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# Inhibition of the renin–angiotensin system ameliorates genetically determined hyperinsulinemia

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**Abstract**

This study was performed in order to assess the potentially different effects of the angiotensin-converting enzyme inhibitor captopril and of the angiotensin II receptor antagonist irbesartan on the metabolic syndrome in an animal model. Male NZO/BL6 F1 mice were treated with captopril, irbesartan, or placebo for 10 months: Control animals treated with placebo developed a metabolic syndrome with obesity ($55.5 \pm 6.3$ g), hypertension ($146 \pm 10$ mm Hg), hyperinsulinemia ($7.2 \pm 5.7$ ng/ml), hypercholesterolemia ($5.1 \pm 0.7$ mmol/l), cardiac hypertrophy ($269 \pm 44$ mg) and atherosclerotic plaques in the ascending aorta ($3.6 \pm 1.5$ $\mu m^2$). Treatment with angiotensin-converting enzyme inhibitor or angiotensin II receptor antagonist significantly ($p < 0.001$) reduces hypertension ($73 \pm 5$ and $78 \pm 11$ mm Hg), cardiac hypertrophy ($203 \pm 26$ and $202 \pm 18$ mg) and atherosclerosis ($2.2 \pm 0.9$ and $1.8 \pm 0.8$ $\mu m^2$). In addition, they prevented the development of obesity ($42.2 \pm 3.5$ and $38.3 \pm 2.8$ g) and hyperinsulinemia ($3.6 \pm 1.5$ and $1.8 \pm 0.4$ ng/ml). In conclusion, long-term treatment with an angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist can ameliorate obesity and hyperinsulinemia in a genetically determined mouse model. 

*Keywords:* Angiotensin $AT_1$ receptor; Angiotensin-converting enzyme inhibitor; Obesity; Hyperinsulinemia; Metabolic syndrome; (Mouse)

## 1. Introduction

Obesity is associated with hypertension, higher levels of cholesterol and insulin resistance, summarised as the metabolic syndrome with a subsequent high risk for atherosclerosis and high cardiovascular morbidity and mortality (Krauss et al., 1998). There is evidence that atherosclerotic lesions contain angiotensin II and angiotensin $AT_1$ receptors and that activation of the renin–angiotensin aldosterone system triggers atherosclerosis (Sugiyama et al., 1997; Potter et al., 1998; Song et al., 1998; Yang et al., 1998; Russel, 1999). Furthermore, it is well established that inhibition of the renin–angiotensin aldosterone system by either angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists prevent or reduce atherogenesis (Sugano et al., 1996; Keidar et al., 1997; Knowles et al., 2000). In clinical practice, angiotensin-converting enzyme inhibitors are implemented for primary and secondary prevention in diabetic patients (Zanetti et al., 1997; Tatti et al., 1998, Estacio et al., 1998; The Heart Outcomes Prevention Evaluation Study Investigators, 2000). Up to now, it remains controversial if angiotensin II antagonists have the same ability compared to angiotensin-converting enzyme inhibitors. The purpose of this study was to examine the influence of the angiotensin II antagonist irbesartan versus the angiotensin-converting enzyme inhibitor captopril on the development of atherosclerosis and the metabolic syndrome in an animal model. New Zealand obese (NZO) and C57/B6 mice exhibit a polygenic metabolic syndrome (Crofford and Davis, 1965; Herberg and Coleman, 1977; Veroni et al., 1991; Igel et al., 1997; Ortlepp et al., 2000) comparable to that in humans. Especially under high fat diet they develop hyperinsulinemia and subsequent diabetes

