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**Publication details:**

Clinical Science

v. 114

pp. 139-147

0143-5221 (ISSN)

**Publication Date:**

2008

**Publisher DOI:**

<http://dx.doi.org/10.1042/CS20070132>

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## Arterial Stiffness and Haemodynamic Response to Vasoactive Medications in Patients with Insulin Resistance Syndrome

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### Key Words

AT2 receptor, AT1 receptor, digital photoplethysmography, insulin resistance, arterial stiffness

### Abbreviations

INSR, insulin resistance syndrome; NC, normal controls; RAS, rennin-angiotensin system; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; AII, angiotensin II; HOMA, homeostasis model assessment; GTN, glyceryl trinitrate; SI, stiffness index; RI, reflection index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SVRI, systemic vascular resistance index; ZI, stroke index; CI, cardiac index

**Abstract:**

The insulin resistance syndrome (INSR) affects 25% of the Australian population, and is associated with increased cardiovascular risk. We postulated that early cardiovascular changes in these individuals may be associated with an activated renin-angiotensin system (RAS).

We studied 26 subjects: 13 with INSR (waist circumference  $99 \pm 6$  cms, HOMA  $2.5 \pm 0.3$ ) and 13 (NC) controls (waist circumference  $77 \pm 2$  cms, HOMA  $1.4 \pm 0.2$ ). All received intravenous glyceryl trinitrate (10, 20, 40  $\mu\text{g}/\text{min}$ ), L-NMMA (3 mg/kg), angiotensin II (AII) (8, 16 ng/min), the selective AII type 2 receptor (AT2R) inhibitor PD123319 (10, 20  $\mu\text{g}/\text{min}$ ) and AII (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 AII (8, 16 ng/min:  $p=0.0004$  for both doses) and to PD123319 (10, 20  $\mu\text{g}/\text{min}$ :  $p=0.004$  and  $p=0.03$  respectively) in INSRs compared with NC. Co-infusion of AII 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.

This study provides evidence of increased AT1R and AT2R-mediated responses in small to medium-sized arteries in INSR. Increased AT1R and AT2R activity may play an early role in the pathogenesis of vascular changes in INSR before haemodynamic changes become apparent.

## **Introduction.**

The insulin resistance syndrome (INSR) affects up to 25% of the Australian population aged > 20 [1]. It is associated with a two-fold increased risk of developing cardiovascular disease [2], and a seven-fold increased risk of developing type II 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 the INSR such as dyslipidaemia and hypertension may cause endothelial damage, which can lead to a reduction in the production of the vasodilator nitric oxide [9]. Arterial stiffness is related to endothelial dysfunction [10], and may be a precursor to atherogenesis [11].

The renin-angiotensin system (RAS) contribute 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 angiotensin II receptors: AT1 and AT2 receptor [15]. The AT1 receptor (AT1R) mediates most of the potentially harmful cardiovascular effects such as vasoconstriction, fluid retention and atherogenesis, while the AT2 receptor (AT2R) is thought to be up-regulated in diseased vascular beds and mediate essentially opposing effects to the AT1R [15].

In support of this hypothesis, our earlier studies on the intravenous infusion of the highly selective AT2 receptor blocker, PD123319 (10 micrograms/min for 3 minutes) [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 > 2 (a measure of insulin resistance), produced a significant increase in the stiffness of

small to medium sized arteries with a concurrent rise in systemic vascular resistance index but no other haemodynamic changes [18]. This suggests functional expression of AT2 receptors in small to medium sized arteries in patients with insulin resistance and therefore infer early microvascular damage in these individuals.

Patients with the INSR have an established increased cardiovascular risk, and have been demonstrated to have arterial stiffness changes [9, 19, 20]. Increased vascular sensitivity to AII-induced vasoconstriction has previously been reported in patients with type 2 diabetes mellitus [21], and AII appears to be an important determinant of large artery stiffness [22]. The role of the angiotensin II 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 patients with INSR [19]. We therefore investigated the role of the AT1 and AT2 receptors, and nitric oxide mediators (as markers of endothelial function) in the production of arterial stiffness and haemodynamic changes in patients with the INSR, compared with age- and sex-matched controls.

## **Methods**

The study was performed at the University of New South Wales St George Clinical School, St George Hospital, Australia. Approval to perform the study was obtained from the South Eastern Sydney and Illawarra Area Health Service Research and Ethics Committee (O'Sullivan 04/111), Clinical Trial Number: 037/2005 (registered with the Australian Government, Department of Health and Ageing, Therapeutic Goods Administration).

