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Insulin resistance and adipokine levels correlate with early atherosclerosis – a study in prediabetic patients

Abstract: Cardiovascular risk of prediabetes is still subject to controversies. We analyzed the associations between insulin resistance, adipokines and incipient atherosclerosis estimated by intima-media thickness (IMT) in a cross-sectional study on 122 prediabetic subjects without clinical signs of atherosclerotic disease. Homeostasis model assessment of insulin resistance (HOMA-IR, calculated as fasting insulin × fasting plasma glucose / 22.5), adiponectin, leptin, leptin-to-adiponectin ratio, carotid and femoral IMT were evaluated. We also assessed other parameters related to insulin resistance and adipokines (HbA_{1c}, anthropometric and lipid parameters), as they may also influence atherosclerosis. Carotid IMT was correlated to adiponectin and leptin-to-adiponectin ratio (all $p < 0.05$), but not with HOMA-IR or leptin, while femoral IMT showed no relationship with these factors. After adjusting for leptin, leptin-to-adiponectin ratio, triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio, triglycerides-to-HDL ratio and HbA_{1c}, IMT values became correlated with HOMA-IR. Adjustment for HOMA-IR induced the appearance of new correlations between adipokines and both IMT values. In conclusion, insulin resistance and adipokines seem related to IMT in prediabetic subjects without clinical signs of arterial obstruction.

Keywords: prediabetes, insulin resistance, adiponectin, leptin, intima-media thickness

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1 Introduction

Hyperglycemic disorders induced by insulin resistance (prediabetes and type 2 diabetes mellitus) have an epidemic rise in the modern world; estimates for 2035 indicate 592 millions type 2 diabetics and 471 millions prediabetic patients [1]. Besides classical cardiovascular risk factors, insulin resistance is also considered to increase the risk for atherosclerosis. While the association between insulin resistance-induced hyperglycemia and an increased cardiovascular risk is beyond any doubt in type 2 diabetes mellitus [2], the atherosclerotic risk of prediabetes is controversial, being considered double by some authors and only moderately increased by others [3], and therefore needs supplementary analysis.

Metabolic disturbances in insulin resistant subjects are multiple and complex. Major changes occur especially in the adipose tissue, which is known today to have an active hormonal production, with its secretion products known as adipokines. A reduced insulin sensitivity is usually associated with changes in adipokine secretion [4]. Adiponectin and leptin account for the best known adipokines and both seem related to clinical cardiovascular disease [5], although previous studies that tried to establish a quantitative relationship provide contradictory results. Notably, up to this moment available data on this issue [6-9] usually derive from analyses upon high risk populations, such as subjects with metabolic syndrome, type 2 diabetes mellitus, obesity, polycystic ovary syndrome, preexisting cardiovascular disease or ethnic predisposition. On the contrary, there is little data about the relationship that might exist between adipokine profile and early atherosclerosis; moreover, the existing results are marked by frequent contradictions [10,11].

Clinical stages of atherosclerosis can be diagnosed by various methods, with contrast arteriography being a gold standard for many locations of atheromatous

plaques. Incipient atherosclerosis is less evaluated in clinical practice and may need other diagnostic methods. Some non-invasive methods developed in the last years, such as intima-media thickness (IMT), allow a reliable estimation of any degree of atherosclerotic lesions [12]. The few previous studies that examined the relationship between insulin resistance, adipokines and IMT [13,14] did not evaluate subjects with only incipient hyperglycemia and no clinical signs of atherosclerosis. Therefore, our research aimed to analyze the associations that might exist between reduced insulin sensitivity, adipokines and incipient atherosclerosis (estimated by IMT) in prediabetic subjects, whose cardiovascular risk profile is not yet fully defined.

