

# Angiotensin converting enzyme inhibition from birth reduces body weight and body fat in Sprague–Dawley rats

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## Abstract

*In vitro* studies have demonstrated that angiotensin II (ANG II) induces adipocyte hyperplasia and hypertrophy. The aim of the present study was to determine the effect of angiotensin-converting enzyme inhibition on body weight, adiposity and blood pressure in Sprague–Dawley rats. From birth half of the animals ( $n=15$ ) were given water to drink, while the remainder were administered perindopril in their drinking water (2 mg/kg/day). Food intake, water intake and body weight were measured weekly. Blood pressure was measured by tail cuff plethysmography at 11-weeks. Body fat content and distribution were assessed using dual energy X-ray absorptiometry and Magnetic Resonance Imaging at 12 weeks. Animals administered with perindopril had a body fat proportion that was half that of controls. This was consistent with, but disproportionately greater than the observed differences in food intake and body weight. Perindopril treatment completely removed hypertension. We conclude that the chronic inhibition of ANG II synthesis from birth specifically reduces the development of adiposity in the rat.

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## 1. Introduction

Obesity is a major health problem, particularly in the Western world. In the US, a recent survey revealed that 9.1% of all medical expenditure was obesity-related [1]. Obesity is strongly associated with the development of hypertension, dyslipidemia, hyperglycemia and, therefore, with higher cardiovascular risk [2–4]. There is mounting evidence that adiposity is, in part, regulated by a local adipose tissue RAS, which is a major factor in hypertension. Indeed, all components of the RAS have been isolated from adipose tissue (see [5] for a

review). ANG II is produced by adipocytes [6], while increased adipocyte AGT expression has been measured in obese Zucker rats [7] and in obese human subjects [8–10].

The notion of a trophic role for ANG II in adipose tissue is supported by several *in vitro* studies. AT<sub>1</sub> receptors, expressed in high concentration in rat epididymal adipose tissue [11], mediate an age-dependent increase in rat adipocyte size [12], while the recruitment of pre-adipocytes is modulated by an AT<sub>1</sub>-mediated pathway [13]. ANG II promotes adipocyte hypertrophy through the induction of fatty acid synthase (FAS) [14,15], and promotes proliferation and differentiation of adipocytes via a prostacyclin-mediated mechanism [16]. Recent work has shown that AT<sub>1</sub> blockade affects the differentiation of mesenchymal stem cells and pre-adipocytes, and the secretion of adipokines such as TNF- $\alpha$  and adiponectin [17,18].

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Paradoxically, in rats, chronic central [19] ANG II infusion decreases body weight, white adipose tissue mass and plasma leptin levels [19]. This is due to decreased food intake and increased thermogenesis through SNS activation [20,21]. These findings highlight the complexity of the role of both central and peripheral ANG II in body weight and body fat regulation.

Study of AGT-knockout mice has revealed impaired diet-induced weight gain and decreased fat mass in these animals [22], whereas over-expression of AGT in adipose of transgenic mice bearing the rat AGT gene caused an increase fat mass [23]. AT<sub>1a</sub>R knockout mice have greater energy expenditure than their wild type littermates [24], AT<sub>2</sub>R knockout mice have reduced adipose cell size [25] and both AT<sub>1a</sub>R and AT<sub>2</sub>R knockout mice have impaired diet-induced weight gain [24,25]. While ACE inhibition results in weight loss in rats [26,27], body composition has not been assessed. ACE inhibition has been shown to have ameliorative effects on insulin resistance and Type 2 Diabetes [28]; phenomena which may be linked to inhibition of ANG II-mediated lipogenesis [29,30,14,31]. Recent studies [32,17] also suggest that the pharmacological blockade of AT<sub>1</sub> receptors, and subsequent activation of AT<sub>2</sub> and peroxisome proliferator activated receptor (PPAR)- $\gamma$  receptors, decreases adipocyte size.

The present study aimed to determine the effect of inhibition of ACE, on blood pressure, body weight and overall body composition. No studies to date have specifically investigated the effect of ACE inhibition on both body weight and body composition.

## 2. Methods and procedures

### 2.1. Animals and treatment

Commencing with the birth of offspring, Sprague-Dawley rat dams (Purchased from the Animal Resource Centre, Canning Vale, Western Australia) were administered with perindopril (P) in their drinking water (2 mg/kg/day; a dose known to inhibit ANG II in brown adipose tissue [33]) or had plain drinking water, control group (C), the dose of perindopril was 2 mg/kg/day (based on the mean weight of the dams, adjusted weekly). After weaning at 3 weeks of age,  $n = 15$  male offspring per group continued on the same treatment as the mother (2 mg/kg/day, based on the mean weight of the group, adjusted weekly) until the end of experiment, female offspring were not used in the experimental phase of this study.