## **Subjects:**

Subjects were recruited through approved public advertisements. Interested volunteers were requested to attend a screening visit. A medical history and examination were obtained. Fasting blood samples were obtained for total cholesterol, triglycerides (TG), calculated LDL-cholesterol, HDL-cholesterol, plasma glucose, fasting plasma insulin, high sensitivity C-reactive protein, full blood count, electrolytes and creatinine. An

estimate of INSR was derived using the homeostasis model assessment (HOMA): fasting plasma glucose (mmol/l) x fasting plasma insulin (mU/L)/ 22.5 [23]. Those with HOMA >1 were requested to go on a 3 day high carbohydrate diet and return for a 75g oral glucose tolerance test (OGTT). Those with an OGTT consistent with the WHO criteria for diabetes were excluded from the study and referred back to their local medical officer for further management of their condition. Non-diabetic subjects who had impaired fasting glycaemia (fasting BSL  $\geq$  5.6 mmol/L) or who had features consistent with the insulin resistance syndrome (INSR) according to the International Diabetes Federation (IDF) criteria ([http://www.idf.org/webdata/docs/IDF\\_Meta\\_def\\_final.pdf](http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf), cited Dec 2006) were included in the INSR group. Subjects who did not have features of INSR, with a normal 2h OGTT were included in the control group. Other eligibility criteria for both the INSR and the NC group include: age between 18 and 60 years, non-smoker, systolic blood pressure < 140 mmHg, diastolic blood pressure < 90 mmHg, no known cardiovascular disease, BMI < 35 kg/m<sup>2</sup>, not on any vasoactive medications, total cholesterol < 7.5 mmol/L, TG < 4.0 mmol/L, alcohol consumption < 20g per 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]. Informed written consent was obtained from all subjects after the nature of the trial were explained.

The study was conducted in an open-label fashion. Subjects were requested to attend clinic at 8 am, after an overnight fast and abstinence from alcohol and caffeinated foods and beverages for 24 hours. They rested supine for 30 minutes in a temperature-controlled room ( $23 \pm 1^{\circ}$  C) to allow acclimatisation. A 22G intravenous cannula was inserted in a right forearm vein.

#### **Measurement of arterial stiffness and haemodynamic parameters:**

Arterial stiffness was assessed from the left index finger using a digital photoplethysmograph (Pulse Trace, Micro Medical, Gillingham, Kent, UK). 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 stiffness index (SI)

and reflection index (RI) as indices of arterial stiffness. SI has been shown to be strongly correlated to accepted indices of large arterial stiffness, such as central pulse wave velocity (aortic and carotid-femoral) [25, 26]. Chowienczyk et al demonstrated that the RI waveform is formed by wave reflection from small arteries in the trunk and lower limbs, proximal to resistance vessels [27]. SI therefore, can be used to infer changes in large arterial stiffness, and RI, 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 = 8%) and RI (CV = 5%). Similarly, interday reproducibility was good, with CV = 6% for SI and CV = 7% for RI.

Baseline SI and RI were measured whilst receiving normal saline infusion (20 mL/h), at 5 minute intervals for a total of 15 minutes. Simultaneous measurements of haemodynamic parameters, including cardiac index (CI), stroke index (ZI), systemic vascular resistance index (SVRI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were taken, using a previously validated [28, 29] thoracic electrical bioimpedance monitor (BioZ system, Cardiodynamics International Corporation, San Diego, CA.), with an in-built oscillometric sphygmomanometer. All values were averaged to give a mean baseline result.

With the exception of L-NMMA, all infusions were given over 3 minutes. Measurements of arterial stiffness indices (SI and RI) and haemodynamic parameters were recorded at minutely intervals for a minimum of 10 minutes. The three maximum response measurements were identified and averaged, and compared with the baseline result. Minutely measurements of arterial stiffness and haemodynamic parameters continued until values returned to baseline (the “washout period”), before infusion of the higher dose.

**Reagents:**

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

Glyceryl trinitrate (David Bull Laboratories, Australia) was infused intravenously at three doses (10, 20, 40 µg/minute). The washout period between each dose was approximately 30-45 minutes.

