

Arterial stiffness and haemodynamic response to vasoactive medication in subjects with insulin-resistance syndrome

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A B S T R A C T

INSR (insulin-resistance syndrome) affects 25 % of the Australian population and is associated with increased cardiovascular risk. In the present study, we postulated that early cardiovascular changes in these individuals may be associated with an activated RAS (renin–angiotensin system). We studied 26 subjects: 13 with INSR [waist circumference, 99 ± 6 cm; HOMA (homoeostasis model assessment) score, 2.5 ± 0.3] and 13 NCs (normals controls; waist circumference, 77 ± 2 cm; HOMA score, 1.4 ± 0.2). All received intravenous GTN (glyceryl trinitrate; 10, 20 and 40 $\mu\text{g}/\text{min}$), L-NMMA (N^G -monomethyl-L-arginine; 3 mg/kg of body weight), AngII (angiotensin II; 8 and 16 ng/min), the selective AT₂R (AngII type 2 receptor) inhibitor PD123319 (10 and 20 $\mu\text{g}/\text{min}$) and AngII (16 ng/min) + PD123319 (20 $\mu\text{g}/\text{min}$). At the end of each infusion, arterial stiffness indices [SI (stiffness index) and RI (reflection index)] and haemodynamic parameters were measured. There was a significantly higher RI response to AngII ($P = 0.0004$ for both 8 and 16 ng/min doses) and to PD123319 ($P = 0.004$ and $P = 0.03$ for 10 and 20 $\mu\text{g}/\text{min}$ doses respectively) in subjects with INSR compared with NCs. Co-infusion of AngII and PD123319 did not lead to additive changes in RI. RI responses to L-NMMA and GTN were not significantly different in both groups. No significant differences in SI and haemodynamic responses were detected. In conclusion, AT₁R (AngII type I receptor) and AT₂R activity produce arterial stiffness changes in subjects with INSR. Evidence of increased AT₁R- and AT₂R-mediated responses in small-to-medium-sized arteries in INSR was found, and may play an early role in the pathogenesis of vascular changes in INSR before haemodynamic changes become apparent.

Key words: angiotensin II (AngII), angiotensin II type 1 receptor (AT₁ receptor), angiotensin II type 2 receptor (AT₂ receptor), arterial stiffness, digital photoplethysmography, insulin resistance.

Abbreviations: ANCOVA, analysis of covariance; AngII, angiotensin II; AT₁R, AngII type 1 receptor; AT₂R, AngII type 2 receptor; CI, cardiac index; CV, coefficient of variation; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GTN, glyceryl trinitrate; HDL-C, high-density lipoprotein cholesterol; HOMA, homoeostasis model assessment; HR, heart rate; IDF, International Diabetes Federation; INSR, insulin-resistance syndrome; LDL-C, low-density lipoprotein cholesterol; NC, normal control; L-NMMA, N^G -monomethyl-L-arginine; OGTT, oral glucose tolerance test; RAS, renin–angiotensin system; RI, reflection index; SBP, systolic blood pressure; SI, stiffness index; SVRI, systemic vascular resistance index; ZI, stroke index.

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INTRODUCTION

INSR (insulin-resistance syndrome) affects up to 25% of the Australian population aged >20 years [1]. It is associated with a 2-fold increased risk of developing cardiovascular disease [2], and a 7-fold increased risk of developing Type 2 diabetes mellitus [3]. With the median age and weight of the Australian population rising, INSR and its complications are emerging to be a major public health problem.

Arterial stiffness is an established independent and powerful predictor of cardiovascular disease [4–6], and has a close correlation with atherosclerotic burden and cardiovascular risk factors [7]. Insulin stimulates the proliferation of vascular smooth muscle cells [8] and may contribute to vascular stiffening. In addition, other components of INSR, such as dyslipidaemia and hypertension, may cause endothelial damage, which can lead to a reduction in the production of the vasodilator NO [9]. Arterial stiffness is related to endothelial dysfunction [10] and may be a precursor to atherogenesis [11].

The RAS (renin–angiotensin system) contributes to the development of arterial stiffness by altering the extracellular matrix in the vascular media [12]. In addition, atherosclerotic lesions have been demonstrated to have an activated local RAS [13,14], with up-regulation of the two principal subtypes of AngII (angiotensin II) receptors: AT₁R (AngII type 1 receptor) and AT₂R (AngII type 2 receptor) [15]. AT₁R mediates most of the potentially harmful cardiovascular effects, such as vasoconstriction, fluid retention and atherogenesis, whereas AT₂R is thought to be up-regulated in diseased vascular beds and mediates essentially opposing effects to AT₁R [15].