2 Materials and methods

A total of 154 subjects aged 30 to 70 years were selected among the patients who visited the Clinical Centre of Diabetes, Nutrition and Metabolic Diseases of the University of Medicine and Pharmacy "Grigore T. Popa" – Iași, Romania, between November 2010 and July 2011 and were diagnosed with prediabetes (impaired fasting glycemia – IFG and impaired glucose tolerance – IGT) in the previous 6 months according to the most recent World Health Organization / International Diabetes Federation criteria [15]. Impaired fasting glycemia was defined as fasting plasma glucose of 6.1 to 6.9 mmol/L and 2-hour plasma glucose during the oral glucose tolerance test below 7.8 mmol/L. Impaired glucose tolerance was defined as fasting plasma glucose below 7.0 mmol/L and 2-hour plasma glucose between 7.8 and 11.0 mmol/L. This cross-sectional study was approved by the Ethics Committee of our University and all procedures were performed in accordance with the guidelines of the Declaration of Helsinki. All subjects gave their written informed consent to participate in the study.

In order to eliminate confounding factors, subjects with at least one of the following characteristics were excluded: 1. diabetes mellitus diagnosed according to World Health Organization / International Diabetes Federation criteria [15]; 2. atherosclerotic cardiovascular disease, as defined by previous or newly discovered myocardial infarction, angina, coronary revascularization, electrocardiogram findings of ischemia, stroke, transient ischemic attack, peripheral arterial disease; 3. history of severe liver or kidney disease; 4. history of endocrine diseases; 5. treatment with biguanides, pioglitazone or lipid-lowering drugs; 6. patients who did not sign the informed consent.

All participants underwent clinical examination, laboratory tests, resting electrocardiogram, echocardiography, carotid and femoral ultrasonography. Based on the results of these investigations we excluded 32 of the 154 subjects, who didn`t met the inclusion and exclusion criteria (newly discovered with type 2 diabetes mellitus, clinical cardiovascular disease, renal or liver disease). We performed our analysis on the remaining 122 subjects.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic digital sphygmomanometer placed on the upper arm at the height of the heart in a sitting position after the subject had rested for 10 minutes. The average value from 3 measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg, according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [16] or self-reported use of antihypertensive medication.

Anthropometric parameters, known to be related to insulin resistance, adipokines and atherosclerosis risk, were determined. Height and weight in light clothing were measured following standardized procedures. Body mass index (BMI) was calculated as body weight (in kilograms) divided by square of the height (in meters). Excess weight was defined as BMI ≥25 kg/m^2 and obesity as BMI ≥30 kg/m^2. The waist circumference (WC) was measured midway between the lower rib margin and the iliac crest after normal expiration and the hip circumference was measured at the widest circumference over the trochanter in standing subjects. Waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated.

Venous blood samples from subjects resting in the sitting position were collected after an overnight fast. Samples were taken into vacuum tubes containing EDTA or a serum separator gel. After sampling, tubes were immediately centrifuged at 3000G for 5 minutes. Routine biochemistry tests and hemoglobin A_{1c} (HbA_{1c}) were performed in the same day; a part of serum was stored at –80°C for immunological measurements. Biochemistry tests (fasting glycemia, total cholesterol, triglycerides and high-density lipoprotein cholesterol – HDL-cholesterol) were determined with a Cobas Integra® 400 Plus analyzer (Roche Diagnostics Ltd., Basel, Switzerland) using absorbance photometry assays. Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated using the formula of Friedewald et al: LDL-cholesterol = total cholesterol – HDL-cholesterol – triglycerides/2.2 (mmol/L). HbA_{1c} was measured with the same analyser using a turbidimetry assay. Lipid profile and HbA_{1c} were evaluated

because of their known relationship to insulin resistance, prediabetes and atherosclerosis progression. Dyslipidemia was defined as total cholesterol values ≥ 5.17 mmol/L and/or serum triglycerides ≥ 1.7 mmol/L, according to American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis [17]. The fasting insulin concentrations were measured using comercially available enzyme-linked immunosorbent assay kits from DiaMetra S.R.L. Milan, Italy, with intra-assay variability ≤ 2.3% and inter-assay variability ≤ 12.4%. The adiponectin and leptin serum concentrations were measured using comercially available enzyme-linked immunosorbent assay kits from BioVendor GmbH, Heidelberg, Germany (adiponectin: intra-assay variability 3.3% and inter-assay variability 6.2%; leptin: intra-assay variability 4.2% and inter-assay variability 4.4%) and leptin-to-adiponectin ratio (LAR, mg/g) was calculated. Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR), calculated as fasting insulin (in microunits per milliliter) × fasting plasma glucose (in millimoles per liter)/22.5.

