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## The renin–angiotensin system is modulated by swimming training depending on the age of spontaneously hypertensive rats

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### ABSTRACT

**Aim:** To investigate the effects of swimming training on the renin–angiotensin system (RAS) during the development of hypertensive disease.

**Main methods:** Male spontaneously hypertensive rats (SHR) were randomized into: sedentary young (SY), trained young (TY), sedentary adult (SA), and trained adult (TA) groups. Swimming was performed 5 times/wk/8wks.

**Key findings:** Trained young and adult rats showed both decreased systolic and mean blood pressure, and bradycardia after the training protocol. The left ventricular hypertrophy (LVH) was observed only in the TA group (12.7%), but there was no increase on the collagen volume fraction. Regarding the components of the RAS, TY showed lower activity and gene expression of angiotensinogen (AGT) compared to SY. The TA group showed lower activity of circulatory RAS components, such as decreased serum ACE activity and plasma renin activity compared to SA. However, depending on the age, although there were marked differences in the modulation of the RAS by training, both trained groups showed a reduction in circulating angiotensin II levels which may explain the lower blood pressure in both groups after swimming training.

**Significance:** Swimming training regulates the RAS differently in adult and young SHR rats. Decreased local cardiac RAS may have prevented the LVH exercise-induced in the TY group. Both groups decreased serum angiotensin II content, which may, at least in part, contribute to the lowering blood pressure effect of exercise training.

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### Introduction

Left ventricular hypertrophy (LVH) and diastolic dysfunction are the early manifestations of cardiac damage in patients with arterial hypertension characterizing hypertensive heart disease (Diamond and Phillips 2005). LVH is widely recognized as a powerful predictor of cardiovascular morbidity and mortality, regardless of blood pressure (BP) levels and other cardiovascular risk factors (Agabiti-Rosei and Muiasan 2002). The presence of LVH increases both cardiovascular risk and poor clinical outcomes in hypertensive patients (Ruilope and Schmieder 2008).

Improvements in lifestyle have been prescribed under different pathological conditions such as hypertension, diabetes, heart failure and obesity. Among these changes, in case of hypertension, physical

activities are used as the first and the most promising step, leading to a reduction in blood pressure, a decrease of lowering medication for blood pressure and a delay of end-organ damage. Dynamic exercise induces physiological LVH, which is characterized by a predominant increase in the left ventricle (LV) chamber, although increases in wall thickness and in the LV mass are also observed (Woodiwiss and Norton, 1995). These changes are associated with an improvement of the LV function (Caso et al. 2000; Pluim et al., 2000). Moreover, dynamic exercise leads to other beneficial adaptations, such as, reduced heart rate (HR) for a given workload, an important marker of a better prognosis (Levine 1997) and blood pressure reduction, particularly in hypertensive individuals (Laterza et al. 2007).

The renin–angiotensin system (RAS) is critical in regulating systemic blood pressure and pathogenesis of the hypertension. The relationship between hypertension to all-cause mortality and the incidence of cardiovascular disease among human beings have been reported to be age-dependent (Franklin et al. 2001; Lewington et al. 2002; Port et al. 2000). Angiotensin II (AngII), a major effector molecule of the renin–angiotensin system, stimulates atherosclerosis through various deleterious effects such as endothelial dysfunction,

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cellular proliferation and inflammation (Sata and Fukuda 2010). Ang II also promotes inflammation, generation of reactive oxygen species and governs the onset and progression of vascular senescence, all of which are associated with functional and structural changes, contributing to age-related diseases (Abadir 2011) and hypertension.

The RAS is a primary treatment for hypertension. The administration of angiotensin converting-enzyme inhibitors and angiotensin receptor antagonists attenuate or prevent the development of hypertension and its consequences on target organs (Freslon and Giudicelli 1983; Oddie et al. 1992; Wu and Berecek, 1993). Since exercise training is one of the most effective non-pharmacological interventions prescribed to hypertensive individuals, it is of extreme importance to understand the effects of regular physical exercise on the RAS.

No previous studies however have provided explanations regarding the RAS modulation during the establishment (young) and the maintenance (adult) of the hypertensive process, as recently reviewed by Schlüter et al. (2010) and even less is known about the effects of the exercise training on the RAS in a hypertensive state. The relevance of this study is to clarify the benefits of changes in lifestyle before or after the hypertensive disease has been already established. This knowledge would be of particular importance to those who have hypertensive parents or have genetic propensity to developing hypertension.

Previously, we investigated the RAS modulation during the time-course of hypertension, and the effects on hemodynamic and morphometric parameters (Zamo et al. 2010). This work was undertaken to investigate the effects of swimming training (ST) in different stages of hypertension on 1) hemodynamic patterns (blood pressure and heart rate); 2) left ventricular hypertrophy and collagen volume fraction; and 3) local and systemic RAS of spontaneously hypertensive rat (SHR).

