Università degli Studi di Milano-Bicocca

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Doctoral program in Hypertension and Cardiovascular Prevention

Effects of Insulin Resistance on Systemic Hemodynamics and Autonomic Cardiovascular Regulation in Normotensive Healthy Adults

Doctoral dissertation

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Abstract

Hemodynamic effects of insulin resistance (IR) are thought to be largely dependent on its relationship with body mass index (BMI) and blood pressure (BP) levels. The first part of the present thesis was aimed at exploring whether IR is associated with hemodynamic indices of cardiovascular function in a large sample of non-diabetic individuals from the general population (n=731) and if so, to explore if such relationship is continuous across different categories of BMI (lean, overweight and obese), and BP (normal BP, high-normal BP and hypertension). IR was assessed with the homeostasis model assessment of IR (HOMA-IR). Based on a value of HOMA-IR of 2.09 (75th percentile of distribution curve), subjects were classified as insulin-sensitive (IS, HOMA<2.09) or insulin-resistant (IR, HOMA≥2.09). Synchronized beat-to-beat recordings of stroke volume (impedance cardiography) and R-R interval (ECG), along with repeated BP measurements were performed over 5 minutes. Stroke index (SI), cardiac index (CI), systemic vascular resistance index (SVRI), left cardiac work index (LCWI), pre-ejection period (PEP) and left ventricular ejection time (LVET) were computed and averaged. In analysis of co-variance allowing for confounders, IR subjects showed significantly higher BP levels and SVRI, and reduced R-R interval, SI, CI, LCWI, PEP and LVET. These differences remained significant when analyses were performed within each BMI and BP category. Overall, these results indicate that effects of IR on hemodynamic indices of cardiovascular function are continuous across different BMI and BP categories, reinforcing the importance of IR in the pathogenesis of cardiovascular alterations beyond its association with obesity and hypertension.

The finding of a significant association between IR and hemodynamic alterations even in lean and normotensive subjects was the rationale to explore potential mechanisms for these alterations in this selected group of subjects. Specific objectives of this second part of the thesis were: 1) To explore the relationship between insulin resistance and systemic hemodynamics, cardiac baroreflex sensitivity and indices of autonomic CV modulation. 2) To explore the relationship of insulin resistance with 24h heart rate, average blood pressure levels and blood pressure variability over the 24h; and 3) To explore the relationship of insulin resistance with central blood pressure levels and with measures of large artery stiffness and wave reflections.

The study population for these analyses was constituted by subjects who were below the 30th percentile of diastolic blood pressure (DBP) distribution curve (DBP ≤72 mmHg) and who had no elevation in systolic BP levels. In addition, subjects were excluded in case of diabetes mellitus (fasting blood glucose ≥126 mg/dL or use of medications for previously diagnosed type 2 diabetes) obesity (BMI≥30) or taking medications with effects on BP. A total of 90 subjects fulfilling inclusion criteria were considered for the present analysis and underwent further assessments. Insulin resistance was assessed with HOMA-index and subjects classified into IR tertiles, based on the distribution of HOMA-index values. 24h Ambulatory BP monitoring was performed. Mean SBP and DBP were averaged for the day, night and 24h, and the respective day-to-night dipping was calculated. BPV was assessed for SBP and DBP as 24h standard deviation (SD), weighted 24h SD (wSD), daytime and night-time SD. Recordings of pulse waveform were obtained by means of a previously validated oscillometric device for ambulatory BP monitoring with in-built transfer-function like method. Aortic pulse wave velocity (PWV, m/s) and other measures derived from pulse wave analysis such as augmentation index (AIx, %), central SBP

(cSBP), central DBP (cDBP) and central pulse pressure (cPP) were computed. Peripheral SBP and DBP, and heart rate (HR) were recorded and pulse pressure (PP) calculated as the difference between SBP and DBP. Non-invasive assessment of beat-to-beat BP, R-R interval (ECG) and stroke volume (by means of impedance cardiography) were performed during 10 min in supine position and specific hemodynamic indices associated with their measurement were computed and averaged: RRI (msec), heart rate (HR, bpm), stroke volume index (SI, mL/beat/m^2), cardiac index (CI, L/min/m^2), SBP (mmHg) and DBP (mmHg), systemic vascular resistance index (SVRI, dyn/sec/cm^{-5}/m^2), left cardiac work index (LCWI, Kg/m/m2), pre-ejection period (PEP, msec), left ventricular ejection time (LVET, msec) and PEP/LVET ratio were calculated. Cardiac autonomic modulation was assessed by computer analysis of 10 min beat-to-beat BP and ECG recordings in resting supine position. Cardiac baroreflex sensitivity (BRS) was estimated by sequence method. Total variance, low-frequency (LF) and high-frequency (HF) spectral components of HR variability (HRV) were assessed by autoregressive analysis. LF/HF ratio was calculated. After multiple regression analysis, adjusting for common confounders such as age, sex, HR and BMI, increasing values of HOMA-IR were associated with reduced RRI, SI, CI, and with increased SVRI, SBP and DBP. IR was also associated with reduced BRS (up, down, and total slopes), decreased parasympathetic indices of autonomic CV modulation (SDRRI, HF-power, total power) and a predominance of sympathetic component of HRV (increased LF/HF ratio). Increasing values of HOMA-IR were also associated with increased HR and average SBP levels (during day, night and 24-h period), with augmented BP variability (Day SBP SD, and SBP wSD) and with a reduced dipping of HR. Finally, insulin resistance was shown to be associated with increasing values of aortic PWV, and with higher central and peripheral SBP and DBP levels. Overall, these results

support significant associations between insulin resistance and changes in hemodynamic and autonomic indices of cardiovascular function, even after accounting for common confounders. These findings suggest that in normotensive healthy adults, increases in insulin resistance may promote alterations in autonomic cardiovascular modulation, in systemic hemodynamics and in arterial stiffness, all of which are known contributors to the pathogenesis of hypertension.

Part 1: Insulin resistance and beat-to-beat cardiovascular dynamics: a constant relationship across different body mass index and blood pressure categories

Introduction

Apart from its metabolic actions to promote glucose uptake in classical target tissues involved in glucose homeostasis, insulin also exerts physiological functions in non-classical targets such as vascular endothelium and the heart, thus playing an important role in coupling metabolic and hemodynamic homeostasis under normal conditions.(1) Conversely, in insulin resistance (IR) states, the concomitant impairment of shared insulin-signaling pathways in metabolic and cardiovascular (CV) targets of insulin, explains the frequent clustering of metabolic and CV alterations (i.e. changes in vascular and cardiac function and structure). (2-5) In the vascular endothelium, IR becomes manifest as a reduced local vasodilator response to insulin (6, 7) as well as an impaired endothelial function. (8) At the heart level, IR-related alterations include left ventricular remodeling (3) as well as an impaired systolic and diastolic performance in the absence of structural changes or coronary artery disease. (9, 11) In addition, the IR-induced hyperinsulinemia produces increases in central sympathetic drive to the heart and peripheral vasculature, and activation of tubular sodium reabsorption, further contributing to the hemodynamic alterations associated with IR. (12, 13) Epidemiological studies have shown a progressive increase in IR and insulin levels accompanying body weight gain and blood pressure raise. (14-17) This has led to consider that hemodynamic effects of IR might depend on its association with body mass index (BMI) and blood pressure (BP) levels. Aim of the present study was to assess whether IR is associated with hemodynamic indices of CV function in a random sample of non-diabetic individuals from the general population and if so, to explore if such a relationship is constant across different categories of BMI (lean, overweight and obese), and BP levels (normal BP, high-normal BP and hypertension).

Research design and Methods

Study population

The present study was embedded in the frame of the Medellin´s Heart Study, a cross-sectional study conducted between years 2008-2009 in the city of Medellin, Colombia, with the aim to assess the behavior of major CV risk factors in a probabilistic sample of the general population of the city aged between 30 and 65 years. Following approval by the institutional ethics committee (CES University), a total of 800 individuals (Female: 55% mean age 50.3±12.1 years) were recruited. More than 90% of individuals fulfilling inclusion criteria that were invited to participate, accepted to take part in the study and gave voluntary oral and written informed consent. Individuals were excluded in case of pregnancy; musculoskeletal disease limiting movement or mental disability impeding autonomous signing of informed consent. Post-hoc exclusion criteria were the presence of atrial fibrillation, tachyarrhythmias or excessive thoracic fluid states, which may introduce inaccuracies in the hemodynamic assessment based on impedance cardiography. (18, 19)

The sample size was estimated using a formula for population proportions previously described (20) by focusing on a major CV risk factor such as arterial hypertension. Considering a reference population of N=1.094.054 subjects between 30-65 years residing in the city of Medellin and a prevalence of hypertension of 18.2% in year 2007, a sample size of 800 subjects was the minimum necessary to estimate the prevalence of hypertension and other CV risk factors with a 95% confidence interval, and a 5% precision. Individuals were selected among all city inhabitants living in different,

randomly selected areas of the city using a multiple stratified sampling procedure, and constituted a representative sample of the general population.

Study assessments

Questionnaire and Anthropometric measurements

A standardized questionnaire was administered including information on demographic factors, socio-economical status (education, income), lifestyles (smoking, physical activity, and dietary patterns), medication use, personal and familiar history of CV disease, and CV risk factors (hypertension, diabetes mellitus, dyslipidemia). Height was measured to the nearest 0.10 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.10 Kg with calibrated scales and BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured with a non-stretchable standard tape measure over the unclothed abdomen at midway between the lateral lower ribs and the iliac crest and expressed rounded to the nearest 0.50 centimeters.

Hemodynamic assessment

Synchronized beat-to-beat recordings of stroke volume (SV) and R-R interval (RRI) were performed using impedance cardiography (ICG) EBI100C and electrocardiogram amplifier ECG100C modules respectively (MP150 acquisition unit, BIOPAC Systems, Inc. Goleta, CA) during a protocol consisting of signal recordings performed for 5 min while resting supine, 1 min in standing position, 30 sec while in sitting position, and 30 sec while in sitting position and holding breath. Blood pressure levels were repeatedly

measured with standard mercury sphygmomanometer during each phase of the protocol. Beat-to-beat stroke volume was calculated (Sramek-Bernstein equation) and specific parameters associated with its measurement were derived from the analysis of the impedance cardiography signal recorded by means of Noninvasive Cardiac Output Module (NICO100C, BIOPAC Systems, Inc. Goleta, CA). A four spot disposable electrode system (Cardiomed® electrodes) was employed: The outer (current injecting) neck top electrode was placed on the central portion of the right sternocleidomastoid (SCM) muscle, while the inner (current sensing) neck top electrode was positioned 3 cm below the former on the left SCM muscle. Inner (current sensing) torso bottom electrode was placed on the spine, 25 cm far from the inner electrode on the neck, and the outer (current injecting) torso bottom electrode was placed 3 cm below the former. A precision, high frequency, small current of 400 μA (rms) was continuously injected through current injecting electrodes. Raw impedance signal (Z) and its first derivative (dZ/dt) were simultaneously recorded at a sample rate of 1000 Hz, and scaled at a magnitude range of 5 Ohms/Volt and 2 Ohms/sec/Volt, respectively. Signals were filtered on-line with a low pass filter, settled at 10 Hz, and a high pass filter settled as DC. Electrocardiographic recordings were performed by two lead electrodes (3M™ Red Dot™ electrodes) coming from the ECG100C module. Signals were processed and analyzed with Acqknowledge v.4.1.1 (BIOPAC Systems, Inc. Goleta, CA) software and beat-to-beat cardiac and hemodynamic indices were computed and averaged: RRI (msec), heart rate (HR, bpm), stroke volume (SV, mL/beat), cardiac output (CO, L/min), systolic (S) BP (mmHg) and diastolic (D) BP (mmHg), systemic vascular resistance (SVR, dyn/sec/cm^{-5}), pre-ejection period (PEP, msec), left ventricular ejection time (LVET, msec) and PEP/LVET ratio and left cardiac work (LCW, Kg/m). Stroke volume index (SI, mL/beat/m^2), cardiac index (CI,

L/min/m^2), SVR index (SVRI, dyn/sec/cm^{-5}/m^2) and LCW index (LCWI, Kg/m/m^2) were calculated by normalizing SV, CO, SVR and LCW respectively, by the body surface area (BSA) as calculated with Mosteller formula. Due to the better stability of impedance signal while in the sitting position, hemodynamic parameters during this period were considered for the present analysis**.**

Blood pressure measurement

BP was measured initially in both arms, to identify subjects who might have a significant between arm BP difference. Since no subject was found to have inter-arm BP differences in systolic BP >10 mmHg, a second conventional sitting BP measurement was obtained from the left arm, 5 min apart, by a trained physician, with the cuff at the heart level, after 5 min rest, using a standard mercury sphygmomanometer, following the European Society of Hypertension guidelines. (21) The average office BP levels of each subject were thus defined by the average of the two measurements obtained from the left arm. Phase I and V (disappearance) Korotkoff sounds were used to identify SBP and DBP, respectively. Pulse pressure (PP) was calculated as the difference between SBP and DBP and mean arterial pressure (MAP) as DBP plus 1/3 of PP. Hypertension was defined as physician's diagnosis, based on either, a mean SBP≥140 mmHg and/or DBP≥90 mmHg, presence of antihypertensive treatment, or any combination of these characteristics.

Laboratory tests

A venous blood sample was drawn from all participants after at least 8 hours fasting, and processed within the first 6 hours. Plasma glucose was determined by the glucose oxidase method and insulinemia by enzyme immunoassay, following standardized procedures in the clinical laboratory. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were also determined. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's formula whenever triglycerides were <400 mg/dL.

Definition of metabolic and blood pressure phenotypes

Insulin resistance was evaluated with the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula ((glycemia (mg/dL)/18) x insulinemia (uU/mL)/22.5). This index, considered a reliable marker of IR, was shown to be closely correlated with the insulin sensitivity index measured through the standard euglycemic hyperinsulinemic glucose clamp method. (22) A value of HOMA-IR corresponding to the 75^{th} percentile of distribution curve (2.09) for the entire study population (n=731) was considered as the threshold to define IR based on the World Health Organization report for diagnosis and classification of diabetes mellitus. (23) According to this value, individuals were classified as insulin-sensitive (IS, HOMA<2.09) or insulin-resistant (IR, HOMA≥2.09). In order to evaluate the relationship of IR with hemodynamic variables across different categories of BMI, individuals were classified as lean (BMI<25.0 Kg/m^2), overweight (BMI 25.0-29.9 kg/m^2), or obese (BMI≥30.0 kg/m^2). To assess the relationship of IR with hemodynamic variables across different BP categories, individuals were further

classified into three subgroups following the ESH guidelines for classification of BP levels (21): Normal BP (<130/85 mmHg), high-normal BP (130-139/85-89 mmHg), and hypertension (≥140 and/or 90 mmHg).

Statistical analysis

On the basis of the standard deviation for stroke index (7.8 mL/beat/m2) obtained for our study population, and a minimum expected difference of 5 mL/beat/m2 between IR and IS subjects, that we have set out to detect, with a 85% power and a $p<0.05$ two-tailed significance level; the study should have included a minimum of 24 subjects in each group. This number is smaller than the actual size of each of the subgroups considered for the present analysis (determined by the presence or absence of insulin resistance and by category of either BMI -lean, overweight or obese- or BP -Normal BP, high-normal BP, and hypertension-); therefore our study was sufficiently powered to detect significant differences in stroke index between IR and IS subjects in any BMI or BP category.

