Effect of angiotensin receptor blockade on insulin sensitivity and endothelial function in abdominally obese hypertensive patients with impaired fasting glucose

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Abstract

AngII (angiotensin II) may contribute to cardiovascular risk in obesity via adverse effects on insulin sensitivity and endothelial function. In the present study, we examined the effects of ARB (angiotensin receptor blocker) therapy (losartan, 100 mg/day) on insulin sensitivity and endothelial function in 53 subjects with stage I hypertension, abdominal obesity and impaired

fasting glucose. The study design was a randomized double-blinded parallel design placebo-controlled multicentre trial of 8 weeks duration. We used the hyperinsulinaemic–euglycaemic clamp technique to measure insulin sensitivity (expressed as the 'M/I' value) and RH-PAT (reactive hyperaemia-peripheral arterial tonometry) to measure endothelial function. Additional measures included HOMA (homoeostasis model assessment)-B, an index of pancreatic β-cell function, and markers of inflammation [e.g. CRP (C-reactive protein)] and oxidative stress (e.g. F_2-isoprostanes). ARB therapy did not alter insulin sensitivity [5.2 (2.7) pre-treatment and 4.6 (1.6) post-treatment] compared with placebo therapy [6.1 (2.9) pre-treatment and 5.3 (2.7) post-treatment; P value not significant], but did improve the HOMA-B compared with placebo therapy ($P = 0.05$). ARB therapy also did not change endothelial function [RH-PAT, 2.15 (0.7) pre-treatment and 2.11 (0.7) post-treatment] compared with placebo therapy [RH-PAT, 1.81 (0.5) pre-treatment and 1.76 (0.7) post-treatment; P value not significant]. Markers of inflammation and oxidative stress were not significantly changed by ARB therapy. In conclusion, ARB therapy did not alter peripheral insulin sensitivity or endothelial function in this cohort of patients with essential hypertension, abdominal obesity and impaired fasting glucose, but did improve pancreatic β-cell function.

Keywords

angiotensin II; clinical trial; hypertension; insulin signalling; obesity; vascular function

INTRODUCTION

Epidemic obesity threatens to reverse progress made in reducing the burden of cardiovascular disease in the United States [1]. Two pathophysiological links underlying obesity and its associated cardiovascular risk are insulin resistance and endothelial dysfunction. Insulin resistance, highly prevalent in obesity, predisposes to diabetes and also adversely affects BP (blood pressure) and lipid metabolism. Endothelial dysfunction, also common in obesity, increases susceptibility to vascular injury by permitting a pro-inflammatory and pro-thrombotic state. Although often considered as distinct entities, insulin sensitivity and endothelial function are in fact integrally linked [2]. Insulin stimulates endothelial NO production via a signalling pathway homologous to that of insulin-stimulated glucose transport in skeletal muscle. In turn, NO activity is essential for effective insulin-stimulated glucose disposal.

Overactivity of the RAAS (renin–angiotensin–aldosterone system), and specifically the vasoactive mediator AngII (angiotensin II), appears to be of particular importance in the genesis of insulin resistance and endothelial dysfunction in obesity. AngII is known to interfere with insulin metabolic signalling at multiple levels [3,4]. In addition, AngII limits NO bioavailability by stimulating vascular ROS (reactive oxygen species) generation [5]. In experimental models, targeting of AngII improves both insulin resistance and endothelial function [6,7].

These observations suggest that pharmacological inhibition of AngII activity may uniquely address the pathophysiological mechanisms related to excess cardiovascular risk in obesity.

Accordingly, we sought to examine the effect of angiotensin receptor blockade with losartan on insulin sensitivity and endothelial function in hypertensive individuals with abdominal obesity and impaired fasting glucose.

MATERIALS AND METHODS

Subjects

Eligible subjects comprised non-diabetic men or women, 18–75 years of age, with mild hypertension and taking 1 antihypertensive medication. Inclusion criteria included a screening visit BMI (body mass index) between 30 and 40 kg/m^2, waist circumference >40 inches in men or >35 inches in women, and fasting glucose 100 mg/dl and <126 mg/dl. At the screening visit, subjects' SBP (systolic BP) had to be <160 mmHg, and a DBP (diastolic BP) of <100 mmHg. At the pre-randomization visit, the SBP had to be 120 and <160 mmHg, and the DBP 80 and <100 mmHg.