Intima-media thickness (IMT) was measured using a full digital ultrasound system (Tellus UF-870AG, Fukuda Denshi Co. Ltd., Japan) with automatic frequency adjustment, connected with a 5.0-12.0 Mhz, 38 mm linear array transducer. All measurements were carried out by the same investigator, who was blinded to the cardiovascular risk factors of the patients. Measurements were made in a quiet, temperature-controlled environment, with the subject resting in the supine position, the head rotated 45° from neutral. The far wall of both the right and left common carotid and femoral arteries was used, because of higher reproducibility and possible overestimation of the IMT of the near wall [18]. For carotid IMT, two measurements were made in each of these standardized points: 1 cm proximal to the carotid dilatation, the carotid bulb and 1 cm distal to bifurcation; the six results were then averaged separately for left and right carotid arteries. Femoral IMT was similarly measured in 3 reference points located 10, 15 and 20 mm next to the bifurcation and the same procedure was followed for obtaining the average value. The mean readings of the left and right arteries were then averaged to obtain a single mean value at carotid and femoral levels for each participant.

2.1 Statistical methods

All previously mentioned data were centralized in a database using Microsoft Excel. Statistical analysis was performed using SPSS version 16.0 (Chicago, Illinois, USA). For descriptive statistics, discrete and continuous variables were expressed as frequencies and percentages, and means, standard deviations and standard errors, respectively. Student t-test (for continuous variables) and χ^2-test (for categorical variables) were used to evaluate differences. Pearson and Spearman correlations were used to analyse associations between variables, and Bonferroni's corrections were applied on the results in order to minimize the probability of a type I statistical error. Because some parameters may vary differently between males and females, analysis was sometimes stratified by gender. Multiple linear regression analysis was performed to disclose independent contributions between variables. A two-sided p-value < 0.05 was considered as statistically significant; precision of the measurements was defined in 95% confidence interval as $p < 0.05$.

3 Results

122 subjects were studied, 50 men (40.98%) and 72 women (59.02%), 52.5% with isolated IFG and 47.5% with IFG+IGT. The age ranged between 30 and 70 years and BMI ranged from 19.3 to 43.8 kg/m^2. Smoking was present in 17.21%, hypertension in 43.44%, dyslipidemia in 41.8% and excess weight in 82.8% of subjects, 55.73% being obese. The main characteristics of the subjects are shown in table 1.

Neither carotid, nor femoral IMT were correlated to the anthropometric data or to the lipid profile (all $p > 0.05$). Age appeared related to carotid IMT ($r = 0.202$, $p = 0.027$), but not to femoral IMT ($r = 0.013$, $p = 0.889$). Neither adiponectin, leptin nor the LAR values were correlated with any of the anthropometric data, except for a positive relationship between leptin and the WHtR. There were no correlations between leptin or LAR and the lipid profile; adiponectin correlated only with triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio. Leptin and LAR correlated with HbA_{1c}, while adiponectin showed no correlation with HbA_{1c}. HOMA-IR was not related to the anthropometric parameters, except for an association with the WHR, but correlated with some components of the lipid profile (triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio) and HbA_{1c}. All these results are detailed in table 2. The adipokines correlated with HOMA-IR, as shown in table 3.

The initial relationships between IMT values and the metabolic profile (HOMA-IR, adipokines and HbA_{1c}) are shown in table 4. In order to minimize type I statistical errors, Bonferroni's corrections were applied on the results of previously presented correlations, without statistically