## Materials and methods

### Animals

Male SHR (from Central Bioterium of the Federal University of São Paulo, Brazil) 4-week old (young) and 12-week old (adult) were used. The animals were randomly divided into four groups: sedentary young (SY,  $n = 13$ ), trained young (TY,  $n = 13$ ), sedentary adult (SA,  $n = 12$ ) and trained adult (TA,  $n = 13$ ). Each group was subdivided into 2 groups: 1) for hemodynamic, biochemical and molecular studies, and 2) for histological studies. The animals were housed in standard cages (4 per cage). Food and water were provided ad libitum. Room temperature was kept at  $23 \pm 1$  °C, and a 12:12 dark-light cycle was maintained throughout the experimental period. Animals were weighed on the first day of each week, every week. This study was conducted in accordance with the guidelines of the Brazilian College of Animal Experimentation (COBEA). This research was approved by the Ethics Committee of the Federal University of Sao Paulo (#118/01).

### Swimming protocol

Swimming training was performed as described previously (Medeiros et al. 2004). The physical training was done for 8 weeks, 1 hour/day, 5 days/week, with a gradual increase of workload (until the weight placed on the tail reached 5% of body weight) in a heated pool (30–32 °C) adapted for rats. The workload correspondent to each animal was corrected every week. Sedentary rats were placed in the swimming apparatus for 10 min, twice a week to simulate the water stress associated with the experimental protocol. This protocol is defined as a low-intensity, long training period, effective for cardiovascular adaptations and an increase in muscle oxidative capacity (Baker and Horvath 1964; Oliveira et al. 2009).

### Measurement of hemodynamic signals – BP and HR

Twenty-four hours after the last training session, sedentary and trained rats were anesthetized (ketamine, 90 mg/kg and xylazine, 10 mg/kg; i.p.) and catheterized in their right femoral artery. Twenty-four hours after catheterization, the signals of resting BP of conscious rats were recorded (2 kHz) on a beat-to-beat basis using AT/CODAS (DataQ Instruments, Ohio, USA). A strain-gauge transducer (Statham P23 Db) was used for arterial pressure measurement. The transducer signal was fed to an amplifier (GPA-4, model 2, Stemtech) and further to a 10-bit analog-to-digital converter, which was interfaced to a computer. The HR was obtained from arterial BP pulses.

### Serum and tissue samples

Twenty four hours after hemodynamic measurements, the animals were sacrificed by decapitation, without prior anesthesia, and blood was collected from the neck wound and centrifuged (1500 g, 15 min at 4 °C). All blood samples were collected into chilled glass tubes: for PRA assay, the tubes contained potassium EDTA; for ACE I activity assay, the tubes had no anticoagulant; and to determine angiotensin II (Ang II) levels, the tubes contained a mixture of potassium EDTA (25 mM), *o*-phenanthroline (0.44 mM), pepstatin A (0.12 mM), and 4-chloromercuribenzoic acid (1 mM) to prevent the *in vitro* production and degradation of angiotensin peptides (Kohara et al. 1991). The blood was centrifuged, and the plasma or serum was separated and stored at  $-20$  °C. Heart tissues were dissected, and the LV was isolated and stored at  $-80$  °C until biochemical and molecular analyses were performed.

### Cardiac hypertrophy

To measure cardiac mass, the heart was removed from the thoracic cavity and dissected to separate the LV. To measure cardiac mass, the LV was dissected from the remaining tissue on removal of the atria and the free wall of the right ventricle. The interventricular septum remained as part of the LV. To evaluate cardiac hypertrophy, left ventricle weight (LVW) was normalized by the length of the animal's tibia (LVW/TL in mg/mm) as suggested by Yin et al. (1982).

For morphometric analysis, rats were killed by decapitation, the chest was opened and the hearts were stopped at diastole by perfusion with 14 mM KCl. After the heart was weighed, the LV was fixed at 6% formaldehyde and embedded in paraffin, cut into 5  $\mu$ m sections at the level of the papillary muscle, and subsequently stained with hematoxylin and eosin for the visualization of cellular structures.

Myocardial interstitial collagen volumetric fraction (CVF) was determined by using polarized light in the Picrosirius red prepared tissues, as previously reported (Junqueira et al. 1979). In short, the section was placed in a projection microscope ( $\times 200$ ), and 20 fields were selected and interstitial collagen was determined by a computer-assisted image analysis system (Quantimet 520; Cambridge Instruments), by a blinded observer for the experimental groups. CVF was calculated as the sum of all connective tissue areas divided by the sum of all muscle areas in all fields. Perivascular collagen was specifically excluded from this determination. The fibrillar collagen data are presented as percentage (%).