Data were analyzed using IBM®SPSS® software v.18.0.0. Normal distribution of variables was assessed with the Smirnov-Kolgomorov test. Differences in the frequency of categorical variables were assessed using $\chi 2$ test. Student´s t-test was applied to assess differences in clinical and hemodynamic variables between IS and IR subjects both in the whole study population and within each BMI and BP category. Differences in hemodynamic parameters between IS and IR subjects were corrected by applying analysis of covariance (ANCOVA) adjusting for BMI, MAP, PP, age, sex and cigarette smoking. Differences in hemodynamic variables already indexed for BSA (SI, CI, SVRI and LCWI) were not adjusted for BMI and waist circumference was considered instead. Differences in

SVRI were not adjusted for MAP and PP as the latter variable was included in the formula to compute SVRI. Since the distribution of HOMA-IR was asymmetric and highly skewed, the relationship between HOMA-IR and hemodynamic variables was assessed with Spearman correlation. To assess the effects of IR (HOMA ≥2.09) and other potential predicting variables (i.e. age, sex, HOMA, MAP, PP, BMI, waist circumference, cigarette smoking, LDL cholesterol, HDL cholesterol, Total cholesterol, tryglicerides and heart rate) on the variation of hemodynamic parameters such as RRI, SI, CI, SVRI (dependent variables), a stepwise multiple linear regression analysis was performed where all predicting variables were modeled together and standardized regression coefficients calculated. The p value for entry of variable in the model was <0.05; while the p value to exclude a variable from the model was > 0.05

Results

Hemodynamic data were available in 790 participants and 59 individuals (7.3% of the study population) with diagnosis of diabetes (fasting blood glucose ≥126 mg/dL or use of medications for previously diagnosed type 2 diabetes) were excluded. Thus a total of 731, non-diabetic subjects were considered for the present analysis. A total of 213 subjects were hypertensive (29.1% of study population) and from them 87 (8.4% of entire study population) were on antihypertensive treatment. Clinical characteristics of the entire population of the study and for the IS and IR subgroups are presented in table 1.

Table 1. Clinical characteristics for the entire study population; and for insulin-sensitive (IS) and insulin-resistant (IR) subjects. Values are presented as means±standard deviation except for sex and cigarette smoking which are shown as percentages (%). BMI: Body mass index; LDL: low-density lipoprotein; HDL: High- density lipoprotein.

Variable	**All (n=731)**	**IS (n=548)**	**IR (n=183)**	**Difference between means (95% CI)**	**p value**
Age (years)	49.44± 11.68	49.13± 11.77	50.36± 11.40	-1.21 (-3.18, 0.73)	0.219
Male sex, %(n)	0.45 (335)	0.47 (257)	0.43(78)	-	0.315
Cigarette smoking, %(n)	0.26 (190)	0.28 (153)	0.20 (37)	-	0.019
BMI (Kg/m^2)	25.97± 4.61	24.87± 4.01	29.40± 4.72	-4.52 (-5.22, -3.82)	<0.0001
Waist circumference (mm)	850.15± 12.21	820.65± 11.61	930.06± 10.66	-100.42 (-120.33, -80.50)	<0.0001
HOMA-Index	1.70± 1.43	1.12± 0.48	3.46± 1.82	-2.34 (-2.51, -2.18)	<0.0001
Fasting blood glucose (mmol/L)	4.75±0.70	4.63±0.67	5.13±0.72	-0.5 (-0.61, -0.39)	<0.0001
Fasting plasma insulin (uU/mL)	7.85± 6.10	5.40±2.30	15.28± 7.71	-9.87 (-10.60, -9.14)	<0.0001
Total serum Cholesterol (mmol/L)	5.66±1.16	5.58±1.15	5.93±1.15	-0.33 (-0.53, -0.14)	0.001
LDL Cholesterol (mmol/L)	3.82±1.03	3.76±1.03	3.92±1.05	-0.13 (-0.30, 0.03)	0.120
HDL Cholesterol (mmol/L)	1.02±0.28	1.05±0.28	0.92±0.26	0.13 (0.09, 0.18)	<0.0001
Tryglicerides (mmol/L)	1.82±1.17	1.62±1.65	2.39± 1.77	-0.77 (-0.96, -0.57)	<0.0001

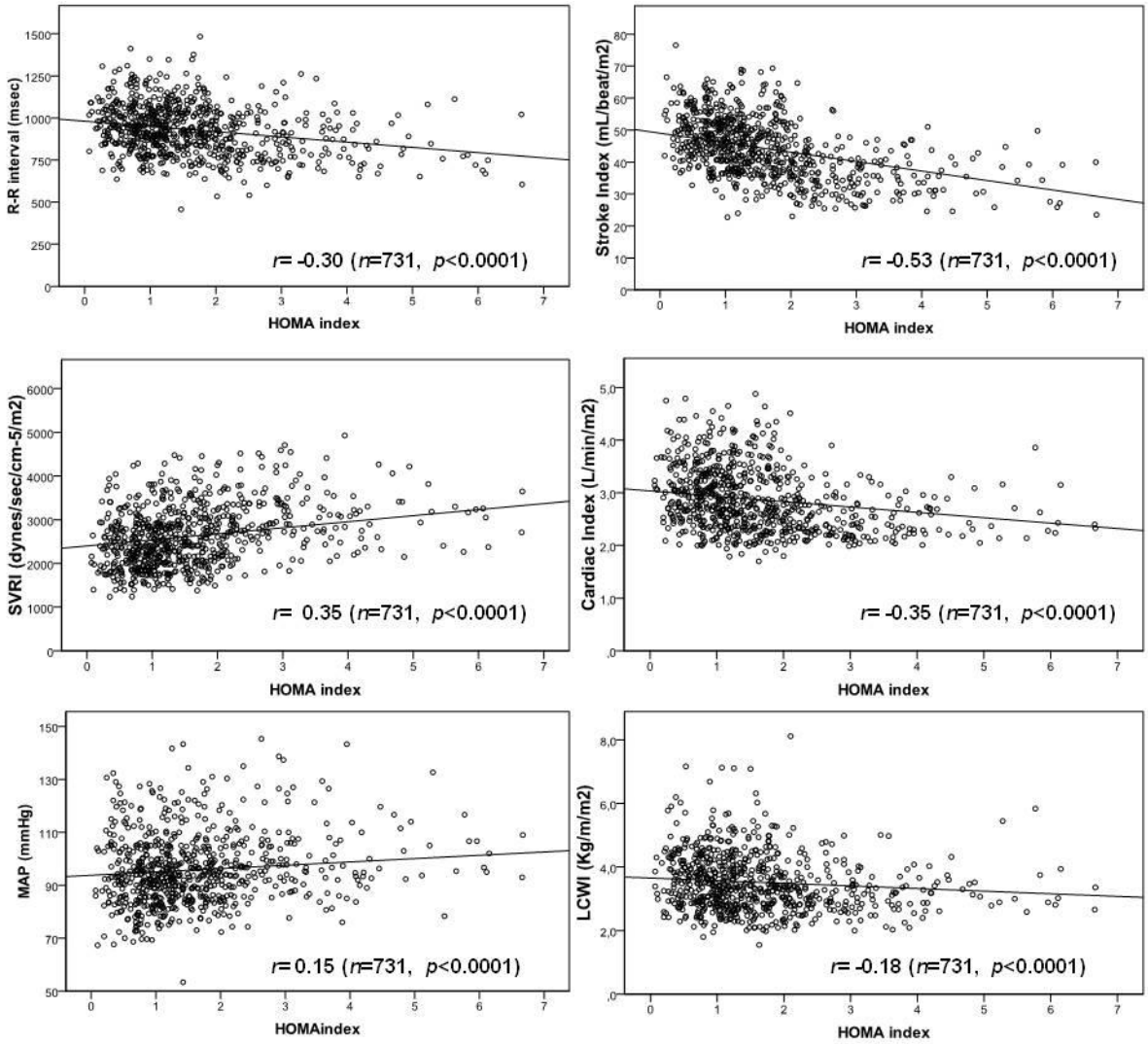
Average age of participants was 49.4±11.6 years; 55.7% were women; and the prevalence of IR was 25.0 %. While age and sex distribution did not differ significantly between IS and IR subgroups, significant differences were observed for all anthropometric measures and biochemical parameters but LDL cholesterol. Analysis of variance showed significant differences in most hemodynamic parameters between IS and IR individuals: IR subjects had significantly higher SVRI and BP levels and a reduced RRI, SI, CI, LCWI, PEP and LVET when compared to IS individuals. These differences remained significant in analysis of covariance, adjusting for BMI, MAP, PP, sex, age, and cigarette smoking (Table 2).

Table 2. Hemodynamic variables for the entire study population; and for insulin-sensitive (IS) and insulin-resistant (IR) subjects. Values are presented as means±standard deviation. Differences in hemodynamic parameters between IS and IR individuals were adjusted for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking and presented with 95% confidence interval.

Variable	All (n=731)	IS (n=548)	IR (n=183)	Adjusted difference* (95% CI)	Adjusted p (ANCOVA)
RRI (msec)	927.12± 149.04	953.22± 143.12	851.03±141.3	110.12 (83.05, 136.11)	<0.0001
MAP (mmHg)	96.03± 13.39	94.51± 13.2	100.12± 13.7	-3.09 (-5.39, -0.79)	0.009
SBP (mmHg)	127.35± 22.01	125.73± 21.9	132.29± 21.5	-4.16 (-7.68, -0.64)	0.020
DBP (mmHg)	80.37± 11.07	79.12± 10.64	84.02± 11.41	-2.90 (-4.84, -0.93)	0.004
SI (mL/beat/m^2)	43.98± 9.44	46.68± 8.57	35.91± 7.04	10.44 (8.94, 11.94)	<0.0001
CI (L/min/m^2)	2.86± 0.59	2.97± 0.60	2.54± 0.42	0.38 (0.27, 0.48)	<0.0001
SVRI (dyn/s/cm^{-5}/m^2)	2636.13± 692.22	2495.32± 644.07	3057.71± 660.02	-432.21 (-542.06, -322.5)	<0.0001
PEP (msec)	105.11± 22.33	106.04± 21.63	100.81± 24.95	5.00 (1.00, 9.00)	0.012
LVET (msec)	314± 42.45	316.0± 42.84	305.84± 43.66	15.00 (7.00, 23.00)	<0.0001
PEP/LVET ratio	0.34± 0.09	0.34± 0.09	0.34± 0.10	0.04 (-0.02, 0.01)	0.673
LCW (Kg/m)	6.05± 1.56	6.05± 1.57	6.04± 1.51	0.45 (0.17, 0.73)	0.001
LCWI (Kg/m/m^2)	3.52± 0.91	3.60± 0.94	3.29± 0.79	0.36 (0.19, 0.52)	<0.0001

In bivariate correlation analysis (Spearman correlation), HOMA was directly correlated with SVRI (r=0.35) and MAP (r=0.15); and inversely correlated with RRI (r=-0.30), SI (r=-0.53), CI (r=-0.35), LVET (r=-0.12) and PEP (r=-0.12) (p<0.001 for all correlations). Figure 1.

Figure 1. Relationship between HOMA and hemodynamic variables in the entire study population (n=731). Spearman correlation coefficients (r), and levels of statistical significance (p values) are shown. SVRI: Systemic vascular resistance index; LCWI: Left cardiac work index; MAP: Mean arterial pressure.



In the stepwise multiple linear regression analysis taking hemodynamic parameters as dependent variables, HOMA had the strongest effect on RRI variation (beta: -0.329, p<0.001), and was the second most important predictor of SI (beta: -0.276, p<0.001), CI (beta: -0.330, p<0.001) and SVRI (beta: 0.303, p<0.001), after heart rate (table 3).

Table 3. Stepwise (backward) multiple linear regression analysis for hemodynamic parameters (dependent variables) and possible predicting variables (n=731). Standardized regression coefficients for each predictor are presented with their respective significance level. *MAP was not included in the model for SVRI, as it was included in the formula to compute SVRI. †BMI was only included in the model for RRI as other variables had already been indexed for BSA. NS: denotes non-significant variables that were excluded from the model during the stepwise regression. NI: denotes variables that were not included in the model (i.e. those used in the formula to compute the hemodynamic variables). ‡ HR was not included in the model for RRI, as it is dependent on the length of RRI.

Predicting variables	Dependent variable											
	RRI (msec)			SI (mL/beat/m^2)			CI (L/min/m^2)			SVRI (dyn/s/cm^{-5}/m^2)		
	Beta coefficient	p value	Partial R^2	Beta coefficient	P value	Partial R^2	Beta coefficient	P value	Partial R^2	Beta coefficient	P value	Partial R^2
Age, y	-	NS	-	-	NS	-	-	NS	-	0.254	<.001	0.252
Sex, male	0.168	<.001	0.153	-0.129	<.001	-0.119	-0.154	<.001	-0.142	0.136	<.001	0.126
HOMA≥2.09	-0.329	<.001	-0.268	-0.276	<.001	-0.234	-0.330	<.001	-0.280	0.303	<.001	0.258
MAP*, mmHg	-0.140	.001	-0.109	-	NS	-	-	NS	-	NI	NI	NI
PP, mmHg	0.136	.002	0.104	-	NS	-	-	NS	-	-	NS	-
BMI †, Kg/m^2	-0.180	.001	-0.122	NI	NI	NI	NI	NI	NI	NI	NI	NI
Waist circumference (cm)	-0.109	.04	-0.058	-0.114	.001	-0.097	-0.108	.005	-0.091	0.147	<.001	0.124
Smoking, yes	-	NS	-	-	NS	-	-	NS	-	-	NS	-
LDL cholesterol, mg/dL	-	NS	-	-	NS	-	-	NS	-	-	NS	-
HDL cholesterol, mg/dL	-	NS	-	-	NS	-	-	NS	-	-	NS	-
Total cholesterol, mg/dL	-	NS	-	-0.071	.019	-0.070	-0.063	.055	-0.062	-	NS	-
Triglycerides, mg/dL	-	NS	-	-	NS	-	-	NS	-	-	NS	-
HR‡, bpm	NI	NI	NI	-0.372	<.001	-0.348	0.362	<.001	0.339	-0.224	<.001	-0.210
R^2 for the model	**0.136**			**0.355**			**0.239**			**0.260**		

When analyses of variance were performed within each BMI category, IR subjects reported significantly higher SVRI and MAP, and reduced RRI, SI, CI and LCWI when compared to IS individuals ($p<0.001$ for all parameters). Despite the higher BMI in IR subjects within each BMI category, differences for hemodynamic parameters remained significant after further adjustment for BMI and other confounders. Although in analysis of variance MAP was significantly higher in IR subjects within each BMI category, after adjustment for BMI, differences remained significant only for obese subjects (Figure 2 and tables 4-6).

Figure 2. Hemodynamic variables by BMI categories: comparison between insulin-sensitive (IS, □) and insulin-resistant (IR, ■) individuals. Data are shown as means±SEM. **p<0.05, and *p<0.0001 after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking. SVRI: Systemic vascular resistance index; LCWI: Left cardiac work index; MAP: Mean arterial pressure. The number of IS and IR subjects in each category is shown between parentheses at the base of each bar.