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Table 1
Baseline characteristics before treatment at the age of 8 weeks

| | Placebo | Irbesartan | Captopril |
|---|---|---|---|
| | $N=15$ | $N=14$ | $N=14$ |
| Body weight (g) | 33.4 ± 3.2 | 33.9 ± 3.9 | 34.1 ± 3.2 |
| Glucose (mmol/l) | 8.1 ± 1.2 | 7.8 ± 1.1 | 7.9 ± 1.4 |
| Heart rate (BPM) | 606 ± 73 | 613 ± 73 | 600 ± 77 |
| Systolic blood pressure (mm Hg) | 109 ± 8 | 109 ± 8 | 111 ± 7 |
| Diastolic blood pressure (mm Hg) | 73 ± 6 | 74 ± 6 | 74 ± 7 |
| Mean blood pressure (mm Hg) | 85 ± 6 | 86 ± 6 | 86 ± 6 |

Data are given as mean ± S.D.

mellitus. This study was initiated as a primary prevention study with medical treatment from adolescence till old age. The primary hypothesis of this study was that blocking of the angiotensin $AT_1$ receptor with irbesartan would prevent the development of atherosclerosis in mice with a metabolic syndrome better than just blocking the angiotensin-converting enzyme by captopril. Hence, hypertension is a crucial parameter, which influences atherosclerosis, both medications were given in a equipotent dosage concerning the blood pressure. The second hypothesis was created during the study: blocking of the angiotensin $AT_1$ receptor would prevent the development of weight gain and hyperinsulinemia in mice with a metabolic syndrome. Analysed parameters were blood pressure, cardiac weight, obesity, insulin resistance, serum cholesterol level and atherosclerosis and kidney function.

## 2. Methods

### 2.1. Animals

NZO and C57Bl/6 mice were obtained from Bomholtgard (Ry, Denmark). Female C57Bl/6 and male NZO mice were used to generate F1 hybrids. Male F1 hybrids were used, because pure NZO mice would not survive the experimental period due to diabetic coma. After weaning (3 weeks of age), mice received a standard chow (No. 1314; Altromin, Lage, Germany) till the 8th week. Between the 8th and 34th and between the 48th and 52nd week, mice received a high cholesterol diet [Altromin, Lage, Germany] with 5.0% crude fat, 1.5% cholesterol, 48% carbohydrates, 22.5% crude protein, 0.4% NaCl and 12.5 kJ/g. Between the 34th and 48th week, mice received a high fat diet to enhance obesity [C1057; Altromin, Lage, Germany] with 16% fat, 46.8% carbohydrates, 17.1% protein and 15.4 kJ/g]. Throughout the study, mice had free access to food and water. Hence, the primary study endpoint was atherosclerosis high cholesterol diet was chosen as the primary diet. During the study, placebo mice gained more weight than animals treated with irbesartan or captopril. To confirm this observation, the diet was switched to the high fat diet. Mice were kept individually in cages (Macrolon, type II, EBECO, Castrop-Rauxel, Germany) on soft wood bedding in a temperature-controlled room (20 °C, 55 ± 5% relative humidity) with a 12-h light–dark cycle and lights on at 0600 AM. Animals were killed at 52 weeks of age by exsanguination in isoflurane anaesthesia. The study was approved by the committee for ethics of animal experimentation at the Regierungspräsidium, Köln, Germany.

### 2.2. Medication

At the age of 8 weeks, mice were randomised into three groups: placebo, captopril and irbesartan treatment. Medication was given orally with the drinking water from the 8th week till the end of the study. The initial doses of captopril were 0.0375 mg/g body weight/day and the initial doses of irbesartan were 0.0625 mg/g body weight/day. During the study period, the irbesartan doses had to be increased to 0.2125 mg/g body weight/day at the age of 16 weeks to maintain an equipotent effect in reduction of the blood pressure compared with captopril treatment.