### Measurement of Ang II in serum

The samples were passed through phenyl silica cartridges (Sep-Pak C18 columns, Waters, Milford, Massachusetts, USA), and the absorbed angiotensin was eluted with methanol. Eluate was dried in a vacuum centrifuge and the pellet was re-suspended in the Enzyme Immunoassay buffer (EIA buffer), mixed and centrifuged at 3000 g for 10 min at 4 °C. Ang II levels were determined by ELISA, according to the

manufacturer's instructions (SPI-BIO #A05880, Montigny-le-Bretonneux, France).

#### Serum and cardiac ACE activity

ACE activity was determined by a fluorometric assay using Abz-FRK(Dnp)P-OH derivatives as substrates by continuously measuring the fluorescence according to Alves et al. (2005). Cardiac SHR tissues were quickly harvested, rinsed, and homogenized in Tris-HCl buffer, pH 7.0, containing 50 mM NaCl and centrifuged at 1000 g for 10 min. The assays were performed at 37 °C in 0.1 M Tris-HCl buffer, pH 7.0, containing 50 mM NaCl and 10 μM ZnCl<sub>2</sub>. The hydrolysis rate of the intramolecularly quenched fluorogenic substrate Abz-YRK-(Dnp)p (10 mM) incubated with aliquots of the homogenate of tissues and serum for 30 min at 37 °C was assessed to obtain ACE enzymatic activity. Fluorescence increments along the time were read at 420 nm emission: 320 nm excitation. Cardiac and serum ACE activities were expressed as arbitrary fluorescence units (AFU)·min<sup>-1</sup>·mg<sup>-1</sup> of protein × 1000. The protein content was determined by the Bradford method (Bradford 1976) by using bovine serum albumin as the standard (Bio-Rad protein assay).

#### Plasma renin activity (PRA) assay

The PRA was measured by angiotensin I radioimmunoassay, using a commercially available kit (REN-CT2, CIS Bio International). This assay was conducted according to the manufacturer's instructions and it permits direct measurement of PRA. Results were quantified using a Gama Counter (Cobra II Autogamma, Packard, Meriden, Connecticut), and the enzyme activity was expressed as ng Ang I/mL/h.

#### MRNA quantitation using real-time PCR

The relative gene expression of cardiac angiotensinogen (AGT) was analyzed by real-time PCR.

Total RNA was isolated from LV tissue with a Trizol reagent (GIBCO Invitrogen). Total RNA concentration and integrity were assessed and the real-time polymerase chain reaction was performed. The mRNA expression of the renin-angiotensin system components were assessed by oligonucleotide primers as follows: for rAgtF: 5'-TCTACCTTTTGGGTGCTG-3', rAgtR: 5'-CAAGGAGGATGCTGTTGAGA-3', expected product size: 88 bp. The expression of *Ppia* (peptidylprolyl isomerase A – cyclophilin A) (rPpiaF: 5'-AATGCTGGACCAACACAAA-3', rPpiaR: 5'-CCITCTTTCACCTCCCAA-3', expected product size: 101 bp) was measured as an internal control for a sample variation in RT reaction. Real-Time PCR amplifications were performed with an ABI Prism 7700 Sequence Detection System (Applied Biosystems) by using SYBR Green PCR Master Mix (Applied Biosystems). The results were quantified as Ct values, where Ct is defined as the threshold cycle of the polymerase chain reaction at which the amplified product is first detected. Values for the control gene (*Ppia*) were used to standardize the results in order to compensate for differences in RNA content among the samples. To compare the level of gene expression in the trained group to the level in the control group, the following formula was applied  $e = 2^{-\Delta\Delta Ct}$ .

#### Statistical analysis

All values are presented as mean ± standard error. The results were analyzed by a Student's *t*-test between groups at the same age, except for BW, which was analyzed using 2-way ANOVA for repeated measures. Tukey's post hoc test was used between means when a significant change was observed with ANOVA. A *p*-value of less than 0.05 was considered statistically significant.