AII and PD123319 were obtained from Sigma-Aldrich Chemical Company (Sydney, Australia). 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 were randomly selected and sent for analysis by an independent accredited private laboratory (AMS Laboratories, Sydney, Australia). 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 AII (8, 16 ng/minute) intravenously. The washout period between each dose was approximately 30-60 minutes.

PD123319 was infused at two doses (10, 20 µg/minute). The washout period between each dose was approximately 30-60 minutes.

Finally, both AII (16 ng/minute) and PD123319 (20 µg/minute) were infused simultaneously.

The above doses were chosen in order to produce arterial stiffness changes, whilst minimizing haemodynamic changes which affect arterial stiffness indices.

L-NMMA was obtained from Alexis Corporation (Lausen, Switzerland). It was aseptically reconstituted with sterile normal saline. Subjects received 3 mg/kg L-NMMA intravenously over 5 minutes. The maximum responses occurred between 15-45 minutes



after commencing the infusion. Three maximal responses values were obtained and averaged, and was used to compare with the baseline value. The washout period for L-NMMA varied between 2 to >6 hours.

### **Statistical analysis.**

All statistical analyses were performed using STATISTICA 7.0 software (StatSoft, Tulsa, OK, USA). Baseline values for SI, RI, SBP, DBP, SVRI, ZI and CI were averaged, and presented as the mean  $\pm$  SEM. Normality of distribution of arterial stiffness indices and haemodynamic parameters were tested using the Shapiro-Wilk test. SI and RI were not normally distributed, whilst all haemodynamic parameters demonstrated normal distribution. The primary end-point of the study was the difference in the mean percentage change from baseline in response to GTN (10, 20, 40  $\mu$ g/minute), L-NMMA (3mg/kg), AII (8,16 ng/min), PD123319 (10, 20  $\mu$ g/minute) and AII (16 ng/min) + PD123319 (20  $\mu$ g/minute) between the INSR subjects and age- and sex-matched controls. The Mann-Whitney *U* test was used for comparison of SI and RI changes, and the independent *t*-test was used for comparison of haemodynamic changes.

Haemodynamic changes are known to affect arterial stiffness. Haemodynamic changes from baseline and after each drug infusion were therefore compared within each group, using the dependent *t*-test, to allow correction for the influence of any significant haemodynamic change with each infusion using ANCOVA.

Age and gender are well-documented predictors of arterial stiffness. Since INSR subjects are 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-cholesterol, HDL cholesterol, triglyceride, HOMA, fasting plasma insulin and glucose on arterial stiffness indices for the group as a whole were further analysed 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 80% statistical power to detect a difference of  $>10\%$  in responsiveness (two-tailed alpha of  $p < 0.05$ ), based on the between person variability observed from previous studies [17,18].

## **Results**

Twenty-eight 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 hour OGTT. Twenty-six subjects completed the study.

The INSR group comprised twelve subjects who fulfilled the IDF criteria for the insulin resistance syndrome, and one with impaired fasting glycaemia. Thirteen age- and sex-matched healthy controls were recruited. All subjects were non-smokers and did not have any significant medical conditions, including cardiovascular disease. None were receiving medications. The demographic features of each group are listed in Table 1.

There was a statistically significant difference in features which defined the INSR subjects, including BMI, waist circumference, HOMA score and HDL. 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 before each infusion were obtained. The mean baseline CV before each infusion for SI was 8.0%, and for RI, 7.1%.

Statistical comparison of the mean percentage change from baseline between the INSR subjects and NCs demonstrated a significant difference in the mean percentage change in RI only after AII (8, 16 ng/min) and PD123319 (10, 20  $\mu\text{g}/\text{minute}$ ), as illustrated in Figures 1 and 2. There were no concurrent significant differences in SI or haemodynamic parameter response to angiotensin II and PD123319 between the two groups.

Infusion of AII (8,16 ng/min) produced significant changes in HR, SVRI and CI. After correction for these factors and DBP changes, the RI response remained significantly different between the two groups at both doses of angiotensin II ( $p = 0.005$  and  $p = 0.01$ , respectively).

A significant increase in SVRI was observed in INSR subjects with the infusion of PD123319 (10, 20  $\mu\text{g}/\text{min}$ ). After correction for this factor and DBP change, the difference in RI response between the two groups remained significantly different ( $p = 0.03$  and  $p = 0.02$ ).