### 2.3. Measurement of blood pressure

Monthly measurement of blood pressure and heart rate were determined by a non-invasive tail cuff and pulse transducer system (Softron blood pressure-98A, Tokyo, Japan) as described previously (Krege et al., 1995). Briefly,

Table 2
Summary of study endpoints at the age of 52 weeks

| | Placebo | Irbesartan | Captopril |
|---|---|---|---|
| | $N=15$ | $N=14$ | $N=14$ |
| *Obesity/insulin resistance* | | | |
| Body weight (g) | 55.5 ± 6.3 | 38.3 ± 2.8[a] | 42.2 ± 3.5[a] |
| Gain of body weight on HCD (g) | 13.1 ± 5.7 | 4.3 ± 3.2[a] | 7.0 ± 4[a] |
| Gain of body weight on HFD (g) | 9.0 ± 3 | 0.1 ± 9[a] | 1.1 ± 5.3[a] |
| Urea (mmol/l) | 6.4 ± 1.3 | 7.9 ± 1.8 | 13.8 ± 5.7[a,b] |
| Cholesterol (mmol/l) | 5.1 ± 0.7 | 4.9 ± 0.8 | 5.9 ± 0.9[b] |
| Triglycerides (mmol/l) | 1.3 ± 0.5 | 0.9 ± 0.4 | 1.2 ± 0.4 |
| Glucose (mmol/l) | 6.7 ± 2.3 | 7.2 ± 2.1 | 8.1 ± 1.7 |
| Insulin (ng/ml) | 7.2 ± 5.7 | 1.8 ± 0.4[a] | 3.6 ± 1.5[b] |
| *Cardiovascular system* | | | |
| Heart rate (BPM) | 730 ± 48 | 742 ± 45 | 728 ± 65 |
| Systolic blood pressure (mm Hg) | 146 ± 10 | 78 ± 11[a] | 73 ± 5[a] |
| Diastolic blood pressure (mm Hg) | 91 ± 12 | 49 ± 9[a] | 49 ± 6[a] |
| Mean blood pressure (mm Hg) | 110 ± 11 | 59 ± 9[a] | 57 ± 6[a] |
| Aortic thickening (μm) | 52 ± 9 | 33 ± 5[a] | 34 ± 4[a] |
| Atherosclerosis ($\mu m^2$) | 3.6 ± 1.5 | 1.8 ± 0.8[c] | 2.2 ± 0.9[c] |
| Cardiac hypertrophy (mg) | 269 ± 44 | 202 ± 18[a] | 203 ± 26[a] |
| *Kidney* | | | |
| Renal weight (mg) | 437 ± 75 | 355 ± 67[c] | 408 ± 53 |
| Creatinine serum (μmol/l) | 107 ± 42 | 94 ± 48 | 101 ± 39 |
| Daily drinking water (ml) | 8.8 ± 2.1 | 7.4 ± 1.1 | 8.0 ± 0.9 |

Data are given as mean ± S.D. HCD = High Cholesterol Diet 2. – 8. Month. HFD = High Fat Diet 9. – 11. Month.
[a] Verum versus placebo, $P<0.001$.
[b] Captopril versus irbesartan, $P<0.01$.
[c] Verum versus placebo, $P<0.01$.

mice were put in a restriction cage that was placed in a 37 °C heated tube for 15 min. The cuff that occluded the flow of blood into the tail was inflated to a maximum of 200 mm Hg and deflated automatically at a rate of 10 mm Hg $s^{-1}$. Animals were habituated to the procedure by 10 cycles of inflation and deflation. Thereafter, six measurements were performed with each animal, and means were calculated (S.D. less than 3 mm Hg). Depending on the length of the adaptation period, the procedure took a total of 30–60 min per mouse. Measurements were performed between 0700 and 1100 AM.

### 2.4. Serum parameters

Blood glucose, serum cholesterol, serum triglycerides, serum urea, serum creatinine and urine creatinine were measured by auto-analyser (Johnson & Johnson, Neckargemünd, Germany). Serum insulin was determined by radio immunoassay (Amersham-Pharmacia, Freiburg, Germany) with anti-rat insulin antiserum and $^{125}$I-labelled rat insulin as tracer. Free and bound radioactivity were separated with an anti-Immunglobulin-G antibody. All samples were assayed in duplicate.