Subjects with known sensitivity or intolerance to ARBs (angiotensin receptor blockers) or a history of angio-oedema were not eligible. Other exclusion criteria included the presence or suspicion of a secondary cause of hypertension, a history of malignant hypertension, smoking within the past year, renal impairment, defined as a serum creatinine >1.5 mg/dl or >2 + proteinuria by dipstick, the use of antioxidant supplements, an inability or unwillingness to practice acceptable methods of birth control, the presence of coronary artery disease, congestive heart failure or stroke, or any other medical condition that, in the investigator's judgment, would alter the conduct or outcome of the study.

Study design

The present study was an investigator-initiated industry-sponsored multi-site double-blinded placebo-controlled randomized parallel design clinical trial. The number of subjects at each site was: four at the University of Texas Southwestern Medical Center at Dallas, Dallas, TX, U.S.A., two at the St. Luke's Roosevelt Hospital, New York, NY, U.S.A., six at the Brigham and Women's Hospital, Boston, MA, U.S.A., 19 at the Veterans Administration Health Care System, San Diego, CA, U.S.A., two at the University of Miami Diabetes Research Institute, Miami, FL, U.S.A., 14 at the Indiana University School of Medicine, Indianapolis, IN, U.S.A., two at the Baylor Clinic, Houston, TX, U.S.A., and four at the University of Pennsylvania School of Medicine, Philadelphia, PA, U.S.A.

The study duration was 12 weeks, with 4 weeks of placebo run-in followed by 8 weeks of double-blind medication. Participants were randomized to either losartan (100 mg/day) or placebo in a 1:1 manner. At the screening visit, subjects gave written informed consent. Each study site institutional review board approved the protocol. The study is registered on ClinicalTrials.gov (http://www.ClinicalTrials.gov) under the identifier NCT 00675987.

Antihypertensive medication was tapered and discontinued after the screening visit. Once off medication, subjects began a 4-week single-blind placebo run-in period. BP and adherence were measured midway and at the end of the run-in period. Subjects were excluded for non-compliance at the end of the placebo period (<75 % or >120 % of correct number of pills taken) or if the BP fell outside accepted limits (see above).

Eligible patients then underwent 8 weeks of double-blind treatment. Endothelial function and insulin sensitivity were measured on day 1 of treatment and on the final day (testing visits). Subjects were instructed to maintain a constant diet throughout the study. Before all study visits, subjects fasted, avoided exercise for >12 h, arrived via motorized transportation, did not use sildenafil, tadalafil or vardenafil for 72 h, and did not use cold medication, nutritional supplements and niacin for >24 h. Study medication was not taken on the morning of a scheduled visit. Study visits all occurred before 10.00 hours. For pre-menopausal women, study visits were scheduled to occur during the follicular phase of the menstrual cycle. Efforts were made to maintain a calm environment throughout study visits, and study measurements were made in a temperature-controlled room (37 °C).

Study measurements

Anthropometric measurements—Body weight and height were measured with the subject wearing light clothing and no shoes. Waist circumference was measured at the level of the umbilicus on the bare abdomen.

BP—A study investigator measured BP using a standard aneroid sphygmomanometer. All site investigators underwent standardized training in accord with American Heart Association guidelines [8]. The average of at least three measurements was taken, and the values were rounded to the nearest whole number. If a single DBP reading deviated >5 mmHg from the average, the BP was considered unstable and the measurements were repeated on another occasion.

Endothelial function—Endothelium-dependent vasodilation was assessed by measuring the flow-induced change in digital PVA (pulse volume amplitude) of the first finger. Digital PVA was measured using a peripheral arterial tonometer (EndoPAT; Itamar-Medical). The peripheral arterial tonometer apparatus comprises a finger-mounted probe that surrounds the distal phalanx with an electronically controlled inflatable pressuring air cushion confined within a rigid external case. The pressure changes within the probe that accompany pulsatile volume changes in the finger are fed to a personal computer where the signal is band-pass-filtered (0.3–30 Hz), amplified, displayed and stored. The PVA is a measure of the height of the pulse wave and is proportional to pressure changes accompanying volume changes within the probe.