Over the course of this study, except where specified, all animals were housed two or three per cage in a stable envi-

ronment with ambient temperature maintained at  $22 \pm 2$  °C with a 12-hour light:dark cycle. All animals had *ad libitum* access to food and water. The diet was based on the American Institute of Nutrition (AIN-93) guidelines [34,35] and contained protein (20%), carbohydrate (63.6%) and fat (7%). Diets were produced to specification (Glenforest Stockfeeders, Perth, Australia), packed under liquid nitrogen and kept frozen until use.

The Animal Ethics Committee of the Howard Florey Institute, University of Melbourne, approved all procedures related to animal care and handling.

### 2.2. Ingestive behavior and body weight

Food and water intakes were manually measured using digital scales weekly. During weeks 6, 8 and 10 animals were single housed for individual food and fluid intake measurements. Body weights were measured manually at birth (data not shown), and weekly from weaning.

### 2.3. Blood pressure and heart rate measurement

Blood pressure was measured using tail cuff plethysmography at 11-weeks of age (IITC Life Science, Woodland Hills, CA). The heat chamber was set at 28–30 °C for optimal tail arterial dilatation to allow the measurement of the pulsatile pressure. A tail cuff/sensor was inflated by the system to a maximum pressure of ~250 mm/Hg and systolic blood pressure and pulse were determined using the optical sensor. Measurements were taken at the same time of the day (1000–1600 h) to minimize the influence of circadian cycles on blood pressure. Rats were acclimatized to the system for 3 consecutive days prior to measurement on day 4, the average of 3 measurements on this day were used.

### 2.4. Dual energy X-ray absorptiometry (DEXA)

At 12 weeks of age, whole body composition and femoral bone mineral status of the animals was assessed using DEXA (Hologic QDR 4500, Hologic Inc. USA) equipped with software optimized for adult rats weighing between 200 and 750 g. The animals were scanned under light anesthesia (Nembutal, 40 mg/kg, I.P.). A whole body scanning mode was used providing information such as percentage body fat, percentage bone mineral content (BMC) and percentage fat-free (i.e. lean) mass (FFM). Animals were placed in the prone position and at the centre of the scan table and parallel to the long axis of the scan table.

Table 1

Food intake (absolute and relative to body weight), fluid intake and body weight at 6-, 8- and 10-weeks for control and perindopril-treated animals

Age	6-weeks		8-weeks		10-weeks	
	Control	Perindopril	Control	Perindopril	Control	Perindopril
Food intake (g/day)	23.8 $\pm$ 1.5	19.4 $\pm$ 0.6*	25.3 $\pm$ 0.6	24.6 $\pm$ 0.5	26.2 $\pm$ 0.6	24.2 $\pm$ 0.3*
Food intake (g/100 g/day)	11.8 $\pm$ 0.5	13.7 $\pm$ 0.4*	8.4 $\pm$ 0.3	10.6 $\pm$ 0.3*	6.8 $\pm$ 0.2	7.8 $\pm$ 0.2**
Fluid intake (ml/day)	24.5 $\pm$ 1.1	34.4 $\pm$ 1.7**	26.1 $\pm$ 1.4	36.8 $\pm$ 1.6**	25.3 $\pm$ 1.2	43.0 $\pm$ 1.7**
Body weight (g)	202.3 $\pm$ 4.1	141.6 $\pm$ 5.0**	302.3 $\pm$ 4.1	232.7 $\pm$ 6.2**	385.0 $\pm$ 13.8	311.8 $\pm$ 6.0**

Significant differences indicated by \*  $p < 0.01$  \*\*  $p < 0.001$  (Control vs Perindopril).

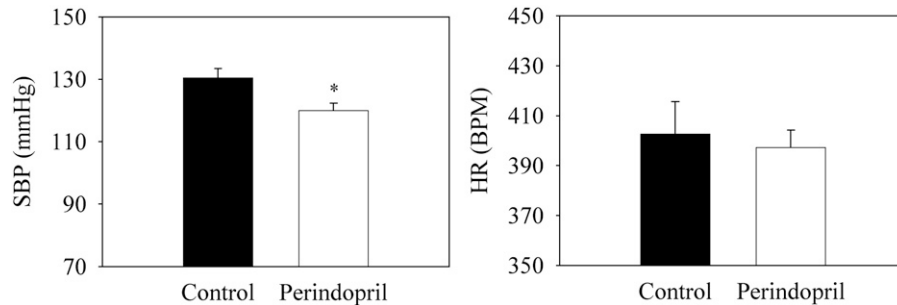


Fig. 1. The systolic blood pressure (SBP) and heart rate (HR) of control and perindopril-treated animals. \* $p < 0.01$ .