In support of this hypothesis, our previous studies on the intravenous infusion of the highly selective AT₂R blocker PD123319 (10 µg/min for 3 min) [16] did not produce any significant haemodynamic or arterial stiffness changes compared with placebo infusion in 16 healthy volunteers [17]. In contrast, infusion of the same dose of PD123319 in ten age- and sex-matched otherwise healthy individuals with HOMA-IR [HOMA (homeostasis model assessment) of insulin resistance] >2 produced a significant increase in the stiffness of small-to-medium-sized arteries with a concurrent rise in SVRI (systemic vascular resistance index), but no other haemodynamic changes [18]. This suggests functional expression of AT₂Rs in small-to-medium-sized arteries in subjects with insulin resistance and, therefore, infers early microvascular damage in these individuals.

Subjects with INSR have an established increased cardiovascular risk and have been demonstrated to have changes in arterial stiffness [9,19,20]. Increased vascular sensitivity to AngII-induced vasoconstriction has been reported previously in patients with Type 2 diabetes mellitus [21], and AngII appears to be an important determinant of large artery stiffness [22]. The role of the

AngII receptors in potential early vascular changes in INSR, however, has not been established. Furthermore, there have been conflicting reports of the presence of endothelial dysfunction in subjects with INSR [19]. We therefore investigated the role of the AT₁Rs, AT₂Rs and NO mediators in the production of arterial stiffness and haemodynamic changes in subjects with INSR compared with age- and sex-matched NCs (normal controls).

MATERIALS AND METHODS

Subjects

Subjects were recruited through approved public advertisements. Interested volunteers were requested to attend a screening visit, where a medical history and examination were obtained. Fasting blood samples were obtained for total cholesterol, triacylglycerols (triglycerides), calculated LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), plasma glucose, FPI (fasting plasma insulin), high-sensitivity CRP (C-reactive protein), full blood count, electrolytes and creatinine. An estimate of INSR was derived using HOMA: FPG [fasting plasma glucose (mmol/l)] × FPI (m-units/l)/22.5 [23]. Those with a HOMA score >1 were requested to go on a 3-day high-carbohydrate diet and return for a 75 g OGTT (oral glucose tolerance test). Those with an OGTT consistent with the WHO (World Health Organization) criteria for diabetes were excluded from the study and were referred back to their local medical officer for further management of their condition. Non-diabetic subjects who had impaired fasting glycaemia (fasting blood sugar level ≥5.6 mmol/l) or who had features consistent with INSR according to IDF (International Diabetes Federation) criteria (http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf) were included in the INSR group. Subjects who did not have features of INSR, with a normal 2 h OGTT, were included in the control group. Other eligibility criteria for both the INSR and the NC groups included: age between 18 and 60 years, non-smoker, SBP (systolic blood pressure) <140 mmHg, DBP (diastolic blood pressure) <90 mmHg, no known cardiovascular disease, BMI (body mass index) <35 kg/m², not on any vasoactive medication, total cholesterol <7.5 mmol/l, triacylglycerol <4.0 mmol/l, alcohol consumption <20 g/day, creatinine <110 mmol/l and haemoglobin >120 g/l. The above parameters were chosen to exclude patients requiring medical therapy according to Australian national guidelines [24].

The study was conducted in an open-label fashion. Subjects were requested to attend the clinic at 08.00 hours, after an overnight fast and abstinence from alcohol and caffeinated foods and beverages for 24 h. The subjects rested supine for 30 min in a temperature-controlled room (23 ± 1 °C) to allow acclimatization. A 22-gauge

intravenous cannula was inserted in a right forearm vein.

Informed written consent was obtained from all subjects after the nature of the trial was explained. The study was performed at the University of New South Wales St George Clinical School, St George Hospital, Kogarah, Australia. Approval to perform the study was obtained from the South Eastern Sydney and Illawarra Area Health Service Research and Ethics Committee. The study was registered with the Australian Government, Department of Health and Ageing, Therapeutic Goods Administration (Clinical Trial Number 037/2005).