Table 1: Clinical, laboratory and ultrasound characteristics of study subjects

Characteristics	Mean	Standard deviation	Standard error
Age (years)	58.99	10.81	0.97
BMI (kg/m^2)	30.86	5.37	0.49
WC (cm)	101.03	13.52	1.24
WHR	0.96	0.09	0.01
WHtR	0.62	0.09	0.01
SBP (mmHg)	132.14	9.62	1.51
DBP (mmHg)	81.16	4.87	0.99
Total cholesterol (mmol/l)	5.42	1.15	4.04
Triglycerides (mmol/l)	1.74	0.98	7.82
HDL-cholesterol (mmol/l)	1.28	0.35	1.25
LDL-cholesterol (mmol/l)	3.36	1.07	1.13
Cholesterol / HDL	4.50	1.45	0.13
Non-HDL cholesterol (mmol/l)	4.14	1.15	1.13
Triglycerides / HDL	1.61	1.40	0.31
HbA_{1c} (%)	5.99	0.42	0.04
HOMA-IR	2.53	1.99	0.18
Adiponectin (μg/ml)	11.68	4.60	3.99
Leptin (ng/ml)	19.58	15.60	1.42
LAR (mg/g)	2.14	2.93	0.27
Carotid IMT (mm)	0.82	0.34	0.03
Femoral IMT (mm)	0.77	0.32	0.02

Abbreviations: BMI = body mass index, WC = waist circumference, WHR = waist-to-hip ratio, WHtR = waist-to-height ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio, IMT = intima-media thickness

changing the data. The results of multiple regression analysis are mentioned in table 5.

Carotid IMT correlated only with adiponectin and LAR, while femoral IMT had no relationships whatsoever with the previously mentioned metabolic factors. Neither carotid, nor femoral IMT had any initial correlations with HbA_{1c} values. When adjusting for age, all initial correlations carotid IMT had with HOMA-IR and with adipokines disappeared and the regression coefficients for femoral IMT were left unchanged, while both IMT values showed moderate positive associations with HbA_{1c}. After using HOMA-IR as the adjustment factor, carotid IMT became negatively correlated with adiponectin and positively correlated with leptin and LAR; all these correlations had moderate intensity. Similar results were noticed when adjustments for HOMA-IR were applied on the relationships between femoral IMT and adipokine values. Both carotid and femoral IMT became positively correlated with HbA_{1c} after adjusting for HOMA-IR values.

Adjustments for adipokines, lipid profile parameters and HbA_{1c} were then applied on the relationships between carotid/femoral IMT and HOMA-IR (table 6).

Both carotid and femoral IMT became correlated with HOMA-IR after adjustments for leptin and LAR; when adjustment for adiponectin was used, the relationships between IMT values and HOMA-IR were marked by a large standard error. As to lipid profile parameters, only adjustments for triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio induced new correlations between both carotid/femoral IMT and HOMA-IR. Significant correlations appeared

Table 2: Correlations of clinical and biochemical data with HOMA-IR and adipokine values

	HOMA-IR	Adiponectin	Leptin	Leptin:Adiponectin Ratio
Age (years)	r = 0.402 p = 0.077	r = 0.056 p = 0.540	r = 0.025 p = 0.788	r = - 0.010 p = 0.913
BMI (kg/m^2)	r = 0.010 p = 0.917	r = - 0.029 p = 0.759	r = - 0.114 p =0.222	r = - 0.035 p = 0.710
WC (cm)	r = 0.103 p = 0.269	r = 0.069 p = 0.463	r = - 0.013 p = 0.891	r = 0.103 p = 0.270
WHR	r = 0.106 p = 0.283	r = - 0.036 p = 0.714	r = 0.055 p = 0.577	r = - 0.091 p = 0.359
WHtR	**r = 0.258** **p = 0.006**	r = - 0.153 p = 0.108	**r = 0.240** **p = 0.011**	r = 0.159 p = 0.094
SBP (mmHg)	r = 0.113 p = 0.566	r = - 0.174 p = 0.071	r = 0.210 p = 0.086	r = 0.183 p = 0.767
DBP (mmHg)	r = 0.126 p = 0.069	r = - 0.214 p = 0.069	r = 0.098 p = 0.616	r = 0.108 p = 0.213
Total cholesterol (mmol/l)	r = 0.054 p = 0.554	r = 0.049 p = 0.594	r = - 0.016 p =0.859	r = - 0.037 p = 0.689
Triglycerides (mmol/l)	**r = 0.393** **p = 0.000**	**r = - 0.316** **p = 0.000**	r = 0.050 p = 0.586	r = 0.078 p = 0.396
HDL-cholesterol (mmol/l)	**r = - 0.326** **p = 0.000**	**r = 0.392** **p = 0.000**	r = - 0.039 p = 0.675	r = - 0.079 p = 0.397
LDL-cholesterol (mmol/l)	r = 0.055 p = 0.562	r = 0.049 p = 0.607	r = - 0.009 p = 0.927	r = - 0.032 p = 0.733
Cholesterol / HDL	**r = 0.354** **p = 0.000**	**r = - 0.369** **p = 0.000**	r = - 0.007 p = 0.944	r = 0.069 p = 0.465
Non-HDL cholesterol (mmol/l)	r = 0.151 p = 0.105	r = - 0.063 p = 0.505	r = - 0.007 p = 0.937	r = - 0.022 p = 0.815
Triglycerides / HDL	**r = 0.399** **p = 0.000**	**r = - 0.378** **p = 0.000**	r = 0.025 p =0.791	r = 0.102 p = 0.280
HbA_{1c} (%)	**r = 0.227** **p = 0.012**	r = 0.004 p = 0.963	**r = 0.322** **p = 0.000**	**r = 0.259** **p = 0.004**