### 2.5. Magnetic resonance imaging (MRI)

Following the assessment of whole body composition by DEXA, regional body fat masses were visualized by MRI. A Bruker BIOSPEC 47/30 scanner, equipped with a horizontal 4.7T Oxford magnet was used. Rats were placed in the anesthetic chamber exposed to 5% isoflurane in medical grade oxygen. Following induction of anesthesia, rats were transferred to a purpose-built Perspex holder and a nose-cone was placed over the front of the head. The isoflurane concentration was then reduced to 1 to 1.5% to maintain anesthesia via the nose-cone for the duration of the procedure. Proton density weighted coronal images were acquired to provide a qualitative measure of differences in body composition.

### 2.6. Statistical analysis

Results are expressed as mean  $\pm$  standard error of mean (SEM). Body weight, food and water intake were analyzed by repeated measures ANOVA, followed by a *post hoc* least significant difference (LSD) test (Statistica, Statsoft).  $p < 0.05$  was considered to be a statistically significant difference.

## 3. Results

### 3.1. Ingestive behavior

Compared with controls, animals in the perindopril group consumed on average 9.4% less food ( $p < 0.01$ ). In contrast to

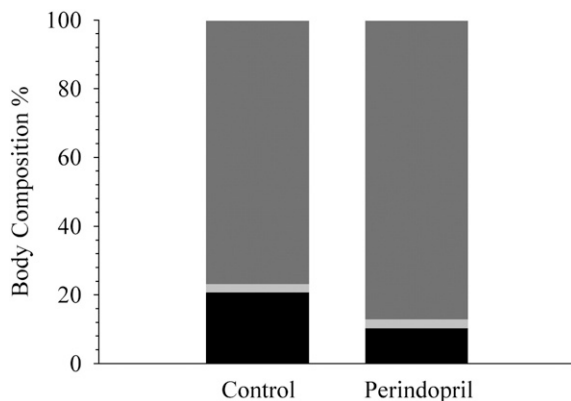


Fig. 2. Relative body composition obtained by DEXA for control and perindopril-treated animals. Fat mass = ■ Bone Mineral Content = ■ Fat Free Mass = ■

the data for absolute food intake, food intake adjusted for body weight was, on average, 15.8% greater in the perindopril-treated animals. A significant difference ( $p < 0.01$ ) was apparent at all three time-points (see Table 1). Animals administered perindopril drank, on average, 50.3% more fluid ( $p < 0.001$ ) than control animals. A significant difference in fluid intake was evident at all three time-points measured.

### 3.2. Systolic blood pressure and heart rate

The systolic blood pressure in animals treated with perindopril was  $120 \pm 2$  mmHg significantly lower than the control animals,  $131 \pm 2$  mmHg ( $p < 0.01$ ). Heart rate was not affected by perindopril treatment (Fig. 1).

### 3.3. Body weight and composition

Compared with those of controls, the body weights of perindopril-treated rats were reduced ( $p < 0.001$ ) 30%, 23% and 19% at 6-, 8- and 10-weeks, respectively. At 10-weeks, body weights were  $385.0 \pm 13.8$  vs.  $311.8 \pm 6.0$  for the control and perindopril groups, respectively.

There were marked differences in body composition between the two experimental groups. Animals in the perindopril group had approximately half the percentage body fat of those in the control group (C  $21.0 \pm 1.1\%$  vs. P  $10.3 \pm 0.4\%$ ;  $p < 0.001$ ). The reduction in relative fat mass was accompanied by a significant increase in the proportion of lean mass in perindopril-treated

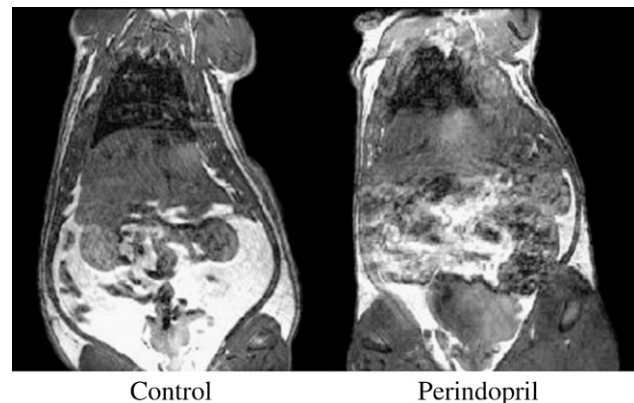


Fig. 3. Proton density weighted axial MRI images representative of the two experimental groups. Note the difference in intra-abdominal fat mass (seen as white).

rats (C  $67.0 \pm 0.7$  vs. P  $81.3 \pm 0.3$ ,  $p < 0.001$ ) (Fig. 2). MRI confirmed, qualitatively, these differences in body composition. As demonstrated in the representative scan in Fig. 3, perindopril-treated rats had markedly less intra-abdominal fat. There was a small, but significant difference in BMC (C  $12.0 \pm 0.3$  g vs. P  $9.4 \pm 0.2$  g,  $p < 0.01$ ).