No incremental increase in SI and RI (Figure 3) was found with the infusion of both AII (16 ng/min) + PD123319 (20  $\mu\text{g}/\text{minute}$ ). Similarly, there were no significant haemodynamic changes observed.

There was an increased RI response to L-NMMA (Figure 4) in INSR subjects (mean % change in RI  $24.3 \pm 6.1\%$ ) compared to NCs (mean % change  $12.3 \pm 3.6\%$ ),  $p = 0.04$ . However, after correction for changes in DBP, HR, SVRI, ZI and CI, the difference was found to be non-significant ( $p = 0.11$ ). No concurrent significant difference in SI or haemodynamic response were found between the two groups.

Infusion of GTN (10, 20, 40  $\mu\text{g}/\text{min}$ ) 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\*dose as the relevant effect of interest.

Results of the correlation analyses are presented in Figure 5. In this cohort, the only factor significantly correlated with RI was BMI. Interestingly, a significant negative correlation was observed ( $r = -0.47$ ,  $p = 0.01$ ). Total cholesterol, calculated LDL levels, SBP and DBP were significantly correlated with SI.

## Discussion

In recent years, there has been 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 patients, including atheroma formation and the loss of arterial compliance. While the roles of AII and aldosterone have clearly been implicated in the pathogenesis of atheroma [30], the role of angiotensin 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 AT1 and AT2 receptors, the latter which appear to become expressed in the presence of arterial damage and play a role in counteracting the deleterious effects of AT1 mediated responses.

We found a significant increase in RI (an index of small to medium-sized arterial stiffness) in response to angiotensin II (8, 16 ng/min) in INSR patients compared with age- and sex-matched controls, with no concurrent significant change 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 INSR subjects, which was not evident in the control group.

Evidence of increased functional AT2R expression in INSR patients was also demonstrated with the intravenous infusion of the highly selective AT2R blocker, PD123319 (10, 20 µg/min). A significant RI increase in INSR patients was observed, with the control group demonstrating minimal change. There were no concurrent significant differences in large arterial stiffness and haemodynamic response.

In total, these findings support the hypothesis that both AT1 and AT2 receptor 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 demonstrate an increased vasoconstrictor response [30, 31] to angiotensin II in the presence of PD123319. This study did not demonstrate an increased RI response to the concurrent infusion of angiotensin II (16 ng/min) and PD123319 (20 µg/min). Repeated or prolonged stimulation of the AT1 receptor causes desensitization, presumed secondary to receptor internalisation [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 angiotensin II in the presence of PD123319 in this study.

We were able to confirm the relationship between arterial stiffness and endothelial nitric oxide activity, with both arterial stiffness indices (SI and RI) responding appropriately to the nitric oxide mediators, GTN and L-NMMA. Any changes in SI and RI therefore, is likely to be influenced by the degree of nitric oxide release in large and small to medium-sized arteries, respectively.

We did not find any significant difference between the INSR and control groups in the endothelium-independent vasodilatory response to GTN. Natali et al had similar findings in their study on hypertensive men who underwent euglycaemic clamp studies to test their insulin sensitivity [34]. No significant difference in the endothelium-independent vasodilatation between the highest and lowest tertiles of insulin sensitivity was found.

We demonstrated a trend towards an increase in RI in response to L-NMMA in INSR patients compared with NCs. This suggests a possible increased expression of basal NO in the INSR subjects, which concurs with other studies on patients with type II 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 nitric oxide activity. A relatively greater activation of AT2 compared to AT1 receptors by endogenous AII may in part explain the significantly lower baseline SI, RI, DBP and SVRI in INSR subjects compared with the control group, although this may also have been a chance finding.

Correlation analysis revealed a negative correlation between BMI and RI. These results make sense in light of the results of the L-NMMA study above, which suggests a higher basal nitric oxide release in INSR subjects.

A significant relationship between SI and established cardiovascular risk factors such as hypertension and dyslipidaemia was also found. While the relationship between SBP and DBP with indices of large arterial stiffness are well-documented, reports of its association with LDL and total cholesterol is controversial [4, 37, 38]. Our data, 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 minutes post-infusion, which is adequate time for equilibration. Furthermore, the dosing appeared adequate, as there was no significant increase in RI with the higher dose of PD123319 (20 µg/min).