Measurement of arterial stiffness and haemodynamic parameters

Arterial stiffness was assessed from the left index finger using a digital photoplethysmograph (Pulse Trace; Micro Medical). The method has been described in detail previously [18]. Briefly, the Pulse Trace system uses a simplified analysis of the digital volume pulse wave, and measures SI (stiffness index) and RI (reflection index) as indices of arterial stiffness. SI has been shown to be strongly correlated with accepted indices of large arterial stiffness, such as central pulse wave velocity (aortic and carotid-femoral) [25,26]. Chowienczyk et al. [27] demonstrated that the RI waveform is formed by wave reflection from small arteries in the trunk and lower limbs, proximal to resistance vessels. SI therefore can be used to infer changes in large arterial stiffness and RI to infer changes in small-to-medium-sized arterial stiffness. An increase in both SI and RI corresponds with an increase in large and small-to-medium-sized arterial stiffness respectively.

Preliminary studies using the method in 115 subjects showed good intra-individual reproducibility for SI [CV (coefficient of variation) = 8%] and RI (CV = 5%). Similarly, inter-day reproducibility was good, with CV = 6% for SI and CV = 7% for RI.

Baseline SI and RI were measured while receiving normal saline infusion (20 ml/h) at 5 min intervals for a total of 15 min. Simultaneous measurements of haemodynamic parameters, including CI (cardiac index), ZI (stroke index), SVRI, SBP, DBP and HR (heart rate) were taken using a previously validated [28,29] thoracic electrical bioimpedance monitor (BioZ system; Cardiodynamics International), with an in-built oscillometric sphygmomanometer. All values were averaged to give a mean baseline result.

With the exception of L-NMMA (N^G -monomethyl-L-arginine), all infusions were given over 3 min. Measurements of arterial stiffness indices (SI and RI) and haemodynamic parameters were recorded at 1 min intervals for a minimum of 10 min. The three maximum response measurements were identified, averaged and compared with the baseline result. Measurements of arterial stiffness and haemodynamic parameters continued

each minute until values returned to baseline (the 'wash-out period') before infusion of the higher dose.

Reagents

The order of drug administration was standardized, so that any carry-over effects (unlikely based on the Results, see later) would be the same for all subjects.

GTN (glyceryl trinitrate; David Bull Laboratories) was infused intravenously at three doses (10, 20 and 40 $\mu\text{g}/\text{min}$). The washout period between each dose was approx. 30–45 min.

AngII and PD123319 were obtained from Sigma-Aldrich. Aliquots were dispensed into autoclaved vials and then freeze-dried. The freeze-dried aliquots were stored for 2 years at 2–8°C before being used for this project. Both at the time of initial aliquot preparation and again immediately prior to the commencement of this project, 10% of the sample was randomly selected and sent for analysis by an independent accredited private laboratory (AMS Laboratories). On both occasions the samples were certified sterile and pyrogen-free. Both reagents were aseptically reconstituted with sterile normal saline. Subjects received two doses of AngII (8 and 16 ng/min) intravenously. The washout period between each dose was approx. 30–60 min. PD123319 was infused at two doses (10 and 20 $\mu\text{g}/\text{min}$). The washout period between each dose was approx. 30–60 min.

Finally, both AngII (16 ng/min) and PD123319 (20 $\mu\text{g}/\text{min}$) were infused simultaneously. The above doses were chosen in order to produce arterial stiffness changes, while minimizing haemodynamic changes which affect arterial stiffness indices.

L-NMMA was obtained from Alexis Corporation. It was aseptically reconstituted with sterile normal saline. Subjects received 3 mg of L-NMMA/kg of body weight intravenously over 5 min. The maximum responses occurred between 15 and 45 min after commencing the infusion. Three maximal response values were obtained and averaged, and the mean value was used to compare with the baseline value. The washout period for L-NMMA varied between 2 and > 6 h.

Statistical analysis

All statistical analyses were performed using STATISTICA 7.0 software (StatSoft). Baseline values for SI, RI, SBP, DBP, SVRI, ZI and CI were averaged and are presented as means \pm S.E.M. Normality of distribution of arterial stiffness indices and haemodynamic parameters were tested using the Shapiro–Wilk test. SI and RI were not normally distributed, whereas all of the haemodynamic parameters were normally distributed. The primary end-point of the study was the difference in the mean percentage change from baseline in response to GTN (10, 20 and 40 $\mu\text{g}/\text{min}$), L-NMMA (3 mg/kg of body weight), AngII (8 and 16 ng/min), PD123319 (10 and 20 $\mu\text{g}/\text{min}$) and

AngII (16 ng/min) + PD123319 (20 µg/min) between the subjects with INSR and age- and sex-matched NCs. A Mann–Whitney *U* test was used for comparison of changes in SI and RI, and an independent Student's *t* test was used for comparison of haemodynamic changes.