#### 4. Discussion

Administration of perindopril, was associated with a lower body weight, such that 10 week old perindopril-treated animals weighed approximately 20% less than controls. This is commensurate with previous reports of body weight reductions in rats, associated with administration of ACE inhibitors or ANG II receptor antagonists [26,17,32]. Perindopril-treated animals consumed approximately 10% less food than controls; however, they actually consumed significantly more when adjusted for body weight. These findings suggest that perindopril-treated animals gained weight with relative inefficiency, rather than experiencing appetite suppression, this is consistent with the increased energy expenditure seen in AT1aR knockout mice [24] and the increased activity in AGT-knockout mice [22].

In agreement with previous studies [36,37], administration of perindopril was associated with a large increase in water intake. Indeed, peripheral administration of perindopril (at doses used in this study) is believed to result in an increase in the level of ANG I arriving at the circumventricular organs [38,39], and, in turn, an increase in the synthesis of brain ANG II by local ACE. Experiments utilizing intracerebroventricular infusion of ANG II have demonstrated that increased brain ANG II causes both decreased food intake and increased water intake [40,41]. In contrast, very high doses of ACE inhibitor universally reduce ACE activity, resulting in abolition of drinking [42]. Reduction in water drinking has also been achieved by central administration of the AT<sub>1</sub> antagonist, losartan [43].

Given the probable increase in brain ANG II in the animals in this study, as evidenced by increased water intake, it may be argued that the body weight loss occurs via the same mechanisms as ANG II infusion [19]. This appears unlikely as ANG II infusion leads to muscle wastage rather than fat loss, in fact pair fed saline treated controls showed no difference in epididymal fat pad mass compared to ANG II infused animals despite significant weight loss [21] indicating that any fat mass differences observed following ANG II infusion are probably due to food intake reductions. In the current study adjusted food intake was actually increased in perindopril-treated animals and the percentage of body fat was markedly decreased implicating the direct actions of ACE inhibition on adipose tissue.

Consistent with previous reports, and its clinical usage, perindopril caused a substantial reduction in SBP [44,45]. The lack of significant effect on heart rate is also consistent with the results in spontaneously hypertensive rats [46]. The lower blood pressure in perindopril-treated animals and the increase in drinking demonstrate that ACE inhibition is occurring in our model of drug delivery.

The most striking finding in this study was the difference in the body composition of perindopril-treated animals, relative to

controls. As illustrated in Fig. 2, perindopril-treated animals had less than half of the body fat (as a proportion of their own mass) and had a relative lean mass some 14% higher than controls. While these findings represent differences in overall body composition, MRI images (as represented in Fig. 2) suggested that the greatest differences in adiposity were intra-abdominal (including visceral and retroperitoneal). Indeed, a recent study using chronic AT<sub>1</sub> blockade demonstrated a reduction in both relative adiposity and adipocyte size in rat retroperitoneal and epididymal adipose tissue [17].

Administration of ANG II, *in vitro*, has also been shown to promote transcription of the key lipogenic enzymes (FAS and GPDH), resulting in increase fatty acid and triglyceride storage [14]. Given that ANG II appears to be necessary for the differentiation of pre-adipocytes to mature adipocytes [18,16,47] and contributes to adipose tissue growth and development [48], it is not surprising that ACE inhibition leads to a reduction in body fat.

As demonstrated in Fig. 1, administration of perindopril was associated with a modest relative decrease in bone mass. This finding is consistent with the established correlation between body fat and bone mineral content, and may relate to decreased plasma leptin concentration. Indeed, adiposity, serum leptin concentrations [49], and bone mass [50] have been shown to be positively correlated. Systemic administration of the ACE inhibitor, captopril, is associated with decreases in serum leptin concentration [51]. However, the RAS is present in haematopoietic bone marrow [52] and it has been reported that the ACE inhibitor, captopril, in pregnant dams can retard foetal ossification in rats [53]. Therefore, the lower bone mass may directly relate to inhibition of ACE activity.

#### 5. Conclusion

The present study demonstrates that chronic administration of perindopril results in a decrease in body adiposity and suggests that ANG II is important for the growth of fat stores. These findings may have implications for the prevention and future treatment of obesity and obesity-related disease.

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