In conclusion, this study provides evidence of a locally activated renin-angiotensin system in small to medium-sized arteries of patients with INSR, which is not present in age- and sex-matched healthy controls. There is an apparent increase in both AT1 and AT2- mediated responses in these arteries, which may represent an interactive homeostatic response to counteract early vascular damage in INSR.

**Acknowledgements**

This project was funded by the Royal Australasian College of Physicians Research Foundation and the National Health and Medical Research Council (NH&MRC), Australia.

There are no conflicts of interest.

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## Figure Legend

**Table 1.** Demographic features of patients with insulin resistance and their age- and sex-matched controls.

**Table 2.**

Factors correlated with SI and RI. Values presented are *r* values, using Spearman rank-order correlation.

**Figure 1.**

Comparison of percentage changes in SI and RI with angiotensin II (8, 16 ng/min) using the Mann-Whitney *U*-test.

**Figure 2.**

Comparison of percentage changes in SI and RI with PD123319 (10, 20 micrograms/min) using the Mann-Whitney *U*-test.

**Figure 3.**

Comparison of percentage changes in SI and RI in patients with INSR and age- and sex-matched controls after intravenous infusion of angiotensin II (16 ng/min) + PD123319 (20 µg/min).

**Figure 4.**

% Change in SI and RI with increasing dose of GTN (10, 20, 40 microgram/min) in INSR and NCs and comparison of percentage changes in SI and RI in response to L-NMMA using the Mann-Whitney *U*-test.