As haemodynamic changes are known to affect arterial stiffness, haemodynamic changes from baseline and after each drug infusion were compared within each group, using a dependent Student's *t* test, to allow correction for the influence of any significant haemodynamic change with each infusion using ANCOVA (analysis of covariance).

Age and gender are well-documented predictors of arterial stiffness. As subjects with INSR were age- and sex-matched with the control population, adjustments for these factors were not required. The influence of such factors as total cholesterol, calculated LDL-C, HDL-C, triacylglycerol, HOMA, FPI and FPG on arterial stiffness indices for the group as a whole were analysed further using Spearman's rank-order correlation. Measurements were not adjusted for waist circumference and BMI, as the IDF considers obesity as an essential feature of INSR.

Power calculation for $n=13$ in each group had an 80% statistical power to detect a difference of >10% in responsiveness (two-tailed α of $P < 0.05$), based on the between-person variability observed from previous studies [17,18].

RESULTS

A total of 28 volunteers were recruited. Two were excluded from the study: one was a current smoker, and the other was diagnosed with diabetes mellitus after the 2 h OGTT. Therefore a total of 26 subjects completed the study.

The INSR group comprised 12 subjects who fulfilled the IDF criteria for INSR and one with impaired fasting glycaemia. The NC group comprised 13 age- and sex-matched healthy controls. All subjects were non-smokers and did not have any significant medical conditions, including cardiovascular disease. None were receiving medication. The demographic features of both groups are listed in Table 1.

There was a statistically significant difference in features which defined subjects with INSR, including BMI, waist circumference, HOMA score and HDL-C. There were baseline differences in SI, SVRI and DBP, with the NC group having higher baseline values. Arterial stiffness responses were therefore adjusted for DBP and SVRI changes using ANCOVA.

Baseline arterial stiffness and haemodynamic values were remeasured after the washout period of each drug and before infusion of the next medication. The mean baseline CV before the infusions for SI was 8.0% and for RI it was 7.1%.

Table 1 Demographic features of patients with INSR and their age- and sex-matched NCs

	Patients with INSR	NCs	<i>P</i> value
<i>n</i>	13	13	
Age (years)	31 ± 2	31 ± 3	0.92
Females (<i>n</i>)	8	8	
BMI (kg/m ²)	29.9 ± 2.0	22.5 ± 0.6	0.0006
SI (m/s)	6.27 ± 0.25	7.93 ± 0.57	0.01
RI (%)	67 ± 4	78 ± 3	0.03
SBP (mmHg)	111 ± 3	116 ± 3	0.32
DBP (mmHg)	61 ± 3	70 ± 3	0.03
HR (beats/min)	66 ± 2	67 ± 2	0.69
SVRI (dyn · s · min)	1976 ± 104	2416 ± 138	0.02
ZI (ml/m)	45 ± 2	41 ± 2	0.21
CI (litre · min ⁻¹ · m ⁻¹)	3.0 ± 0.1	2.8 ± 0.1	0.23
Total cholesterol (mmol/l)	4.9 ± 0.3	5.3 ± 0.4	0.37
Triacylglycerol (mmol/l)	1.2 ± 0.2	1.4 ± 0.3	0.59
HDL-C (mmol/l)	1.5 ± 0.1	1.8 ± 0.1	0.05
Calculated LDL-C (mmol/l)	2.8 ± 0.2	2.8 ± 0.3	0.93
HOMA	2.5 ± 0.3	1.4 ± 0.2	0.004
FPI (m-units/l)	12 ± 2	7 ± 1	0.007
FPG (mmol/l)	4.9 ± 0.1	4.6 ± 0.1	0.06
Waist circumference (cm)	99 ± 6	77 ± 2	0.0006

Statistical comparison of the mean percentage change from baseline between the subjects with INSR and NCs demonstrated a significant difference in RI after AngII (8 and 16 ng/min) and PD123319 (10 and 20 µg/min), as shown in Figures 1 and 2. There were no concurrent significant differences in SI or haemodynamic response to AngII and PD123319 between the two groups.