Investigators at each site were trained and had to demonstrate competence with the technique prior to subject enrolment. The baseline PVA was measured over a period of at least 5 min to ensure stability. Thereafter, a cuff was rapidly inflated to occlusive pressure (at least 50 mmHg >SBP) on the upper arm to induce limb ischaemia. After exactly 5 min, the cuff was rapidly deflated and pulse volume measurements were repeated during this reactive hyperaemic phase. The increase in PVA during RH (reactive hyperaemia) following cuff release is in large part caused by NO [9]. Accordingly, endothelium-dependent vasodilation was calculated from the stored digitized data as the ratio of PVA during RH over a 1-min interval starting 1 min after cuff deflation and divided by the baseline PVA obtained over the 1-min interval immediately preceding cuff inflation. The PVA from the same finger of the contralateral non-ischaemic hand was used to adjust for any drift in the

magnitude of the signal due to systemic factors, and the PVA ratio was normalized to the control contralateral finger [RH-PVA (PVA during RH relative to baseline)]. The measurement of endothelial function was repeated once after at least 15 min of rest, and the average of the two measurements was calculated.

Insulin sensitivity—A hyperinsulinaemic–euglycaemic clamp was performed to assess peripheral insulin sensitivity after the measurement of endothelial function. An intravenous catheter was placed in each arm, one for blood withdrawal (the hand) and the other for infusion (the contralateral forearm). The hand of the blood-withdrawal arm was placed in a box heated to 50 °C to ensure arterialization of venous blood. The subject's weight was measured at each clamp visit, and the BSA (body surface area) was determined by the Gehan and George equation:

$$BSA \quad = \quad 0.0235 \times (height \quad in \quad cm)^{0.42246} \\ \times (weight \quad in \quad kg)^{0.51456}]$$

A 2.5 h hyperinsulinaemic–euglycaemic clamp was performed using a primed continuous insulin infusion. The priming was 160 m-units/m^2 per min for the first 10 min, followed by 80 m-units/m^2 per min for 140 min. Plasma glucose was assessed every 5 min at the bedside, and 20 % dextrose was infused at a variable rate to maintain plasma glucose of 90 mg/dl $\pm$ 5 % [10]. Samples for insulin measurement were obtained in triplicate at baseline, every 30 min for the first 120 min, and then every 10 min for the last 30 min. The volume remaining from the 20 % dextrose infusion pump was recorded every 15 min. Insulin sensitivity (M) was calculated as the average glucose infusion rate (mg/kg of body weight per min) over the last 30 min of the clamp. The infusion rate is approximately equivalent to the GDR (glucose disposal rate), as endogenous production is suppressed or at least greatly minimized at this dose of insulin administration [11]. Steady-state insulin levels varied among subjects and we therefore calculated the insulin-adjusted insulin sensitivity or insulin sensitivity index (M/I; where I is mean level of insulin), which is the GDR/I ratio during the last 30 min of the clamp multiplied by 200 (arbitrary units). HOMA-IR [HOMA (homoeostasis model assessment) of insulin resistance], an index that correlates inversely with peripheral insulin sensitivity, and HOMA-B (HOMA of β-cell function), an index that correlates directly with pancreatic β-cell function, were calculated as described previously [12].

Additional assays—A fasting venous blood specimen was obtained at the initial visit for standard safety labs, such as blood count (LH750; Beckman Coulter), and hepatic and renal profiles (Modular Biochemical Analyser; Roche Diagnostics), as well as for glucose and insulin levels (ADVIA Centaur; Siemens Healthcare Diagnostics). A urine dipstick pregnancy test was performed at the initial visit as well as the two testing visits. At the two testing visits, blood was withdrawn for measurements of hsCRP (high-sensitivity C-reactive protein) (Modular P analyser; Roche), VCAM-1 (vascular cell-adhesion molecule-1) (ELISA; R&D Systems), MCP-1 (monocyte chemoattractant protein-1) (ELISA; R&D Systems), ox-LDL (oxidized low-density lipoprotein) (ELISA; Mercodia), F_2-isoprostane

(GC–MS; Vanderbilt University, Nashville, TN, U.S.A.) and E-selectin (ELISA; R&D Systems), and urine was obtained to assess for the presence of microalbuminuria (Modular P Analyser; Roche Diagnostics).

Statistical methods

The primary approach to the statistical analysis of the efficacy data was a modified intention-to-treat approach. This analysis included all patients who received at least one dose of the double-blind study drug and had a valid measurement of insulin sensitivity and/or endothelial function at baseline and a valid measurement after baseline. Patients were analysed based on the treatment group to which they were randomized.