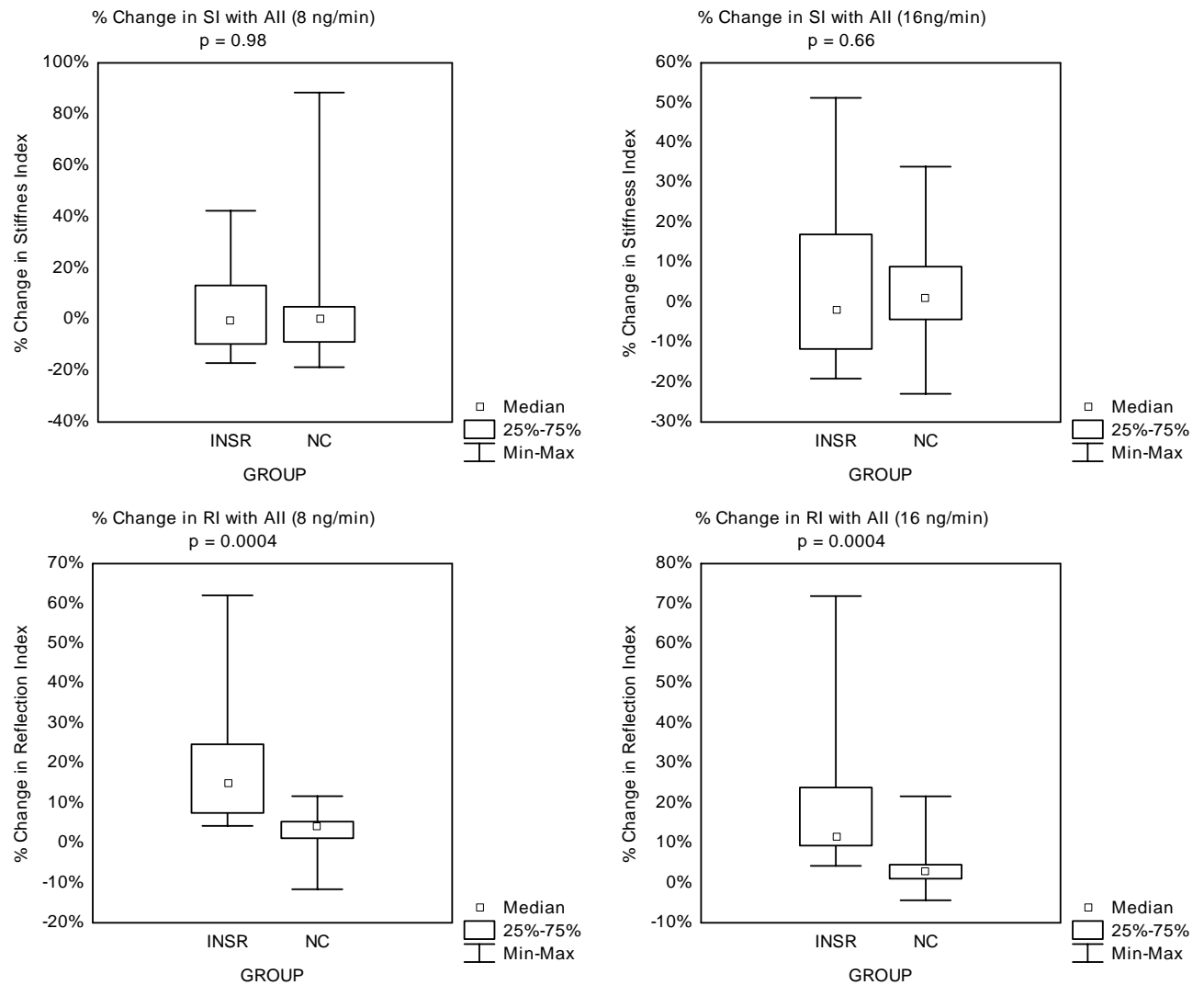
**Table 1.**

	INSR (n=13)	NC (n=13)	<i>p</i>
AGE (yrs)	31 ± 2	31 ± 3	0.92
Females	8	8	
Body Mass Index (kg/m <sup>2</sup> )	29.9 ± 2.0	22.5 ± 0.6	0.0006*
Stiffness Index (m/s)	6.27 ± 0.25	7.93 ± 0.57	0.01*
Reflective Index (%)	67 ± 4	78 ± 3	0.03*
SBP (mmHg)	111 ± 3	116 ± 3	0.32
DBP (mmHg)	61 ± 3	70 ± 3	0.03*
Heart rate (bpm)	66 ± 2	67 ± 2	0.69
Systemic Vascular Resistance Index (dyne s min)	1976 ± 104	2416 ± 138	0.02*
Stroke Index (mL/min/m)	45 ± 2	41 ± 2	0.21
Cardiac Index (L/min/m)	3.0 ± 0.1	2.8 ± 0.1	0.23
Total Cholesterol (mmol/L)	4.9 ± 0.3	5.3 ± 0.4	0.37
Triglyceride (mmol/L)	1.2 ± 0.2	1.4 ± 0.3	0.59
HDL Cholesterol (mmol/L)	1.5 ± 0.1	1.8 ± 0.1	0.05*
Calculated LDL Chol (mmol/L)	2.8 ± 0.2	2.8 ± 0.3	0.93
HOMA	2.5 ± 0.3	1.4 ± 0.2	0.004*
FPI (mU/L)	12 ± 2	7 ± 1	0.007*
FPG (mmol/L)	4.9 ± 0.1	4.6 ± 0.1	0.06
Waist Circumference (cms)	99 ± 6	77 ± 2	0.0006*

**Table 2.**

	<b>Stiffness Index</b>	<b>Reflection Index</b>
Body Mass Index	-0.26, p = 0.20	-0.47, p = 0.01
HOMA	-0.29, p = 0.15	-0.19, p = 0.35
Fasting plasma Glucose	-0.07, p = 0.73	0.17, p = 0.41
Fasting Plasma Insulin	-0.29, p = 0.15	-0.18, p = 0.37
SBP	0.48, p = 0.01	0.23, p = 0.25
DBP	0.63, p = 0.006	0.33, p = 0.10
Triglyceride	0.35, p = 0.08	0.02, p = 0.93
Total Chol	0.54, p = 0.004	0.21, p = 0.30
HDL	0.09, p = 0.64	0.07, p = 0.73
LDL	0.42, p = 0.04	0.23, p = 0.25
Waist Circumference	-0.24, p = 0.24	-0.37, p = 0.07