### 2.5. Histology

As described previously (Paigen et al., 1985, 1987), the ascending aorta extending from the upper part of the left ventricle to the aortic arch was dissected and cleaned of peripheral fat under a dissecting microscope. The ascending aorta was fixed with 4% formalin and embedded in OCT-compound. Fifteen serial 10-μm sections caudal of the distal sinus aorticus were generated with a cryostat for each mouse. Sections were stained with hemotoxylin and red oil O. For quantitative analysis of atherosclerosis, sections were coded and scored blindly. The length and the average thickness were determined by using a grid ocular and multiplied to obtain a cross-sectional area in $\mu m^2$. For electron microscopy, small tissue samples were fixed in 3% 0.1 M phosphate-buffered glutardialdehyde (pH 7.2). After dehydration in an ascending alcohol series and propyleneoxide, they were embedded in epoxy resin (glycide ether 100, Serva). Sections (1 μm) were cut and toluidin blue-stained for light microscopy pre-screening. Light golden ultrathin sections (80–100 nm) were then cut on an ultramicrotom (Reichert Ultracut S, Germany), stained with uranyl acetate and lead citrate, and viewed and photographed in a Philips TEM 400 transmission electron microscope. Unfixed kidneys were snap-frozen, embedded and stained with hemotoxylin.

### 2.6. Statistical analysis

Continuous data are presented as mean ± S.D. Difference between the three treatment groups were tested using Analysis of variance (ANOVA) or cross tabs $Chi^2$ analysis

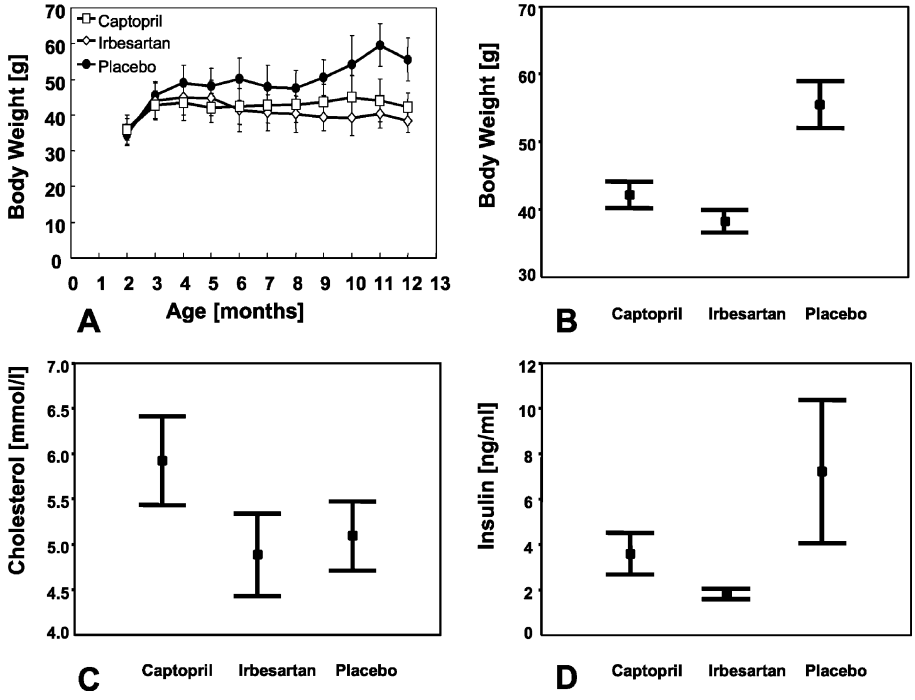


Fig. 1. Amelioration of obesity and insulin resistance by captopril and irbesartan. The figure illustrates the gain of body weight (A) over the study period. Irbesartan- and captopril-treated mice did not show the degree of obesity as control mice treated with placebo (B). Compared to control mice and irbesartan treated mice cholesterol was slightly elevated in Captopril treated mice (C). Whereas control mice showed hyperinsulinemia, captopril treated mice showed a reduced level of insulin and irbesartan mice had normal insulin level (D).