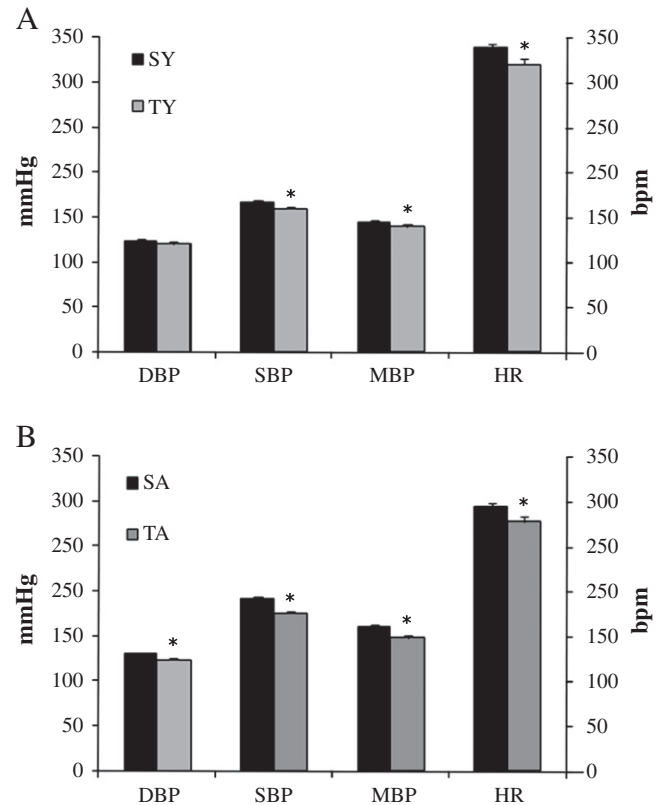
## Results

### Hemodynamic parameters

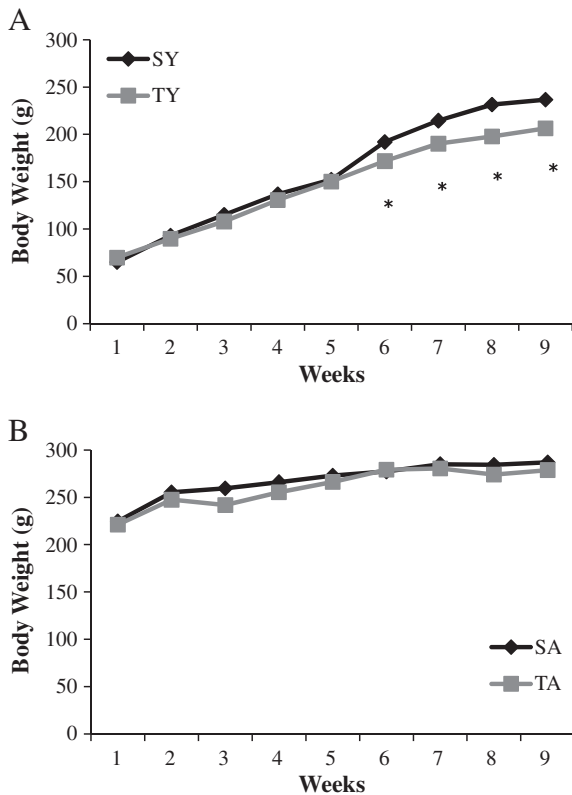
The resting values of diastolic blood pressure (DBP), systolic blood pressure (SBP), mean blood pressure (MBP) and heart rate (HR) are shown in Fig. 1. Swimming training decreased SBP and MBP both in TY (SBP: SY, 168 ± 2 mmHg vs TY, 160 ± 2 mmHg, *p* < 0.05; MBP: SY, 145 ± 2 mmHg vs TY, 140 ± 2 mmHg, *p* < 0.05) and TA groups (SBP: SA, 191 ± 3 mmHg vs TA, 175 ± 3 mmHg, *p* < 0.05; MBP: SA, 161 ± 2 mmHg vs TA, 149 ± 2 mmHg, *p* < 0.05) when compared to their controls. Swimming training decreased significantly DBP only in the adult group (DBP: SA, 131 ± 1 mmHg vs TA, 124 ± 2 mmHg, *p* < 0.05), while no reduction was observed in the young group (SY, 123 ± 2 mmHg vs TY, 120 ± 2 mmHg, *p* > 0.05). In Fig. 1, it can also be observed that both young (SY, 339 ± 4 bpm vs TY, 321 ± 6 bpm, *p* < 0.05) and adult (SA, 294 ± 5 bpm vs TA, 278 ± 5 bpm, *p* < 0.05) groups showed resting bradycardia after the swimming training, which indicates that aerobic conditioning was achieved by the exercise protocol (Medeiros et al. 2004; Tipton 1965).

### Body weight and cardiac hypertrophy

Body weight (BW) was measured weekly whose values are shown in Fig. 2. The BW at the beginning of the training protocol was similar among groups of the same age (SY, 65 ± 1 g vs TY, 70 ± 2 g; SA, 225 ± 4 g vs TA, 221 ± 2 g). However, in the sixth week



**Fig. 1.** Hemodynamic parameters after swimming training protocol. A) SY, sedentary young (*n* = 6); TY, trained young (*n* = 7). B) SA, sedentary adult (*n* = 7); TA, trained adult (*n* = 7). Blood pressures as diastolic (DBP), systolic (SBP) and mean (MBP) and the heart rate (HR). SY compared to TY, and SA compared to TA. Values are shown as means ± SEM. \**p* < 0.05.



