

Effects of Different Degrees of Insulin Sensitivity on Endothelial Function in Obese Patients

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Abstract

Background: Obesity derived from intra-abdominal fat deposition tends to increase hormonal and cytokine production, thus worsening insulin sensitivity and leading to endothelial dysfunction. Hyperinsulinemia is considered an independent risk factor for ischemic heart disease and cause of endothelial dysfunction in healthy individuals.

Objective: To assess the impact of different degrees of insulin resistance, measured by HOMA-IR (Homeostasis Model Assessment of Insulin Resistance), on endothelial function in obese, non-diabetic patients without prior history of cardiovascular events and different metabolic syndrome components.

Methods: Forty obese individuals were submitted to anthropometric measurements, BP measurements at office and ABPM and laboratory tests, in addition to non-invasive ultrasound assessment of endothelial function. Patients were divided into 3 groups according to the level of insulin resistance: patients with HOMA-IR values from 0.590 to 1.082 were assigned to Group 1 (n=13), from 1.083 to 1.410 to Group 2 (n=14) and from 1.610 to 2.510 to Group 3 (n=13).

Results: We found a significant difference in flow-mediated dilation in group 3 compared to group 1 (9.2 ± 7.0 vs 18.0 ± 7.5 %, $p=0.006$). There was a negative correlation between endothelial function and insulin, HOMA-IR and triglycerides.

Conclusion: Our data suggest that mild changes in insulin resistance levels assessed by HOMA-IR may have an impact on vasodilatory endothelial function in uncomplicated obese individuals with different cardiovascular risk factors. (Arq Bras Cardiol 2012;98(1):45-51)

Keywords: Insulin resistance, endothelium, obesity, diabetes mellitus, hypertension.

Introduction

Obesity results from a combination of genetic factors and daily life behavior such as poor diet and sedentary lifestyle and is defined as a body mass index (BMI) higher than 30 kg/m². The main clinical consequences of obesity are the development of type 2 diabetes mellitus, high blood pressure, dyslipidemia and cardiovascular diseases¹. Obesity derived from intra-abdominal or visceral fat deposition tends to increase hormonal and cytokine production worsening insulin sensitivity and leading to endothelial dysfunction due to several mechanisms^{2,3}. Peripheral insulin resistance is one of the key metabolic features of obesity⁴ playing a significant role in pathophysiology of arterial hypertension⁵ and atherosclerosis⁶.

Hyperinsulinemia is the key factor in insulin resistance in normoglycemic and non-diabetic patients, and is considered an independent risk factor for ischemic heart disease⁷ and cause of endothelial dysfunction in healthy individuals⁸. Steinberg et al⁹ showed that normoglycemic obese, insulin-resistant patients present endothelial dysfunction similar to type 2 diabetics compared to lean controls⁹.

Thus, changes in endothelium-dependent vascular relaxation described in essential hypertensive^{10,11} and uncomplicated obese patients may represent a link between insulin resistance and the development of atherosclerosis.

The presence of metabolic syndrome (MS) where insulin resistance is the main component was identified as an independent predictor of endothelial dysfunction in asymptomatic individuals with MS versus normal controls. It was also shown that the degree of endothelial dysfunction increases as components of MS also increase, probably indicating a cumulative effect of these risk factors on endothelial function¹².

The aim of this study was to assess the impact of different degrees of insulin resistance, measured by HOMA-IR

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(Homeostasis Model Assessment of Insulin Resistance), on endothelial function in obese, non-diabetic patients without prior history of cardiovascular events and different metabolic syndrome components.

Methods

Forty patients were eligible in this cross-sectional study (11 men and 29 women), with age ranging from 19 to 70 years. The main inclusion criteria were: male and female individuals, aged between 18 and 70 years, diagnosed with obesity stage 1 or 2 - BMI between 30 and 39.9 kg/m²,¹³ either normotensive or stage 1 essential hypertension. Hypertensive individuals received non-pharmacologic treatment alone or antihypertensive medication. Those under treatment underwent a 1-month wash-out period. Main exclusion criteria were: stage 3 obesity (BMI ≥ 40 kg/m²), use of drug treatment for obesity, use of angiotensin converting enzyme inhibitors (ACE) or AT1 angiotensin receptor blockers (ARB), statins or any other drug with a potential effect on endothelial function, secondary forms of arterial hypertension, history of cardiovascular event, tobacco use, chronic renal disease and type 1 or 2 diabetes mellitus. The individuals were classified as diabetic either in the presence of fasting plasma glucose ≥ 126mg/dL or hours plasma glucose ≥ 200mg/dL (data collected from patient's records). Patients evolving to stage 2 hypertension — SBP ≥ 160 and/or DBP ≥ 100 mmHg —¹⁴ or any symptoms attributed to drug withdrawal during the wash out period were excluded from study.