There were two co-primary end points. The first was the change in peripheral insulin sensitivity after losartan therapy compared with after placebo therapy. The second was the change in endothelial function after losartan therapy compared with after placebo therapy. Secondary end points included the changes in microalbuminuria, hsCRP, VCAM-1, MCP-1, ox-LDL, F_2-isoprostane and E-selectin after losartan compared with placebo therapy.

It was anticipated that a total enrolment of 60 subjects randomized into the losartan and placebo groups at a 1:1 ratio would yield approximately 25 evaluable subjects in each group. On the basis of two published studies demonstrating a positive effect of losartan on insulin resistance [13,14], the losartan treatment effect was estimated to be 1.38 mg/kg of body weight per min. Assuming an S.D. of 1.5 mg/kg of body weight per min and assuming no change in the GDR in the placebo group, 25 evaluable patients per group would then provide 89 % power to detect a significant difference between placebo and losartan treatment. Losartan was expected to improve endothelial function by 24.5 % with an S.D. of 21 % [15–18]. Assuming a similar treatment effect size, 25 evaluable patients per group would provide 98 % power to detect a significant difference between losartan and placebo.

An ANCOVA (analysis of covariance) model was used to compare the treatments on the primary and secondary efficacy variables. No adjustment for multiple hypothesis testing was made. The ANCOVA included terms for treatment, investigator sites and the corresponding baseline measurement. Investigator sites with low enrolment were combined into one or more groups for analysis. Baseline characteristics are described as means (S.D.) or medians (S.D.) for parametric and non-parametric data respectively. The S.D. of the median was calculated as S.D. = (quartile 3 – quartile 1)/1.075.

RESULTS

A total of 171 potential subjects were screened. Of these, 118 were disqualified for the reasons listed in Figure 1, leaving 53 subjects who were randomized, 26 to losartan therapy and 27 to placebo. Of those enrolled, 51 subjects completed the trial, 25 in the losartan group and 26 in the placebo group. Quality review of the data resulted in three subjects being excluded from the analysis of insulin sensitivity due to poor quality insulin clamps. In one subject, the serum insulin level did not increase from baseline, in one subject the serum insulin level inexplicably increased ~5-fold mid-clamp, and in one subject steady-state was not achieved. No digital endothelial function study was considered inadequate. These

determinations were made in a blinded manner prior to locking of the database. Baseline characteristics of the 53 randomized subjects are presented by treatment assignment in Table 1.

Insulin sensitivity

At the baseline visit, insulin sensitivity was 5.65 (2.7) mg/kg of body weight per min among all subjects, confirming the presence of insulin resistance in the study population in comparison with historical healthy populations [19]. Insulin sensitivity at baseline was not different between the losartan- and placebo-treated subjects (Table 2). The primary end point, the change in insulin sensitivity, was not different between the losartan-treated and the placebo-treated groups. Of note, HOMA-B tended to increase from baseline in the losartan-treated group { + 26.1 [95 % CI (confidence interval), −2.75, 55.0]} and decrease in the placebo-treated group [−9.6 (95 % CI, −38.8, 19.5)], a difference on the margin of statistical significance ($P = 0.05$).

Endothelial function

At the baseline visit, the RH-PVA was 2.0, a result similar to what has been observed previously in healthy populations [20]. Baseline endothelial function was not different between the losartan- and placebo-treated subjects (Table 3). The change in endothelial function was not different after losartan therapy compared with placebo therapy (P value not significant).

Secondary outcomes

In addition to changes in insulin sensitivity and endothelial function, we also examined a series of measures of inflammation, oxidative stress and endothelial injury (Table 4). There were no significant changes in any of these after losartan compared with after placebo therapy.

BP and heart rate

BP was not different between the two treatment groups at baseline (Table 1). The change in BP after losartan therapy was not significantly different than after placebo therapy (Table 5). SBP tended to fall 3.3 (13.0) mmHg in the losartan group and to increase 1.4 (8.4) mmHg in the placebo group. DBP tended to fall 1.5 (8.5) mmHg in the losartan group and to increase 1.2 (7.4) mmHg in the placebo group. There was no difference in the change in heart rate in the losartan-compared with the placebo-treated patients.

Renal function and potassium

Baseline renal function, measured as the serum creatinine level, was within the normal range and did not differ between the two treatment groups (Table 5). There was no significant difference in the change in renal function after losartan compared with placebo therapy. Baseline and post-treatment serum potassium levels were within the normal range and did not differ between the two treatment groups.

