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Long term treatment with ACE inhibitor enalapril decreases body weight gain and increases life span in rats

Edson Lucas Santos [a], Kely de Picoli Souza [a], Elton Dias da Silva [b], Elice Carneiro Batista [b], Paulo J. Forcina Martins [c], Vânia D'Almeida [c], João Bosco Pesquero [b,*]

[a] School of Environmental and Biological Science, Federal University of Grande Dourados, Rodovia dourados - Itahum, Km 12, Dourados, MS, Brazil
[b] Department of Biophysics, Federal University of São Paulo, Rua Botucatu 862/7o. andar, Vila Clementino, 04023-062 São Paulo, SP, Brazil
[c] Department of Psychobiology, Federal University of São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Renin–angiotensin system is involved in homeostasis processes linked to renal and cardiovascular system and recently has been linked to metabolic syndrome. We analyzed the influence of long term angiotensin I converting enzyme (ACE) inhibitor enalapril treatment in normotensive adult Wistar rats fed with standard or palatable hyperlipidic diets. Our results show that long term enalapril treatment decreases absolute food intake, serum leptin concentration and body weight gain. Moreover, in adipose tissue, enalapril treatment led to decreased ACE activity, enhanced the expression of peroxisome proliferator activated receptor gamma, adiponectin, hormone-sensitive lipase, fatty acid synthase, catalase and superoxide dismutase resulting in prolonged life span. On the other hand, the ACE inhibitor was not able to improve the transport of leptin through the blood brain barrier or to alter the sensitivity of this hormone in the central nervous system. The effect of enalapril in decreasing body weight gain was also observed in older rats. In summary, these results extend our previous findings and corroborate data from the literature regarding the beneficial metabolic effects of enalapril and show for the first time that this ACE inhibitor prolongs life span in rats also fed with palatable hyperlipidic diet, an action probably correlated with adipose tissue metabolic modulation and body weight reduction.

1. Introduction

Obesity is considered a worldwide problem of public health showing an increased prevalence in developing countries. It is largely responsible for the epidemic surge of type 2 diabetes and cardiovascular disease, with urgent need for new and more efficient drugs and therapies. Since the adverse effects of obesity can be reversed by weight loss, which is essentially a reduction in adipose tissue mass, they are likely related to the massive accumulation of fat, especially visceral (omental) adipose tissue [1]. Yudkin et al. [2] suggested that obesity is associated with a low-level chronic inflammatory state that induces insulin resistance and endothelial dysfunction. In obesity, human adipose tissue presents enhanced release of TNF-α, interleukin-8 (IL-8), IL-10, IL-6, and IL-1β. Further, a large number of fibroblasts, mast cells, macrophages, leukocytes, and other cells involved in inflammation are embedded within the adipose tissue structure [1].

In humans and rodents, obesity is usually linked to fat mass gain, resistance to leptin action and hyperleptinaemia [3]. Leptin is the product of the *ob* gene which is secreted mainly by adipocytes and plays an important role in the regulation of body weight by controlling food consumption, sympathetic nervous system activation and thermogenesis [4]. This hormone is also involved in regulatory processes of immunity, inflammation and hematopoiesis [5].

The renin–angiotensin system (RAS) has angiotensin II (AngII), a potent vasoconstrictor, as the principal effector peptide [6]. The cleavage of angiotensinogen by renin generates angiotensin I, which is cleaved by angiotensin converting enzyme (ACE), generating AngII. A broad range of physiological actions of AngII occurs through its interaction with the receptor type 1 (AT_1) [7]. Traditionally, the RAS has been involved in physiological and pathophysiological processes linked to renal and cardiovascular system. Additionally, AngII has been implicated in the control of adiposity through synthesis and storage of lipid in adipocytes. More recently, RAS has also been linked to metabolic syndrome [8].

ACE inhibitors (ACEi) block the ACE mediated conversion of angiotensin I to AngII and the inactivation of kinins. Thus, the

Abbreviations: AngII, angiotensin II; ACE, angiotensin converting enzyme; ACEi, ACE inhibitors; FAS, fatty acid synthase; GLUT4, glucose transporter-4; GPX, glutathione peroxidase; HSL, hormone-sensive lipase; icv, intracerebroventricular; PPARγ, peroxisome proliferator activated receptor gamma; RAS, renin–angiotensin system; AT_1, receptor type 1; Cu/Zn-SOD, copper/zinc-superoxide dismutase; Mn-SOD, manganese-superoxide dismutase.

* Corresponding author. Tel.: +55 11 5572 4583; fax: +55 11 5571 5780.
E-mail address: jbpesq@biofis.epm.br (J.B. Pesquero).

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antihypertensive effects of ACEi are therefore believed to be a result of both decreased levels of vasoconstrictor peptide AngII and accumulation of kinins [9]. Many publications have demonstrated that enalapril, an ACEi, is able to reduce cardiovascular mortality and morbidity in patients with heart failure [10,11]. These protective roles of enalapril were shown to be related to protective effects on kidney [12,13] and cardiovascular system [14] in humans and rodents. In addition, the protective effect of enalapril on target organs was associated with a prolonged life span in rodents [13,14]. Carter et al. [15] have shown that enalapril improves physical performance in aged rats. In addition, it has been shown that enalapril is able to decrease body weight and body fat in young and adult rats [14,16]. In humans enalapril was also shown to reduce body weight [17]. Moreover recent investigation has revealed that mice lacking ACE have lower body weight and reduced fat mass [18]. Further, downregulation or disruption of the AngII type 1 receptor attenuate oxidative stress and stimulate the expression of prosurvival genes nicotinamide phosphoribosyltransferase and sirtuins [19,20]. However, despite much effort, the precise mechanisms responsible for the effects of enalapril in metabolic parameters, as well as in longevity, are still unclear.