The response to AngII (8 and 16 ng/min) was corrected for HR, SVRI, CI and DBP changes in each group. After correction for these factors, the RI response remained significantly different between the two groups at both doses of AngII ($P = 0.005$ and $P = 0.01$ respectively).

The RI response to PD123319 (10 and 20 µg/min) was corrected for SVRI and DBP change. After correction for these factors, the difference in RI response between the two groups remained significantly different ($P = 0.03$ and $P = 0.02$ respectively).

No incremental increase in SI and RI (Figure 3) was found with the infusion of both AngII (16 ng/min) + PD123319 (20 µg/min). Similarly, there were no significant haemodynamic changes observed.

RI increased significantly from baseline in both subjects with INSR and NCs (results not shown). The RI response to L-NMMA (Figure 4) in subjects with INSR was significantly greater compared with NCs (mean percentage change in RI was 24.3 ± 6.1 and $12.3 \pm 3.6\%$ respectively; $P = 0.04$). However, after correction for

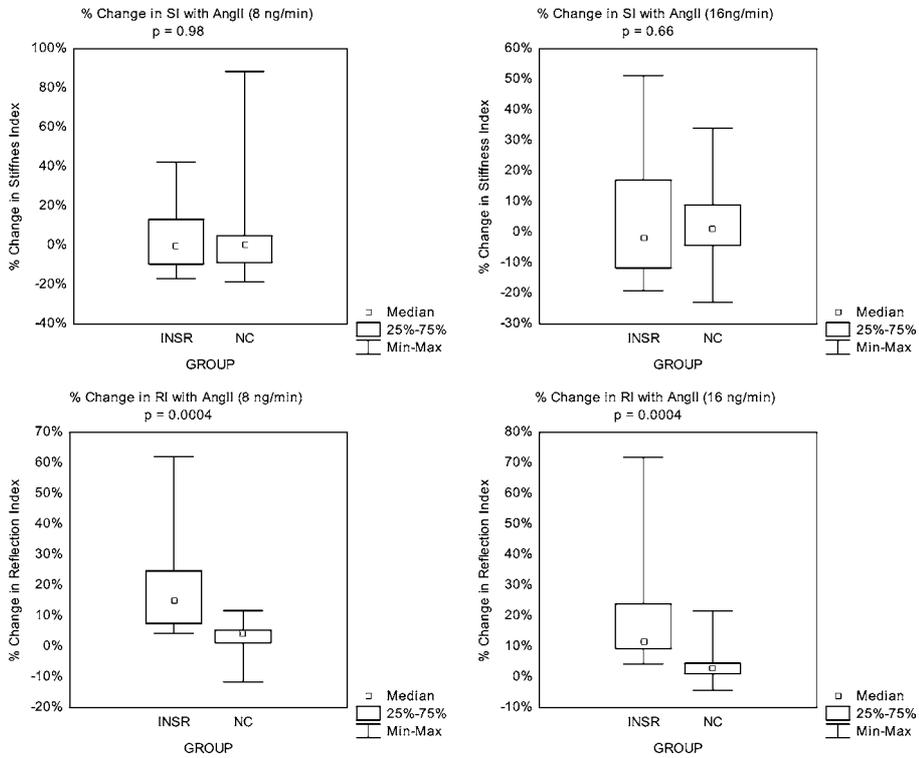


Figure 1 Comparison of percentage changes in SI and RI with AngII (8 and 16 ng/min) using the Mann–Whitney *U* test

