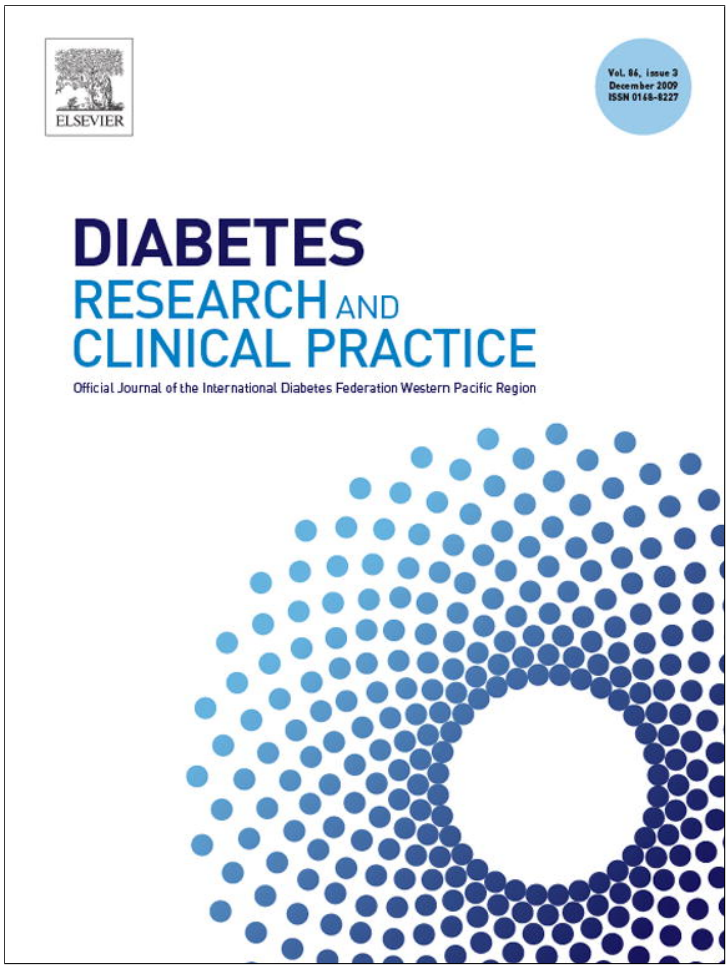

DIABETES RESEARCH AND CLINICAL PRACTICE 86 (2009) 177–185

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Intra-renal hemodynamics and carotid intima-media thickness in the metabolic syndrome

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ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form
5 September 2009
Accepted 15 September 2009
Published on line 7 October 2009

Keywords:
Metabolic syndrome
Obesity
Intima-media thickness
Renal resistances

ABSTRACT

Aims: Metabolic syndrome (MetS) is associated with increased cardiovascular risk. We hypothesize that early vascular changes are already present at the time of diagnosis of MetS. The relationship of different measures of early vascular impairment with body fat distribution and the natural progression of MetS was examined in newly diagnosed subjects non-pharmacologically treated.

Methods: 246 consecutively enrolled subjects were categorized according to the presence of MetS and type 2 diabetes (T2D). Intra-renal Doppler flow was used to ascertain resistive (RI) and pulsatility (PI) indices as markers of vascular resistance. Carotid intima-media thickness (IMT), cutis-rectis (CR) and rectis-aorta (RA) thicknesses were measured by ultrasonography; RA/CR ratio was used as measure of body fat distribution. Pro-inflammatory cytokines, C-reactive protein, oxidative markers insulin and adiponectin blood concentrations were also measured.

Results: Baseline characteristics demonstrated increasing trends in biochemical, inflammatory, and oxidative parameters from MetS−, MetS+, to MetS+/T2D ($p < 0.001$). After adjusting for age, the same increasing trends across the groups were observed in both sexes in IMT ($p < 0.001$), RI ($p < 0.001$) and PI ($p < 0.001$). IMT correlated with RI ($r = 0.25$; $p < 0.001$), PI ($r = 0.26$; $p < 0.001$), and RA/CR ratio ($r = 0.43$; $p < 0.001$).