The present study was designed to follow up our previous findings [16] and to investigate the metabolic effects of long term enalapril treatment in young and old normotensive rats. During six months of enalapril treatment, we evaluated body weight gain, food ingestion, serum insulin and leptin level. Additionally, we also investigated the transport of leptin across the blood brain barrier (BBB) and the sensitivity of central nervous system to exogenous leptin. In order to gain insight into the molecular mechanism of enalapril effect, we investigated the influence of the ACEi treatment in epididymal adipose tissue expression of genes involved in metabolic control like peroxisome proliferator activated receptor gamma (PPARγ), adiponectin, hormone-sensitive lipase (HSL), glucose transporter-4 (GLUT4), fatty acid synthase (FAS), catalase, copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPX) mRNAs. Finally, to rule out any detrimental effect of enalapril on the health status of the rats, we also followed life span of animals fed with standard or palatable hyperlipidic diet from 6 to 26 months of age.

2. Materials and methods

2.1. Animals and experimental procedure

All aspects of animal care and experimentation performed in this study agree with ethical principles in animal research adopted by Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethical Committee for Animal Research (Protocol 1554/05) from the Federal University of São Paulo.

Wistar male rats were obtained from the *Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia* (CEDEME) at the Federal University of São Paulo. The rats were maintained during 7 days in a temperature controlled room (23 ± 1 °C) with a 12 h light–dark cycle (lights on at 07:00 h), with free access to food and water. This first week was considered as an adaptation period. The experimental groups designed for each study were the following:

I Effect of long term treatment with enalapril on body weight gain, caloric intake, arterial blood pressure and serum measurements: 30-day-old Wistar male rats were randomly assigned in two diet groups (n = 20/group). The first group was fed with commercial standard diet (16 kJ/g), composed of 22% protein, 4.5% fat, 55% carbohydrate and 6.5% fiber (Nuvital Nutrientes S/A, Colombo, PR). The second group was fed with a palatable hyperlipidic diet (21 kJ/g, 35% of calories as fat), composed of 20% protein, 20% fat, 48% carbohydrate and 4% fiber [16,21]. The palatable hyperlipidic diet consisted of commercial rat chow plus peanuts, milk chocolate, and sweet biscuit in the proportion 3:2:2:1. All components were powdered, mixed and made into pellets. The caloric density of the diets was determined with an adiabatic calorimeter (IKA-C400). The use of palatable hyperlipidic diet has been previously shown to increase circulating leptin levels and lipogenesis, resulting in white adipose tissue accumulation [16,21]. Both groups were divided and received pure drinking water (control group) or solution containing enalapril (Aché-Biosintética, São Paulo, BR) 10 mg/kg per day, a dose currently used in the literature [14,22], generating four groups of 10 rats each. To ensure delivery of the correct drug doses, drug concentrations were adjusted in the drinking water each week based on the average water consumption and body weights in each group. These groups were monitored for 26 weeks (six months).

II *Effect of enalapril on life span*: Other four groups of 20 rats each were pooled as described in the previous experiment. However, these animals were checked for as long as 26 months (n = 20/group) and were used to analyze life span. During life span studies, the condition of all rats was monitored weekly by a veterinarian that checked for fur state, bone morphology alterations, food intake, locomotor activity in cage, foot ulcers, nasal bleeding, respiratory infections and presence of tumors by palpation.

III Effect of long term enalapril treatment in one-year-old rats: two groups of eight rats (one-year old) were treated for 20 weeks with standard diet and water with or without enalapril.

IV Intracerebroventricular (icv) administration of leptin in enalapril treated rats: three-month-old Wistar male rats (n = 7–9), were implanted with icv cannulas. Following the rats were fed with standard or palatable hyperlipidic diet and treated with enalapril for one month.

V Measurement of BBB permeability to intravenous ^{125}I-leptin in enalapril treated rats: three-month-old Wistar male rats were fed standard or palatable hyperlipidic diet and treated with or without enalapril for one month (n = 5/group).

VI *Effect of enalapril on gene expression and ACE activity*: Three-month-old Wistar male rats fed standard diet were treated with or without enalapril for one month (n = 10/group) for gene expression study and ACE activity in adipose tissue.

2.2. Serum measurements

Blood was collected by tail venipuncture monthly between 9 and 12 a.m. Leptin and insulin in serum samples were measured using the rat radioimmunoassay kits from Linco Research (St. Charles, MO).

2.3. Mean arterial blood pressure

Blood pressure was measured in conscious rats at 30 °C by tail-cuff plethysmography using a pressure transducer (PE-300 – Narco Biosystems, Austin, TX) connected to a polygraph recorder (Gould Recorder, Cleveland, OH).

2.4. Icv administration of leptin

Wistar male rats were implanted with icv cannulas as previously described [23]. Following, cannula placement was confirmed by monitoring drinking behavior in response to injection of 100 pmoles AngII. The rats were injected icv either with 3.5 μg leptin (R&D System, Inc., Mineapolis, USA) in 3 μl of saline or with saline alone. Food intakes were recorded 24 h after injection.