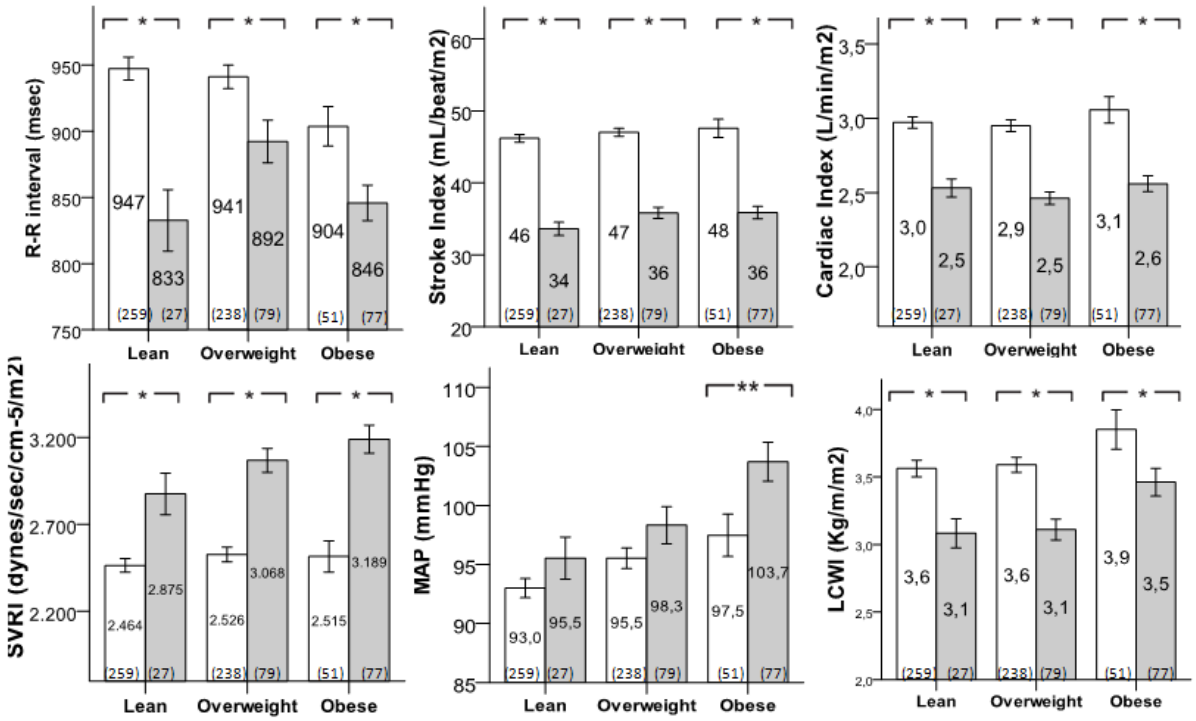


Table 4. Differences in hemodynamic variables between lean-insulin-sensitive (IS) and lean-insulin-resistant (IR) subjects. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between lean-IS and lean-IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for age, sex BMI, waist circumference, MAP, PP, and cigarette smoking using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted P value
No (%)	286 (100)	259 (89.6)	27 (10.4)	-	-	<0.0001	-
RRI, msec	930 (913, 947)	943 (926, 951)	802 (746, 858)	141 (85, 197)	135 (79, 193)	<0.0001	<.001
MAP, mmHg	93.2 (91.7, 94.7)	92.9 (91.4, 94.6)	95.5 (91.8, 99.2)	-2.54 (-7.6, 2.5)	-2.75 (-7.4, 1.9)	0.324	.24
SBP, mmHg	123.7 (121.4, 125.1)	123.5 (121.0, 125.1)	126.0 (120.0, 131.8)	-2.4 (-10.5, 5.7)	-3.9 (-10.9, 3.1)	0.561	.27
DBP, mmHg	78.1 (76.9, 79.3)	77.9 (76.6, 79.2)	80.3 (77.5, 83.2)	-2.4 (-6.5, 1.6)	-2.2 (-5.2, 1.9)	0.242	.29
SV, mL/beat	71.7 (70.0, 73.4)	73.5 (71.9, 75.2)	54.1 (51.5, 56.7)	19.4 (14.1, 24.7)	18.5 (13.1, 23.9)	<0.0001	<.001
SI, mL/beat/m^2	45.0 (43.9, 46.0)	46.2 (45.1, 47.2)	33.6 (31.7, 35.5)	12.6 (9.3, 15.9)	11.6 (8.3, 15.0)	<0.0001	<.001
CO, L/min	4.66 (4.55, 4.76)	4.72 (4.60, 4.83)	4.08 (3.89, 4.27)	0.53 (0.28, 0.99)	0.51 (0.24, 0.97)	<0.0001	.001
CI, L/min/m^2	2.93 (2.85, 3.00)	2.97 (2.89, 3.04)	2.53 (2.40, 2.65)	0.44 (0.20, 0.57)	0.40 (0.17, 0.53)	<0.0001	.001
SVR, dyn/s/cm^{-5}	1568 (1521, 1514)	1545 (1497, 1592)	1787 (1620, 1953)	-241 (-397, -85)	-244 (-394, -94)	0.002	.002
SVRI, dyn/s/cm^{-5}/m^2	2502 (2427, 2578)	2454 (2386, 2542)	2874 (2629, 3120)	-410 (-554, -157)	-391 (-527, -155)	0.002	.001
PEP, msec	109 (106, 111)	109 (107, 112)	103 (95, 111)	5.0 (4.0, -23.0)	7.0 (-2.0, 15.0)	0.154	.11
LVET, msec	311 (305, 315)	311 (306, 316)	305 (291, 319)	5.0 (-9.0, 22.0)	4.0 (-12.0, 21.0)	0.435	.61
PEP/LVET ratio	0.36 (0.34, 0.37)	0.36 (0.34, 0.37)	0.34 (0.31, 0.37)	0.018 (-0.02, 0.06)	0.02 (-0.02,0.05)	0.350	.26
LCW, Kg/m	5.59 (5.42, 5.76)	5.65 (5.47, 5.84)	4.95 (4.66, 5.25)	0.70 (0.12, 1.27)	0.63 (0.48, 1.23)	0.017	.03
LCWI, Kg/m/m^2	3.51 (3.40, 3.63)	3.56 (3.44, 3.68)	3.08 (2.86, 3.30)	0.48 (0.1, 0.86)	0.41 (0.03, 0.79)	0.014	.03

Table 5. Differences in hemodynamic variables between overweight-insulin-sensitive (IS) and overweight-insulin-resistant (IR) subjects. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between overweight-IS and overweight -IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for BMI, waist circumference, MAP, PP, age, sex and cigarette smoking. using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted p value
No (%)	317 (100)	238 (66.8)	79 (33.2)	-	-	<.001	-
RRI, msec	943 (926, 960)	966 (947, 985)	875 (841, 908)	91 (52, 129)	90 (50, 130)	<.001	<.001
MAP, mmHg	95.4 (94.0, 96.9)	94.8 (93.1, 96.4)	97.6 (94.5, 100.6)	-2.80 (-6.19, 0.57)	-1.7 (-5.1, 1.6)	.10	.31
SBP, mmHg	127.8 (125.4, 130.2)	127.5 (124.6, 130.3)	128.7 (124.3,133.1)	-1.25 (-5.8, 4.3)	-0.05 (-5.4, 5.4)	.65	.99
DBP, mmHg	80.6 (79.4, 81.9)	79.8 (78.4, 81.2)	83.1 (80.2, 85.9)	-3.31 (-6.2, -0.42)	-2.52 (-5.5, 0.4)	.02	.09
SV, mL/beat	77.7 (76.0, 79.4)	81.8 (80.1, 83.5)	65.4 (62.2, 68.5)	16.5 (13.0, 19.9)	16.3 (12.8, 19.9)	<.001	<.001
SI, mL/beat/m^2	44.2 (43.2, 45.3)	47.0 (45.9, 48.1)	35.8 (34.2, 37.3)	11.2 (9.1, 13.2)	10.2 (8.2, 12.2)	<.001	<.001
CO, L/min	4.97 (4.86, 5.07)	5.13 (5.01, 5.25)	4.47 (4.32, 4.62)	0.65 (0.42, 0.88)	0.65 (0.42, 0.88)	<.001	<.001
CI, L/min/m^2	2.82 (2.76, 2.89)	2.95 (2.87, 3.02)	2.46 (2.37, 2.54)	0.48 (0.34, 0.52)	0.42 (0.29, 0.56)	<.001	<.001
SVR, dyn/s/cm^{-5}	1505 (1463, 1546)	1443 (1397, 1490)	1689 (1608, 1769)	-245 (-337, -153)	-225 (-314, -135)	<.001	<.001
SVRI, dyn/s/cm^{-5}/m^2	2661 (2585, 2736)	2526 (2442, 2609)	3067 (2930, 3204)	-541 (-705, -377)	-452 (-610, -295)	<.001	<.001
PEP, msec	104 (101, 106)	105 (102, 108)	99 (93, 106)	5.5 (-0.5, 11.0)	5.0 (-2.0, 11.0)	.07	.14
LVET, msec	314 (309, 319)	318 (312, 324)	304 (293, 314)	14.0 (2.9, 25.0)	17.0 (5.0, 28.0)	.01	.005
PEP/LVET ratio	0.34 (0.32, 0.35)	0.34 (0.32, 0.35)	0.34 (0.31, 0.37)	-0.009 (-0.03, 0.02)	0.023 (-0.02, 0.05)	.84	.52
LCW, Kg/m	6.10 (5.94, 6.25)	6.25 (6.06, 6.43)	5.65 (5.37, 5.92)	0.60 (0.24, 0.95)	0.67 (0.31, 1.04)	0.001	<.001
LCWI, Kg/m/m^2	3.47 (3.37, 3.56)	3.59 (3.48, 3.70)	3.11 (2.95, 3.26)	0.47 (0.25, 0.69)	0.45 (0.23, 0.66)	<.001	<.001

Table 6. Differences in hemodynamic variables between obese-insulin-sensitive (IS) and obese-insulin-resistant (IR) subjects. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between obese-IS and obese-IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted p value
No (%)	128 (100)	51 (33.8)	77 (66.2)	-	-	<.001	-
RRI, msec	882 (859, 906)	938 (905, 972)	845 (816, 875)	92 (47, 137)	100 (54, 146)	<.001	<.001
MAP, mmHg	101.1 (98.6, 103.7)	97.2 (93.3, 101.0)	103.8 (100.4,107.1)	-5.5 (-11.0, -1.5)	-4.03 (-8.6, 0.62)	.01	.08
SBP, mmHg	134.6 (130.0,138.6)	128.5 (121.7,135.2)	138.3 (132.8,143.9)	-9.8 (-18.5, -1.2)	-5.1 (-12.1, 1.9)	.02	.15
DBP, mmHg	84.5 (82.6, 86.4)	81.9 (79.0, 84.8)	86.2 (83.7, 88.7)	-4.26 (-8.0, -0.4)	-3.5 (-7.4, 0.4)	.02	.07
SV, mL/beat	77.0 (73.6, 80.4)	88.7 (83.3, 94.1)	69.3 (65.8, 72.7)	19.4 (13.4, 25.4)	19.2 (13.2, 25.3)	<.001	<.001
SI, mL/beat/m^2	40.5 (38.7, 42.2)	47.6 (45.0, 50.1)	35.8 (34.1, 37.5)	11.7 (8.7, 14.5)	11.6 (8.6, 14.7)	<.001	<.001
CO, L/min	5.24 (5.04, 5.45)	5.69 (5.33, 6.05)	4.95 (4.73, 5.17)	0.74 (0.34,1.13)	0.71 (0.31, 1.11)	<.001	.001
CI, L/min/m^2	2.75 (2.65, 2.86)	3.05 (2.87, 3.23)	2.55 (2.45, 2.66)	0.49 (0.30, 0.69)	0.48 (0.28, 0.68)	<.001	<.001
SVR, dyn/s/cm^{-5}	1541 (1468, 1613)	1364 (1256, 1471)	1658 (1568, 1748)	-294 (-433, -154)	-240 (-372, -108)	<.001	<.001
SVRI, dyn/s/cm^{-5}/m^2	2920 (2788, 3052)	2515 (2334, 2696)	3189 (3029, 3349)	-674 (-917, -431)	-581 (-819, -344)	<.001	<.001
PEP, msec	103 (96, 111)	105 (88, 122)	102 (97, 107)	2.0 (-12.0, 18.0)	7.0 (-8.0, 23.0)	.70	.35
LVET, msec	315 (308, 322)	325 (316, 334)	308 (298, 318)	16.0 (2.3, 30.9)	18.0 (3.0, 32.0)	.02	.01
PEP/LVET ratio	0.32 (0.1, 0.34)	0.30 (0.28, 0.32)	0.34 (0.31, 0.36)	-0.03 (-0.05, -0.005)	-0.03 (-0.06,-0.002)	.02	.03
LCW, Kg/m	6.87 (6.55, 7.19)	7.15 (6.60, 7.69)	6.68 (6.29, 7.08)	0.45 (-0.18, 1.11)	0.61 (0.13, 0.81)	.15	.06
LCWI, Kg/m/m^2	3.61 (3.44, 3.78)	3.85 (3.55, 4.14)	3.46 (3.25, 3.66)	0.39 (0.045, 0.73)	0.47 (0.23, 0.66)	.02	.007

When analyses of variance were performed within each BP category, IR subjects, showed significantly higher SVRI and a reduced RRI, SI, CI, LVET and LWCI when compared to IS individuals ($p<0.001$ for all parameters). These differences remained significant after adjustment for BMI, MAP, age, sex and cigarette smoking. Although analysis of variance showed significantly higher MAP in IR subjects within each BP category, differences lost significance when adjusting for BMI (Figure 3 and Tables 7-9).

Figure 3. Hemodynamic variables by BP categories: Comparison between insulin-sensitive (IS, □) and insulin-resistant (IR, ■) individuals. Data are shown as means±SEM. *$p<0.0001$ after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking. SVRI: Systemic vascular resistance index; LCWI: Left cardiac work index; MAP: Mean arterial pressure. The number of IS and IR subjects in each category is shown between parentheses at the base of each bar.