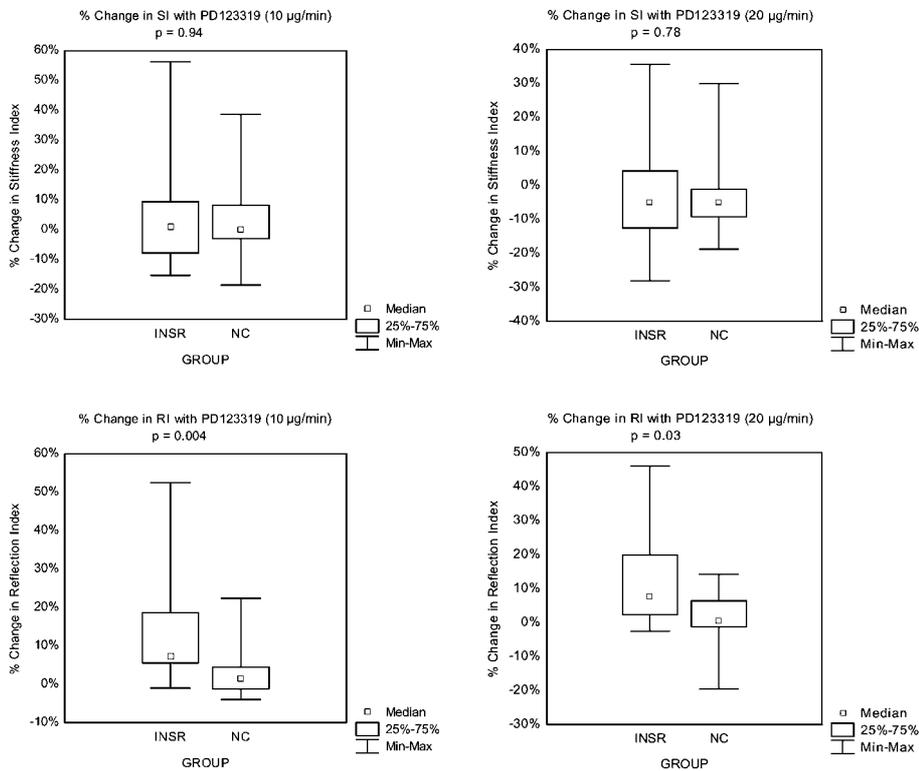


Figure 2 Comparison of percentage changes in SI and RI with PD123319 (10 and 20 µg/min) using the Mann–Whitney *U* test

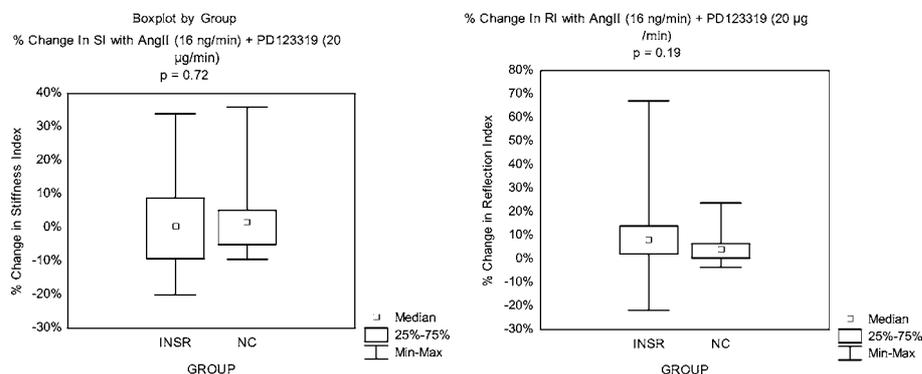


Figure 3 Comparison of percentage changes in SI and RI in subjects with INSR and NCs after intravenous infusion of AngII (16 ng/min) + PD123319 (20 µg/min)