2.5. Measurement of BBB permeability to intravenous ^{125}I-leptin

Leptin was radioactively labeled by the iodobead method (Pierce Rockford, IL) with ^{125}I (Amersham-Pharmacia, Piscataway, NJ). ^{125}I-leptin was purified from free iodine on a Sephadex G-10 column. ^{125}I-leptin transport across the BBB was evaluated according to previously established method [24]. Briefly, rats were anesthetized with intraperitoneal injection of urethane (1.25 g/kg). ^{125}I-leptin (10^6 cpm) was injected intravenously, the rats were sacrificed and the blood and brain were removed. Serum was obtained from the whole blood by centrifugation (5000 × *g*, 10 min, 4 °C) and the level of radioactivity measured in 50 μl of the resulting serum or in the homogenized brain, measured in a gamma counter. The brain/serum ratio (μl/g) was calculated by the formula: (cpm/g of brain)/(cpm/μl serum) and was taken as an index of ^{125}I-leptin passage to the brain.

2.6. Analyses of gene expression by quantitative real time PCR

After sacrifice of rats, samples of epididymal adipose tissue were isolated, homogenized in TRIzol reagent (Invitrogen, USA) and processed according to the instructions of the manufacturer. Reverse transcription was performed using 2 μg of total pure RNA, 50 ng of random hexamers and 200 U of Maloney murine leukemia virus reverse transcriptase (Invitrogen, USA), as described by the manufacturers. First-strand cDNA synthesized from total RNA was used as the template for each reaction. Real time PCR reactions were done in duplicate and performed in a 10 μl reaction mixture consisting of 5 μl SYBR Green PCR Master Mix (Applied Biosystems), 1 μl cDNA and 0.3 μmol/l of each primer for a specific target on an Opticon II cycler (Bio-rad). The iCycle iQ Real Time PCR Detection System (Bio-Rad) was used for the signal detection, and all the PCR reactions were performed using QuantiTec SYBR Green PCR (QIAGEN GmbH, Germany) using 300 nmol/l of each primer. For standardization and quantification, rat β-actin was amplified simultaneously. The following primer sequences were employed: 5′-AACATCCCCAACTTCAGCAG-3′ (forward) and 5′-GGAAGAGGTACTGGCTGTCG-3′ (reverse) for the amplification of PPARγ, 5′-ATCTGATTACACCAAAAGT-3′ (forward) and 5′-TCAGTTGGTATCATGGTAG-3′ (reverse) for the amplification of adiponectin, 5′-CCCATAAGACCCCATTGCCTG-3′ (forward) and 5′-CTGCCTCAGACACACTCCTG-3′ (reverse) for the amplification of HSL, 5′-CTTGGGTGCCGATTACAACC-3′ (forward), and 5′-GCCCTCCCGTACACTCACTC-3′ (reverse) for the amplification of FAS, 5′-GGGCTGTGAGTGAGTGCTTTC-3′ (forward) and 5′-CAGCGAGGCAAGGCTAGA-3′ (reverse) for the amplification of GLUT4, 5′-AGCGGATTCCTGAGAGAGTG-3′ (forward) and 5′-GAGAATCGAACGGCAATAGG-3′ (reverse) for the amplification of catalase, 5′-CCAGTGCAGGACCTCATTTT-3′ (forward) and 5′-CCTTTCCAGCAG TCACATTG-3′ (reverse) for the amplification of Cu/Zn-SOD, 5′-ACACATTAACGCGCAGATCA-3′ (forward) and 5′-AATATGTCCCCCACCATTGA-3′ (reverse) for the amplification of Mn-SOD, 5′-GGTTTCCCGTGCAATCAG-3′ (forward) and 5′-CTCACCATTCACTTCGCACTT-3′ (reverse) for the amplification of GPX, 5′-AGAGGGAAATCGTGCGTGAC-3′ (forward), and 5′-CATAGTGATGACCTGTCCGT-3′ (reverse) for the amplification of rat β-actin. The initial step in the reaction was 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min. To obtain a calibration curve, serial dilutions of rat cDNA were used. The individual targets for each sample were quantified by determining the cycle threshold (Ct) and by using a calibration curve. Gene expression levels were normalized relative to the expression of β-actin, which served as the internal control, with results being determined in duplicate.

2.7. ACE activity

Adipose tissue ACE activity on Abz-FRK(Dnp)P-OH was determined under the optimal experimental conditions (buffer, pH, chloride and zinc ions) previously established for recombinant wild-type ACE [25]. Tissues were homogenized in Tris–HCl buffer, pH 7.0, containing 50 mM NaCl. Homogenates were centrifuged at 1000 × *g* for 10 min and the supernatant was frozen at −20 °C. The protein contents of the samples were measured using bovine serum albumin as standard. Enzymatic activity was continuously monitored with a Hitachi F-2005 fluorometer by measuring the fluorescence (λ_{ex} = 320 nm and λ_{em} = 420 nm) for 5–10 min. Before starting the reaction by the addition of the substrate, the plasma was preincubated for 5 min in a thermostated cuvette at 37 °C in the assay buffer. The sensitivity of the assay was tested with 1–10 μl homogenates incubated in a final volume of 200 μl. For ACE determination, 10 μl homogenates was incubated with 10 μM Abz-FRK(Dnp)P-OH at 37 °C in 0.1 M Tris–HCl, pH 7.0, containing 50 mM NaCl and 10 μM ZnCl2, in a final volume of 200 μl. The slope was converted into μM substrate hydrolyzed per minute based on a calibration curve obtained by complete hydrolysis of the peptide as reported previously by Araujo et al. [25]. ACE activity is reported as μM of Abz-FRK(Dnp)P-OH hydrolyzed per minute per μg of protein. The measurements were performed in triplicate.