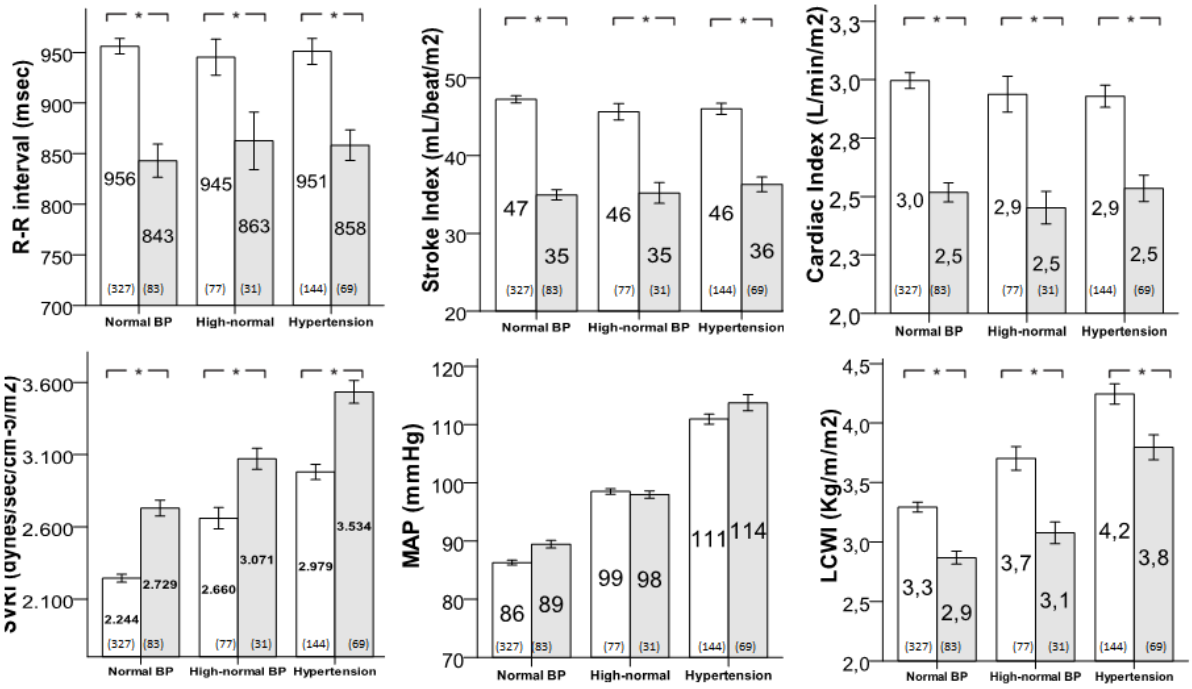


Table 7. Hemodynamic variables in individuals with normal BP, and differences in hemodynamic parameters between insulin-sensitive (IS) and insulin-resistant (IR) individuals. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between IS and IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted p value
No (%)	410 (100)	327 (79.8)	83(20.2)	-	-	<.001	-
RRI, msec	933 (919, 947)	956 (941, 971)	843 (810, 875)	113 (79, 145)	122 (85, 158)	<.001	<.001
MAP, mmHg	86.8 (86.1, 87.6)	86.2 (85.4, 87.0)	89.1 (87.5, 90.8)	-3.2 (-4.9, -1.4)	-0.96 (-2.89, 0.97)	<.001	.32
SBP, mmHg	113.2 (112.3,114.2)	112.4 (111.4,113.4)	116.7 (114.8,118.7)	-4.4 (-5.6, -2.1)	-2.34 (-4.66, -0.02)	<.001	.04
DBP, mmHg	74.0 (73.4, 74.7)	73.6 (72.8, 74.3)	75.9 (74.6, 77.1)	-2.3 (-3.8, -0.7)	-1.07 (-2.80, 0.66)	.05	.22
SV, mL/beat	76.1 (74.6, 77.6)	79.2 (77.6, 80.9)	63.9 (61.2, 66.6)	15.3 (11.8, 18.8)	19.1 (15.4, 22.6)	<.001	<.001
SI, mL/beat/m^2	44.7 (43.8, 45.6)	47.2 (46.3, 48.1)	34.9 (33.6, 36.2)	12.3 (10.3, 14.2)	12.1 (10.1, 14.2)	<.001	<.001
CO, L/min	4.93 (4.84, 5.02)	5.02 (4.90, 5.13)	4.60 (4.43, 4.77)	0.41 (0.18, 0.65)	0.62 (0.38, 0.87)	<.001	<.001
CI, L/min/m^2	2.90 (2.84, 2.96)	3.00 (2.93, 3.06)	2.51 (2.43, 2.60)	0.47 (0.34, 0.61)	0.44 (0.29, 0.58)	<.001	<.001
SVR, dyn/s/cm^{-5}	1370 (1341, 1400)	1338 (1305, 1370)	1500 (1434, 1566)	-152 (-234, -90)	-180 (-258, -103)	<.001	<.001
SVRI, dyn/s/cm^{-5}/m^2	2342 (2290, 2394)	2244 (2189, 2299)	2729 (2620, 2838)	-484 (-605, -363)	-399 (-524, -274)	<.001	<.001
PEP, msec	106 (104, 108)	107 (104, 109)	102 (97, 107)	2.0 (-0.3, 10.0)	5.0 (-1.0, 11.0)	.05	.10
LVET, msec	311 (307, 315)	312 (307, 316)	305 (297, 314)	5.0 (-3.0, 16.0)	14.0 (4.0, 24.0)	.19	.009
PEP/LVET ratio	0.35 (0.34, 0.36)	0.35 (0.34, 0.36)	0.34 (0.32, 0.36)	-0.09 (-0.01, 0.033)	0.001 (-0.03, 0.026)	.45	.95
LCW, Kg/m	5.47 (5.34, 5.59)	5.52 (5.38, 5.66)	5.25 (5.01, 5.48)	0.27 (-0.03, 0.57)	0.55 (0.33, 0.97)	.08	<.001
LCWI, Kg/m/m^2	3.21 (3.13, 3.28)	3.29 (3.21, 3.37)	2.87 (2.76, 2.97)	0.42 (0.25, 0.59)	0.46 (0.28, 0.65)	<.001	<.001

Table 8. Hemodynamic variables in individuals with high-normal BP, and differences in hemodynamic parameters between insulin-sensitive (IS) and insulin-resistant (IR) individuals. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between IS and IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted p value
No (%)	108 (100)	77 (59.8)	31 (40.2)	-	-	<.001	-
RRI, msec	921 (891, 952)	945 (909, 980)	862 (804, 920)	82 (16, 148)	83 (13, 154)	.01	.02
MAP, mmHg	98.4 (97.6, 99.1)	98.5 (97.6, 99.5)	98.0 (96.7, 99.3)	0.61 (-1.7, 2.9)	0.86 (-1.55, 3.3)	.60	.48
SBP, mmHg	129.2 (127.9,130.6)	129.9 (128.3,131.4)	127.7 (124.8,130.6)	2.15 (-0.83, 5.16)	1.45 (-1.58, 4.48)	.15	.34
DBP, mmHg	82.9 (81.8, 84.0)	82.8 (81.5, 84.2)	83.1 (81.3, 84.9)	0.29 (-2.6, 2.1)	0.21 (-2.19, 2.61)	.80	.86
SV, mL/beat	75.2 (71.7, 78.6)	78.9 (74.9, 82.9)	65.7 (59.7, 71.3)	13.2 (5.9, 20.5)	19.2 (12.1, 25.9)	<.001	<.001
SI, mL/beat/m^2	42.6 (40.7, 44.5)	45.6 (43.5, 47.7)	35.2 (32.5, 37.9)	10.4 (6.7, 14.1)	11.5 (7.5, 15.4)	<.001	<.001
CO, L/min	4.93 (4.70, 5.14)	5.07 (4.79, 5.36)	4.55 (4.27, 4.84)	0.52 (0.04, 1.0)	0.91 (0.46, 1.37)	.03	<.001
CI, L/min/m^2	2.80 (2.70, 2.92)	2.93 (2.78, 3.08)	2.45 (2.31, 2.59)	0.48 (0.23, 0.73)	0.55 (0.29, 0.82)	<.001	<.001
SVR, dyn/s/cm^{-5}	1586 (1513, 1659)	1554 (1460, 1649)	1665 (1566, 1764)	-110 (-271, 50.6)	-233 (-384, -83)	.17	.003
SVRI, dyn/s/cm^{-5}/m^2	2777 (2659, 2895)	2659 (2512, 2807)	3070 (2922, 3219)	-410 (-660, -161)	-470 (-733, -207)	.001	.001
PEP, msec	105 (101, 109)	107 (102, 111)	101 (92, 110)	5.2 (-3.4, 14)	2.0 (-0.7, 11.0)	.23	.64
LVET, msec	308 (299, 317)	309 (299, 320)	304 (287, 322)	5.0 (-14.0, 24)	7.0 (-14.0, 27.0)	.60	.52
PEP/LVET ratio	0.35 (0.33, 0.37)	0.35 (0.33, 0.38)	0.34 (0.30, 0.37)	0.01 (-0.03, 0.06)	0.003 (-0.04, 0.05)	.47	.90
LCW, Kg/m	6.20 (5.91, 6.48)	6.40 (6.03, 6.77)	5.70 (5.34, 6.06)	0.69 (0.74, 1.31)	1.22 (0.63, 1.80)	.02	<.001
LCWI, Kg/m/m^2	3.52 (3.36, 3.68)	3.70 (3.50, 3.90)	3.07 (2.89, 3.26)	0.62 (0.29, 0.95)	0.73 (0.40, 1.07)	<.001	<.001

Table 9. Hemodynamic variables in individuals with hypertension, and differences in hemodynamic parameters between insulin-sensitive (IS) and insulin-resistant (IR) individuals. Values for hemodynamic parameters before and after indexing for body surface area (BSA) are presented as means (95% confidence interval). Mean differences for hemodynamic parameters between IS and IR individuals are shown with their respective 95% CI, before (T-Test) and after adjustment for BMI, MAP, PP, waist circumference, age, sex and cigarette smoking using analysis of covariance (ANCOVA).

Variable	All	IS	IR	Difference between means (95% CI)	Adjusted difference (95% CI)	p value	Adjusted p value
No (%)	213 (100)	144 (52.1)	69 (47.9)		-	<.001	-
RRI, msec	921 (900, 941)	951 (925, 976)	858 (828, 888)	92 (50, 134)	100 (52, 148)	<.001	<.001
MAP, mmHg	111.5 (110.0,113.0)	110.4 (108.6,112.3)	113.8 (111.1,116.5)	-3.33 (-5.55, -0.12)	-2.05 (-5.73, 1.64)	.04	.27
SBP, mmHg	153.3 (150.6,156.0)	153.5 (150.1,156.8)	152.9 (148.2,157.6)	0.61 (-5.23, 5.44)	2.42 (-3.67, 8.52)	.83	.43
DBP, mmHg	91.1 (89.6, 92.6)	89.7 (87.9, 91.4)	94.2 (91.6, 96.7)	-4.5 (-7.5, -1.4)	-2.63 (-5.99, 0.72)	.005	.12
SV, mL/beat	73.6 (71.5, 75.7)	76.8 (74.5, 79.1)	67.0 (63.1, 70.7)	9.8 (5.6, 14.1)	13.8 (9.1, 18.4)	<.001	<.001
SI, mL/beat/m^2	42.8 (41.6, 44.1)	46.0 (44.6, 47.4)	36.3 (34.4, 38.2)	9.7 (7.3, 12.1)	8.9 (6.2, 11.8)	<.001	<.001
CO, L/min	4.81 (4.70, 4.94)	4.88 (4.73, 5.03)	4.67 (4.44, 4.90)	0.21 (-0.05, 0.47)	0.42 (0.13, 0.71)	.11	.004
CI, L/min/m^2	2.80 (2.72, 2.87)	2.93 (2.83, 3.02)	2.53 (2.42, 2.65)	0.39 (0.23, 0.54)	0.32 (0.14, 0.49)	<.001	<.001
SVR, dyn/s/cm^{-5}	1828 (1778, 1877)	1778 (1721, 1836)	1931 (1837, 2024)	-152 (-256, -47)	-194 (-311, -77)	.005	.001
SVRI, dyn/s/cm^{-5}/m^2	3159 (3066, 3251)	2979 (2875, 3083)	3533 (3376, 3691)	-554 (-738, -370)	-423 (-628, -217)	<.001	.001
PEP, msec	105 (100, 110)	108 (101, 114)	100 (94, 107)	7.3 (-3.0, 18)	8.0 (-4.0, 20.0)	.18	.20
LVET, msec	320 (315, 326)	327 (320, 334)	307 (296, 318)	20.0 (7.6, 32.0)	19.0 (5.0, 34.0)	.002	.009
PEP/LVET ratio	0.33 (0.32, 0.35)	0.33 (0.31, 0.34)	0.34 (0.31, 0.36)	-0.01 (-0.04, 0.07)	-0.02 (-0.05, 0.01)	.46	.25
LCW, Kg/m	7.04 (6.83, 7.26)	7.07 (6.81, 7.33)	6.99 (5.58, 7.39)	0.08 (-0.38, 0.55)	0.45 (0.07, 0.97)	.72	.08
LCWI, Kg/m/m^2	4.10 (3.96, 4.23)	4.24 (4.07, 4.41)	3.79 (3.58, 4.00)	0.45 (0.16, 0.73)	0.38 (0.06, 0.70)	.002	.02

Discussion

Besides the finding of an independent relationship of IR with hemodynamic indices of CV function, obtained in a random sample of non-diabetic individuals from the general population, the key novel contribution of our study consists in the demonstration of the constant character of such a relationship across different categories of BMI and BP levels, and independently of its association with obesity and hypertension.

In the whole population of our study HOMA was directly correlated with SVRI and BP levels; and inversely correlated with RRI, SI, CI, PEP, LVET and LCWI. Besides, in a stepwise multiple linear regression analysis where IR and other potential predicting variables such as age, sex, BMI, and BP levels were modeled together, IR showed a significant contribution to the variation of hemodynamic parameters such as RRI, SI, CI and SVRI. Apart from the significant differences in hemodynamic indices (higher SVRI and BP levels and reduced RRI, SI, CI, PEP, LVET and LWCI), IR subjects were also characterized by hyperinsulinemia and higher fasting plasma glucose concentrations (classical manifestations of IR) as well as by clinical alterations (i.e. elevated waist circumference, BMI and BP levels) and lipid abnormalities (elevated total serum cholesterol, hypertrigliceridemia and low levels of HDL cholesterol). This clustering of metabolic and hemodynamic phenotypes -most of which are currently considered as defining criteria of the cardiometabolic syndrome- (24), has been previously documented by other studies and is thought to be a manifestation of the concomitant impairment of vascular and metabolic insulin-signaling pathways in IR states. (3-5)

When analysis of covariance was performed within each BMI and BP category, the association between IR and hemodynamic indices initially found in the entire study population, was replicated and remained significant. Regardless of BMI and BP category,

hemodynamic profiles in IR individuals were very similar, being characterized by higher SVRI and BP levels and by reduced RRI, PEP, LVET, SI, CI, and LCWI when compared to IS individuals. This constitutes the main finding of our study, and based on it we conclude that the relationship between HOMA and hemodynamic indices of CV function is constant across different BMI and BP categories and independent of the presence of obesity or hypertension.

Although BMI was higher in IR subjects within each BMI category, differences in hemodynamic parameters between IS and IR subjects remained significant even after further adjustment for BMI. Differences in SI, CI, SVRI and LCWI, were not adjusted by BMI as these variables had already been indexed for BSA. Besides, analysis of co-variance within each BP category, also allowing for BMI and MAP, showed significant differences for most hemodynamic parameters between IS and IR subjects.