DISCUSSION

We conducted an 8-week randomized double-blinded placebo-controlled trial of the ARB losartan (100 mg/day) in 53 patients with stage I hypertension, abdominal obesity and impaired fasting glucose, with co-primary end points of insulin sensitivity and endothelial dysfunction. We did not observe a significant effect of ARB therapy on either peripheral insulin sensitivity or endothelial function compared with placebo therapy. However, we did observe an improvement in pancreatic β-cell function attributable to ARB therapy. In addition, we did not observe an effect of losartan treatment on a panel of biomarkers of inflammation, oxidative stress and endothelial injury. Losartan did tend to lower BP to a similar extent to that observed in a related trial of 261 patients with essential hypertension randomized to either losartan or placebo for 8 weeks; however, the present study was not powered to detect differences in BP [21].

Insulin resistance

There is extensive experimental evidence suggesting that AngII induces insulin resistance. Specifically, AngII directly interferes with insulin metabolic signalling. AngII inhibits IRS (insulin receptor substrate)-1 and IRS-2 associated PI3K (phosphoinositide 3-kinase) activity and reduces the ability of IRS-1 to associate with the activated insulin receptor [4,22,23]. AngII also decreases IRS-1 protein levels in vascular smooth muscle cells and induces SOCS-3 (suppressor of cytokine signalling-3), which further inhibits insulin signalling and insulin-stimulated glucose disposal [3,24]. Thus the biology supports a potential role for ARB therapy to improve insulin sensitivity via improved insulin metabolic signalling.

In the present study, however, losartan did not improve peripheral insulin sensitivity, as measured using a hyperinsulinaemic–euglycaemic clamp. This differs with findings in some, but not all previous, reports. Paolissi et al. [14] reported an ~25 % improvement in GDR with losartan compared with placebo therapy in 16 mild-to-moderate hypertensive subjects, but, of note in that study, an unusually large reduction in BP (16/10 mmHg) was attributed to losartan therapy. Moan et al. [13] described a 27–30 % improvement in GDR and GDR/I with losartan (100 mg/day for 6 weeks), but this was an uncontrolled study of only five subjects with severe (DBP 115 mmHg) hypertension. In a subsequent controlled study by the same authors involving 20 mildly hypertensive subjects [25], losartan had no effect on insulin sensitivity. Interestingly, Moan et al. [25] and others [26–28] found that losartan, when compared with calcium channel blocker therapy, improved insulin sensitivity in hypertensive subjects. Laakso et al. [29] found that 12 weeks of treatment with losartan compared with metoprolol in hypertensive patients with hyperinsulinaemia had no effect on insulin-stimulated GDR. Finally, Fogari et al. [30–32] have reported three studies of different populations of hypertensive patients in which the effect of losartan on insulin-stimulated GDR was compared with ACE (angiotensin-converting enzyme) inhibitor therapy; in each trial, losartan failed to improve GDR, whereas the ACE inhibitor did. Our present controlled study of mild hypertensive subjects did not detect an effect of losartan on insulin sensitivity. On balance, these trials fail to support the notion that ARB therapy with

losartan in mild hypertension improves insulin resistance, as measured using the euglycaemic clamp technique.

This failure to improve insulin sensitivity seems in conflict with the reported reduction in risk for diabetes attributed to ARB therapy [33]. This discordance may be due to the fact that the GDR during a euglycaemic clamp is mostly determined by skeletal muscle insulin sensitivity, whereas the ARB therapy may be improving glycaemia by beneficial effects on the pancreatic β-cells. Santoro et al. [34], who found that ACE inhibitor therapy improved glycaemia in essential hypertension not by improvement in peripheral insulin sensitivity, but rather by preservation of pancreatic islet β-cell function, suggested this.

In fact, experimental data suggests that AngII contributes to β-cell dysfunction via adverse effects on islet blood flow, and by promoting islet cell fibrosis, inflammation and oxidative stress [35–37]. Indeed, ARB therapy was shown to improve early-phase insulin responses in hypertensive subjects with impaired glucose tolerance [38]. Our present study confirms that ARB therapy may preserve β-cell function in hypertension.