Conclusions: Carotid IMT and intra-renal resistances are elevated at an early stage in MetS and are associated with a dysregulated production of fat-derived hormones and cytokines.

1. Introduction

Metabolic syndrome (MetS) is characterized by increased visceral fat and insulin resistance, both of which are associated with an increased risk of developing type 2 diabetes (T2D) and cardiovascular disease (CV) [1]. Despite the increased prevalence of MetS worldwide [2], the underlying pathophysiology in the development of CV disease is still incompletely understood. Recent studies have clearly established the association of MetS with markers of systemic inflammation, including C-reactive protein (CRP), tumor necrosis factor (TNF)-α and interleukin (IL)-6, all of which may play a role in the development of obesity-related insulin resistance and other co-morbidities including diabetes, hypertension and CV diseases [3].

Atherosclerosis is characterized by a long clinically silent phase before overt disease manifestation and frequently precedes the diagnosis of MetS [4] or T2D [5]. Identifying

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0168-8227/$ – see front matter
doi:10.1016/j.diabres.2009.09.015

clinical markers of early vascular change in the initial stages of MetS and T2D may improve the understanding of the mechanisms involved in the development of atherosclerosis [6]. High-resolution B-mode pulsed-wave Doppler ultrasound, which measures carotid intima-media thickness (IMT) and pulsatility (PI) and resistive (RI) indices calculated from the blood flow velocity in intra-renal interlobar arteries is promising [7,8]. Elevated RI and PI suggest increased intra-renal resistances, which are correlated with carotid IMT [9] and are increased in patients with T2D, hypertension or chronic renal failure [8–10]. This may also be associated with endothelial dysfunction [11] as subjects with hypertension [12], diabetes [13], or obesity [14] often exhibit a higher carotid IMT than control subjects. An increased carotid IMT is a well-known marker of subclinical atherosclerosis, and strongly predicts the occurrence of myocardial infarction and stroke [7,15]. Furthermore, increased intra-renal RI and PI values also reflect end-organ damage, characterized by glomerular and arteriolar sclerosis [16]. Increased intra-renal resistances might indicate, at least at an early stage, a functional and reversible modification consequent to intra-renal vasoconstriction due to endothelial dysfunction [11]. This activity might be antecedent to the appearance of structural changes consequently causing arterial atherosclerosis and glomerular ischemia [17].

We hypothesize that early markers of vascular impairment and subclinical atherosclerosis are already present in subjects at time of first diagnosis of MetS, and that measures of body fat distribution are associated with such different stages. We speculate that there is a continuum in obese patients without MetS to those with MetS and diabetes. Studies investigating the relationships between carotid IMT, intra-renal resistances, body fat distribution and markers of endothelial function and inflammation at the time of diagnosis of MetS are lacking. In the present study, we selected newly diagnosed and untreated subjects with MetS, with or without T2D, and measured the intra-renal RI and PI, carotid IMT and serum blood concentrations of inflammatory and oxidative stress markers to investigate any possible relationships with early atherosclerosis.

2. Patients and methods

2.1. Subjects

Three hundred forty-one consecutive overweight or obese subjects (age range: 30–60 years; BMI range: 25–39.9 kg/m^2) were recruited among the Obesity and Related Diseases Outpatient Department seen at the Department of Internal Medicine, Cardiovascular and Kidney Diseases of the University of Palermo between January 2006 and June 2008. Patients were referred to the clinic by their physicians for management of obesity or for a new diagnosis of hypertension, T2D, or MetS. Patients had no other systemic or end-organ disease on the basis of clinical history and physical examination, routine blood tests and electrocardiogram, nor were they on pharmacological treatment for obesity-related disorders, including anti-hypertensive, blood-glucose lowering and antiplatelet drugs. Nineteen subjects refused to participate in the study for personal reasons, 39 cases were excluded due to technical difficulties in performing the echo-Doppler renal hemodynamic measurements, and 37 cases due to logistic and administrative difficulties. The remaining 246 subjects with complete data were classified into three groups on the basis of their clinical and laboratory data: no MetS (MetS−); MetS present (MetS+); and MetS with T2D (MetS+/T2D). Metabolic syndrome was defined according to both the NCEP-ATP-III [18] and additionally determined using the International Diabetes Foundation criteria [19]; T2D was defined according to the American Diabetes Association criteria [20]. The study protocol was approved by the Ethics Committee of our Institution. All study participants were informed about aims and methods and gave voluntary written consent.