Abbreviations: BMI = body mass index, WC = waist circumference, WHR = waist-to-hip ratio, WHtR = waist-to-height ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure, HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio

Table 3: Correlations between HOMA-IR and adipokine values

	HOMA-IR	
	r	**p**
Adiponectin	**- 0.329**	**0.0001**
Leptin	**0.298**	**0.001**
LAR	**0.252**	**0.006**

Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio

Table 4: Correlations of IMT values with the metabolic profile

	Carotid IMT		Femoral IMT	
	Pearson	**p**	**Pearson**	**p**
HOMA-IR	-0.016	0.859	0.015	0.873
Adiponectin	**0.190**	**0.036**	0.041	0.661
Leptin	-0.098	0.287	-0.080	0.390
LAR	**-0.212**	**0.021**	-0.174	0.058
HbA_{1c}	-0.023	0.803	0.131	0.154

Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio, IMT = intima-media thickness

Table 5: Multiple linear regression models: the relationships between IMT and the metabolic parameters adjusted for age and HOMA-IR

		Carotid IMT			Femoral IMT		
		Beta	**Sign.**	**Std. Err.**	**Beta**	**Sign.**	**Std. Err.**
Adjustments for age	HOMA-IR	-0.059	0.519	10.628	-0.069	0.451	10.780
	Adiponectin	0.096	0.297	10.587	0.109	0.240	10.707
	Leptin	0.055	0.548	10.620	0.044	0.634	10.761
	LAR	0.006	0.952	10.637	-0.018	0.850	10.770
	HbA_{1c}	**0.234**	**0.009**	10.337	**0.228**	**0.012**	10.518
Adjustments for HOMA-IR	Adiponectin	**-0.328**	**0.0001**	1.902	**-0.330**	**0.0001**	1.900
	Leptin	**0.300**	**0.001**	1.920	**0.304**	**0.001**	1.917
	LAR	**0.257**	**0.006**	1.948	**0.265**	**0.004**	1.942
	HbA_{1c}	**0.271**	**0.003**	1.932	**0.271**	**0.003**	1.933

Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio, IMT = intima-media thickness

Table 6: Multiple linear regression models: the relationships between IMT values and HOMA-IR adjusted for adipokines, lipid profile and HbA1c

		Carotid IMT			Femoral IMT		
		Beta	**Sign.**	**Std. Err.**	**Beta**	**Sign.**	**Std. Err.**
HOMA-IR	**Adjustments for:**						
	Adiponectin	-0.326	0.0001	49.211	-0.330	0.0001	41.747
	Leptin	**0.297**	**0.001**	14.984	**0.303**	**0.001**	14.985
	LAR	**0.246**	**0.006**	2.803	**0.257**	**0.004**	2.816
	Total cholesterol	0.052	0.570	45.020	0.057	0.536	45.038
	HDL-cholesterol	**-0.325**	**0.0001**	12.850	**-0.326**	**0.0001**	12.859
	LDL-cholesterol	0.052	0.582	41.401	0.057	0.546	41.477
	Non-HDL-cholesterol	0.148	0.115	44.309	0.153	0.103	44.418
	Triglycerides	**0.391**	**0.0001**	80.469	**0.392**	**0.0001**	80.491
	Cholesterol-to-HDL ratio	**0.348**	**0.0001**	1.356	**0.355**	**0.0001**	1.363
	Triglycerides-to-HDL ratio	**0.396**	**0.0001**	2.974	**0.400**	**0.0001**	2.978
	HbA_{1c}	**0.271**	**0.003**	0.406	**0.270**	**0.003**	0.406

Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance, LAR = leptin-to-adiponectin ratio, IMT = intima-media thickness

when the relationships between carotid/femoral IMT and HOMA-IR were adjusted for HbA_{1c}.