Endothelial function

AngII increases vascular ROS generation by induction of vascular NADPH oxidase [39]. As a consequence of this, the AngII-infused rat and the rat doubly transgenic for human renin and angiotensinogen develop hypertension, vascular oxidative stress and endothelial dysfunction, each of which is remedied by ARB therapy [5,40]. However, in our present study of obese hypertensive patients with impaired fasting glucose, losartan therapy did not improve endothelial function, as assessed by the change in digital PVA during RH. This is consistent with some, but not all, published reports. For example, ACE inhibitor therapy had no effect on forearm resistance vessel endothelial function compared with placebo therapy in 24 subjects with essential hypertension [41]. In contrast, Koh et al. [42] found that each of losartan, candesartan and irbesartan improved FMD (flow-mediated dilation) of the brachial artery compared with placebo in patients with mild-to-moderate essential hypertension. We did not detect an effect of ARB therapy on endothelial function measured by RH-PVA; however, our subjects' RH-PVA response prior to intervention was similar to that reported previously in healthy subjects [9,20,43]. Although the present study did not have an internal control group of healthy individuals, these historical data suggest that our selection criteria did not effectively identify a group of subjects with endothelial dysfunction.

Inflammation and oxidative stress

As a secondary aim of the present study, we examined the effect of losartan therapy on markers of inflammation, oxidative stress and endothelial damage. We anticipated that abnormalities in these biomarkers would provide insight into the mechanisms of AngII-mediated insulin resistance and endothelial dysfunction had these findings been confirmed. For each of these markers there was biological plausibility [40,44]; however, in the present study, losartan did not cause a significant improvement in any of these markers.

Limitations

The results of the present study should be considered in light of a few study limitations. First, the hyperinsulinaemic–euglycaemic clamp technique, as performed, measures skeletal muscle insulin resistance, but does address β-cell function. Secondly, our sample size may have been insufficient to detect changes in many of the examined biomarkers. Thirdly, both Bonetti et al. [43] and Liu et al. [20] found low interday reproducibility of the RH-PVA measurements. These reports suggest that our power calculation, based upon the repeatability of the brachial artery ultrasound technique, may have overestimated the power of our sample size. In addition, although subjects fulfilled entry criteria for BP and fasting glucose level, average BPs and glucose levels decreased somewhat (mean values, 135–136/88–89 mmHg and 96–98 mg/dl respectively) after the placebo run-in period. Greater baseline abnormalities may be necessary to observe a positive effect of ARBs on insulin resistance or endothelial dysfunction.

Conclusions

In summary, we did not observe an effect of ARB therapy on either insulin sensitivity or endothelial dysfunction in stage I hypertension characterized by abdominal obesity and insulin resistance. Our results do suggest though that ARB therapy improves pancreatic β-cell function in this population.

ACKNOWLEDGEMENTS

Abbreviations

ACE	angiotensin-converting enzyme
ANCOVA	analysis of covariance
AngII	angiotensin II
ARB	angiotensin receptor blocker
BMI	body mass index
BP	blood pressure
BSA	body surface area
CI	confidence interval
DBP	diastolic BP
GDR	glucose disposal rate

GDR/*I* ratio	GDR/mean level of insulin ratio
HOMA	homoeostasis model assessment
HOMA-B	HOMA of pancreatic β-cell function
HOMA-IR	HOMA of insulin resistance
hsCRP	high-sensitivity C-reactive protein
IRS	insulin receptor substrate
MCP-1	monocyte chemoattractant protein-1
ox-LDL	oxidized low-density lipoprotein
PVA	pulse volume amplitude
RH	reactive hyperaemia
RH-PVA	PVA during RH relative to baseline
ROS	reactive oxygen species
SBP	systolic BP
VCAM-1	vascular cell-adhesion molecule-1

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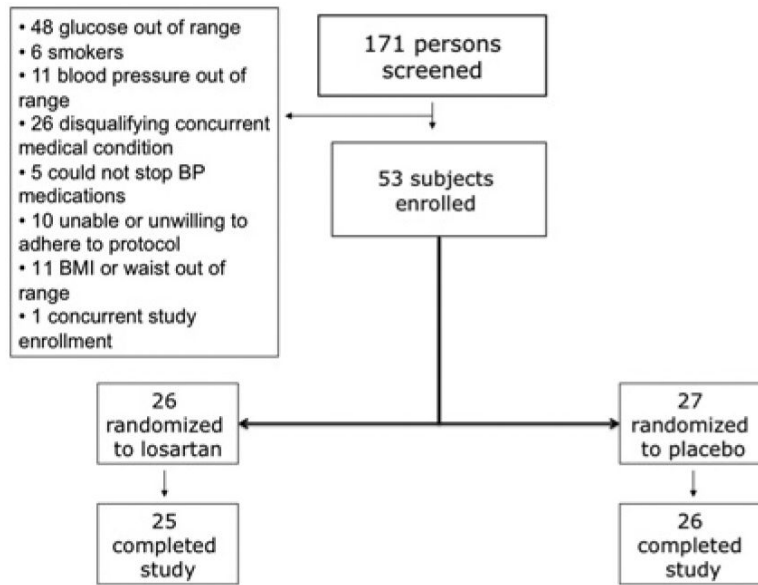
Figure 1.
CONSORT flow diagram for the trial