In our study, RRI was always shorter in IR individuals independently of BMI and BP category. These findings are in line with previous reports showing a linear increment in resting HR along with insulin levels during IR states, likely as a result of the insulin-induced increase in central sympathetic drive to the heart, which may be further enhanced, indirectly, by hyperinsulinemia-induced reduction in glycemia. (14, 24)

Regardless of BMI and BP category, SI was consistently reduced in IR individuals. Although the shorter RRI and the concomitant reductions in LVET could be major explanatory factors for these reductions in SI, the finding of a lower CI in these individuals despite the higher HR, might depend on increased SVRI but possibly, also on an impaired left ventricular (LV) systolic performance. Although our study cannot provide conclusive evidence on this issue, because we did not include echocardiographic assessment of cardiac function in all participants; clinical studies in humans have shown

IR to be associated with LV remodeling and with impaired systolic and diastolic performance even in the absence of structural alterations or coronary artery disease. (9, 11) In addition, induced IR in rats has been associated with an impaired contractile function and with reduced end-diastolic volume, ejection fraction, SV and CO; (3) as well as with cardiac structural alterations (increased LV mass and relative wall thickness). (26)

Unlike other hemodynamic indices, SVRI showed a progressive significant increase from lean to overweight and obese subjects; and from normal BP to high-normal BP and hypertension categories, and was always higher in IR subjects. This linear trend seems to suggest a dose-response effect relationship between IR and SVRI and BP levels and points towards an increased vascular tone as a primary mechanism for BP elevation in subjects with IR. Supporting these findings, experimental studies have shown that vascular actions of insulin are context dependent, and mediated by two major signaling branches(1): While in normal conditions insulin-stimulated production of nitric oxide (NO) through phosphatidylinositol 3-kinase-dependent signaling pathways produces vasodilatation, capillary recruitment and increases local blood flow (27); during IR states the predominant stimulation of mitogen-activated protein kinase-dependent pathways by the compensatory hyperinsulinemia, leads to increased production of endothelin-1, a potent vasoconstrictor which opposes vasodilator actions of NO (28) causing a shift towards a predominant vasoconstrictor state. In addition, the insulin-induced increase in central sympathetic drive to the peripheral vasculature, may further contribute to these increases in SVRI. (12) In line with these observations, clinical studies have shown a reduced local vasodilatatory response during insulin infusion in cases of chronic hyperinsulinemia, (6, 7, 29) and an excessive forearm vasoconstriction in the context of severe insulin resistance. (30)

Limitations of the study

While our study has several points of strength, among which the large sample of subjects selected in a representative manner from a general population, in all of whom beat-by-beat hemodynamic assessment was performed, we have also to acknowledge some limitations, mostly related to the technique employed for hemodynamic assessment, i.e. impedance cardiography. The acknowledged main limitations of impedance cardiography are related to the biophysical basis of this technique, which may lead to SV overestimation in cases of low impedance values, and to its high sensitivity to movement artifacts. (18, 19) However, these potential problems can be in most cases controlled by proper equipment calibration and correct positioning of electrodes. Other inaccuracies of this method may occur in specific clinical conditions, such as presence of atrial fibrillation, tachyarrhythmias or excessive thoracic fluid states, all of which represent contraindications for use of this method, and were among the exclusion criteria of our study. Despite these theoretical problems, the validity of impedance cardiography in measuring SV, its changes following a variety of interventions, and the reproducibility of the data so obtained, are supported by a number of studies. (31-34) Moreover, its use has been considered as acceptable in particular for the assessment of large samples of individuals in research studies on a population basis (34-36) where other potentially more precise methods, such as echocardiography, can hardly be employed due to the possibility of a significantly large inter-observer variability.

Although hemodynamic effects of IR are often clustered with metabolic alterations and largely explained by an increased body weight or elevated BP levels, the findings of our study indicate that effects of IR on hemodynamic indices of CV function are constant across different BMI and BP categories and independent of BMI and BP levels, thus

reinforcing the importance of IR in the pathogenesis of CV alterations beyond its association with obesity and hypertension.

Perspectives

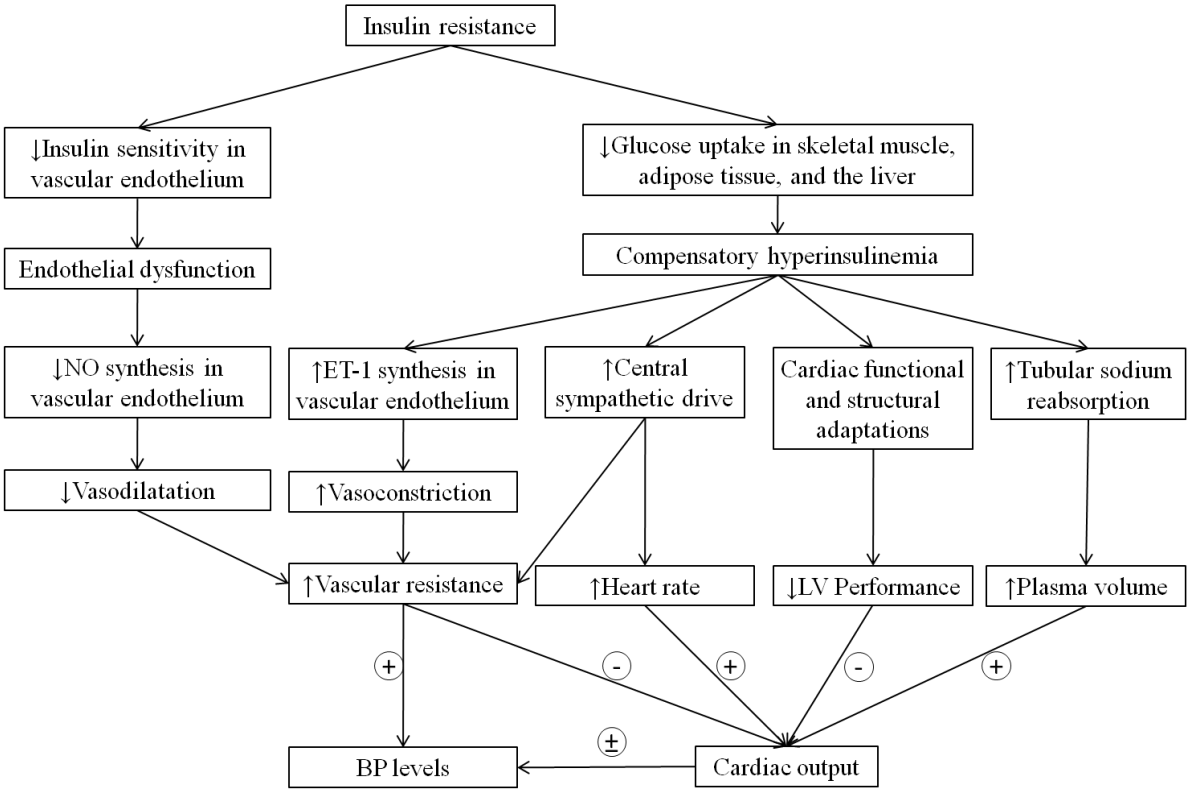
Most previous studies investigating the cardiovascular effects of insulin resistance have focused on specific populations or clinical settings, such as type 2 diabetes mellitus, congestive heart failure, elderly subjects or overweight/obese subjects. Upcoming studies should therefore extend our observations on the important independent contribution of insulin resistance to the pathogenesis of CV alterations also to lean, normotensive or otherwise healthy subjects, if possible on a population basis. They should also consider the possibility of a prospective follow-up to better define the role of IR in development, establishment and progression of CV disease, thus further testing the hypothesis that IR itself might be considered as a target for CVD prevention. The questions on whether insulin resistance in lean subjects is worth of medical treatment, and how to treat these subjects, represent issues still in need of a satisfactory answer.

Part 2: Effects of Insulin Resistance on Systemic Hemodynamics and Autonomic Cardiovascular Regulation in Normotensive Healthy Adults

Introduction

Insulin exerts pleiotropic effects on the cardiovascular system. In addition to its metabolic effects on glucose homeostasis, insulin also exerts physiological functions in the vascular endothelium, the heart, the autonomic nervous system, and the kidneys thus playing an important role in coupling metabolic and hemodynamic homeostasis under normal physiological conditions (1) Figure 4.

Figure 4. Proposed mechanisms for the hemodynamic alterations associated with insulin resistance. NO: Nitric oxide; ET-1: Endothelin-1; LV: Left ventricular; BP: Blood pressure.



On the other hand, in pathophysiological conditions associated with insulin resistance (i.e. obesity, type 2 diabetes) a cluster of hemodynamic and autonomic alterations accompanying metabolic alterations has been reported. (2-5) It has been suggested that alterations in autonomic cardiovascular modulation associated with insulin resistance (i.e. increased in central sympathetic drive) (12, 13) may represent a potential mechanism for the hemodynamic alterations associated with IR.

In normal physiological conditions, a very precise control of BP levels is achieved through a complex combination between central neural and reflex influences, leading to a continuous modulation of efferent sympathetic and parasympathetic nerve activity and the associated activity of neuro-hormonal systems primarily regulated by the hypothalamus (37). It is thus likely that alterations in the autonomic nervous system associated with insulin resistance (i.e. increased in central sympathetic, reductions in parasympathetic modulation, and impaired baroreflex sensitivity) may contribute to elevation of blood pressure levels and to alterations in heart rate and blood pressure variability over the 24 hours, even before the establishment of arterial hypertension. Of note, recent studies have provided evidence that insulin resistance may induce significant funtional and structural changes in large arteries (i.e. increased arterial stiffness) (38), (39), (40) which in turn might further contribute to elevation in BP levels and alterations in BP variability.

It should be considered however, that most studies exploring the hemodynamic and autonomic effects of insulin resistance have been conducted in obese, type 2 diabetes mellitus, and hypertensive populations.

The first part of the present thesis consistently showed that IR is associated with significant increases in blood pressure levels and other important alterations in systemic

hemodynamics even in lean and normotensive subjects. This was the rationale to explore potential mechanisms for these alterations in this selected group of subjects. Specific objectives of this second part of the thesis were:

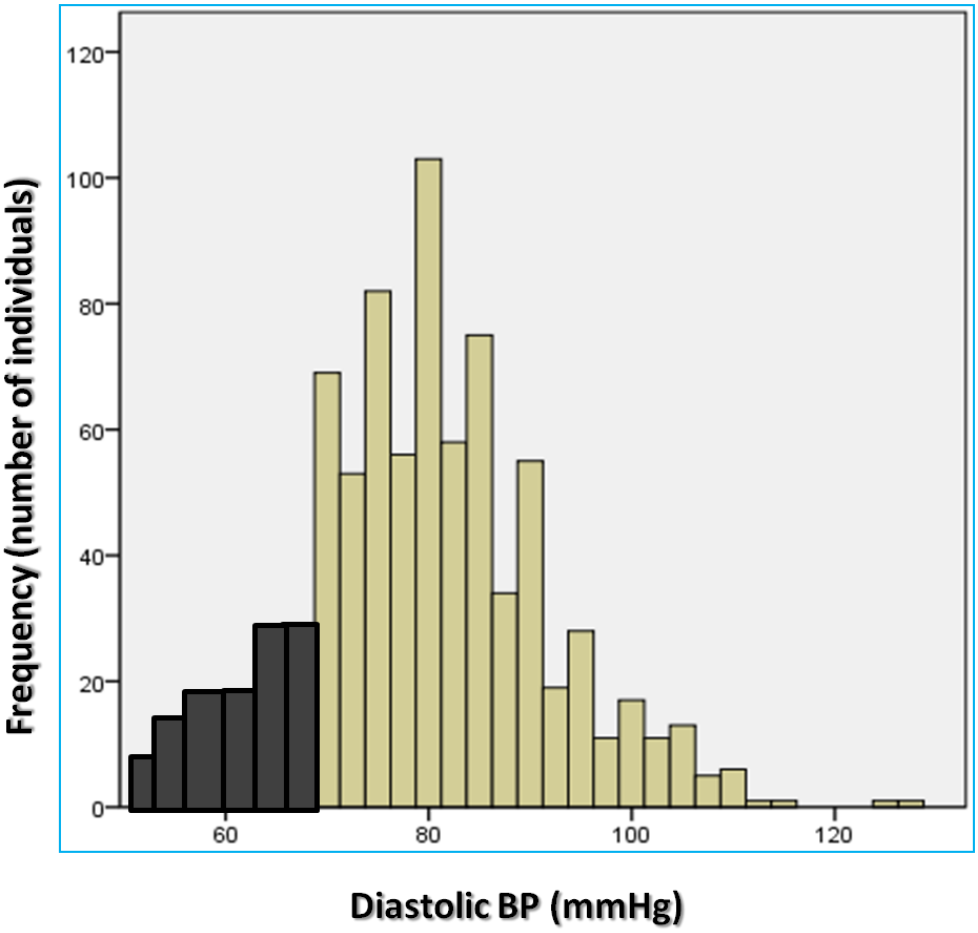
1) To explore the relationship between insulin resistance and systemic hemodynamics, cardiac baroreflex sensitivity and indices of autonomic CV modulation.
2) To explore the relationship of insulin resistance with 24h heart rate, average blood pressure levels and blood pressure variability over the 24h.
3) To explore the relationship of insulin resistance with central blood pressure levels and with measures of large artery stiffness and wave reflections.

Research design and methods

Study population

In order to explore the association of insulin resistance with systemic hemodynamics and autonomic cardiovascular regulation independently of the presence of hypertension, we selected subjects who were below the 30^{th} percentile of diastolic blood pressure (DBP) distribution curve (≤72 mmHg) and who had no elevation in systolic BP. DBP was chosen as selection criterion instead of SBP in order to reduce the selection bias of excluding older subjects (i.e. systolic BP increases with age, mostly as a result of stiffening of large arteries with increased pulse wave velocity). Figure 5.

Figure 5. Diastolic blood pressure distribution curve in the whole population of the Medellin's Heart Study (n=800). Darker bars indicate the subjects with diastolic blood pressure levels below the 30th percentile of diastolic blood pressure (DBP) distribution curve (≤72 mmHg).



In addition, subjects were excluded in case of diabetes mellitus (fasting blood glucose ≥126 mg/dL or use of medications for previously diagnosed type 2 diabetes) obesity (BMI≥30), taking medications with effects on BP, pregnancy; musculoskeletal disease, limiting movement or mental disability impeding autonomous signing of informed consent. A total of 90 subjects fulfilling inclusion criteria were considered for the present analysis and underwent further assessments.

Study assessments

Questionnaire and anthropometric measures

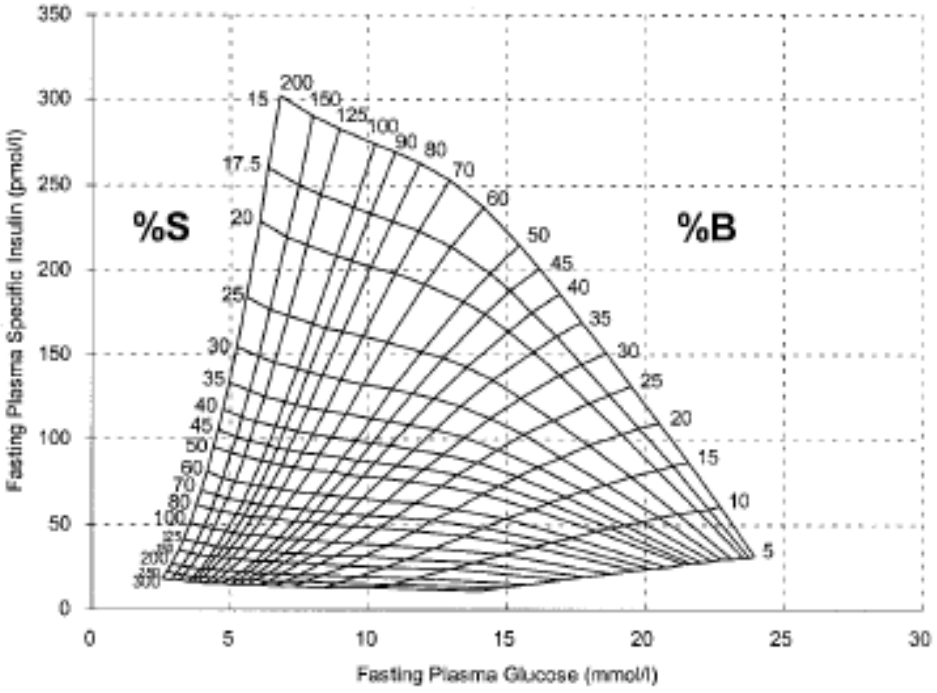
A standardized questionnaire was administered including information on demographic factors, socio-economical status (education, income), lifestyles (smoking, physical activity, and dietary patterns), medication use, personal and familiar history of CV disease, and CV risk factors (hypertension, diabetes mellitus, dyslipidemia). Height was measured to the nearest 0.10 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.10 Kg with calibrated scales and BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured with a non-stretchable standard tape measure over the unclothed abdomen at midway between the lateral lower ribs and the iliac crest and expressed rounded to the nearest 0.50 centimeters.