The following anthropometric measurements were recorded at the baseline visit: body weight (kg), height (cm), BMI (weight/height²) and waist circumference (cm). Waist circumference was measured at a midway level between the lower rib margin and the iliac crest. Blood pressure was measured three times within a 1-minute interval according to AHA guidelines¹⁵ and the mean of these three values was used in subsequent analyses.

Laboratory tests performed in study individuals agreed with conventional methods used at the Central Laboratory of the Kidney and Hypertension Hospital. The following tests were made on a Roche-Hitachi 912 chemistry analyzer (Hitachi, Nakakojo, Japan): creatinine (automated kinetic alkaline picrate), blood fasting glucose (automated colorimetric method), cholesterol/triglycerides (colorimetric enzymatic method), HDL-cholesterol (homogenous method) and LDL-cholesterol (indirect calculation). Insulin levels were determined on a 1277 Gamma Master device (Wallac, Turku, Finland) using radioimmunoassay based on I-125 (DPC Kit) measures. The level of insulin sensitivity was calculated by HOMA-IR formula [glucose (mMol/L) x insulinemia (μU/mL) / 22.5]¹⁶. Patients were classified as having metabolic syndrome based on criteria of the National Cholesterol Education Program Adult Treatment Panel III - NCEP ATP III¹⁷.

Blood pressure levels were also determined by Ambulatory Blood Pressure Monitoring – ABPM (Spacelabs, Issaquah, WA) with Blood Pressure and Heart Rate recorded every 15 minutes during daytime and every 20 minutes during nighttime).

Brachial artery B mode ultrasound

Endothelial function test was performed by high-resolution B-mode ultrasound images using a non-invasive methodology described by Celermajer et al¹⁸ with modifications¹⁹. The equipment used in this study was an Ultramark HDI 3000 (ATL ultrasound incorporation), with a linear transducer of L7– 4MHz. The test consisted of four phases, as follows: rest, after reactive hyperaemia (endothelium-dependent phase – FMD), again with the individual at rest, and finally, after administration of sublingual nitrate (endothelium-independent phase – NMD). All measurements were performed in the same place, in longitudinal section 5–10cm above the antecubital fossa of the right upper arm. At the end of the first phase of resting when a satisfactory position was found to carry on the endothelial study, the skin was marked and the arm remained in the same position throughout the test. At this moment, the diameter and blood flow velocity were determined in triplicate for the first phase. To obtain an increased flow, a cuff was placed on the right upper arm and it was inflated to 300mm Hg, resulting in a complete interruption of blood flow during a 5-min period, and then the cuff was deflated. Second and third scans were obtained after 15 and 90 s after the cuff was released (known as reactive hyperaemia phenomenon that was followed by a brachial FMD). The maximum blood flow (mm/min) was determined in the first 15 s after the cuff release. Ninety seconds after ischaemia, three measurements of the brachial artery diameter were taken at the diastolic period (FMD). A 10-min rest was then allowed for recovery of the vessel and at the end of this period a sublingual tablet of isosorbide dinitrate 5.0 mg was given to the individuals. Five minutes after isosorbide dinitrate administration, three measurements were obtained for brachial artery diameter and blood flow velocity to determine nitrate-induced vasodilation of the arterial wall (known as endothelium-independent vasodilation). The mean of these values was used in subsequent analyses. FMD response was expressed as the change in end-diastolic diameter of the brachial artery during reactive hyperaemia compared with the baseline (rest) measurement and used as a measure of endothelium-dependent vasodilation. The mean of these values was used in subsequent analyses. To determine the confidence of results, vascular tests (vasodilation after reactive hyperaemia and nitrate-stimulated dilation) were performed by two independent observers in 10 healthy volunteers. Intra- and interobserver variability for repeated measurements of the same recording of the brachial artery diameter was 2.1671.7, 2.4171.9 and 6.774.0%, respectively.

Patients were divided into 3 groups (tertiles) according to the level of insulin resistance determined by HOMA-IR, since most of them had at least one additional criterion for metabolic syndrome, and therefore insulin resistance, despite blood pressure values, as follow: patients with HOMA-IR values from 0.590 to 1.082 were assigned to Group 1 (n=13), from 1.083 to 1.410 to Group 2 (n=14) and from 1.610 to 2.510 to Group 3 (n=13).

