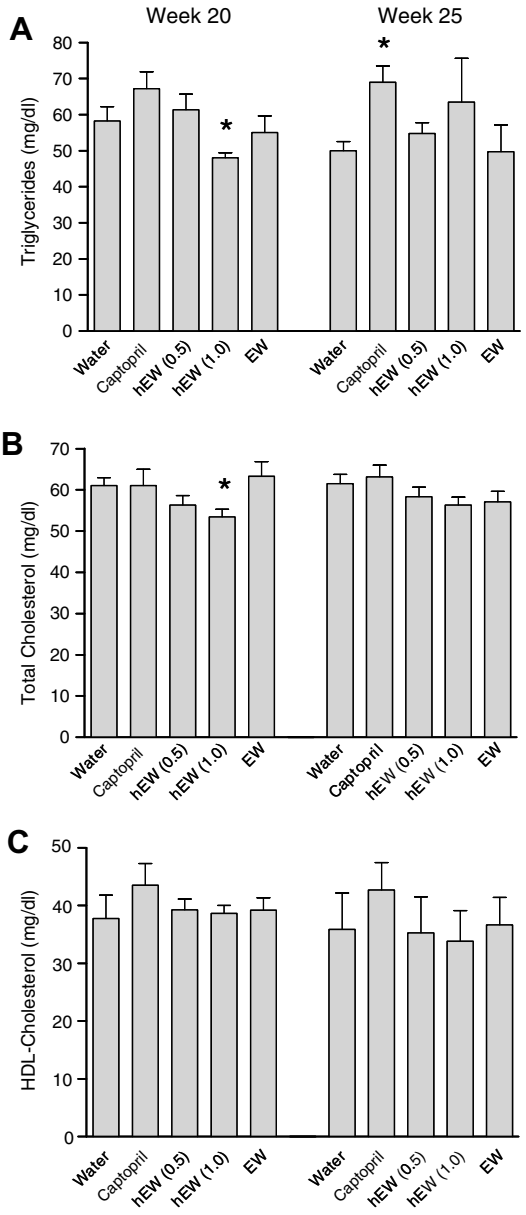

Fig. 3. Lipid profile in plasma of SHR after long-term treatments with $hEW_{(0.5)}$ (0.5 g/kg/day), $hEW_{(1.0)}$ (1 g/kg/day), EW (1 g/kg/day) and captopril (100 mg/kg/day). The animals drank different fluids from weaning until the 20th week of life (treatment period) and tap water from the 20th to the 25th week of life (follow-up period). (A) Triglycerides, (B) total cholesterol and (C) HDL-cholesterol. Data are mean values ± SEM for 8 rats. $^{*}P < 0.05$ vs. water, as estimated by Student *t*-test.

ACE-inhibitors have positive effects on lipid homeostasis (Ernsberger & Koletsky, 2006). Captopril decreased triglyceride levels, total cholesterol and non-HDL-cholesterol in a spontaneously hypertensive obese rat model (Ernsberger, Johnson, Rosenthal, Mirelman, & Koletsky, 2007). Regarding food-derived peptides, fermented milk supplemented with a whey protein concentrate was reported to exert both a serum lipid improvement effect and a hypotensive effect on animals and humans, that were mainly attributed to the activity of the lactic acid bacteria used as starters (Kawase, Hashimoto, Hosoda, Morita, & Hosono,

2000). Nevertheless, it has also been demonstrated that food protein hydrolysates and peptides decrease the micellar solubility of cholesterol (Zhong, Liu, Ma, & Shoemaker, 2007), which is associated with an inhibition of cholesterol absorption in the jejunum that was confirmed *in vivo* in mice (Zhong, Zhang, Ma and Shoemaker, 2007). In humans, Pins and Keenan (2006) found that the daily intake of a hydrolysed whey protein supplement (20 g) significantly reduced blood pressure, total cholesterol and LDL-cholesterol.

4. Conclusions

The main finding of this study is that the long-term treatment with hEW increased the ORAC value in plasma and reduced the MDA levels in aorta of SHR. This suggests that hEW may prevent oxidative stress by increasing plasma radical-scavenging capacity and inhibiting lipid peroxidation. This antioxidant activity could act together with the ACE-inhibitory activity and the vasodilator properties, contributing to the antihypertensive effect of hEW. In addition, this product showed hypolipidemic properties and thus, the consumption of hEW may provide an extra benefit by controlling blood lipid levels in certain diseases.

Acknowledgement

This study was supported by the projects CM-S0505-AGR-0153 and AGL2007-65035.

References

Akpaffiong, M. J., & Taylor, A. A. (1998). Antihypertensive and vasodilator actions of antioxidants in spontaneously hypertensive rats. *American Journal of Hypertension, 11*, 1450–1460.