**Fig. 2.** Body weight changes during the swimming training protocol. A) SY, sedentary young ( $n=13$ ); TY, trained young ( $n=13$ ). B) SA, sedentary adult ( $n=12$ ); TA, trained adult ( $n=13$ ). SY compared to TY, and SA compared to TA. Values are shown as means  $\pm$  SEM. \* $p<0.05$ .

of the swimming training protocol, the SY group became significantly heavier than the TY group and at the end of the swimming training protocol, the groups showed different BW (SY,  $237 \pm 5$  g vs TY,  $206 \pm 4$  g,  $p<0.05$ ). Previously, our group using this same training protocol had already observed that this lower weight gain

in trained rats was associated with a decreased intraperitoneal fat mass (Rocha et al. 2007).

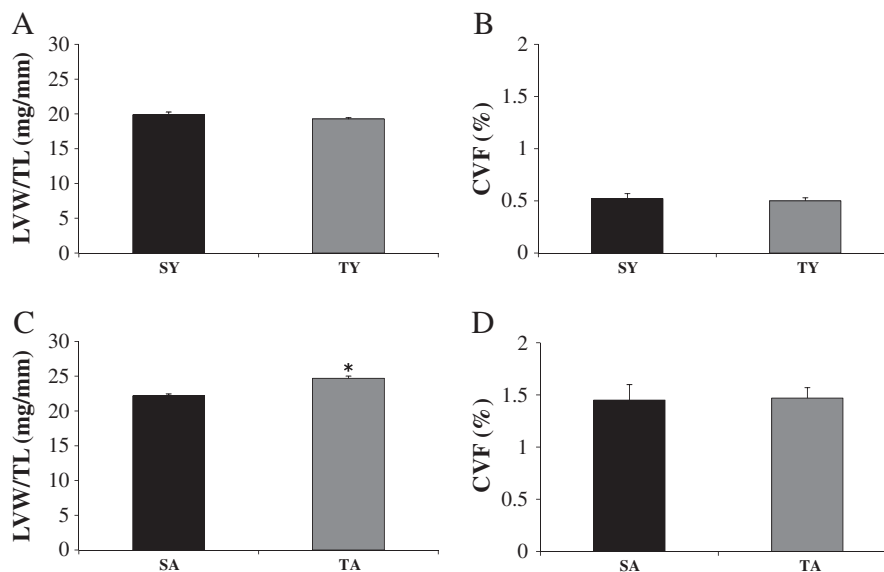
Due to this significant difference of BW, the left ventricular hypertrophy (LVH) was evaluated by using the left ventricular weight and tibia length ratio (LVW/TL) as suggested by Yin et al. (1982). Fig. 3 shows the data referring to the LVH (3A and 3C) and cardiac fibrosis (3B and 3D) evaluated by the CVF method. The increase of the LVH can be observed only in the adult group (3C, 12.7%) (SY,  $19.87 \pm 0.43$  mg/mm vs TY,  $19.28 \pm 0.19$  mg/mm,  $p>0.05$ ; SA,  $22.16 \pm 0.29$  mg/mm vs TA,  $24.70 \pm 0.33$  mg/mm,  $p<0.05$ ).