**Figure 1**



**Figure 2.**

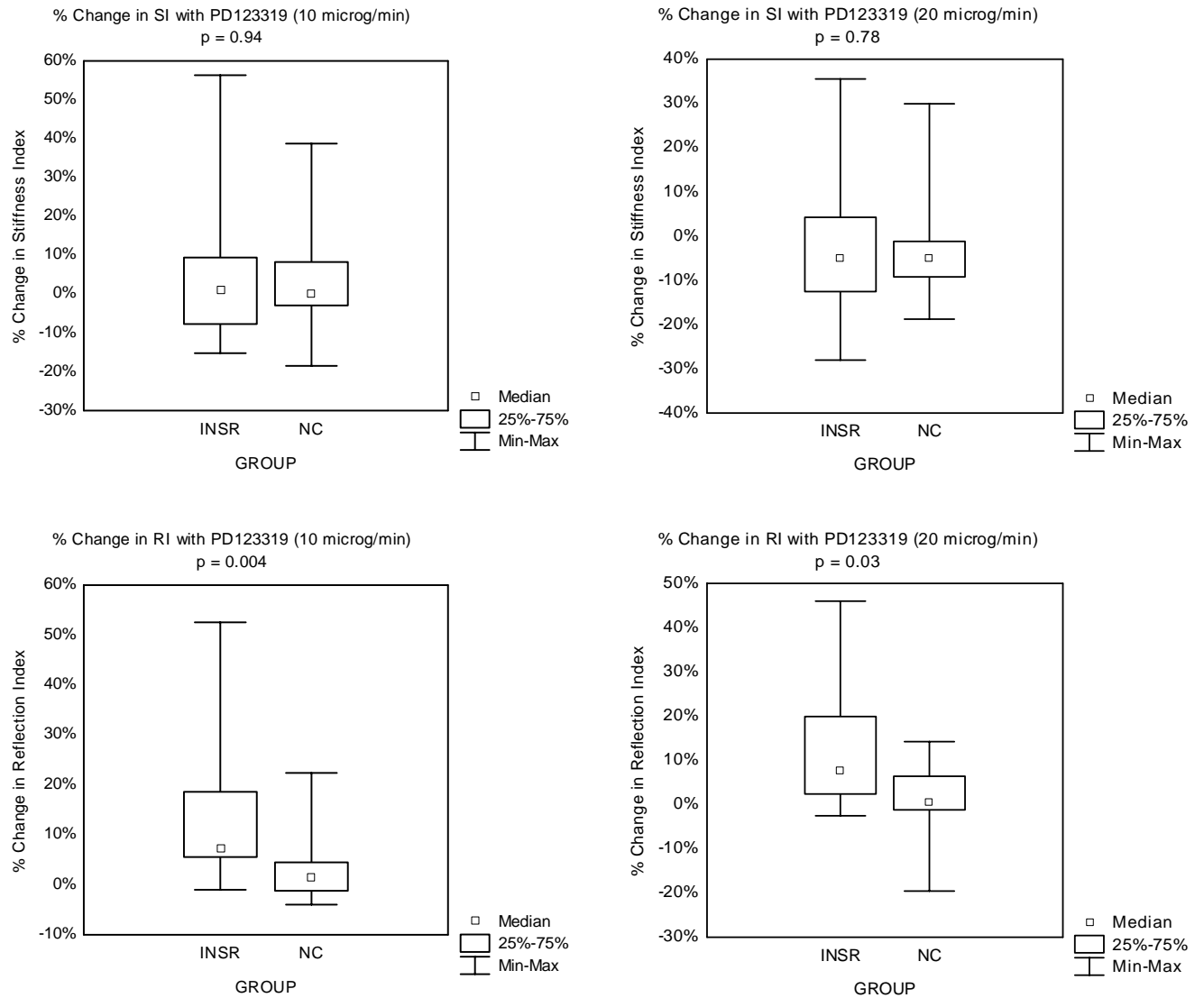
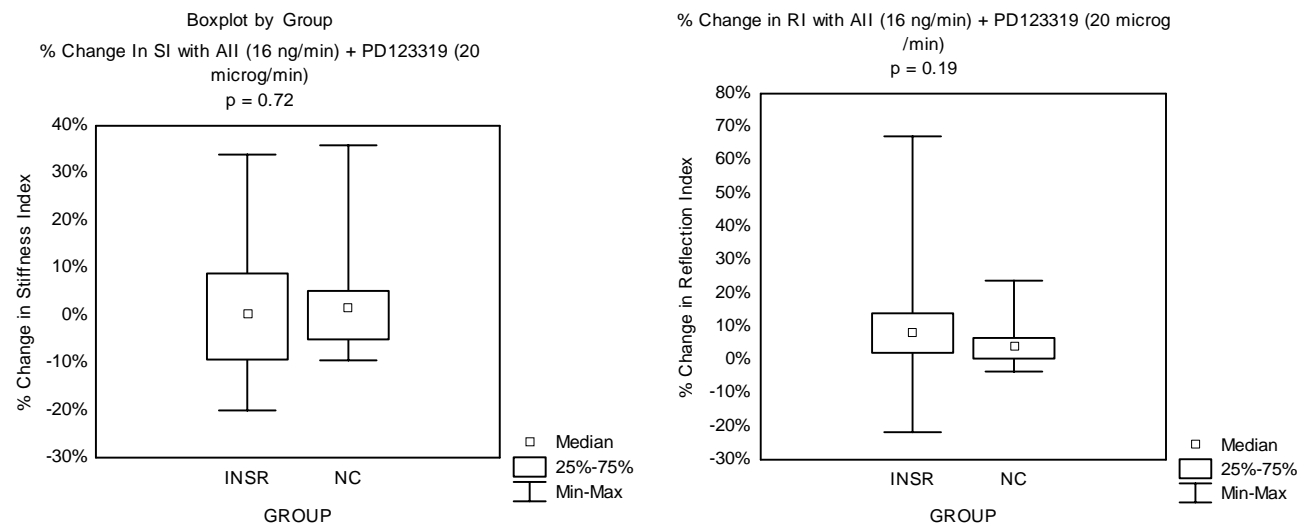