Table 1

Characteristics of all study participants at screening and by treatment assignment at completion of placebo run-in

Characteristic	Screening visit	Baseline visit	
	All subjects ($n = 53$)	Placebo ($n = 27$)	Losartan ($n = 26$)
Age (years)	52.5 (9.5)	53.8 (8.3)	51.1 (10.5)
Sex (female) (n)	26 (49.1 %)	12 (44.4 %)	14 (53.8 %)
Race			
White (n)	40 (75.4 %)	24 (88.9 %)	16 (61.5 %)
Black (n)	10 (18.9 %)	1 (3.7 %)	9 (34.6 %)
Other (n)	3 (5.7 %)	2 (7.4 %)	1 (3.8 %)
Taking antihypertensive therapy (n)	1 (1.9 %)	0	1 (3.8 %)
Weight (kg)	101.2 (13.5)	101.6 (14.1)	101.0 (13.7)
BMI (kg/m^2)	34.7 (3.8)	34.7 (3.8)	34.8 (3.8)
Waist circumference (cm)[*]	111.2 (9.9)	n/a	n/a
SBP (mmHg)	136.0 (10.8)	135.0 (9.6)	136.3 (10.9)
DBP (mmHg)	87.2 (6.4)	86.6 (5.3)	88.7 (5.3)
Heart rate (beats/min)	71.5 (10.6)	74.8 (12.2)	72.4 (9.2)
Potassium (mmol/l)	4.3 (0.3)	4.0 (3.3, 4.7)	4.1 (3.6, 4.9)
Glucose (mmol/l)	104.8 (8.6)	97.5 (1.7)	97.5 (1.7)
Insulin (μ-units/ml)	14.3 (8.0)	10.2 (0.9)	11.7 (1.2)
HOMA-IR	3.8 (2.3)	2.5 (0.2)	2.9 (0.3)
Triacyglycerols (mmol/l)	n/a	136.5 (56.0, 309.0)	113.0 (51.0, 235.0)
HDL-cholesterol (mmol/l)	n/a	44.0 (22.0, 77.0)	43.0 (31.0, 83.0)
Creatinine (mg/dl)	1.0 (0.2)	0.93 (0.03)	0.88 (0.04)
Urine albumin/creatinine ratio (mg/mmol)	n/a	1.22 (0.35)	1.02 (0.18)

Continuous variables as means (S.E.M.) or medians (range). HDL, high-density lipoprotein; n/a, not available.

[*] Measured at the screening visit only.

Table 2

Insulin clamp data in losartan- and placebo-treated patients

Parameter	Treatment assignment	Pre-treatment	Post-treatment	Change from baseline	P value
Fasting glucose (mg/dl)	Losartan	97.5 (9.5)	98.2 (6.5)	2.3 (−1.38, 5.92)	0.69
	Placebo	97.5 (8.6)	97.9 (10.5)	1.4 (−2.26, 5.04)	
Fasting insulin (μ-units/ml)	Losartan	11.7 (6.1)	14.5 (8.3)	3.5 (0.56, 6.34)	0.07
	Placebo	10.2 (4.3)	10.9 (7.2)	0.2 (−2.69, 3.04)	
HOMA-IR	Losartan	2.9 (1.7)	3.3 (2.1)	0.7 (−0.02, 1.41)	0.20
	Placebo	2.5 (1.2)	2.7 (2.2)	0.1 (−0.61, 0.85)	
HOMA-B	Losartan	127.3 (71)	150.0 (90)	26.1 (−2.75, 55.0)	0.05
	Placebo	108.3 (44)	108.1 (50)	−9.6 (−38.76, 19.52)	
Insulin sensitivity corrected for the steady-state insulin level [mg/kg of body weight per min/(μ-units/ml)]	Losartan	5.2 (2.7)	4.5 (1.7)	−0.8 (−1.62, −0.07)	0.62
	Placebo	6.1 (2.9)	5.3 (2.8)	−0.6 (−1.39, 0.19)	

Values are means (S.D.) or least-squares mean (95 % CI). *P* values are a comparison between the groups for a change from the baseline value adjusted for baseline value and study site.