#### Biochemical analysis of the renin–angiotensin system

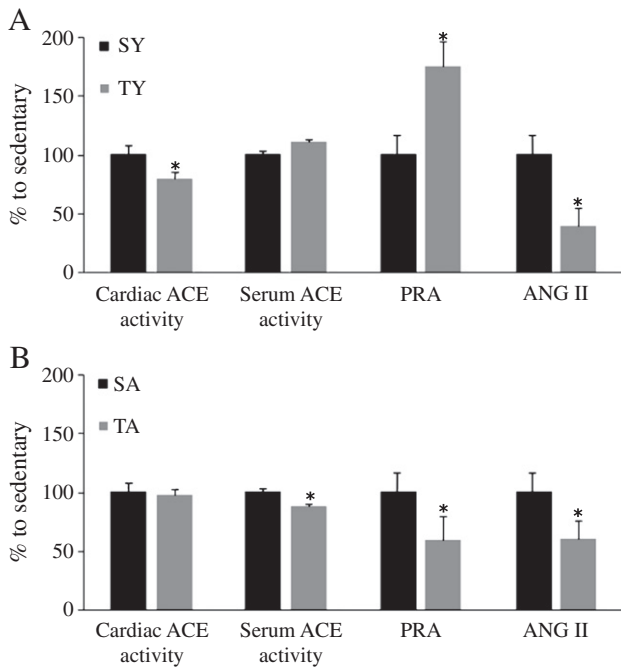
##### ACE, PRA and Ang II

Renin and ACE are the two main enzymes that regulate the formation of the peptide angiotensin II. Fig. 4 shows local (cardiac) and systemic ACE activities, Ang II and PRA. Swimming training decreased 20% of the cardiac ACE activity (SY,  $1579 \pm 108$  UF $\cdot$ min $^{-1}\cdot$ mg $^{-1}$  vs TY,  $1266 \pm 87$  UF $\cdot$ min $^{-1}\cdot$ mg $^{-1}$ ,  $p<0.05$ ) while it increased 75% of the PRA in the TY group compared to the SY group (SY,  $0.45 \pm 0.07$  ng AngI $\cdot$ ml $^{-1}\cdot$ h $^{-1}$  vs TY,  $0.79 \pm 0.09$  ng AngI $\cdot$ ml $^{-1}\cdot$ h $^{-1}$ ,  $p<0.05$ ). On the other hand, swimming training decreased 11% of the serum ACE activity (SA,  $28041 \pm 712$  UF $\cdot$ min $^{-1}\cdot$ mg $^{-1}$  vs TA,  $24,893 \pm 807$  UF $\cdot$ min $^{-1}\cdot$ mg $^{-1}$ ,  $p<0.05$ ) and 39% of the PRA in the TA group compared to the SA group (SA,  $1.35 \pm 0.05$  ng AngI $\cdot$ ml $^{-1}\cdot$ h $^{-1}$  vs TA,  $0.83 \pm 0.05$  ng AngI $\cdot$ ml $^{-1}\cdot$ h $^{-1}$ ,  $p<0.05$ ). No difference was observed in either serum ACE activity of young animals or cardiac ACE activity of adult animals after the swimming training protocol.

Serum Ang II concentration was evaluated by ELISA. Ang II levels were lower in both trained groups when compared to their respective sedentary groups (SY,  $77.6 \pm 13$  vs TY,  $31.4 \pm 11$  pg/mL; SA,  $58.8 \pm 5.1$  vs TA,  $36 \pm 5.2$  pg/mL, both  $p<0.05$ ). Although there were marked differences in the modulation of the RAS by training depending on the age, both young and adult trained groups showed a reduction of 60% and 39%, respectively in angiotensin II levels.



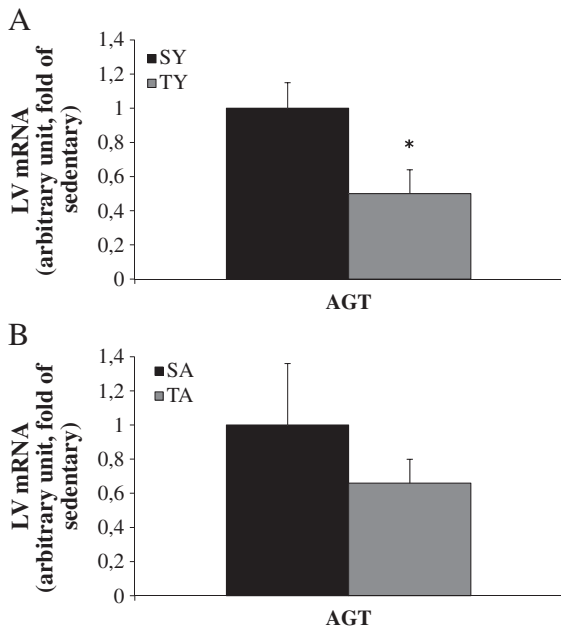
**Fig. 3.** Left ventricular hypertrophy and collagen volume fraction. A) Left ventricle weight/tibia length ratio of young groups. B) Collagen volume fraction of young groups. C) Left ventricle weight/tibia length ratio of adult groups. D) Collagen volume fraction of adult groups. SY, sedentary young ( $n=5$ ); TY, trained young ( $n=4$ ); SA, sedentary adult ( $n=5$ ); TA, trained adult ( $n=4$ ). SY compared to TY, and SA compared to TA. Values are shown as means  $\pm$  SEM. \* $p<0.05$ .



**Fig. 4.** Cardiac and serum ACE activity, serum angiotensin II levels and plasma renin activity (PRA). A) SY, sedentary young (n = 8), TY, trained young (n = 9). B) SA, sedentary adult (n = 7), TA, trained adult (n = 9). SY compared to TY, and SA compared to TA. \*p < 0.05.

*Gene expression by real time-PCR*

Fig. 5 shows the relative gene expression of AGT. AGT gene expression was decreased in TY compared with SY (50%, p < 0.05). Otherwise, AGT gene expression was not significantly decreased in the TA group compared with the SA group.



**Fig. 5.** Gene relative expression to cardiac ACE and AGT. A) SY, sedentary young (n = 8), TY, trained young (n = 7). B) SA, sedentary adult (n = 8), TA, trained adult (n = 7). SY compared to TY, and SA compared to TA. \*p < 0.05.