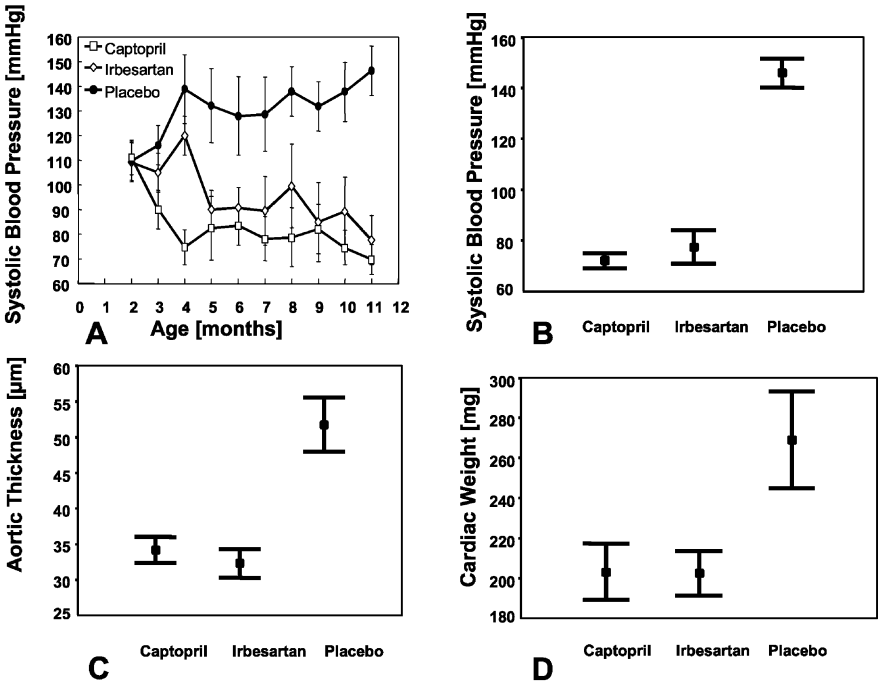


Fig. 2. Reduction of hypertension, cardiac hypertrophy and aortic thickening by captopril and irbesartan. The figure illustrates the systolic blood pressure over the study period (A). Irbesartan- and captopril-treated mice did not develop the degree of hypertension (B), aortic thickness (C), and cardiac hypertrophy (D) as control mice.