Ames, B.-N., Shigenaga, M.-K., & Hagen, T.-M. (1993). Oxidants, antioxidants, and the degenerative diseases if aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7915–7922.

Bagchi, D., Prasad, R., & Das, D.-K. (1989). Direct scavenging of free radicals by captopril, an angiotensin converting enzyme inhibitor. *Biochemistry and Biophysical Research Communications, 158*, 52–57.

Bain, S.-C., Le Guen, C.-A., Lunec, J., & Barnett, A.-H. (1991). Comparison of the free radical scavenging activity of captopril versus enalapril: A three month in vivo study in hypertensive diabetic patients. *Journal of Human Hypertension, 5*, 511–515.

Bolterman, R.-J., Manriquez, M. C., Ortiz Ruiz, M.-C., Juncos, L.-A., & Romero, J.-C. (2005). Effects of captopril on the renin angiotensin system, oxydative stress, and endothelin in normal and hypertensive rats. *Hypertension, 46*, 943–947.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*, 48–54.

Dávalos, A., Miguel, M., Bartolomé, B., & López-Fandiño, R. (2004). Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. *Journal of Food Protection, 67*, 1939–1944.

de Cavanagh, E.-M., Inserra, F., Ferder, L., & Fraga, C.-G. (2000). Enalapril and captopril enhance glutathione-dependent antioxidant defences in mouse tissues. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 278*, R572–R577.

Erdmann, K., Grosser, N., Schipporeit, K., & Schröder, H. (2006). The ACE inhibitory dipeptide Met-Tyr diminishes free radical formation in human endothelial cells via induction of heme Oxyenase-1 and ferritin. *Journal of Nutrition, 136*, 2148–2152.

Ernsberger, P., Johnson, J. L., Rosenthal, T., Mirelman, D., & Koletsky, R. J. (2007). Therapeutic actions of allylmercaptocaptopril and captopril in a rat model of metabolic syndrome. *American Journal of Hypertension, 20*, 866–874.

Ernsberger, P., & Koletsky, R. J. (2006). Metabolic effects of antihypertensive agents: Role of sympathoadrenal and rennin–angiotensin systems. *Naunyn-Schmiedebergs's Archives of Pharmacology, 273*, 245–258.

Finkel, T., & Holbrook, N.-J. (2000). Oxidants, oxidative stress and the biology of aging. *Science, 408*, 239–247.

Griendling, K.-K., & Ushio-Fukai, M. (2000). Reactive oxygen species as mediators of angiotensin II signalling. *Regulatory Peptides, 9*, 21–27.

Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., & Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from alpha-lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *Journal of Agriculture and Food Chemistry, 53*, 588–593.

Kawase, M., Hashimoto, H., Hosoda, M., Morita, H., & Hosono, A. (2000). Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. *Journal of Dairy Science, 83*, 255–263.

Kedziova-Kornatowska, K.-Z., Luciak, M., & Paszkowski, J. (2000). Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: Effect of treatment with angiotensin convertase inhibitors. *IUBMB Life, 49*, 303–307.

Kim, S.-Y., Je, J.-Y., & Kim, S.-K. (2007). Purification and characterization of antioxidant peptide from hoki (Johnius belengerii) frame protein by gastrointestinal digestion. *Journal of Nutritional Biochemistry, 18*, 31–38.

Kim, S.-K., Kim, Y.-T., Byun, H.-G., Nam, K.-S., Joo, D.-S., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. *Journal of Agriculture and Food Chemistry, 49*, 1984–1989.

Kojsova, S., Jendekova, L., Zicha, J., Kunes, J., Andriantsitohaina, R., & Pechanova, O. (2006). The effect of different antioxidants on nitric oxide production in hypertensive rats. *Physiological Research, 55*, S3–S16.

Liu, Y. H., You, Y., Song, T., Wu, S. J., & Liu, L. Y. (2007). Impairment of endothelium-dependent relaxation of rat aortas by homocysteine thiolactone and attenuation by captopril. *Journal of Cardiovascular Pharmacology, 50*, 155–161.

Mervaala, E. M., Cheng, Z. J., Tikkanen, I., Lapatto, R., Nurminen, K., Vappatalo, H., et al. (2001). Endothelial dysfunction and xanthine oxidoreductase activity in rats with human renin and angiotensinogen genes. *Hypertension, 37*, 414–418.

Miguel, M., Álvarez, Y., López-Fandiño, R., Alonso, M.-J., & Salaices, M. (2007). Vasodilator effects of peptides derived from egg proteins. *Regulatory Peptides, 140*, 131–135.