Statistical Analysis

Continuous variables are expressed as mean \pm standard deviation and analyzed using the One-Way ANOVA test. Categorical variables are expressed as percentages and were analyzed by the Chi-square test. Pearson's correlation coefficient was used to assess correlation among variables. Models of linear regression were constructed based on the correlation analysis, with the endothelial-dependent phase considered as the dependent variable. P-values <0.05 were considered statistically significant. Data were analyzed using version 13.0 of SPSS software (SPSS Corp, Chicago, IL) for Windows.

This protocol was performed according to the Declaration of Helsinki and Good Clinical Practices and was approved by the Ethics Committee of the Kidney and Hypertension Hospital registered by the Oswaldo Ramos Foundation's Research and Ethics Committee and registered under protocol No. 057. All patients signed an informed consent, after having been informed of the procedures involved in this clinical study.

Results

Table 1 shows that demography, clinical and laboratory values of the study population had a homogeneous distribution among tertiles. Office blood pressure (OBP) values were slightly lower in tertile 1 than in the other tertiles, although this difference did not reach statistical significance and was not confirmed by ABPM.

Regarding laboratory tests, those individuals in the upper tertile presented higher levels of total cholesterol and LDL-cholesterol which were statistically different from the other two groups ($p < 0.05$). Comparing insulin levels among the three tertiles, we noted that tertile 3 had the highest value, which was statistically different from two other groups ($p < 0.001$), while we also detected a statistically significant difference between tertile 1 and 2 ($p < 0.001$). The same findings were observed on HOMA-IR, reflecting similar results for fasting glucose in the 3 groups.

Intima-media thickness of carotid artery (IMT-C) did not show any significant difference among groups (0.054 ± 0.02 , 0.066 ± 0.02 and 0.064 ± 0.02 mm in tertiles 1, 2 and 3, respectively). Figure 1 shows a progressive decrease in flow-mediated dilation (endothelial-dependent phase) among the 3 groups, with a statistical significance in tertile 3 compared to tertile 1 (9.2 ± 7.0 vs. 18.0 ± 7.5 %, respectively, $p = 0.006$). However, the difference observed on the nitrate-mediated test (endothelial-independent phase) did not reach significance (15.1 ± 9.8 vs. 19.4 ± 6.3 %, ns).

In a pooled analysis of the 3 tertiles, Pearson's coefficient revealed negative correlation between endothelial function and insulin (Table 2). These measurements were the only significant independent predictors of endothelial-dependent phase in the linear regression model (Table 3).

Discussion

Our data revealed an association between the degree of insulin resistance and endothelium-dependent vasodilation

in a metabolically uncompromised obese population with few cardiovascular risk factors.

Despite all efforts to select obese individuals with no additional cardiovascular risk factors, the study group presented a significant prevalence of high blood pressure (54%, 57% and 61%) and metabolic syndrome (38.5%, 35.7% and 46.1%) in the 3 tertiles, respectively, reflecting the increasingly prevalent interaction of these components²⁰. Nevertheless, the impact of these risk factors on endothelial function did not reach significance in the 3 tertiles, except for triglycerides alone. ABPM measurements showed that blood pressure values remained in stage 1, even after the 1-month wash out period.

The normal thickness of the intima-media complex, coupled with preserved independent endothelial function across the 3 groups demonstrates the low impact of high blood pressure on muscular layer of arteries in these individuals. We sought to recruit normotensive volunteers for this study given that an earlier study from our group showed significant correlation between endothelial dysfunction and slight increases in systolic blood pressure among high-normal hypertensive individuals²¹.

In terms of degree of insulin resistance, we observed significantly higher HOMA-IR values in tertile 3 than in tertile 2 and the same for tertile 1, characterizing steadily rising insulin levels across tertiles. Therefore, HOMA-IR scores presented independent significant correlation with endothelial dysfunction, even in this normoglycemic population. We should stress that in spite of the significant difference in degree of insulin sensitivity among the groups, HOMA-IR scores remained within normal ranges according to the literature, and in our data the highest value observed was 2.51. Despite a lack of standardization for routine use in clinical practice, HOMA-IR is highly applicable in epidemiologic population-based studies²². Average HOMA-IR values of 2.0 were observed in a white American, non-diabetic population with no history of cardiovascular events²³. In the Brazilian Metabolic Syndrome Study HOMA-IR, threshold values greater than 2.71 were observed in healthy individuals without metabolic syndrome components²⁴.

A previous study showed an early association between insulin resistance and endothelial dysfunction in a population of first degree relatives with type 2 diabetics²⁵. Data from our study suggests that even small changes in insulin may have a significant effect on endothelial function in obese populations who are not yet classified as insulin resistant.