**Discussion**

In this study it is important to consider that age refers to the time of hypertension. Once hypertension in SHR is established at 2-month old animals, at the end of the protocol, the young groups had already passed the age of becoming hypertensive, and primarily because of the chronic effects of exercise. The young trained group did not reach the genetically programmed elevation of blood pressure levels nor the adaption observed in the hypertensive state (adult group).

The main finding of this study is that although swimming training induces hemodynamic adjustments in SHR regardless of the animal's age, the cardiac hypertrophy and the RAS modulation were distinctive.

The similar hemodynamic adaptive responses observed in young and adult SHR, bradycardia and reduced BP are probably associated with the decrease in the sympathetic activity and with the increase in the vagal tone to the heart (Medeiros et al. 2004). Indeed, other studies in trained mice (Evangelista et al. 2003) and rats (Negrão et al. 1992) have shown that these animals presented a resting bradycardia due to an attenuation of the intrinsic activity of pacemaker cells.

At the end of the swimming training protocol, the blood pressure was decreased in both TY and TA groups, which suggests that swimming training may be an alternative non-pharmacological treatment of hypertension regardless of whether the disease is already established or not. This decrease in BP may be attributed to the attenuation of sympathetic activity to the heart leading to bradycardia and, consequently, to a reduction of cardiac output (Negrão et al. 1993; Vêras-Silva et al., 1997. This lower sympathetic activity induced by exercise training could also be attributed to the improvement of arterial baroreflex and chemosensitive cardiopulmonary baroreflex sensitivity in SHR (Krieger et al. 1999; Silva et al. 1997), which modulate the peripheral autonomic nervous system.

In addition, other peripheral mechanisms cannot be excluded. In fact, Azevedo et al. (2003) suggest that the observed BP decrease in moderate intensity treadmill-trained SHR is due to the attenuation in total peripheral vascular resistance. The probable mechanisms of this reduction include improvement of endothelial function by increasing shear stress and stimulating an effect over the production of nitric oxide (NO) by the endothelium (Sherman, 2000). Also, the pressure-lowering effect of exercise training can be associated with peripheral structural adaptations such as growth and proliferation of small venules and regression of hypertrophied arteriole wall/lumen ratio in exercised skeletal muscle (Melo et al. 2003).

Modulations of the renin-angiotensin system by exercise training, in adult or young rats, resulted in decreased serum angiotensin II levels, which may, at least in part, contribute to a pressure-lowering effect of exercise training. It has already been described that circulating RAS has a main role on blood pressure control, while local RAS modulates mainly tissue remodeling. (Bader and Ganten 2008; Li et al. 2008; Paul et al. 2006). A treatment of SHR with an ACE inhibitor or blockers of angiotensin receptors lowers the blood pressure suggesting that this system is up-regulated and that the blockade of the angiotensin II may prevent or control hypertension (Lundie et al. 1997; Paull and Widdop, 2001)

The renin-angiotensin system is one of the most studied hormonal mechanisms involved in the regulation of blood pressure in hypertensive disease. Its blockade can prevent the LVH, thereby reducing the significant and independent cardiovascular risk conferred by pathological LVH. Regression of left ventricular mass is also achieved by other medication classes, but the RAS blockers have an additional beneficial effect for the same

blood pressure reduction, for which, the mechanism is not entirely clear (Cowan and Young 2009). Our group as well as others has already shown the efficacy of exercise training as a non-pharmacological therapeutic treatment of hypertension (Chobanian et al. 2003; Laterza et al. 2007; Pescatello et al. 2004; Silva et al. 1997).

Recently our group demonstrated that the physiological LVH induced by swimming training is regulated by local RAS regardless of the systemic components, because the hypertrophy response was maintained even when PRA was inhibited (Barauna et al. 2008; Oliveira et al. 2009). In our data, even with the down-regulation of the PRA, we observed increased left ventricle mass in the TA group which is in agreement with the result that exercise did not downregulate the cardiac components of the RAS. In addition, the usual LVH induced by exercise was prevented in the TY group and we also observed downregulation of both ACE cardiac activity and ATG cardiac gene expression in this group. Although in elite human athletes, the training-induced left ventricular hypertrophy was shown to be influenced by ACE gene polymorphism (Montgomery et al. 1997). The same results have not been confirmed in animal models (Evangelista and Krieger 2006). The number of ACE gene copies per se had no influence on basal cardiac mass or on the magnitude of swimming-induced cardiac hypertrophy. This result suggests that small isolated genetic disturbance in ACE cardiac levels can be compensated under physiological stress such as exercise training.