4 Discussion

We performed a cross-sectional study on a relatively homogenous prediabetic population without clinical signs of cardiovascular disease, in order to analyse the relationships between insulin resistance, adipokines and incipient atherosclerosis. Up to this moment, the association between low insulin sensitivity and IMT measured both at carotid and femoral levels was rarely analysed [19,20] and none of these previous studies concentrated on prediabetic patients as to analyse their adipokine profile.

4.1 Relationships of classical risk factors for atherosclerosis with insulin resistance, adipokine and IMT values

Our data indicate that age is correlated to carotid IMT, but not to femoral IMT. This result is different from a previous research in a nondiabetic healthy geriatric population, where no relationship between carotid IMT and age was identified [21]. On the other hand, some studies found a correlation between carotid IMT and age both in adult and geriatric nondiabetic populations [22,23]. However, as this correlation was not yet analysed in prediabetic patients, we might hypothesize that the metabolic anomalies that characterize this specific population might potentiate the effect of age on IMT values. As the association between

age and femoral IMT was rarely analysed before and other data on prediabetic populations are unavailable, the lack of correlation we found between these two variables needs supplementary analyses on other prediabetic samples, but may indicate a lower or delayed sensibility of femoral IMT to the subclinical atherosclerotic modifications induced by age in the incipient hyperglycemic milieu.

We did not observe any correlation between IMT values and the anthropometric data or the lipid profile of our subjects. On the other hand, some previous reports on healthy subjects mention a direct relationship between carotid IMT and some anthropometric measurements, especially those related to visceral adiposity; similarly, an association between the risk for ischemic heart disease and WHtR was reported on a large Lithuanian population sample [24-26]. Correlations of carotid IMT with different components of the lipid profile in healthy individuals are inconstantly reported [27,28]. In all previous studies regarding IMT in relation to anthropometric and lipid parameters, measurements of femoral IMT were not done and separate analyses on prediabetic subjects are lacking. Therefore, one possible explanation for our data might be that mild hyperglycemia induces specific metabolic changes that contribute to atherosclerosis through separate pathways than those secondary only to adipose excess or to classical lipid anomalies, thus reducing their relative influence upon IMT values. It is also possible that, by excluding subjects with clinical cardiovascular disease, we set aside some individuals in which the anthropometric or lipid anomalies might have influenced more the IMT and might have been better correlated to its values.

Blood lipids also presented variable relationships with insulin resistance and adipokines levels in our subjects. Leptin and LAR were not correlated to the lipid profile. Adiponectin was inversely correlated with markers of atherogenic dyslipidemia (triglycerides, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio) and positively correlated to HDL-cholesterol. Inversely, HOMA-IR was positively associated to triglycerides, cholesterol-to-HDL ratio and triglycerides-to-HDL ratio and negatively associated to HDL-cholesterol. This is the first study to examine the relationships HOMA-IR and these adipokines have with lipid parameters in a prediabetic population, so results must be confirmed by subsequent research. Previous analyses found inconstant correlations between leptin and blood lipids in healthy subjects [29,30], while low adiponectin and high HOMA-IR levels were seldom reported as being associated to atherogenic dyslipidemia in non-diabetic subjects with different cardiovascular risk profiles [31-34]. On the contrary, leptin and LAR, but not adiponectin, were correlated to HbA_{1c} levels in our study, suggesting a greater sensitivity of leptin than adiponectin to the small HbA_{1c} increases induced by prediabetes. Up to this moment, reports about the relationship between leptin and HbA_{1c} in diabetic populations provided contradictory results [35,36], while no previous study tested it in prediabetes subjects.