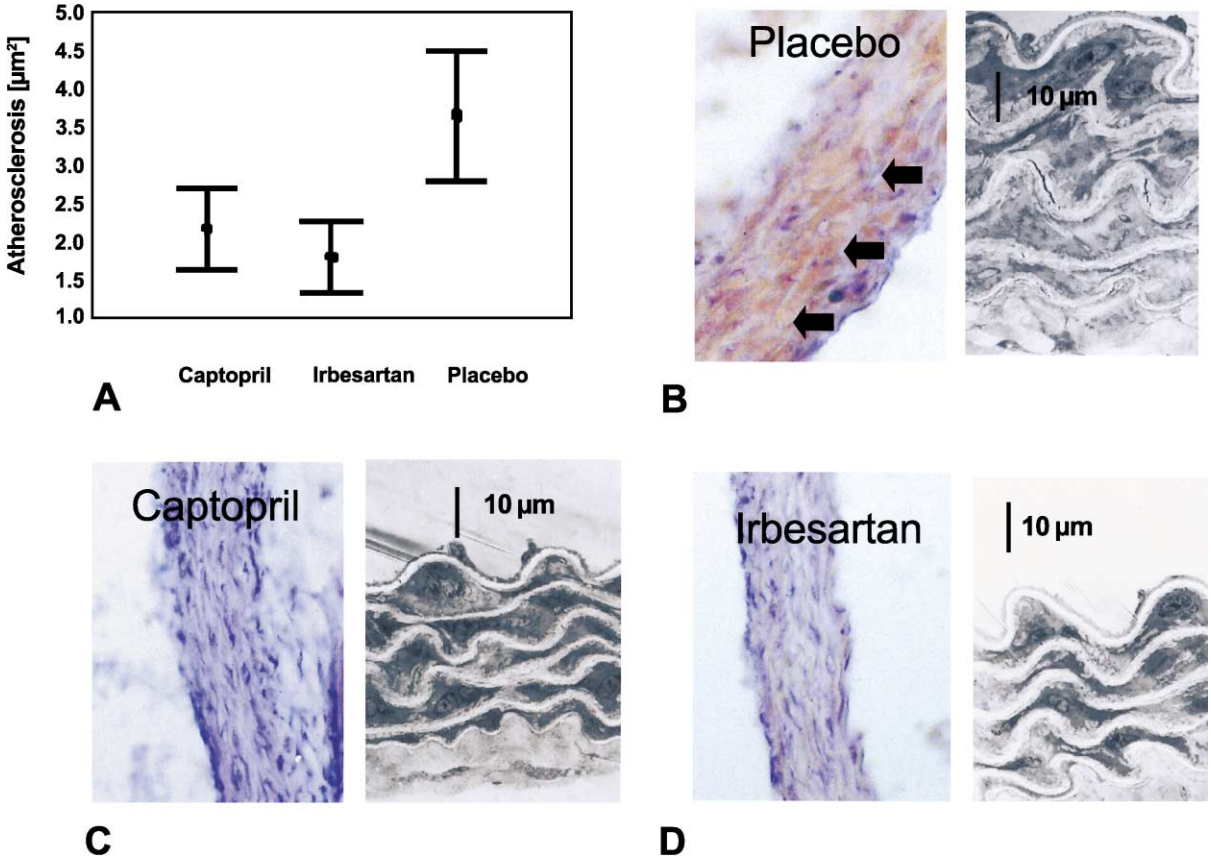


Fig. 3. Placebo mice had a thicker aortic wall and revealed more lipid accumulation in the aorta (arrows in B) than captopril or irbesartan treated mice. (B–D) illustrates representative cross-sections of the ascending aorta (light microscopy with red-oil O stain) and descending aorta (electro microscopy) of Placebo- (B), Captopril- (C), and Irbesartan- (D) treated mice.

as appropriated. All statistical analysis were calculated with the computer programs SPSS Version 9.0. A $P$ value $< 0.05$ was considered significant.

## 3. Results

### 3.1. Baseline characteristics

As given in Table 1, randomised mice had comparable baseline parameters with the age of 8 weeks before starting medical treatment.

### 3.2. Control mice treated with placebo

Control mice developed the full spectrum of a metabolic syndrome. They showed severe obesity ($55.5 \pm 6.3$ g) and insulin resistance with three to four times elevated serum insulin ($7.2 \pm 5.7$ ng/ml). In addition to hypertension, cardiac hypertrophy, and hypercholesterolemia, they showed lipid accumulation in the aorta (see also Table 2 and Fig. 3).

### 3.3. Influence of irbesartan and captopril on the development of a metabolic syndrome

The traits of the metabolic syndrome obesity, hypertension and hyperinsulinemia were ameliorated by blocking the renin–angiotensin aldosterone system. Mice treated with irbesartan and captopril exhibit a lower weight gain over time. This effect was more pronounced on high fat diet, on which control mice gained 9 g, whereas mice treated with irbesartan or captopril failed to gain weight. The development of obesity and cholesterol and hyperinsulinemia are illustrated in Fig. 1. Captopril mice showed slightly elevated cholesterol compared to placebo and irbesartan mice. All mice were normoglycemic and glucose levels did not differ statistically significant ($P = 0.17$). Corresponding the lower degree of obesity captopril- or irbesartan-treated mice exhibit reduced or normal insulin levels, respectively (Table 2).