2.8. Statistical analysis

The data were subjected to *Student's t*-test. Data were expressed as means ± standard error of means (SEM). To evaluate life span, the Kaplan–Meyer log rank test was performed. Differences were considered significant at $p < 0.05$. For the time-course body weight, caloric intake, leptin and insulin data repeated ANOVA test was applied.

3. Results

3.1. Body weight gain and caloric intake

The effectiveness of long term enalapril treatment in decreasing body weight gain in rats fed with standard or palatable hyperlipidic diets was evaluated by a time-course study performed during six months after weaning (Fig. 1). Our data show that in control rats receiving standard diet treated with enalapril there was a significant decrease in percentage of body weight gain (15% reduction, $p < 0.001$) when compared to control rats (Fig. 1A). An even stronger effect of enalapril on body weight could be observed in the rats receiving the palatable hyperlipidic diet (Fig. 1B), which exhibited a significant smaller body weight gain (22% reduction – $p < 0.001$), when compared to control rats.

Caloric intake in the presence of enalapril was significantly lower when analyzed as absolute food intake (Fig. 1C and D), but it was not different when adjusted for body weight (data not shown). In addition, long term enalapril treatment of one-year-old rats was also able to decrease the gain in body weight as compared to control rats receiving only water (Fig. 2).

3.2. Mean arterial blood pressure

Fig. 3A shows that, after six months of enalapril, the rats had significantly lower mean blood pressure values when compared to control rats in both regimens receiving only water. The observed values were 112 ± 6 mmHg versus 76 ± 5 mmHg ($p < 0.05$) and 106 ± 5 mmHg versus 68 ± 5 mmHg ($p < 0.05$), in control and palatable diets, respectively. However, no difference was observed in heart rates after enalapril treatment (Fig. 3B).

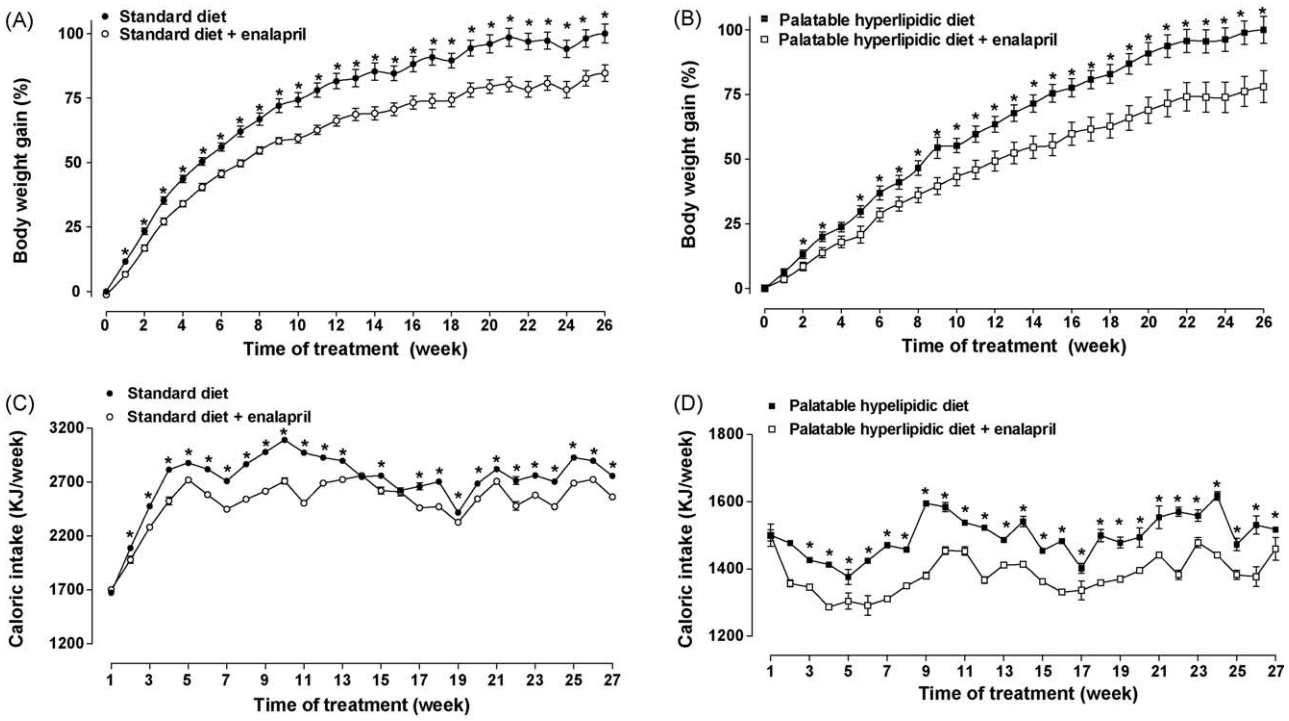

Fig. 1. Percentage of body weight gain of rats fed for 26 weeks (six months) with standard (A) or palatable hyperlipidic (B) diets. Food intake evaluation (in kJ) of rats fed with standard (C) or palatable hyperlipidic (D) diets. Rats received only drinking water (●, ■, for standard and palatable hyperlipidic diets, respectively) or enalapril (10 mg/kg per day) in the drinking water (○, □ for standard and palatable hyperlipidic diets, respectively). Data are expressed as mean ± SEM of 10 animals/group. $^{*}p < 0.05$.