The mechanisms involved in this association remain a topic of intense debate. Insulin acts in the activation of the phosphatidylinositol 3-kinase pathway, regulating NO expression in endothelial cells; the model of insulin resistance would cause dysfunction of this pathway worsening dependent-endothelial vascular relaxation²⁶. Reciprocally, endothelial dysfunction can increase insulin resistance by reducing blood flow in the tissues, caused by an imbalance between NO and endothelin-1 expression. Studies on therapeutic interventions in animal models have shown that optimizing endothelial function promotes improved insulin resistance and vice-versa²⁷. Winkler et al proposed that a-TNF is a link between endothelial dysfunction and insulin resistance in obese normotensives²⁸.

Although the use of fasting data may have some limitation, it is widely accepted that HOMA-IR is a feasible alternative

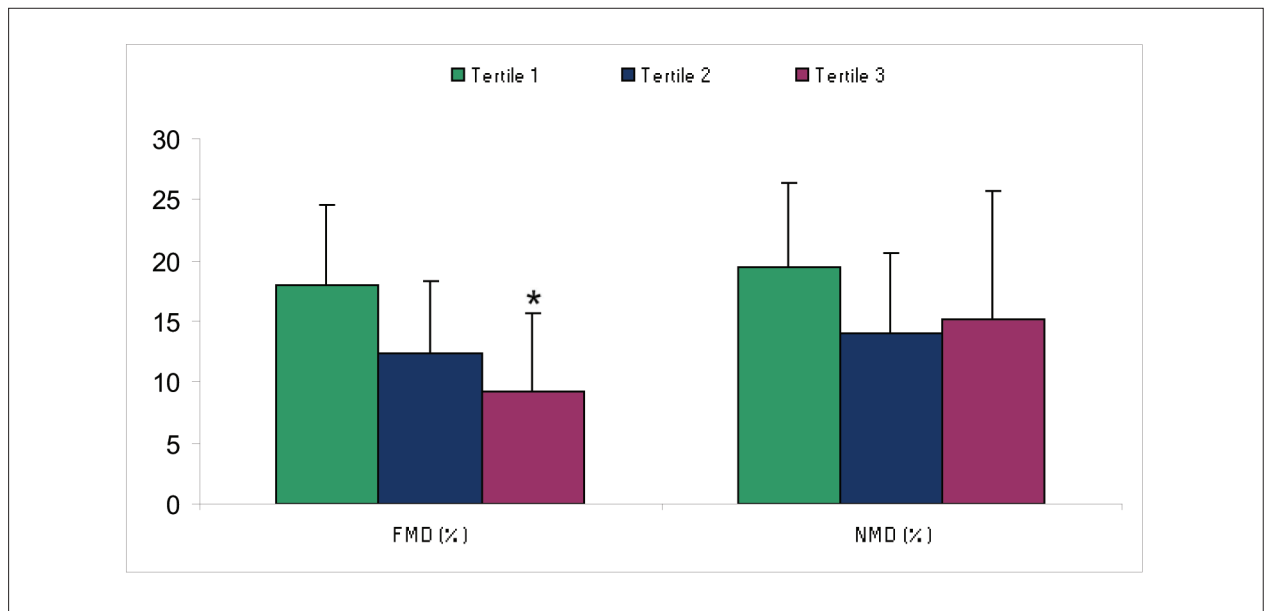


Figure 1 - Comparison of flow-mediated and nitrate dilation among the groups; FMD – Flow-mediated dilation; NMD - nitrate mediated dilation. $p = 0.006$ vs Tertile 1.

Table 1 - Variables of Demography and Chemical Parameters

Characteristic	Tertile 1 (N=13)	Tertile 2 (N=14)	Tertile 3 (N=13)
Clinical			
Age (yr)	43.7±10.6	47.2±13.0	51.8±13.0
Male (%)	23	29	31
BMI (kg/m ²)	33.3±2.2	32.5±1.8	32.6±2.4
Waist (cm)	98.3±8.5	98.4±7.7	101±5.5
Hypertension (%)	54	57	61
Metabolic Syndrome (%)	38.5	35.7	46.1
Office blood pressure			
SBP (mmHg)	130±19	136±12	137±15
DBP (mmHg)	86±10	88±8	88±6
24-h Ambulatory blood pressure			
24-h SBP (mmHg)	127±13	130±12	126±9
24-h DBP (mmHg)	81±9	80±9	78±9
Chemical Variables			
Creatinine (mg/dL)	0.9±0.2	0.9±0.1	0.9±0.1
Total cholesterol (mg/dL)	190±27	182±28	224±21 *
LDL-C (mg/dL)	113±31	107±24	143±23 *
HDL-C (mg/dL)	53±13	51±15	49±8
Fasting triglycerides (mg/dL)	126±60	120±54	162±80
Fasting glucose (mg/dL)	85±10	90±10	92±10
Fasting insulin (mU/L)	4.1±0.7	5.7±0.6 ‡	9.5±1.1 †
HOMA-IR	0.9±0.2	1.3±0.1 ‡	2.1±0.3 †

BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; LDL-C - low-density lipoprotein cholesterol; HDL-C - high-density lipoprotein cholesterol; * $p < 0.05$ vs Tertiles 1 and 2; † $p < 0.001$ vs Tertiles 1 and 2; ‡ $p < 0.001$ vs Tertile 1.

Table 2 - Correlation Analysis between Variables of Demography and Chemical Parameters (n= 40)

	Pearson's coefficient
	FMD
FMD	1
Age	-0.21
BMI	0.09
Waist	0.06
SBP	-0.28
DBP	-0.31
Creatinine	-0.03
LDL-C	-0.19
HDL-C	0.16
Fasting triglycerides	-0.40*
Fasting glucose	-0.02
Fasting insulin	-0.38*
HOMA-IR	-0.35*

FMD - Flow-mediated dilation; BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; LDL-C - low-density lipoprotein cholesterol; HDL-C - high-density lipoprotein cholesterol. * $p < 0.05$.

Table 3 - Predictors of FMD in the linear regression model (n=40)

	β	Std. Error
Fasting triglycerides	-0.03*	0.02
Fasting insulin	-0.99*	0.46
HOMA-IR	-3.91*	1.93

FMD - Flow-mediated dilation (Dependent Variable). * $p < 0.1$

when the clamp technique is not available and, considering all criticism, it has demonstrated a good correlation with clamp data²⁹, being frequently used in large clinical trials. The large population investigation, *The San Antonio Heart Study*, found correlation between HOMA-IR scores and increased risk for cardiovascular disease after adjusting for multiple co-variables, possibly as a result of increased proliferation of vascular smooth-muscle cells and due to subclinical chronic inflammation mechanisms²³. Just like this study, *The San Antonio Heart Study* divided participants into HOMA-IR quintiles which correlated progressively with cardiovascular risk. It could be speculated that, among several mechanisms involved, these patients may have presented different degrees of endothelial dysfunction, similar to those observed in our study.

With regard to lipid profile, we observed a significantly greater difference in total and LDL cholesterol levels in tertile 3 compared to the other tertiles, albeit within a range considered clinically borderline. Notwithstanding the importance of LDL as a cardiovascular risk factor³⁰, the levels observed in

this study were unlikely to have been sufficient to influence endothelial function as observed in the correlation analysis. In contrast, despite the clinically borderline values, triglycerides are presented as another independent variable for endothelial dysfunction in this population. Triglyceride level is directly related to the presence of central obesity, causing increased lipolysis and leading both to insulin resistance and endothelial dysfunction through increased expression of markers of inflammation such as C-reactive protein, IL-6, soluble adhesion molecules, von Willebrand factor and endothelin-1³¹. In addition, clinical and experimental studies have shown that free fatty acids change vasodilatory response of endothelium by inhibiting eNOS and stimulating anion expression by endothelial and vascular cells via NADPH oxidase, thereby lowering NO bioavailability³². In spite of the controversy on their role as an independent cardiovascular risk factor³³, triglycerides are directly related to other non-lipid cardiovascular factors such as microalbuminuria and insulin resistance³⁴. The association found in our study suggests a role of triglycerides in endothelial dysfunction and mechanisms of insulin resistance.

In this study, we aimed to assess an obese population mostly free of target organ damages and low cardiovascular risk factors. We noted, however, some limitations in our study mainly due to the small sample size and cumulative risk factors comparing tertile 3 with tertile 1, that is, elderly individuals, mostly males, higher prevalence of high blood pressure and metabolic syndrome. While individually these factors did not reach statistical significance, together they may have impact on endothelial function and on the degree of insulin resistance. Finally, tertile 3 higher LDL-C levels, though borderline, may have confused the interpretation of our findings.

Conclusions

We have observed that even mild changes in insulin resistance levels assessed by HOMA-IR may have an impact on vasodilatory endothelial function in uncomplicated obese individuals with few cardiovascular risk factors. Although we were unable to establish a clear cause-effect mechanism in this study, a significant involvement in endothelial function related to small changes in HOMA-IR scores was observed. Therefore, we suggest that insulin resistance mechanisms play an early role in endothelial dysfunction, although several studies in the literature have previously reported a reciprocal relationship between these processes.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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