Food consumption was similar in all three groups with approximately 35 g/week. However, it should be noted that all mice wasted a considerable portion of their food during the activity period. Thus, small differences in food intake might not have been detected.

Hypertension, aortic thickening and cardiac weight was reduced equally in mice treated with irbesartan and captopril as given in Fig. 2. Captopril- and irbesartan-treated mice had less accumulation of fatty streaks in the ascending aorta as given in Fig. 3 and Table 2.

Urea was elevated in captopril-treated mice. However, as assessed by histology and serum creatinine, all mice (placebo versus verum-treated, $P = 0.73$) had a normal kidney function (Table 2, histology not shown). Daily water intake was also similar between all groups (placebo $8.8 \pm 2.1$ ml; irbesartan $7.4 \pm 1.1$ ml; captopril $8.0 \pm 0.9$ ml; $P = 0.72$).

## 4. Discussion

The metabolic syndrome has a relevant contribution to cardiovascular morbidity and mortality in the western world. Primary pharmacological prevention might reduce the prevalence of this disease. The ratio of this study was to examine the different effect of the angiotensin II blocker irbesartan versus the angiotensin-converting enzyme inhibitor captopril on the development of lipid accumulation in the aorta and the metabolic syndrome in a polygenic animal model. Non-treated mice in this study developed the full spectrum of a metabolic syndrome including obesity, insulin resistance, hypercholesterolemia, hypertension, cardiac hypertrophy, and lipid accumulation in the aorta. The medication was given at a dosage with a profound effect on the systemic blood pressure. However, treated mice were not relevantly hypotensive, because other mice strains exhibit same low levels of blood pressure values (Ortlepp et al., 2000), the treated mice did not show reflex tachykardia, demonstrated normal activity and have survived over a long study period of 10 months. The study demonstrated that irbesartan and captopril had the same beneficial effect on cardiovascular traits (systolic blood pressure, cardiac hypertrophy, aortic thickening and lipid accumulation in the aorta).

The new findings of this study were that both medication have the potential in preventing weight gain and hyperinsulinemia. Hence, food and water intake did not differ significantly, blocking of the renin–angiotensin system might influence insulin-secretion or insulin/insulin-receptor gene expression, which could lead to more catabolism. This would explain the high level of urea in captopril treated mice with a normal kidney function.

There is evidence for a contribution of the renin–angiotensin system on the development of obesity: In mice and humans, obesity is associated with a higher activation of the renin–angiotensin system (Barton et al., 2000; Schorr et al., 1998). Obese people develop hyperinsulinemia and insulin itself upregulates angiotensinogen expression in human adipose tissue (Jones et al., 1997a) and vascular smooth muscle cells (Kamide et al., 1998). Moreover, angiotensin II seems to trigger adipocyte cell cycle regulation and lipogenesis (Jones et al., 1997b; Crandall et al., 1999). This might cause a vitious circle of obesity, hyperinsulinemia, activation of the renin–angiotensin system and weight gain. Blocking of the renin–angiotensin system might stop this process. This hypothesis is conclusive with the observation that patients treated with insulin gain more weight (UK Prospective Diabetes Study Group, 1998). As an assumption, blocking of the angiotensin receptor type 1 might alter the tyrosine kinase phosphorylation with a subsequent higher translocation of the Glut-4 transporter and thus a reduced demand for insulin secretion. However, many other interactions of the renin–angiotensin system with other systems (e.g., leptin, PPAR) might be possible and the effect of irbesartan and captopril on weight gain and hyperinsulinemia might be non-specific. Blocking the renin–angioten-

sin system might alter the profile of gene expression of gene from different metabolic systems. From data of this study, no firm conclusion can be drawn. This has to be addressed in further animal and in vitro studies. Nevertheless, we conclude that blocking of the renin–angiotensin system might be a therapeutic chance in prevention of weight gain and hyperinsulinemia.

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