3.3. Leptin and insulin measurements

Serum leptin and insulin levels were evaluated during the six-month period with enalapril treatment. As it can be observed, enalapril was able to decrease significantly leptin levels in both groups fed with the standard and the palatable hyperlipidic diet during the whole treatment (Fig. 4A and B). However, the levels of serum insulin were not altered during the treatment in both diets (Fig. 4C and D).

3.4. Adipose tissue ACE activity

ACE activity in the adipose tissue of enalapril treated rats was significantly reduced when compared with the control group. The observed values were 2.20 ± 0.19 μM/min/μg versus 1.64 ± 0.16 μM/min/μg ($p < 0.05$) for control and enalapril treated rats, respectively.

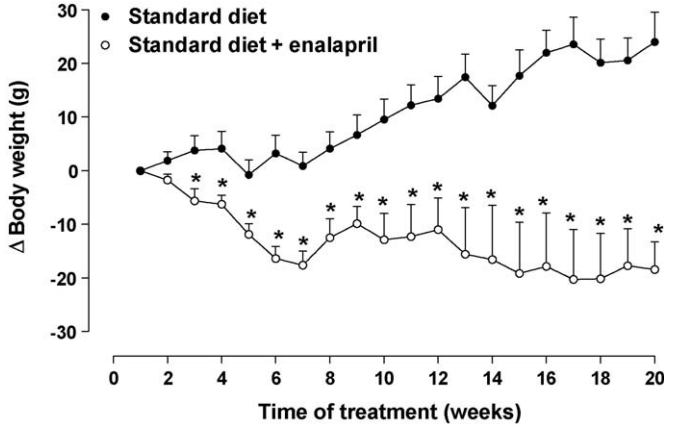

Fig. 2. Effect of enalapril on body weight of one-year-old Wistar rats treated with standard diet for 20 weeks. Data are expressed as mean ± SEM of eight animals/group. $^{*}p < 0.05$.

3.5. Gene expression in the epididymal adipose tissue

Enalapril increased significantly the levels of PPARγ, adiponectin, HSL and FAS mRNA in epididymal adipose tissue of treated rats when compared with control group (Fig. 5A–D, respectively). However, no alteration on the expression of GLUT4 was observed (Fig. 5E). Fig. 6 shows that enalapril treatment increased gene expression of the antioxidant enzymes catalase, Cu/Zn-SOD and Mn-SOD (Fig. 6A–C, respectively). However, no difference in GPX mRNA expression was observed after treatment (Fig. 6D).

3.6. Measurement of BBB permeability to intravenous ^{125}I-leptin and effect of icv injected leptin

In order to evaluate alterations in leptin resistance in enalapril treated rats, labeled leptin was injected intravenously in awaken rats and radioactivity was measured in serum and brain. Enalapril did not change the passage of intravenously injected ^{125}I-leptin across the BBB in any regimen (data not shown). In addition, administration of leptin icv decreased significantly and similarly the caloric intake in the standard and palatable hyperlipidic diet, as well as in control or enalapril treated rats (data not shown). No difference was observed in the effect of leptin when compared to the control with the enalapril treated groups in both regimens.

3.7. Effect of enalapril on life span

Life span was evaluated in rats receiving enalapril during 26 months. Over the time course of the study, the mortality rate reached 80% in the vehicle-treated rats in both diets (Fig. 7A and B). On the other hand, enalapril reduced mortality to 45% in the group fed with standard diet and 40% in the animals fed with palatable hyperlipidic diet ($p < 0.05$ vs. vehicle-treated rats). Eleven rats fed

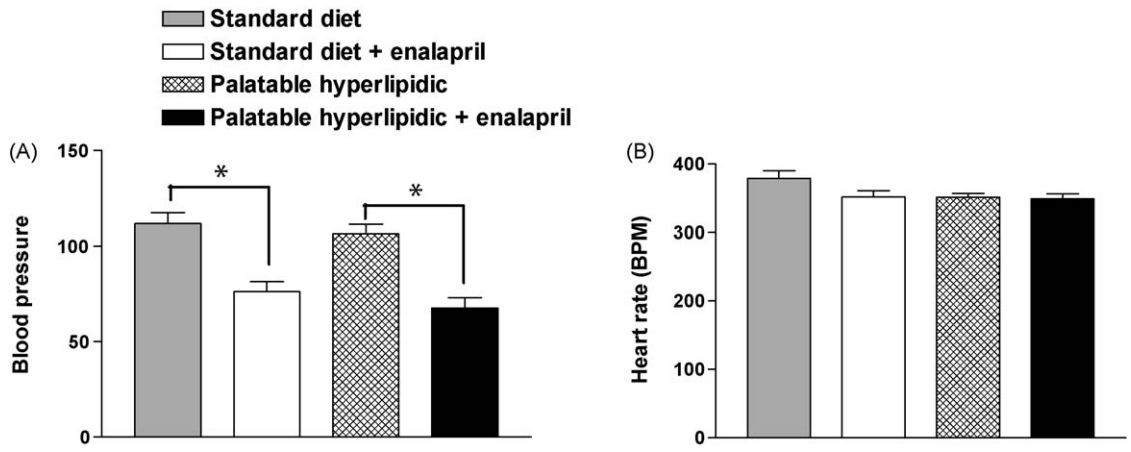

Fig. 3. Mean arterial blood pressure (A) and heart rate (B) after six months of treatment of rats with standard or palatable hyperlipidic diets and drinking water with or without enalapril. Data (in mmHg or beats per min, BPM) are expressed as the mean ± SEM of 10 animals/group. $^{*}p < 0.05$.