2.2. Blood sampling

A blood venous sample was obtained after an overnight fast to assess biochemical data including high-sensitivity C-reactive protein (Diagnostic Biochem, London, Ontario, Canada; CRP), insulin (radioimmunoassay; Insik-5, DiaSorin, Saluggia, Italy; μU/ml), IL-6 (Endogen Human IL-6 ELISA; Pierce Biotech. Rockford, IL, USA; pg/ml), TNF-α (Amersham Biosciences ELISA kit; Little Chalfont, UK; pg/ml), 8-iso-prostaglandin F2α (Assay Design Inc ELISA, Ann Arbor, MI, USA; 8-iso-PGF2α, pg/ml) and adiponectin (Human Adiponectin ELISA Kit; B-Bridge International CA, USA; μg/ml). Low-density lipoprotein (LDL) cholesterol concentration was calculated according to the Friedewald formula [21]. Glomerular filtration rate (GFR) was calculated according to MDRD equations [22]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. [23].

2.3. Body composition and fat distribution

Fat mass (FM, % body weight) was estimated as previously described [24] by means of bioelectrical impedance analysis (BIA-103, RJL, Detroit, MI, USA/Akern, Florence, Italy). Anthropometric measurements were obtained at the umbilicus (waist circumference) and at the most prominent buttock level (hip circumference); their ratio (waist-to-hip ratio, WHR) was considered as a measure of body fat distribution. Abdominal visceral and subcutaneous adipose sizes were also measured by means of high-resolution B-mode ultrasound (Sonoline G50; Siemens; Germany) [25]. Transverse scans were obtained 5 cm above the umbilicus along the xipho-umbilical line. A 10-MHz linear probe was used to measure the distance between the cutis and the conjunction of rectus muscles at the linea alba (cutis-rectis thickness; CR), as a measure of the subcutaneous fat. A 3.5-MHz convex probe measured the distance between the linea alba and the anterior wall of the abdominal aorta (rectis-aorta thickness, RA), as a measure of visceral abdominal fat. The RA to CR ratio (RA/CR) was also considered an indirect measure of body fat distribution. A single physician (SB) was responsible for performing the ultrasonographic body fat thickness examinations. Our laboratory intra-observer coefficient of variation for CR is 1.2% and that for RA is 3.9% including subjects with a BMI range of 18–45 kg/m^2.

2.4. Carotid and renal ultrasound analysis

Images of the right and left extracranial carotid artery walls were obtained in several projections by a high-resolution ultrasonographic 10-MHz linear array probe; end-diastolic intima-media thickness of the far wall of both common carotid arteries was measured 10 mm caudal to the bulb, from the anterior, lateral and posterior approaches using two-dimensional longitudinal sections of the vessel and the distance from the first echogenic line to the second echogenic line; the mean of both sides measurements was considered for calculations [26]. A single physician (SB) was responsible for performing the carotid and renal ultrasonographic examinations who was not blinded to the study hypothesis. Renal Doppler flow was obtained (3.5 MHz probe) of the interlobar arteries by placing the Doppler sample volume at three different positions (superior, mid and inferior) in each of both kidneys, guided by color flow mapping [27]. The mean of both kidneys resistive indices [RI; (peak systolic velocity – end-diastolic velocity)/peak systolic velocity] and pulsatility index [PI; (peak systolic velocity – end-diastolic velocity)/mean velocity] were obtained. The measured values were assessed by a different physician (SV).

2.5. Statistical analysis

All data are presented as means ± standard error of means. All variables with skewed distribution were log-transformed

Table 1 – Characteristics of the study population – using NCEP-ATP-III criteria [18].