Laboratory assessment

A venous blood sample was drawn from all participants after at least 8 hours fasting, and processed within the first 6 hours. Plasma glucose was determined by the glucose oxidase method and insulinemia by enzyme immunoassay, following standardized procedures in the clinical laboratory. Insulin resistance was evaluated with the homeostasis model

assessment of insulin resistance (HOMA) using the formula ((glycemia (mg/dL)/18) x insulinemia (uU/mL)/22.5). This index, considered a reliable marker of IR, was shown to be closely correlated with the insulin sensitivity index measured through the standard euglycemic hyperinsulinemic glucose clamp method. Figure 6 (22) Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were also determined. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's formula whenever triglycerides were <400 mg/dL.

Figure 6. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Taken from Matthews DR, et al. (22).



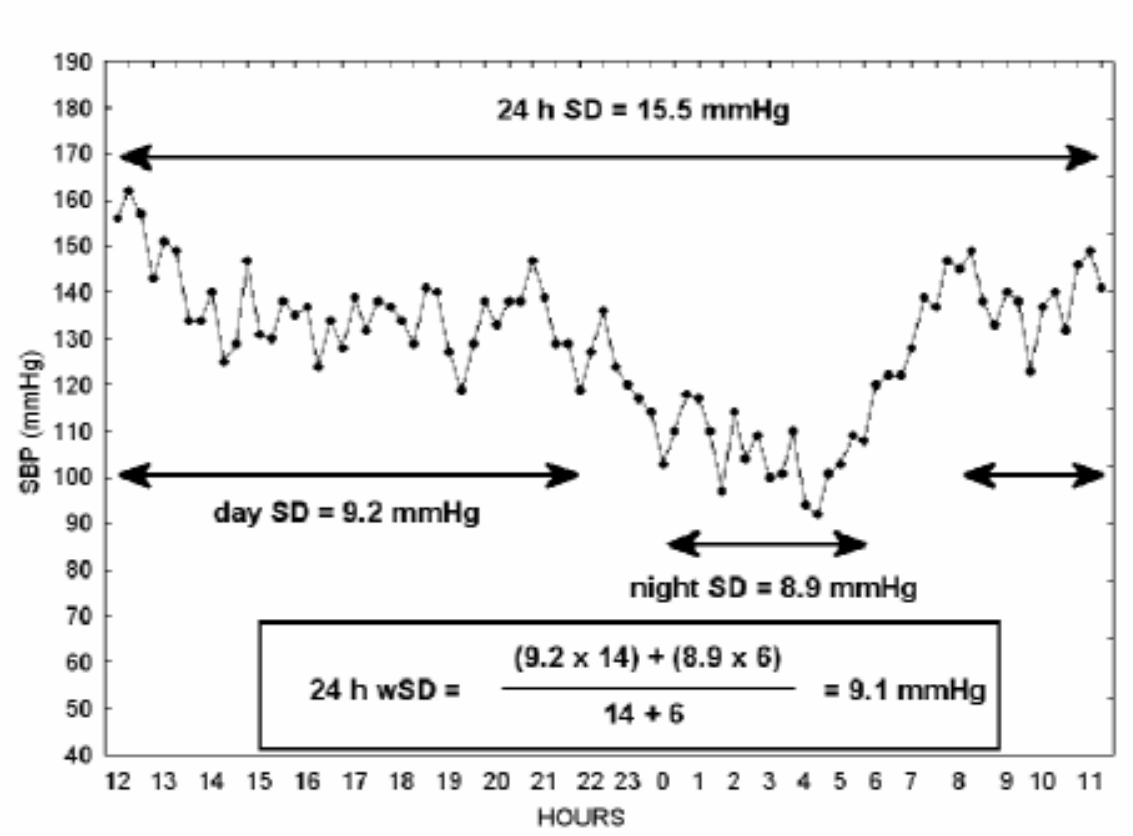
Assessment of average blood pressure levels and blood pressure variability over the 24 hours

Ambulatory BP monitoring (ABPM) was performed with a validated oscillometric device for 24h ambulatory blood pressure monitoring (Mobil-O-Graph 24h PWA Monitor, I.E.M. GmbH, Stolberg, Germany) (41), (42). All patients underwent 24-h ABPM on a usual working day. They were instructed to act and work as usual and to keep their nondominant arm still and relaxed at the side during measurements. The between measurement intervals were 15 min during the day (7.00-23.00 h) and 20 min during the night (23.00-7.00) in all subjects considered. The day period was identified as the interval from 8.00 h to 22.00 h and the night period as the interval from 12:00 to 6.00 h. From these recordings, HR, SBP and DBP levels were averaged for the day, night and 24-hour periods, and the respective dipping from day-to-night calculated. BP variability was assessed separately for SBP and DBP as 24h, daytime and night-time standard deviation (SD), and as recently proposed with the weighted 24h SD (wSD) (43). (Figure 7) 24 h "weighted" standard deviations (wSD) i.e. the mean of day and night SD values weighted for the number of hours included in each of day and nighttime sub-periods, is calculated according to the formula:

$$wSD = \frac{(\text{daytime SD} \times 14) + (\text{night-time SD} \times 6)}{20}$$

where 14 and 6 are the number of hours included in the daytime and in the night-time subperiods, respectively.

Figure 7. A representative 24 h profile of SBP values. 24 h SBP SD is much higher than the corresponding daytime or night-time SD values, separately computed, due to the contribution of a pronounced nocturnal SBP fall.



Assessment of central BP levels, arterial stiffness and wave reflections

By directly reflecting arterial stiffness, having the best predictive value for cardiovascular events and the ease of its measurement, carotid-femoral pulse wave velocity (cfPWV) is currently considered the gold standard for arterial stiffness assessment in daily practice (44). However, in recent years different measurement procedures have been proposed for its estimation. One of such techniques is the aortic PWV approximation technique that is based on a single non-invasive pressure reading which should ease the determination of PWV and should reduce the chance of measuring errors. This novel method calculates the

pulse wave velocity from a single pressure reading using pulse wave analysis and impedance wave separation. The characteristic impedance is calculated in the frequency range from 4-10 Hz and the required flow is approximated by a model based approach considering Windkessel theory (45). A validated algorithm permits transformation of peripheral arterial to central aortic waveforms (46, 47).

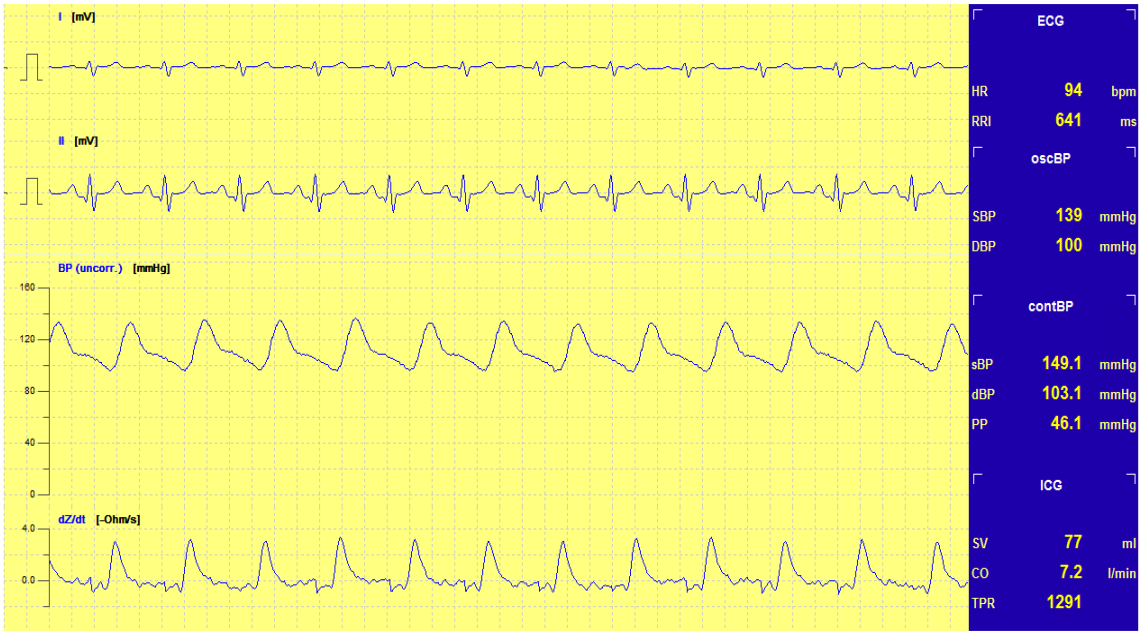
For the present study, we employed a previously validated oscillometric device for ambulatory BP monitoring which allows recording of pulse waveform (Mobil-O-Graph 24h PWA, IEM, Stolberg, Germany). This device has in-built a transfer-function like method (ARCSolver application) and has been recently validated for pulse wave analysis of the central blood pressure and measuring of aortic PWV (48), (49). Using this method, aortic pulse wave velocity (PWV, m/s) and other measures derived from pulse wave analysis such as augmentation index (AIx, %), central SBP (cSBP), central DBP (cDBP) and central pulse pressure (cPP) were computed. Peripheral SBP and DBP, and heart rate were recorded and pulse pressure (PP) calculated as the difference between SBP and DBP.

Non-invasive Hemodynamic Assessment

Non-invasive assessment of beat-to-beat BP, R-R interval (ECG) and stroke volume (by means of impedance cardiography) were performed during 10 min in supine position.

Continuous BP was measured at the finger level using a refined version of the vascular unloading technique (continuous non-invasive arterial blood pressure, CNAP®, module) and corrected to absolute values with oscillometric BP measurement performed in the contralateral upper arm. R-R interval was recorded by means of a high resolution 3-channel-ECG from two separate adhesive monitoring electrodes which were placed on the thorax, to give maximal amplitude of the R-wave. (Figure 8).

Figure 8. Non-invasive assessment of beat-to-beat BP, R-R interval (ECG) and stroke volume (Task Force Monitor®, Graz, Austria).



Synchronized beat-to-beat recordings of stroke volume (SV) and R-R interval (RRI) were performed using impedance cardiography (Task Force Monitor®, Graz, Austria) and specific hemodynamic indices associated with its measurement were computed and averaged: RRI (msec), heart rate (HR, bpm), stroke volume (SV, mL/beat), cardiac output (CO, L/min), systolic (S) BP (mmHg) and diastolic (D) BP (mmHg), systemic vascular resistance (SVR, dyn/sec/cm^{-5}), pre-ejection period (PEP, msec), left ventricular ejection time (LVET, msec) and PEP/LVET ratio and left cardiac work (LCW, Kg/m). Stroke volume index (SI, mL/beat/m^2), cardiac index (CI, L/min/m^2), SVR index (SVRI, dyn/sec/cm^{-5}/m^2) and LCW index (LCWI, Kg/m/m^2) were calculated by normalizing SV, CO, SVR and LCW respectively, by the body surface area (BSA).

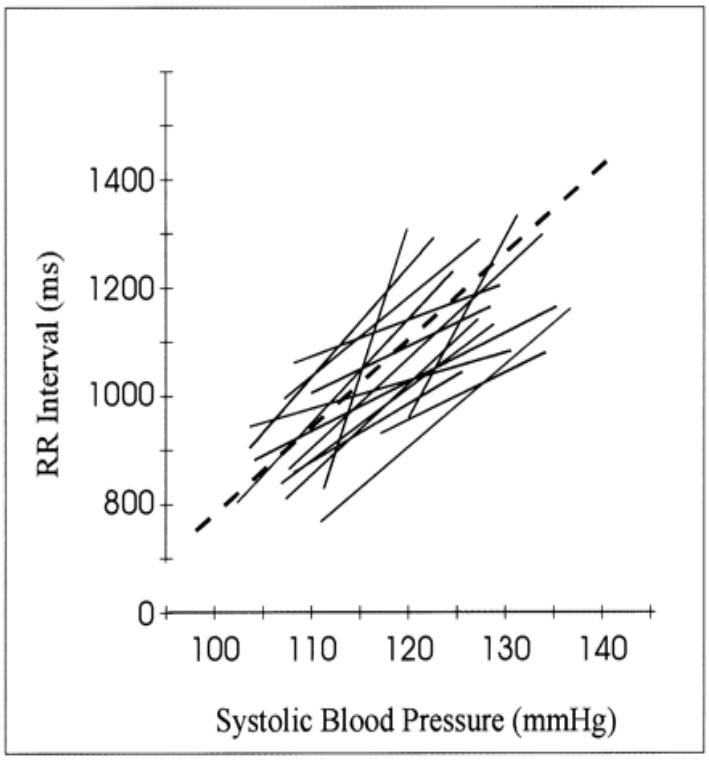
Assessment of indices of autonomic modulation

Cardiac autonomic modulation was estimated from heart rate variability (HRV) analysis and quantification of spontaneous cardiac baroreflex sensitivity (BRS), both in the time and in the frequency domain, by computer analysis of 10 min beat-to-beat BP and ECG recordings obtained while in resting supine. Indices of cardiac autonomic modulation in the time domain such as RRI length, standard deviation of RRI (SDRRI), root mean square of the successive RRI differences (RMSSD RRI, ms), the number of pairs of successive RR that differ by more than 50 ms (NN50 count), and the proportion of NN50 divided by total number of RRs (pNN50, %) were calculated.

Autonomic indices reflecting both sympathetic and parasympathetic cardiac modulation were derived from spectral analysis of HRV in the frequency domain using adaptive autoregressive modelling. Total power spectral density (PSD) of HRV as well as relative PSD for the very low (VLF, 0.025-0.05 Hz), low (LF, 0.05-0.15 Hz) and high frequency (HF, 0.15-0.5 Hz) regions of the HR spectra were quantified and expressed as absolute units [ms^2]. In order to minimise the effect of the changes in total PSD on the values of LF and HF, these powers were normalized by dividing the absolute power of each oscillatory component by total PSD minus the VLF component and multiplying by 100 and expressed as normalized units (nu, %). The ratio between the powers reflecting sympathetic and vagal drive (i.e. LF/HF ratio) was computed. Indices derived from the analysis of HRV reflect both sympathetic and parasympathetic cardiac modulation. (50-54) These indices have proved to be useful to characterize the alterations in cardiac autonomic modulation occurring in essential hypertension (55) and even in pre-hypertensive states. (56) However, their specificity should not be taken for granted. (57).

Cardiac BRS was estimated using the sequence method (58) as the slope of the regression line between RRI and SBP concomitant increases (up-slope) or decreases (down-slope) for at least four consecutive heartbeats, averaged and expressed as total slope of BRS (ms/mmHg). (Figure 9)

Figure 9. Assessment of Cardiac baroreflex sensitivity using the sequence method as proposed by Parati *et al.* (58)



Statistical analysis

Data were analyzed using IBM®SPSS® software v.18.0.0. Normal distribution of variables was assessed with the Smirnov-Kolgomorov test. For statistical analysis subjects were classified into tertiles based on values of HOMA-IR: T1 (HOMA <0.94, n=32), T2 (HOMA 0.94-1.90, n=28) and T3 (HOMA >1.90, n=30). Differences in the frequency of categorical variables were assessed using χ^2 test. Analysis of variance was applied to assess differences in clinical, hemodynamic and autonomic variables among HOMA-IR tertiles. Differences in hemodynamic parameters among HOMA-IR tertiles were corrected by applying analysis of covariance (ANCOVA) adjusting for known confounders such as BMI, MAP, PP, age, sex and cigarette smoking. Differences in hemodynamic variables already indexed for BSA (i.e. stroke index, cardiac index, systemic vascular resistance index, and left cardiac work index) were not adjusted for BMI and waist circumference was considered instead. Differences in SVRI were not adjusted for MAP and PP as the latter variable was included in the formula to compute SVRI.