4.2 Relationships between insulin resistance, adipokine and IMT values

In our data, IMT values initially had inconstant correlations with the metabolic parameters. One possible explanation might be that the influence of insulin resistance and adipokines on this atherosclerosis marker was diminished by excluding from our study all individuals with a history of clinical cardiovascular disease. The lack of any initial correlations between IMT values and HOMA-IR can be interpreted in line with other studies, which found that in patients with incipient hyperglycemia, prediabetes is accompanied by an increased cardiovascular risk only if associated with metabolic syndrome [37], in other words if plain insulin resistance is present. It is therefore possible that a part of the subjects in our sample, having no sign of clinical atherosclerotic disease and maybe only minimally increased HOMA-IR values, to be the reason for the initial lack of correlations between IMT and HOMA-IR. Other studies also found that IMT does not correlate to insulin resistance measurements, but to postprandial hyperglycemia [38,39], which was not tested in our research; one possible explanation is that hyperglycemia can also induce atherosclerosis independently of insulin, through glycation of proteins and lipids and by increasing oxidative stress [40]. Nevertheless, this hypothesis is not yet sufficiently tested in subjects with incipient hyperglycemia and needs to be tested in future research.

On the other hand, adjustment of IMT for HOMA-IR and adipokine levels was necessary in our study to allow disentangling confounding effects by these variables, since both insulin resistance and secretion products of adipocytes are known to be associated with cardiovascular risk in subjects with advanced atherosclerosis [41,42]. In our case, adjustments of IMT values based on leptin, LAR, triglycerides, HDL-cholesterol, cholesterol-to-HDL ratio, triglycerides-to-HDL ratio and HbA_{1c} induced the appearance of statistically significant correlations with HOMA-IR, with similar regression coefficients for carotid and femoral IMT. First, our results agree to observations from other studies, which found insulin resistance measured by HOMA-IR to be directly associated to carotid IMT values in non-diabetic subjects [43] or in obese

adolescents [44]. Second, our data do not suggest that femoral IMT is better correlated with insulin resistance than carotid IMT, as other studies did [20]. Hence, we cannot share the idea that femoral IMT would make a more useful tool than carotid IMT to evaluate the connexions between the cardiovascular outcomes and the metabolic status of prediabetic patients. Third, appearance of new correlations between IMT and HOMA-IR after adjustments for some components of the lipid profile and HbA_{1c} suggests a residual relationship between low insulin sensitivity and atherogenesis, which is not mediated by the influence of insulin resistance-induced lipid and glucose metabolism changes upon IMT. In the previous literature, only one other study advances the hypothesis of separate influences exerted by HOMA-IR and glycemic variations on IMT values [45], but that research was performed in nondiabetic elderly subjects and no data are available for prediabetic patients.

When adjusting for HOMA-IR, moderate correlations of carotid and femoral IMT with adiponectin, leptin and LAR emerged. This adjustment was also necessary, given the relationships between adiponectin, leptin or LAR and insulin resistance indexes identified both in our subjects and in other studies [46].

The inverse relationship between IMT and adiponectin, also identified in studies on healthy subjects [47-49], confirms that such negative correlations are also found in prediabetic patients, independently of the insulin resistance level. It might be possible that low adiponectin levels would induce atherogenesis by favouring inflammation and consequently abnormal changes in glucose and lipid metabolism [49]. Chronic subclinical inflammation is known to be a predictor of both hyperglycemia [50] and cardiovascular disease [51]. However, other observations in healthy individuals suggest that the inverse relationship between adiponectin and IMT is not an independent one, being mostly mediated by other cardiovascular risk factors associated with metabolic syndrome [52]. Hence, we can only speculate that prediabetic patients might manifest supplementary metabolic abnormalities that would induce lowering of adiponectin values parallel to atherogenesis. This hypothesis would explain results of other researches, where adiponectin was identified as a risk factor for atherosclerotic heart disease, in particular the myocardial infarction [53]. Moreover, our study only analyzed the total adiponectin level, without measuring its isoforms, therefore no conclusion is possible on relationships between subclinical atherosclerosis and alterations in adiponectin oligomerization [54] that might exist in prediabetic patients.