Miguel, M., López-Fandiño, R., Ramos, M., & Aleixandre, A. (2005). Short-term effect of egg-white hydrolysate products on the arterial blood pressure of hypertensive rats. *British Journal of Nutrition, 94*, 731–737.

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

Miguel, M., Manso, M. A., Aleixandre, A., Alonso, M.-J., Salaices, M., & López-Fandiño, R. (2007). Vascular effects, Angiotensin I-Converting Enzyme (ACE)-inhibitory activity and antihypertensive properties of peptides derived from egg white. *Journal of Agricultural and Food Chemistry, 55*, 10615–10621.

Miguel, M., Manso, M. A., Alvarez, P., Aleixandre, M.-A., & López-Fandiño, R. (2007). Angiotensin converting enzyme activity in plasma and tissues of spontaneously hypertensive rats after short- and long-

term intake of an egg white hydrolysate. *Molecular Nutrition and Food Research, 51*, 555–563.

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

Nagai, T., Inoue, R., Suzuki, N., & Nagashima, T. (2006). Antioxidant properties of enzymatic hydrolysates from royal jelly. *Journal of Medicinal Food, 9*, 363–367.

Okada, Y., & Okada, M. (1998). Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. *Journal of Agricultural and Food Chemistry, 46*, 401–406.

Ou, B., Hampsch-Woodill, M., & Prior, R. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Palmieri, V.-O., Grattagliano, I., Portincasa, P., & Palasciano, G. (2006). Systemic oxidative alterations are associated with visceral adiposity and liver steatosis in patients with metabolic syndrome. *Journal of Nutrition, 136*, 3022–3026.

Pasini, A. F., Garbin, U., Nava, M. C., Stranieri, C., Pellegrini, M., Boccioletti, V., et al. (2007). Effect of sulfhydryl and non-sulfhydryl angiotensin-converting enzyme inhibitors on endothelial function in essential hypertensive patients. *American Journal of Hypertension, 20*, 443–450.

Pihlanto, A. (2006). Antioxidative peptides derived from milk proteins. *International Dairy Journal, 16*, 1306–1314.

Pins, J. J., & Keenan, J. M. (2006). Effects of whey peptides on cardiovascular disease risk factors. *The Journal of Clinical Hypertension, 8*, 775–782.

Rodríguez-Martínez, M. A., & Ruiz-Torres, A. (1992). Homeostasis between lipid peroxidation and antioxidant enzyme activities in healthy human aging. *Mechanisms of Ageing and Development, 66*, 213–222.

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry, 51*, 3661–3667.

Sawada, M., Sester, U., & Carlson, J.-C. (1992). Superoxide radical formation and associated biochemical alterations in the plasma membrane of brain, heart and liver during the lifetime of the rat. *Journal of Cellular Biochemistry, 48*, 296–304.

Schiffrin, E.-L., & Touyz, R.-M. (2004). From bedside to bench to bedside: Role of renin–angiotensin–aldosterone system in remodeling of resistance arteries in hypertension. *American Journal of Physiology – Heart and Circulatory Physiology, 287*, H435–H446.

Schnackenberg, C.-G., Welch, W.-J., & Wilcox, C.-S. (1998). Normalization of blood pressure and renal vascular resistance in SHR with a membrane permeable superoxide dismutase mimetic: Role of nitric oxide. *Hypertension, 32*, 59–64.

Suetsuna, K., Ukeda, H., & Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. *The Journal of Nutritional Biochemistry, 11*, 128–131.

Suzuki, S., Sato, H., Shimada, H., Takashima, N., & Arakawa, M. (1993). Comparative free radical scavenging action of angiotensin-converting enzyme inhibitors with and without the sulfhydryl radical. *Pharmacology, 47*, 61–65.

Tsuge, N., Eikawa, Y., Nomura, Y., Yamamoto, M., & Sugisawa, K. (1991). Antioxidative activity of peptides prepared by enzymic hydrolysis of egg-white albumin. *Journal of the Agricultural Chemical Society of Japan, 65*, 1635–1641.

Virtanen, T., Pihlanto, A., Akkanen, S., & Korhonen, H. (2007). Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. *Journal of Applied Microbiology, 102*, 106–115.

Zhong, F., Liu, J., Ma, J., & Shoemaker, C.-F. (2007). Preparation of hypocholesterol peptides from soy protein and their hypocholesterolemic effect in mice. *Food Research International, 40*, 661–667.

Zhong, F., Zhang, X., Ma, J., & Shoemaker, C.-F. (2007). Fractionation and identification of a novel hypocholesterolemic peptide derived from soy protein alcalase hydrolysates. *Food Research International, 40*, 756–762.

Zhu, K.-X., Zhou, H.-M., & Qian, H.-F. (2006). Antioxidant and free radicals scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. *Process Biochemistry, 41*, 1296–1302.