Results

Clinical characteristics of the entire study population, and by tertiles of HOMA are presented in Table 10. Average age of participants was 48 ± 10 yrs, and 50% were female. While age and sex distribution did not differ significantly between tertiles of HOMA-IR, significant differences were observed for all anthropometric measures and biochemical parameters but LDL cholesterol.

Table 10. Clinical characteristics of the entire study population and by tertiles of HOMA-IR. Values are expressed as mean ± SD.

Variable	All	T1 (<0.94) (n=32)	T2 (0.94-1.90) (n=28)	T3 (>1.90) (n=30)	P value
Age (years)	48.27 ± 10.5	48.3 ± 9.6	46.8 ± 8.1	46.9 ± 11.1	0.833
BMI	25.40 ± 3.66	22.7 ± 2.9	25.8 ± 2.9	27.2 ± 3.6	0.001
Sex (Male, %)	50	50.1	41.9	58.0	0.452
Smokers (%)	16.4	17.0	29.1	7.7	0.072
Fasting glucose (mg/dL)	76.17± 10.0	70.4 ± 7.5	75.5 ± 7.3	82.4 ± 8.7	0.001
Insulin (mU/mL)	8.7 ± 5.8	3.6 ± 1.0	7.3 ± 1.5	15.1 ± 5.5	0.001
HOMA-Index	1.69 ± 1.26	0.62 ± 0.2	1.35 ± 0.3	3.07 ± 1.2	0.001
Total Cholesterol (mg/dL)	201.9± 37.1	203 ± 41	205 ± 38	200 ± 33	0.879
HDL Cholesterol (mg/dL)	45.3 ± 12.24	51.8 ± 11	43.9 ± 11	39.5 ± 10	0.001
LDL Cholesterol (mg/dL)	129.2± 34	131 ± 37	136 ± 35	125 ± 29	0.462
Triglycerides (mg/dL)	153.6± 79	119 ± 55	142 ± 71	201 ± 89	0.001

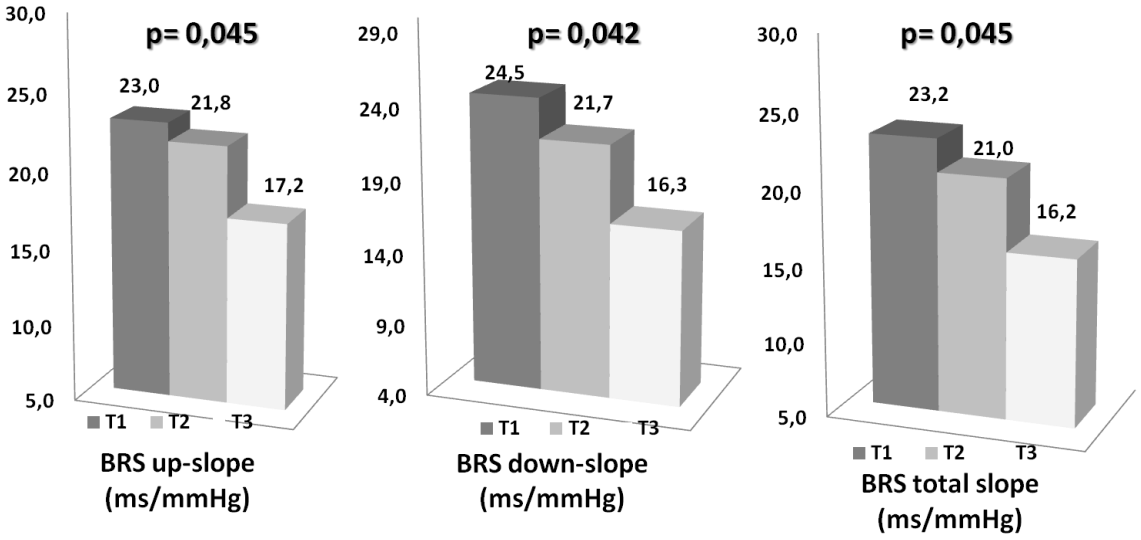
Effects of insulin resistance on systemic hemodynamics and autonomic cardiovascular regulation

Analysis of variance showed a significant overall effect of IR on most hemodynamic parameters: IR was associated with reduced RRI, SI, and CI; and with increased SVRI, SBP and DBP. These differences remained significant in analysis of covariance, adjusting for BMI, MAP, PP, sex, age, and cigarette smoking (Table 11). IR was also associated with reduced BRS (up, down, and total slopes), (Figure 10) decreased parasympathetic indices of autonomic CV modulation (SDRRI, HF-power, total power) and a predominance of sympathetic component of HRV (increased LF/HF ratio). (Table 11).

Table 11. Autonomic and hemodynamic variables by tertiles of HOMA index. Values are expressed as mean ± SEM. *p values were adjusted for age, sex, and BMI.

Variable	T1 (<0.94) (n=32)	T2 (0.94-1.90) (n=28)	T3 (>1.90) (n=30)	P value*
RR interval (ms)	1070 ± 26	998 ± 23	953 ± 26	0.016
Stroke index (mL/beat/m^2)	47.1 ± 1.6	41.3 ± 1.4	38.4 ± 1.6	0.001
Cardiac index (L/min/m^2)	2.70 ± 0.15	2.55 ± 0.10	2.3 ± 0.11	0.071
SVRI (dyn/sec/cm^{-5}/m^2)	2550 ± 119	2707 ± 107	2928 ± 120	0.038
Continuous SBP (mmHg)	103.2 ± 1.7	105.4 ± 1.5	111.3 ± 1.7	0.008
Continuous DBP (mmHg)	67.5 ± 1.4	69.3 ± 1.2	71.1 ± 1.4	0.050
BRS Down-Slope (ms/mmHg)	24.5 ± 2.2	21.7 ± 2.0	16.3 ± 2.2	0.042
BRS Up-Slope (ms/mmHg)	23.0 ± 1.2	21.8 ± 1.1	17.2 ± 1.2	0.045
BRS total slope (ms/mmHg)	23.2 ± 1.2	21.7 ± 1.1	16.8 ± 1.2	0.045
SDRRI (ms)	80.1 ± 7.2	73.6 ± 6.6	58.0 ± 6.9	0.020
HF-power (ms^2)	1291 ± 252	1108 ± 226	478 ± 224	0.036
LF-power (ms^2)	890 ± 122	670 ± 109	390 ± 123	0.003
Total power (ms^2)	2823 ± 455	2218 ± 355	1208 ± 398	0.020
LF/HF ratio	0.88 ± 0.1	0.90 ± 0.2	1.45 ± 0.2	0.040

Figure 10. Cardiac baroreflex sensitivity by tertiles of HOMA-IR.



Association of insulin resistance with 24 hour heart rate, ambulatory blood pressure levels and Blood Pressure variability

In multiple regression analysis and after adjustment for age, sex and BMI, increasing values of HOMA-IR were associated with increased HR and average SBP levels (during day, night and 24-h period), with increased BP variability (Day SBP SD, and SBP wSD) and with a reduced dipping of HR. (Tables 12 and 13)

Table 12. 24 hour, daytime and night-time blood pressure and heart rate average values by tertiles of HOMA index. Values are expressed as least square means ± standard error.

Variable	T1 (<0.94) (n=32)	T2 (0.94-1.90) (n=28)	T3 (>1.90) (n=30)	p value
Day HR (bpm)	71.8 ± 1.6	74.0 ± 1.7	77.1 ± 1.4	0.008
Day SBP (mmHg)	108.0 ± 1.8	111.6 ± 1.6	116.4 ± 1.8	0.007
Day DBP (mmHg)	73.0 ± 0.9	74.8 ± 0.8	76.0 ± 0.9	0.081
Day PP (mmHg)	34.8 ± 1.1	36.8 ± 1.0	40.3 ± 1.1	0.007
Night HR (bpm)	59.3 ± 1.3	64.7 ± 1.2	66.1 ± 1.4	0.001
Night SBP (mmHg)	93.6 ± 1.8	97.5 ± 1.6	102.1 ± 1.8	0.009
Night DBP (mmHg)	63.5 ± 1.1	65.8 ± 1.0	67.0 ± 1.1	0.080
Night PP (mmHg)	30.1 ± 1.0	32.2 ± 0.9	35.1 ± 1.0	0.008
24h HR (bpm)	68.3 ± 1.4	71.5 ± 1.3	73.0 ± 1.3	0.04
24h SBP (mmHg)	103.7 ± 1.7	106.7 ± 1.5	111.3 ± 1.6	0.008
24h DBP (mmHg)	70.2 ± 1.1	71.7 ± 0.8	73.1 ± 0.9	0.091
24h PP (mmHg)	33.5 ± 1.0	35.0 ± 0.9	38.4 ± 1.0	0.004

Table 13. 24-h, day- and night-time blood pressure variabilities and heart rate variabilities by tertiles of HOMA index. Values are expressed as least square means ± standard error

Variable	**T1 (<0.94) (n=32)**	**T2 (0.94-1.90) (n=28)**	**T3 (>1.90) (n=30)**	**p value**
Day SBP SD (mmHg)	9.7 ± 0.5	10.7 ± 0.4	11.3 ± 0.5	0.044
Day DBP SD (mmHg)	6.4 ± 0.4	7.3 ± 0.3	8.1 ± 0.4	0.075
Day HR SD (bpm)	10.1 ± 0.5	9.5 ± 0.5	8.3 ± 0.6	0.090
Night SBP SD (mmHg)	9.6 ± 0.6	9.9 ± 0.5	10.4 ± 0.6	0.626
Night DBP SD (mmHg)	6.5 ± 0.4	6.7 ± 0.4	6.9 ± 0.4	0.709
Night HR SD (bpm)	5.3 ± 0.4	5.5 ± 0.4	5.4 ± 0.5	0.082
24h SBP SD (mmHg)	11.9 ± 0.6	12.5 ± 0.5	13.4 ± 0.6	0.201
24h DBP SD (mmHg)	8.0 ± 0.4	8.3 ± 0.3	8.7 ± 0.4	0.509
24h HR SD (bpm)	11.1 ± 0.5	10.3 ± 0.4	8.6 ± 0.5	0.010
SBP wSD	9.7 ± 0.4	10.3 ± 0.4	11.2 ± 0.5	0.043
DBP wSD	6.5 ± 0.3	7.11 ± 0.4	7.4 ± 0.5	0.090
HR dipping (bpm)	13.1 ± 1.2	11.6 ± 1.1	8.1 ± 0.5	0.012
SBP dipping (mmHg)	14.4 ± 1.7	14.0 ± 1.5	14.4 ± 1.7	0.971
DBP dipping (mmHg)	9.5 ± 0.9	9.0 ± 0.8	8.6 ± 1.7	0.811

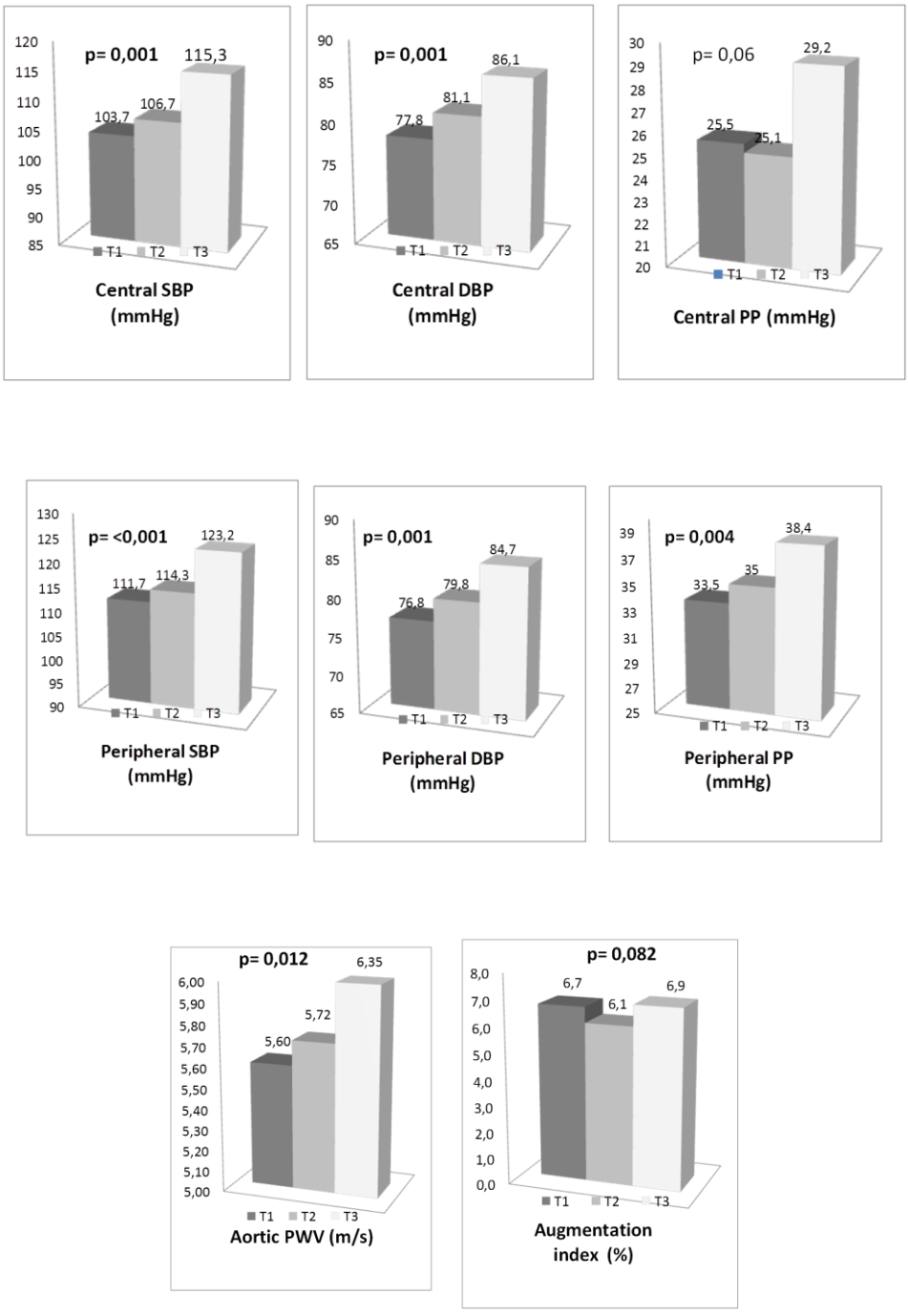
Effects of insulin resistance on central blood pressure levels and in arterial stiffness

In analysis of variance adjusting for age, sex, smoking, and BMI (ANCOVA) among tertiles of HOMA-Index, there was a significant overall effect of IR on measures of large artery stiffness and in central and peripheral BP levels. Increasing values of HOMA-IR were associated with increasing values of aortic PWV, and with higher central and peripheral SBP and DBP levels. (Table 14 and figure 11)

Table 14. Measures of large artery stiffness, central and peripheral BP levels by tertiles of HOMA-IR. Values are expressed as least square means ± standard error.