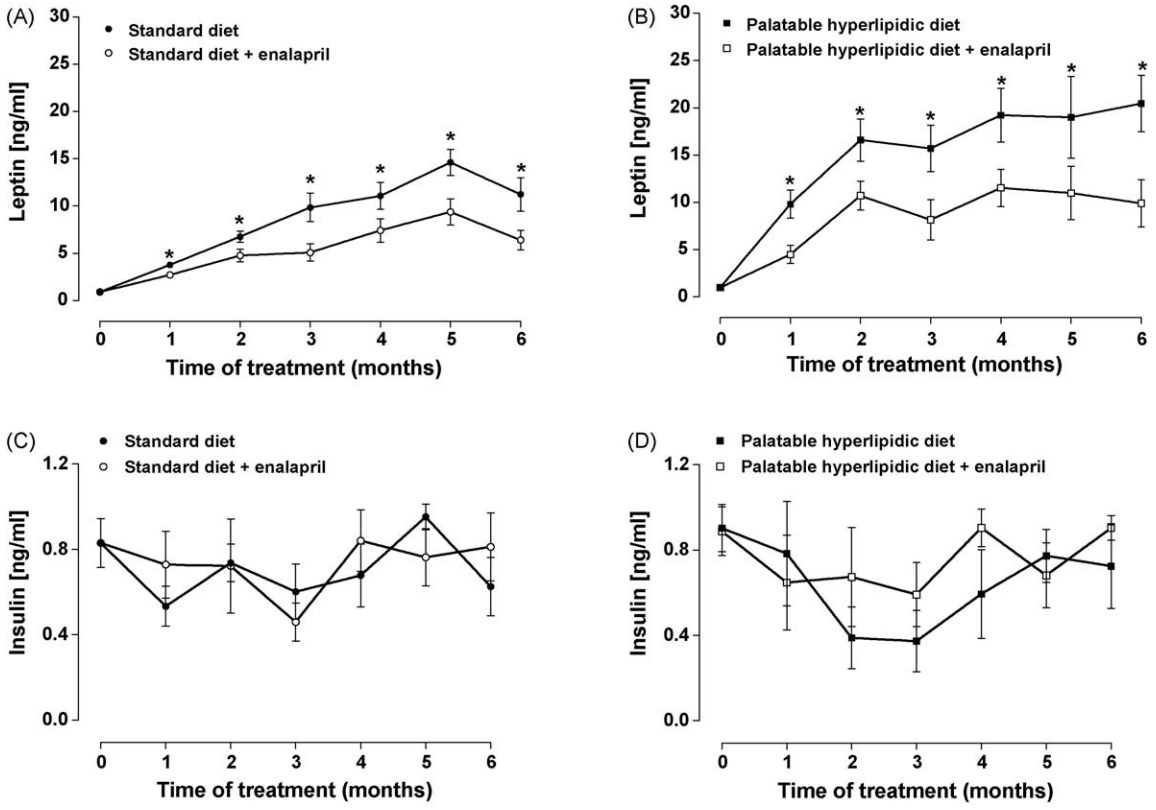

Fig. 4. Leptin (A and B) or insulin (C and D) levels in serum of rats treated for six months with standard (A and C) or palatable hyperlipidic (B and D) diets. Animals received only drinking water (●, ■, for standard and palatable hyperlipidic diets, respectively) or enalapril (10 mg/kg per day) in the drinking water (○, □ for standard and palatable hyperlipidic diets, respectively). Data are expressed as mean ± SEM of 10 animals/group. $^{*}p < 0.05$.

with standard diet and 12 fed with palatable hyperlipidic diet (from 20 animals each group) survived when treated with enalapril in comparison to four rats in the control groups.

4. Discussion

In the present study we analyzed the effect of long term enalapril treatment on metabolic parameters and life span. These data extend our previous findings using the same model, which showed similar effect of enalapril after a short period of treatment (one month) in young rats [16].

The pharmacologic or genetic blockade of RAS decrease its lipogenic effects, as reported by decreased body fat in rodents produced by ACE inhibition [14–16,26], genetic deletion of ACE [18] and adipose tissue hypotrophy by long term AT_1 receptor blockade [27]. In line with these findings, our study shows that enalapril produces an enhancement in the mRNA expression of the lipolytic genes PPARγ and HSL in the adipose tissue. The expression of PPARγ has been associated with increased energy expenditure, decreased glucose, insulin and triglycerides levels and decreased weight gain [18,27,28]. Consequently, PPARγ was shown to up-regulate the expression of HSL [29], an intracellular neutral lipase involved in triacylglycerol degradation [30]. Therefore, the enhanced expression of these genes, regulating an important metabolic pathway in white adipose tissue, might be a mechanism of action for enalapril, resulting in low body weight evolution and