Table 3

EndoPAT results in losartan- and placebo-treated patients

EndoPAT RH	Pre-treatment	Post-treatment	Change from baseline	*P* value
Treatment assignment				
Losartan	2.15 (0.7)	2.11 (0.7)	−0.06 (−0.33, 0.21)	0.31
Placebo	1.81 (0.5)	1.76 (0.7)	−0.23 (−0.51, 0.04)	

Values are means (S.D.) or least-squares mean (95 % CI). *P* values are a comparison between the groups for a change from the baseline value adjusted for baseline value and study site.

Table 4

Blood and urine markers in losartan- and placebo-treated patients

Marker	Treatment assignment	Pre-treatment	Post-treatment	Change from baseline	P value
hsCRP (mg/l)[*]	Losartan	2.6	2.0	−34 % (−50 %, −13 %)	0.07
	Placebo	3.3	2.9	−10 % (−32 %, 20 %)	
VCAM-1 (ng/ml)	Losartan	598	585	−21 (−70, 28)	0.10
	Placebo	663	680	29 (−20, 79)	
MCP-1 (pg/ml)	Losartan	335	338	−24 (−65, 18)	0.62
	Placebo	373	352	−37 (−80, 6.8)	
oxLDL (units/l)	Losartan	50.2	39.8	−5.5 (−11.9, 0.9)	0.37
	Placebo	41.4	39.8	−2.0 (−8.1, 4.1)	
F_2-isoprostanes (ng/mg of creatinine)	Losartan	2.1	3.1	0.9 (−0.4, 2.2)	0.51
	Placebo	1.7	2.5	0.4 (−0.9, 1.6)	
E-selectin (ng/ml)	Losartan	41.8	41.6	−0.6 (−3.5, 2.3)	0.55
	Placebo	43.4	42.7	−1.6 (−4.5, 1.3)	
Urine albumin/creatine (mg/mmol)	Losartan	1.0	1.4	0.2 (−0.3, 0.8)	0.69
	Placebo	1.2	1.5	0.4 (−0.1, 0.9)	

Values are means (S.D.) or least-squares mean (95 % CI), unless otherwise stated. *P* values are a comparison between the groups for a change from the baseline value adjusted for baseline value and study site.

[*] Geometric means and the geometric mean of the percentage change from baseline are reported.

Table 5

Select clinical characteristics in losartan- and placebo-treated patients

Characteristic	Placebo			Losartan			P value
	Pre-treatment	Post-treatment	Change from baseline	Pre-treatment	Post-treatment	Change from baseline	
SBP (mmHg)	135.0 (9.8)	136.4 (11.3)	1.4 (8.4)	136.2 (11.1)	132.9 (12.3)	−3.3 (13.0)	0.36
DBP (mmHg)	86.3 (5.3)	87.5 (9.9)	1.2 (7.4)	88.6 (5.4)	87.1 (10.2)	−1.5 (8.5)	0.26
Heart rate (beats/min)	74.8 (12.2)	73.2 (10.5)	−1.7 (11.7)	72.4 (9.2)	71.4 (9.3)	−1.0 (9.9)	0.81
Blood urea nitrogen (mg/dl)	13.5 (6.0, 19.0)	14.0 (6.0, 21.0)	0.5 (−7.0, 7.0)	12.0 (6.0, 20.0)	12.0 (6.0, 17.0)	0.0 (−7.0, 4.0)	0.48
Creatinine (mg/dl)	0.9 (0.7, 1.3)	0.9 (0.6, 1.3)	0.0 (−0.2, 0.1)	0.9 (0.7, 1.4)	0.8 (0.7, 1.4)	0.0 (−0.1, 0.3)	0.83
Serum potassium (mmol/l)	3.9 (0.3)	4.1 (0.3)	0.2 (0.5)	4.1 (0.3)	4.1 (0.3)	0.0 (0.3)	0.39

Values are means (S.D.) or medians (95 % CI). P values are the comparison of the change after losartan therapy with that after placebo therapy, using ANCOVA with terms for treatment, investigator sites and the corresponding baseline measurement.