Although the mechanism involved in cardiac hypertrophy by exercise training is still unclear, we have already shown that it is regulated independently by the local RAS from the systemic RAS. The hypertrophic response was maintained even when PRA was inhibited (Steiner et al. 2005; Barauna et al. 2008; Oliveira et al. 2009). Moreover, recent evidences have indicated that the cardiac Ang II level is implicated in fibrosis induction. Cardiac Ang II is not required for left ventricle hypertrophy (Adams et al., 2004). Transgenic animal models for RAS demonstrated that an accentuated formation of the local Ang II in the heart does not develop hypertrophy; it presents an increase of fibrosis and it develops hypertrophy only when an excess of cardiac Ang II enters the circulation and causes an increase in BP (Adams et al. 2004; Steiner et al. 2005; Dimmeler et al. 2001). Recently, Xiao et al. (2008) reported that mice expressing ACE only in the heart showed that the increase of cardiac Ang II is not associated with cardiac hypertrophy. This indicates that the augmentation of cardiac Ang II is not sufficient to induce hypertrophy. These results suggest that Ang II is not a direct hypertrophic factor in the cardiac tissue. Moreover, it was already shown that the cardiac hypertrophy induced by exercise training is still observed even in the absence of changes in the cardiac Ang II levels (Filho et al., 2008) and can be prevented by angiotensin receptor blockers (Jiang et al. 2002).

Furthermore, we may not discard the possibility that other non-Ang II mechanisms of physiological hypertrophy could still be activated in the adult group to explain the cardiac hypertrophy observed, such as the phosphoinositide-3-kinase pathway through the insulin-like growth factor-1 receptor (Adams et al. 2004) and the kinin B2 receptor (Steiner et al. 2005).

At the moment, it is not clear why young and adult SHR differently modulate the RAS in response to swimming training. The results however are clearly the same as our previous works where the local RAS is responsible for the exercise-induced cardiac hypertrophy. In addition, we have already demonstrated that RAS is not downregulated by exercise training under normal conditions (Barauna et al. 2008; Rocha et al. 2007). However, Pereira et al. (2009) have recently demonstrated that  $\beta$ -adrenergic receptor KO mice, which develop severe heart failure, when submitted to

exercise training protocol negatively regulate the RAS. It leads us to speculate that exercise training may downregulate the RAS only in pathological conditions in which this system is upregulated (Zamo et al. 2010).

As stated before, although LVH is a powerful predictor of cardiovascular morbidity and mortality, it is not the case for the exercise-induced physiological LVH (Bernardo et al. 2010). In the adult trained group, we observed the LVH without an increase of the cardiac fibrosis, one of the molecular markers which distinguishes the physiological LVH from the pathological. Boissiere et al. (2008) also described in adult rats that exercise training induces additional physiological LV hypertrophy, which does not worsen cardiac function in hypertensive rats. Moreover, the cardiac hypertrophy observed in swimming-trained rats is a physiological and a beneficial cardiac adaptation and is usually associated with increased cardiac mass and performance (Colan 1997; Fagard 1997; McMullen and Jennings 2007). Garciaarena et al. (2009) described that exercise training increases the cardiac hypertrophy observed in SHR rats as well as being able to convert the pathological into physiological cardiac hypertrophy. However, as recently pointed out in a meta-analysis (Schlüter et al. 2010), only few studies have attempted to understand which age is the best to start the exercise training.

## Conclusion

In summary (Fig. 6), our results demonstrate that when young SHR group is submitted to the swimming training protocol, blood pressure decreases, local RAS downregulates and does not develop LVH; while the adult SHR trained group also decreases blood pressure but do not downregulate cardiac RAS and consequently develops increased LVH. The decrease in the circulating angiotensin II content may help the lowering effect of blood pressure due to exercise training.

These results are complementary to our previous work in which we also showed that the management of the hypertensive disease must begin before blood pressure reaches the highest stable levels and before the end-organ damage is reached (Zamo et al. 2010). Altogether, these data indicate that swimming training may also be a useful tool in the early management of cardiovascular damage in hypertension, preventing high pressure-induced end-organ damage.

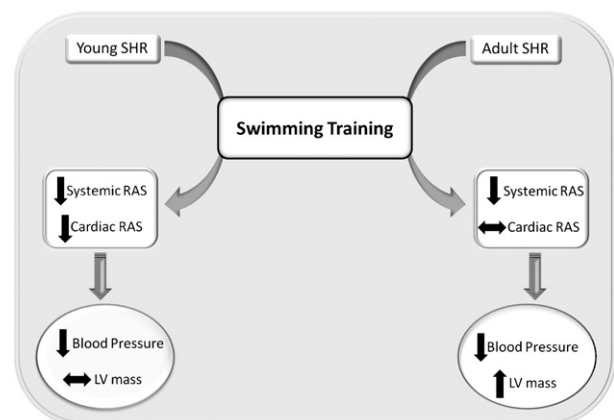


Fig. 6. Summary of the effects of aerobic training on the renin-angiotensin system and cardiac remodeling at two different stages of the hypertension development.

## Conflict of interest statement

No conflict of interest.

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