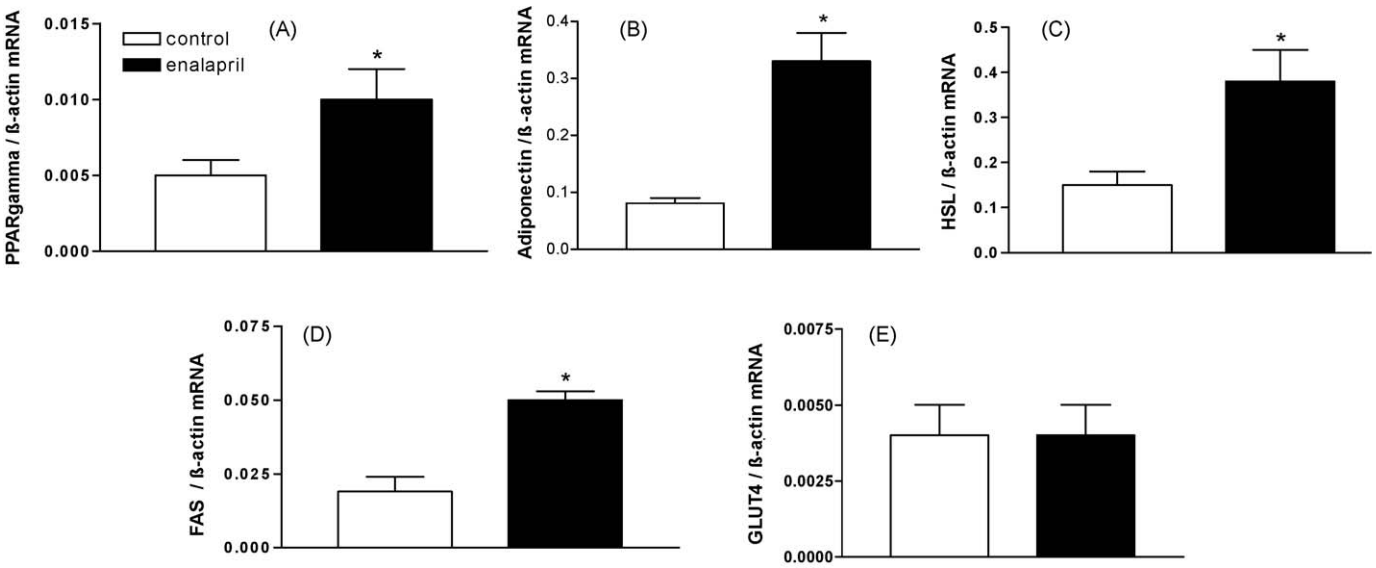

Fig. 5. Effect of enalapril on the expression of peroxisome proliferator activated receptor gamma (PPARγ), adiponectin, fatty acid synthase (FAS) and hormone-sensitive lipase (HSL) in epididymal adipose tissue. Expression of PPARγ (A), adiponectin (B), HSL (C), FAS (D) and GLUT4 (E) mRNA was normalized to the expression of β-actin. Data are expressed as mean ± SEM of 10 animals/group. $^{*}p < 0.05$.

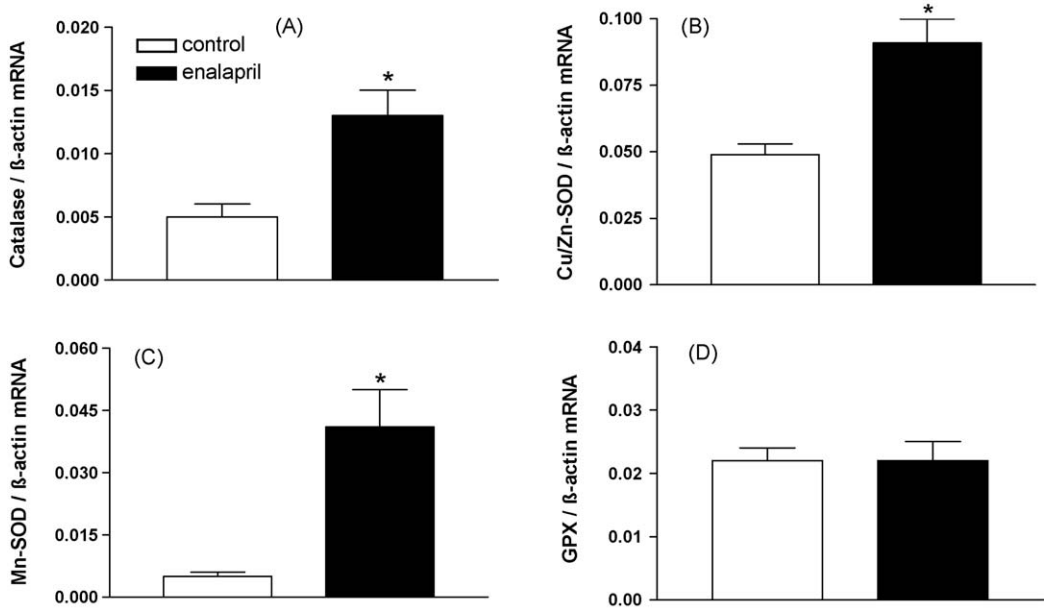

Fig. 6. Effect of enalapril on the expression of catalase, copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPX) in epididymal adipose tissue. Expression of catalase (A), Cu/Zn-SOD (B), Mn-SOD (C) and GPX (D) mRNA was normalized to the expression of β-actin. Data are expressed as mean ± SEM of five animals/group. $^{*}p < 0.05$.

the other metabolic alterations observed after treatment. On the other hand, the expression of FAS was also up-regulated in the adipose tissue of rats after enalapril treatment. This enzyme is involved in the conversion of acetyl-CoA into palmitate, which can subsequently be esterified into triacylglycerols and then stored in the adipose tissue [31]. Thus, the increased expression of this lipogenic enzyme after enalapril treatment, which is in agreement with the findings of Zorad et al. [27] using the AT_1 antagonist candesartan, could be a compensatory mechanism for the low levels of triglyceride content in the adipose tissue. Additionally, enalapril treated rats show increased expression of the antioxidant enzymes catalase, Cu/Zn-SOD, Mn-SOD and the hormone adiponectin, which are stimulated by PPARγ [32,33]. The antioxidant enzymes catalase and SOD eliminate hydrogen peroxide and superoxide radical, respectively, thereby generating the first line of intracellular, enzymatic, and antioxidative defense [34,35], which has been largely described in the literature for their relation with longevity in different animal models [36].