After adjustment by HOMA-IR, our data suggested a positive association between leptin and IMT values, showing a possible influence of body fat mass on the incipient atherosclerotic changes. These results are in agreement with other observations on normal subjects, which found plasma leptin to be correlated to carotid IMT [55]. As adjustment by HOMA-IR allowed us to eliminate the influence of insulin resistance on the relationship between leptin and IMT, we cannot exclude the hypothesis that leptin *per se* may have a direct atherogenic effect on the arterial wall. Moreover, our study seems to be the first to analyse the relationship that plasma leptin has with both carotid and femoral IMT.

In our data, all the adjusted correlations between adipokines and IMT values had the same intensity. These are the first data about the comparative utility of adiponectin, leptin and LAR in prediabetic patients and seem similar to those from studies conducted on high cardiovascular risk individuals [13]. On the contrary, in other types of patients, such as healthy middle-aged Italian men or Japanese type 2 diabetics, LAR seemed to be better correlated than adiponectin or leptin alone to subclinical atherosclerosis evaluated by IMT [56,57]. As our findings do not support the use of LAR as a preferable estimate of atherosclerosis susceptibility compared to adiponectin or leptin alone, these apparent discrepancies need to be verified on larger samples, but might suggest a different pattern of associations between IMT values and adipokine profile in prediabetic patients. Moreover, as adiponectin did not seem to be substantially better correlated with IMT than leptin, we can speculate that both adipokines are related to this marker of subclinical atherosclerosis through a common underlying mechanism.

4.3 Limitations and strengths of the study

Our research has a few limitations. The first one is the small sample size we used; nevertheless, as our study is the first to simultaneously investigate the relationships between insulin resistance markers, adipokine levels and IMT values in subjects with incipient hyperglycemia and no clinical atherosclerosis, it must be credited for the novelty of its idea. Second, we are limited in interpretation given the cross-sectional design of our study, which precludes defining causal relationships. Therefore, we cannot state that abnormal adipokine profile or insulin sensitivity really precede the development of subclinical atherosclerosis measured by IMT values. Another limit in our research was represented by the use of a surrogate marker for insulin resistance (HOMA-IR); however, the value of this marker is already validated by numerous

studies [58], while more complex measurements, as the hyperinsulinemic euglycemic clamp, have costs and technical difficulty levels that make them less accessible for usual clinical practice. Furthermore, our choice of estimating subclinical atherosclerosis based on IMT values led to accurate results, as IMT is considered a direct measure for the arterial wall status [59] and therefore directly reflects the extension of atheroma. Based on our data, which prove an association between metabolic characteristics and IMT even in mildly hyperglycemic but otherwise healthy individuals, the next logical step would be to analyse the cause-effect relationships lying between these parameters. Altogether, further prospective studies following clinical cardiovascular events in relation to the metabolic data are needed in prediabetic patients in order to establish their real cardiovascular risk profile. Strengths of our research include the exclusion of subjects treated with glitazones, metformin or lipid-lowering drugs, which would have influenced the lipid and adipokine profiles, insulin sensitivity or HbA_{1c} levels. Besides, this study adds to the limited information about the associations between metabolic profile (insulin resistance indexes, adipokines and HbA_{1c}) and subclinical atherosclerosis in prediabetic patients.

5 Conclusions

This is the first study to investigate the associations between insulin resistance, adipokines and carotid and femoral IMT in prediabetic subjects without clinical signs of cardiovascular disease. Relationships between this atherosclerosis marker and the metabolic profile seem to exist even if manifest arterial obstructions are not yet present. The associations between IMT values and HbA_{1c} that emerge after adjusting for age prove that hyperglycemic changes, although minor, might have a significant effect on the arterial wall structure. Correlations of IMT with adipokines after HOMA-IR adjustments suggest that secretion anomalies in the adipose cells may modulate the evolution of atheromatous plaques independently of the insulin resistance level. Finally, the relationships identified between IMT and HOMA-IR after adjustments for leptin, LAR, HbA_{1c} and some lipid parameters indicate that correlations between insulin resistance and atherogenesis are not entirely mediated by adipokine, lipid and glycemic abnormalities.

Conflicts of interest: The authors have no conflicts of interest to declare in relation to this article.

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