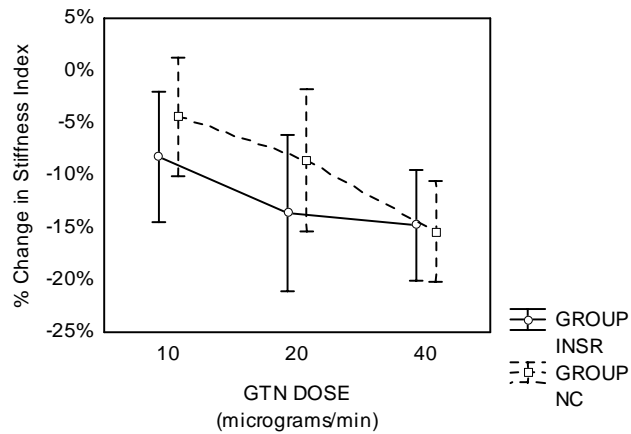
Figure 3.



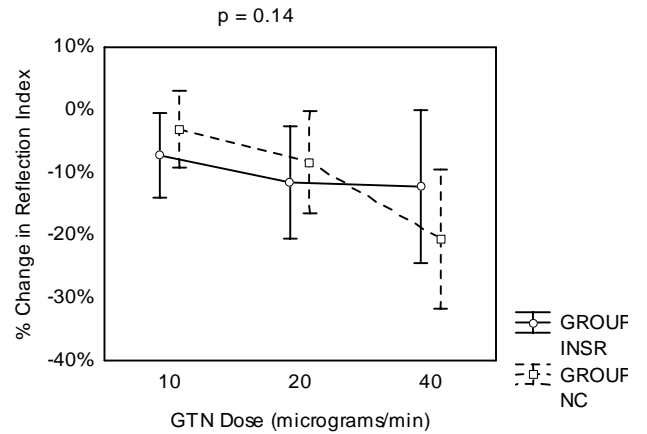


**Figure 4**

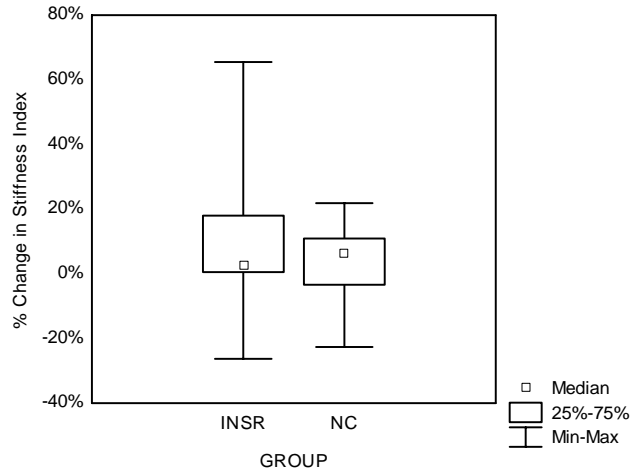
% Change in SI with Increasing Dose of GTN in INSRs and NCs, corrected for DBP and SVRI Changes  
 $p = 0.33$



% Change in Reflection Index with Increasing Dose of GTN in INSRs and NCs, corrected for DBP and SVRI Changes  
 $p = 0.14$



% Change in Stiffness Index with L-NMMA  
 $p = 0.52$



% Change in RI with L-NMMA  
 $p = 0.04$