AngII induces growth of many cell types, including adipocytes [37], and influences the morpho-functional development of adipose tissue [38]. In addition, it stimulates the proliferation of preadipocytes and increases the number of mitotic cells [39], increases significantly triglyceride content in 3T3-L1 cells and human adipocytes by increasing activity and gene expression of the two key lipogenic enzymes, fatty acid synthase and glycerol-3-phosphate dehydrogenase [40]. Thus, the decreased ACE activity in

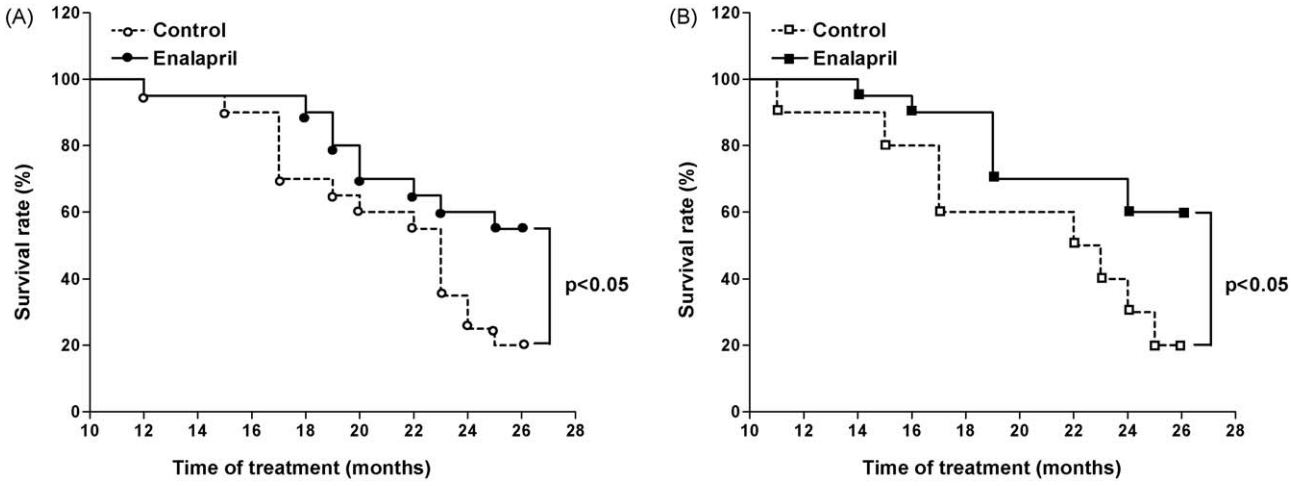

Fig. 7. Kaplan–Meier analysis of life span rate (%) of 26-month enalapril treated rats fed with standard diet (A) or palatable hyperlipidic (B) diets. Data are expressed as the mean ± SEM of 20 animals/group. $^{*}p < 0.05$.

the adipose tissue of enalapril treated rats could explain the reduced lipogenic effect resulting in lower body weight gain observed in our study. Furthermore, enalapril also decreased the food intake in both diets, an important factor to increase life span in diverse animal models [41].

Another important finding of this work was the decreased level of circulating leptin during the whole treatment with enalapril. The ACE inhibitor was not able either to improve the transport of leptin through the BBB or to alter its sensitivity in the central nervous system, conditions which could also lead to lower levels of circulating leptin and reduce caloric intake. Leptin is a hormone mainly produced by adipocytes in proportion to fat size stores [42]. Therefore, the lower serum leptin levels observed in this study probably reflect the decreased degree of fat accumulation promoted by ACEi in rats [15,16,26]. Low leptin levels can lead to a significant increase in daily activity, and therefore, decreased leptin levels can be excellent predictors for activated metabolism [43]. In this direction, enalapril has also been described to improve physical performance in aged rats [15].

In contrast to the effect on leptin levels, enalapril treatment led to an increase in adiponectin expression in the white adipose tissue, as also observed by Kohlstedt et al. [44]. This elevated adiponectin contributes to a lower inflammatory process and cardioprotection [45] and might also be a mechanism to explain the increased life span observed in the enalapril treated rats. In addition, increased life span is also linked to low oxidative stress, produced by increased expression of antioxidant enzymes, and a low inflammatory status produced by decreased caloric intake [46,47]. Furthermore, as shown for AngII type 1 receptor ablation [20], our data show that inhibition of AngII formation by reducing ACE activity decreases blood pressure and attenuates oxidative stress, resulting probably in target organs protection [14] and prolonging life span.

In summary, our results show beneficial effects of chronic enalapril treatment on metabolic homeostasis and life span in Wistar rats fed with standard and palatable hyperlipidic diets, supporting the potential use of this drug in metabolic diseases treatment. The mechanism of action of enalapril seems to be mediated by reduced ACE activity in the adipose tissue and modulation of PPARγ, followed by overexpression of adiponectin and antioxidant genes.

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