	MetS− (N = 123)	MetS+ (N = 87)	MetS+/T2D (N = 36)	P[a]			
				ANOVA	MetS− vs. MetS+	MetS− vs. MetS+/T2D	MetS+ vs. MetS+/T2D
Males/females	53/70	45/42	16/20				
Age (years)	45 ± 1	47 ± 1	53 ± 1	<0.001	NS	<0.001	0.011
Smokers (%)	14.6	14.9	13.8				
Systolic (mmHg)	130 ± 2	141 ± 2	142 ± 2	<0.001	<0.001	0.001	NS
Diastolic (mmHg)	83 ± 1	90 ± 1	86 ± 2	<0.001	<0.001	NS	NS
Hypertension (%)	54.5	67.8	63.9				
Biochemical parameters							
Creatinine (mg/dl)	0.91 ± 0.02	0.95 ± 0.02	0.91 ± 0.03	NS			
GFR (ml/min 1.73 m^2)	109 ± 2	112 ± 3	109 ± 5	NS			
Cholesterol (mg/dl)	203 ± 4	203 ± 5	216 ± 8	NS			
HDL cholesterol (mg/dl)	52.7 ± 1.3	39.9 ± 0.9	43.4 ± 1.5	<0.001	<0.001	<0.001	NS
LDL cholesterol (mg/dl)	120 ± 3	133 ± 4	140 ± 8	NS			
Triglycerides (mg/dl)	105 ± 5	150,4 ± 8,5	154 ± 12	<0.001	<0.001	<0.001	NS
Uric acid (mg/dl)	5.0 ± 0.2	5.5 ± 0.2	5.7 ± 0.3	0.023	0.029	NS	NS
Glucose (mg/dl)	91.8 ± 0.9	98.0 ± 1.2	148.9 ± 7.2	<0.001	NS	<0.001	<0.001
Glycated hemoglobin (%)	5.4 ± 0.1	5.5 ± 0.1	7.8 ± 0.5	<0.001	NS	< 0.001	< 0.001
Insulin (μU/ml)	28.4 ± 0.9	35.5 ± 1.5	36.7 ± 1.6	<0.001	<0.001	0.001	NS
HOMA-I	6.5 ± 0.2	8.5 ± 0.4	12.1 ± 0.9	<0.001	<0.001	<0.001	<0.001
Ultrasonographic parameters							
RA (mm)	64 ± 3	89 ± 4	110 ± 12	<0.001	<0.001	<0.001	NS
CR (mm)	38 ± 1	32 ± 2	23 ± 3	<0.001	0.016	<0.001	0.009
RA/CR	1.7 ± 0.1	2.8 ± 0.3	4.8 ± 0.8	<0.001	<0.001	<0.001	<0.001
Anthropometric measurements							
Body weight (kg)	82.4 ± 1.5	90.2 ± 2.1	89.6 ± 3.4	0.008	0.010	NS	NS
BMI (kg/m^2)	30.5 ± 0.4	33.0 ± 0.6	34.3 ± 0.9	<0.001	0.004	<0.001	NS
FM (%)	34.4 ± 0.9	32.4 ± 0.9	32.6 ± 2.0	NS			
Waist circumference (cm)	99 ± 1	112 ± 2	108 ± 3	<0.001	<0.001	<0.001	NS
Hip circumference (cm)	110 ± 1	117 ± 2	112 ± 3	0.007	0.020	NS	NS
WHR	0.91 ± 0.01	0.97 ± 0.01	0.96 ± 0.02	<0.001	0.002	0.001	NS
Cytokines + oxidative stress markers							
Adiponectin (μg/ml)	6.8 ± 0.2	5.8 ± 0.2	5.3 ± 0.3	<0.001	<0.001	<0.001	NS
CRP (mg/l)	1.61 ± 0.04	1.89 ± 0.06	2.26 ± 0.11	<0.011	<0.001	<0.001	0.002
IL-6 (pg/ml)	73.9 ± 2.4	88.9 ± 2.6	110.3 ± 3.9	<0.001	<0.001	<0.001	<0.001
TNF-α (pg/ml)	3.1 ± 0.1	3.3 ± 0.1	3.7 ± 0.1	<0.001	0.002	<0.001	<0.001
8-iso-PGF2α (pg/ml)	162.8 ± 5.0	202.2 ± 6.4	248.8 ± 13.2	<0.001	<0.001	<0.001	0.001

All values expressed as mean ± SEM, or count (%).