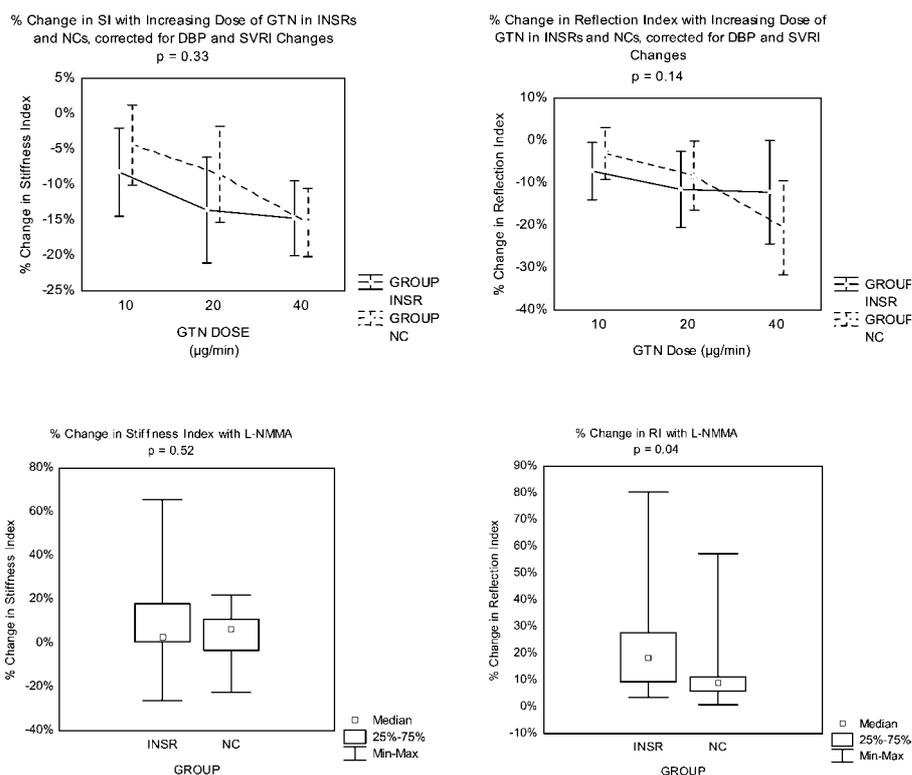


Figure 4 Percentage change in SI and RI with increasing doses of GTN (10, 20 and 40 µg/min) in subjects with INSR and NCs, and a comparison of percentage changes in SI and RI in response to L-NMMA using the Mann-Whitney *U* test

changes in DBP, HR, SVRI, ZI and CI, the difference was found to be non-significant ($P=0.11$). No concurrent significant differences in SI or haemodynamic response were found between the two groups.

Both SI and RI reduced significantly with the highest dose of GTN (40 µg/min) in subjects with INSR and NCs (results not shown). Infusion of GTN (10, 20 and 40 µg/min), however, did not produce any significant difference in arterial stiffness or haemodynamic response between the two groups. This result remained robust

after further analysis using a general linear model with dose as a within-subject factor, group as a between-subject factor, DBP and SVRI as continuous predictors and group \times dose as the relevant effect of interest.

Results of the correlation analyses are shown in Table 2. In this cohort, the only factor correlated significantly with RI was BMI. Interestingly, a significant negative correlation was observed ($r = -0.47$, $P = 0.01$). Total cholesterol, calculated LDL-C levels, SBP and DBP were significantly correlated with SI.

Table 2 Factors correlated with SI and RIValues presented are *r* values, using Spearman rank-order correlation.

Factor	SI	RI
BMI	-0.26, <i>P</i> = 0.20	-0.47, <i>P</i> = 0.01
HOMA	-0.29, <i>P</i> = 0.15	-0.19, <i>P</i> = 0.35
FPG	-0.07, <i>P</i> = 0.73	0.17, <i>P</i> = 0.41
FPI	-0.29, <i>P</i> = 0.15	-0.18, <i>P</i> = 0.37
SBP	0.48, <i>P</i> = 0.01	0.23, <i>P</i> = 0.25
DBP	0.63, <i>P</i> = 0.006	0.33, <i>P</i> = 0.10
Triacylglycerol	0.35, <i>P</i> = 0.08	0.02, <i>P</i> = 0.93
Total cholesterol	0.54, <i>P</i> = 0.004	0.21, <i>P</i> = 0.30
HDL	0.09, <i>P</i> = 0.64	0.07, <i>P</i> = 0.73
LDL	0.42, <i>P</i> = 0.04	0.23, <i>P</i> = 0.25
Waist circumference	-0.24, <i>P</i> = 0.24	-0.37, <i>P</i> = 0.07

DISCUSSION

In recent years, there has been an increasing interest in the detection of subclinical atherosclerotic vascular disease in an effort to reduce the increasing burden of cardiovascular morbidity and mortality. Specific attention has been focused on non-invasive methods, such as measurement of arterial stiffness, to allow early detection and intervention before the establishment of cardiovascular disease.

The clustering of cardiometabolic risk factors in INSR produce a pro-atherosclerotic milieu leading to increased cardiovascular risk. Several mechanisms have been proposed to contribute to the development of early cardiovascular changes in these patients, including atheroma formation and the loss of arterial compliance. Although the roles of AngII and aldosterone have clearly been implicated in the pathogenesis of atheroma [30], the role of AngII receptors and the manner in which they interact in the early stages of insulin resistance has not been established. However, there is increasing evidence of a complex interplay between AT₁Rs and AT₂Rs, the latter of which appear to become expressed in the presence of arterial damage and play a role in counteracting the deleterious effects of AT₁R-mediated responses.