Variable*	**T1 (<0.94) (n=32)**	**T2 (0.94-1.90) (n=28)**	**T3 (>1.90) (n=30)**	**p value**
Peripheral SBP (mmHg)	111.7 ± 2.0	114.3 ± 1.8	123.2 ± 2.0	<0.0001
Periphera lDBP (mmHg)	76.8 ± 1.4	79.8 ± 1.3	84.7 ± 1.5	0.001
Peripheral PP (mmHg)	33.5 ± 1.0	35.0 ± 0.9	38.4 ± 1.0	0.004
Central SBP (mmHg)	103.7 ± 1.7	106.7 ± 1.5	115.3 ± 1.6	0.001
Central DBP (mmHg)	77.8 ± 1.5	81.1 ± 1.3	86.1 ± 1.5	0.001
Central PP (mmHg)	25.5 ± 1.5	25.1 ± 1.3	29.2 ± 1.0	0.071
MAP (mmHg)	92.5 ± 1.5	95.7 ± 1.3	102.3 ± 1.5	<0.0001
HR (bpm)	61.3 ± 1.9	65.0 ± 1.7	66.0 ± 1.9	0.212
Aortic PWV (m/s)	5.64 ± 0.17	5.71 ± 0.10	6.34 ± 0.9	0.012
Augmentation index (%)	6.7 ± 3.2	6.1 ± 3.8	6.9 ± 2.2	0.082

Figure 11. Central and peripheral systolic and diastolic blood pressure levels, pulse wave velocity and augmentation index by tertiles of HOMA-IR. Pulse wave velocity differences were corrected by mean arterial pressure, heart rate, sex , age and BMI. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; PP: pulse pressure; PWV: Pulse wave velocity.



Discussion

Association of IR with hemodynamic indices of cardiac function

Our results indicate that IR, independently of the presence of obesity or hypertension, may induce significant changes in systemic hemodynamics possibly through its effects on cardiac baroreflex sensitivity and CV autonomic modulation. In our study increasing values of HOMA-IR were associated with shorter RRI. These findings are in line with previous reports showing a linear increment in resting HR along with insulin levels during IR states, likely as a result of the insulin-induced increase in central sympathetic drive (14, 25). HOMA was associated with reduced SI. This is in line with clinical studies in humans showing IR to be associated with LV remodeling and with impaired systolic and diastolic performance even in the absence of structural alterations or coronary artery disease. (9-11) Increasing HOMA was associated with higher BP and SVRI which points towards an increased vascular tone as a primary mechanism for BP elevation with increasing HOMA (1). In addition, the insulin-induced increase in central sympathetic drive to the peripheral vasculature, may further contribute to these increases in SVRI. (12).

Association of IR with indices of cardiac autonomic modulation

Previous studies have reported on the association between IR and impaired autonomic cardiovascular modulation either in obese (59) or in normal subjects (60). In turn, an impaired CV autonomic modulation has been proposed as a likely mechanism for the hemodynamic alterations associated with insulin resistance (IR). (61) The present study assessed the association of IR with indices of autonomic CV modulation derived from the

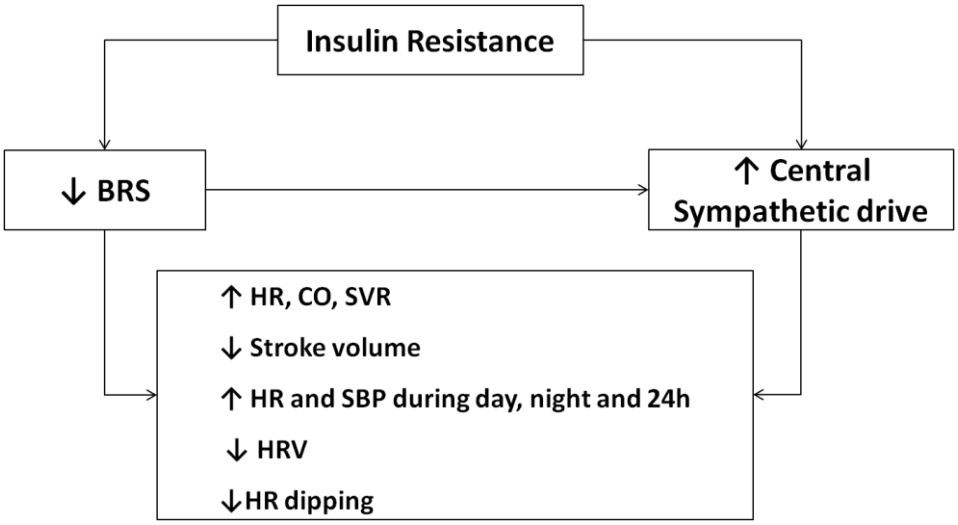
analysis of HRV and cardiac BRS. Although a direct assessment of central sympathetic drive requires the use of dedicated techniques (i.e. direct recording of efferent postganglionic muscle sympathetic nerve activity, MSNA, via microneurography). (62), (63), (64), an indirect assessment of the alterations in CV autonomic modulation is also possible through the analysis of BP variability (BPV) and heart rate variability (HRV), which represent a simple, non-invasive means to quantify the amplitude of BP and HR fluctuations occurring at specific frequency regions which are known to reflect BP and HR modulation by neural autonomic influences (50), (51), (52), (53), (54) , (57).

In the present study, subjects in the upper tertile of HOMA-IR showed significantly higher resting HR, mean BP levels, increased indices of sympathetic CV modulation (LF/HF ratio) and reduced indices of parasympathetic CV modulation (RRI, SDRRI, HF and total power). A large impairment on sympatho-vagal balance was also observed among subjects with higher values of HOMA-IR (mainly related to the progressive reduction in HF component of HRV). Some caution is required, however, for a proper interpretation of these results, because LF and HF powers, despite being considered indices of sympathetic and parasympathetic cardiac modulation, respectively, may be influenced by a number of other cardiovascular regulatory mechanisms. (52, 53, 65) Although changes in HF (0.15-0.5 Hz) spectral power of HRV seem to be primarily caused by modulation of cardiac parasympathetic efferent activity, (51, 65-67) at respiratory frequencies below 9 breaths per minute, sympathetic modulation of respiratory-induced HR changes also occurs, together with some degree of mechanical stimulation of the sinus node by ventilation cycles; thus HF power is considered a satisfactory, but incomplete, measure of parasympathetic cardiac control. The specificity of LF power (0.05-0.15 Hz) in reflecting sympathetic cardiac modulation of HR, has been reported to be even lower as it may also

depend on vagal influences (as demonstrated by the reductions in LF power either by parasympathetic or sympathetic pharmacological blockade) (51), (66) and on a variety of other factors, including thermoregulation, atrial stretch by periodic breathing, and hemodynamic instability. (68). Autonomic modulation of the CV system takes place in a network of intricate interactions between several regulatory systems, and a general concern has been raised that for a comprehensive assessment of autonomic CV modulation, information provided by HRV analysis might not be specific enough, and should be integrated with the analysis of variability of other biological signals. By applying modeling approaches considering the relationship between fluctuations on HR and BP (either in the time or in the frequency domain) (58), (69) it is also possible to assess other mechanisms of major importance for autonomic CV control such as the spontaneous cardiac baroreflex sensitivity. Such an approach may provide a more comprehensive analysis of cardiovascular regulation mechanisms than that represented by the separate analysis of BP and HR variability alone. (70). In the present analysis, increasing values of HOMA-IR were associated with a progressive reduction in cardiac baroreflex sensitivity assessed either as up-slope, down-slope or total slope. These reductions in cardiac BRS were particularly evident in subjects in the upper tertile of HOMA-IR.

Taken together, the findings of our analysis suggest that hemodynamic alterations associated with IR might be explained not only by the effects of IR on vascular tone and cardiac performance but also through its effects on indices of autonomic modulation and cardiac baroreflex sensitivity (Figure 12).

Figure 12. Effects of insulin resistance on hemodynamic indices of cardiac function, and on indices of cardiac autonomic modulation and baroreflex sensitivity. BRS: cardiac baroreflex sensitivity; HR: heart rate; CO: cardiac output; SVR: systemic vascular resistance; SBP: systolic blood pressure; HRV: heart rate variability.

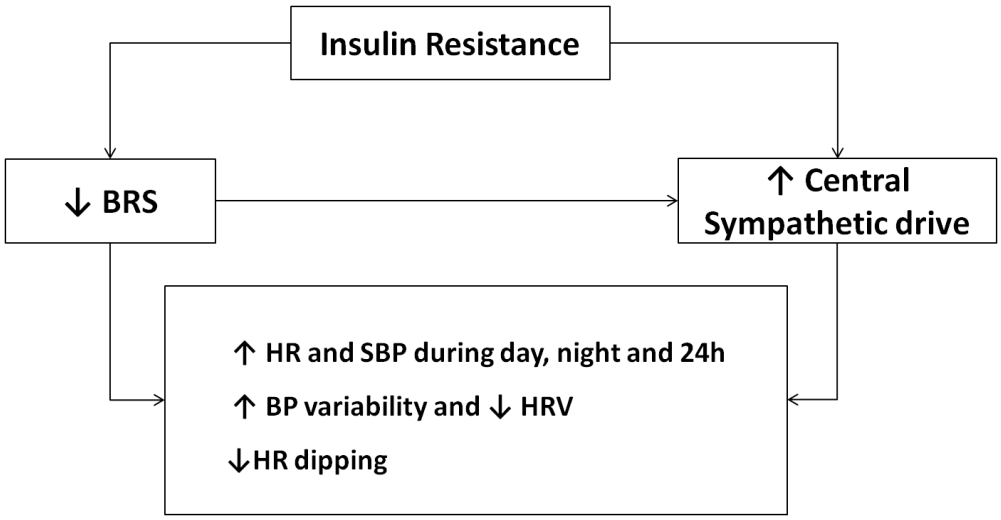


Association of insulin resistance with 24h heart rate, ambulatory blood pressure levels and Blood Pressure variability

Overall, the present analysis showed that increasing values of HOMA-IR are associated with significant increases in average ambulatory HR and SBP levels during day, night and 24h; and with significant increases in indices of SBP variability (Day SBP SD, and 24h SBP wSD). In another analysis conducted in the same subjects, significant associations between HOMA-IR and indices of cardiac autonomic modulation were reported. In particular, increasing values of HOMA-IR were associated with significant reductions in cardiac BRS and increased indices of sympathetic CV modulation (LF/HF ratio) as well as

with significant reductions in indices of parasympathetic CV modulation (HF and TP). Our results thus suggest that IR might contribute to modulation of heart rate and blood pressure levels over the 24h period, possibly through its effects on autonomic cardiovascular modulation (Figure 13)

Figure 13. Effects of insulin resistance in 24h heart rate, ambulatory blood pressure levels and blood pressure variability in normotensive healthy adults. BRS: cardiac baroreflex sensitivity; HR: heart rate; SBP: systolic blood pressure; HRV: heart rate variability.

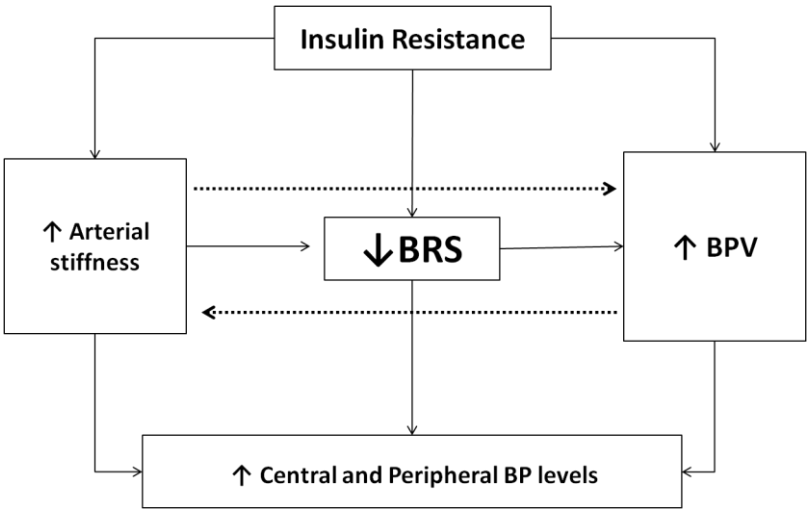


Effects of insulin resistance on central blood pressure levels and in arterial stiffness

The results obtained in the present analysis indicate that in normotensive, otherwise healthy adults, IR may induce significant increases in large artery stiffness (as assessed with aortic PWV) and in central and peripheral BP levels. Of note, the marked increases in PWV in subjects in the upper tertile of HOMA-IR (i.e. HOMA-IR >1.90) seem to suggest

a threshold value for the effects of insulin resistance in arterial stiffness. In the same subjects of the present analysis, significant reductions in cardiac baroreflex sensitivity were observed. This is in line with previous studies showing an increased arterial stiffness to be an independent determinant of impaired baroreceptor reflex function (71). When large arteries become stiffer and less compliant, impairment in elastic recoil of the aorta, and less buffering of BP changes may occur thus resulting in a wider SBP oscillation for any given change in the stroke volume. The association of insulin resistance with an increased variability in systolic BP levels observed in the same subjects of the present analysis might suggest that through its effects on arterial stiffness and BRS, IR resistance may also influence heart rate and BP variabilities over the 24h period. (Figure 14) This finding might suggest possible mechanisms (i.e. insulin resistance) for the association between short-term BPV and arterial stiffness reported in recent studies (72).

Figure 14. Through its effects on arterial stiffness and BRS, IR resistance might importantly influence heart rate and blood pressure variabitities over the 24h period. BRS: cardiac baroreflex sensitivity; BPV: blood pressure variability; BP: blood pressure.



Conclusions

Although hemodynamic and autonomic effects of IR are often clustered with metabolic alterations and largely explained by an increased body weight or elevated BP levels, all the analyses presented above were performed in a population of nondiabetic, nonobese subjects with office and ambulatory BP levels within the normal range. Besides, insulin resistance was associated with significant differences in hemodynamic and autonomic indices even after accounting for common confounders. This suggests that increases in IR, independently of the presence of hypertension, diabetes and obesity, may promote alterations in autonomic cardiovascular modulation and increases in arterial stiffness, which in turn might contribute to the pathogenesis of arterial hypertension.

Most previous studies investigating the cardiovascular effects of insulin resistance have focused on type 2 diabetes mellitus, congestive heart failure, elderly subjects or overweight/obese subjects. Upcoming studies should therefore extend our observations on the important independent contribution of insulin resistance to the pathogenesis of CV alterations also to lean, normotensive or otherwise healthy subjects, if possible on a population basis. They should also consider the possibility of a prospective follow-up to better define the role of IR in development, establishment and progression of CV disease, thus further testing the hypothesis that IR itself might be considered as a target for CVD prevention. The questions on whether insulin resistance in nonobese, nondiabetic subjects is worth of medical treatment, and how to treat these subjects, represent issues still in need of a satisfactory answer.

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APPENDIX

List of publications by Dr. Juan Eugenio Ochoa during his PhD Course

Articles in Journals

Parati G, Liu X, **Ochoa JE,** Bilo G. Prognostic Relevance of Blood Pressure Variability. Role of long-term and very long-term blood pressure changes. Hypertension. 2013. In press.

Parati G, Liu X, **Ochoa JE.** What matters is not only how often but also how much blood pressure rises. Limitations of blood pressure load " Journal of Hypertension. 2013. In press.

Parati G, **Ochoa JE,** Bilo G. Renal Sympathetic Denervation and Daily Life Blood Pressure in Resistant Hypertension: Simplicity or Complexity? Circulation. 2013 Jun 18. [Epub ahead of print]

Parati G, **Ochoa JE,** Bilo G, Mattaliano P, Salvi P, Kario K, Lombardi C. Obstructive sleep apnea syndrome as a cause of resistant hypertension. Hypertension Research, 2013. In press.

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