In the present study, we found a significant increase in RI (an index of small-to-medium-sized arterial stiffness) in response to AngII (8 and 16 ng/min) in subjects with INSR compared with age- and sex-matched NCs, with no concurrent significant difference in large arterial stiffness (SI) and haemodynamic parameters. This suggests a predominant AT₁R-mediated response, and, thereby, increased AT₁R expression/activity in small-to-medium-sized arteries in subjects with INSR, which was not evident in the NC group.

Evidence of increased functional AT₂R expression in subjects with INSR was also demonstrated with the intravenous infusion of the highly selective AT₂R blocker PD123319 (10 and 20 µg/min). A significant

increase in RI in subjects with INSR was observed, with the NC group demonstrating a minimal change. There were no concurrent significant differences in large arterial stiffness and haemodynamic response.

In total, these findings support the hypothesis that both AT₁R and AT₂R expression is increased in young people with INSR, before any other obvious manifestations of vascular disease or BP changes occur, and may therefore be a very early step in the process of vascular damage associated with INSR. Some studies performed on isolated vascular beds have demonstrated an increased vasoconstrictor response [30,31] to AngII in the presence of PD123319. The present study did not demonstrate an increased RI response to the concurrent infusion of AngII (16 ng/min) and PD123319 (20 µg/min). Repeated or prolonged stimulation of the AT₁R causes desensitization, presumed secondary to receptor internalization [32,33]. In contrast, AT₂R-mediated vasorelaxation has been shown not to undergo desensitization [32]. This may therefore explain the lack of an additive effect to AngII in the presence of PD123319 in the present study.

In the present study, we were able to confirm the relationship between arterial stiffness and NO activity in subjects with INSR and NCs, with both arterial stiffness indices (SI and RI) changing in response to the NO mediators GTN and L-NMMA. Changes in SI and RI, therefore, are influenced by the degree of NO release in large and small-to medium-sized arteries respectively. We did not, however, find any significant difference between the INSR and control groups in the endothelium-independent vasodilatory response to GTN. Natali et al. [34] had similar findings in their study on hypertensive men who underwent euglycaemic clamp studies to test their insulin sensitivity. No significant difference in the endothelium-independent vasodilation between the highest and lowest tertiles of insulin sensitivity was found.

We have demonstrated in the present study a trend towards an increase in RI in response to L-NMMA in subjects with INSR compared with NCs. This suggests a possible increased expression of basal NO in subjects with INSR, which concurs with other studies on patients with Type 2 diabetes mellitus [35]. Indeed, there is evidence in rat models that AT₂R-mediated relaxation occurs by stimulation of bradykinin B₂ receptors with downstream activation of the NO pathway [36]. Increased AT₂R expression in the INSR group may therefore account for the increase in basal NO activity. A relatively greater activation of AT₂Rs compared with AT₁Rs by endogenous AngII may, in part, explain the significantly lower baseline SI, RI, DBP and SVRI in subjects with INSR compared with the NC group. Alternatively, a higher basal NO release in subjects with INSR may, in itself, explain these differences, as well as the negative correlation observed between BMI and RI.

We found a significant relationship between SI and established cardiovascular risk factors, such as hypertension and dyslipidaemia. Although the relationship between SBP and DBP with indices of large arterial stiffness are well-documented, reports of its association with LDL-C and total cholesterol are controversial [4,37,38]. Our present results, which suggested functional expression of AT₂Rs in small-to-medium-sized arteries, but not in large arteries, is consistent with the findings of Batenburg et al. [39] in patients with coronary artery disease, where they were able to demonstrate functional expression of AT₂Rs in human micro-arteries, but not in large epicardial coronary arteries.

It is possible that a longer infusion time for PD123319 may have resulted in better equilibration to its receptors. All subjects, however, were observed for a period of at least 30–60 min post-infusion, which is adequate time for equilibration. Furthermore, the dosing appeared adequate, as there was no further increase in RI with the higher dose of PD123319 (20 µg/min).

In conclusion, AT₁R and AT₂R activity, as well as basal NO activity, produce arterial stiffness changes in subjects with INSR. Specifically, we provide evidence of a locally activated RAS in small-to-medium-sized arteries of subjects with INSR, which is not present in age- and sex-matched healthy NCs. There is an apparent increase in both AT₁R- and AT₂R-mediated responses in these arteries, which may represent an interactive homeostatic response to counteract early vascular damage in INSR.

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