BMI, body mass index; CR, cutis-rectis thickness; CRP, C-reactive protein; FM, fat mass; GFR, glomerular filtration rate; HOMA-I, homeostasis model assessment-insulin resistance; IL-6, interleukine-6; 8-iso-PGF2α, 8-iso-prostaglandin F2α; MetS, metabolic syndrome; RA, rectis-aorta thickness; T2D, type 2 diabetes; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

[a] p values calculated using analysis of variance (ANOVA); when ANOVA was significant ($p < 0.05$), pairwise comparisons among groups were tested using the Bonferroni's t-test.

when appropriate. Data are presented by sex and in aggregate where indicated. A one-way ANOVA was used to compare the group effect. When the ANOVA was statistically significant ($p < 0.05$) pairwise comparisons among groups were tested and a Bonferroni adjustment was performed. Linear regression analysis was performed to assess the primary outcomes of IMT, intra-renal RI and PI, after adjusting for age and sex, on the three groups (MetS−, MetS+, MetS+/T2D). Exploratory analyses were performed to assess the predictors of IMT, PI and RI according to the number of MetS categories but also to assess the strength and independency of associations between variables using multiple regression analysis (stepwise forward selection). Correlations are expressed by the Pearson's correlation coefficient. A two-tailed $p < 0.05$ was considered significant. All analyses were performed using SYSTAT (Windows version 11.0; San Jose, CA, USA).

3. Results

Baseline characteristics are reported in Table 1. There were 123 patients in the MetS− group, 87 patients in the MetS+ group, and 36 patients in the MetS+/T2D group applying the ATP-III criteria for MetS. When the IDF diagnostic criteria of MetS were considered, fewer subjects were classified as MetS− ($n = 90$) while an increased number of MetS+ subjects ($n = 120$) was included (Table 2). Physical, clinical, biochemical data, RI, PI, and IMT did not change differently across the three groups of studied subjects according to classification criteria of the IDF or the ATP-III.

Age increased from the MetS− to MetS+ to MetS+/T2D group. Patients with MetS+ or MetS+/T2D had a higher BMI than MetS− patients. All measures of body fat distribution demonstrated that body fat significantly increased towards a more visceral pattern of distribution from the MetS− to the

Table 2 – Characteristics of the study population – using IDF criteria [19].

	MetS− (N = 90)	MetS+ (N = 120)	MetS+/T2D (N = 36)	P[a]
Males/females	42/48	56/64	16/20	
Age (years)	45 ± 1	46 ± 1	53 ± 1	<0.001
Smokers (%)	13.3	12.1	13.8	
Systolic (mmHg)	130 ± 2	142 ± 2	142 ± 2	<0.001
Diastolic (mmHg)	82 ± 1	89 ± 1	86 ± 2	<0.001
Hypertension (%)	47.8	53.2	63.9	
Biochemical parameters				
Creatinine (mg/dl)	0.91 ± 0.02	0.94 ± 0.02	0.91 ± 0.03	NS
GFR (ml/min 1.73 m^2)	105 ± 5	103 ± 4	109 ± 5	NS
Cholesterol (mg/dl)	204 ± 4	202 ± 4	216 ± 8	NS
HDL cholesterol (mg/dl)	54.3 ± 1.4	41 ± 0.8	43.4 ± 1.5	<0.001
LDL cholesterol (mg/dl)	131 ± 4	131 ± 4	140 ± 8	NS
Triglycerides (mg/dl)	96 ± 4	143 ± 6	154 ± 12	<0.001
Uric acid (mg/dl)	5.1 ± 0.1	5.6 ± 0.1	5.7 ± 0.3	0.040
Glucose (mg/dl)	90.4 ± 1.0	97.1 ± 1.0	148.9 ± 7.2	0.001
Glycated hemoglobin (%)	5.3 ± 0.1	5.4 ± 0.1	7.8 ± 0.5	<0.001
Insulin (μU/ml)	28.3 ± 1.1	33.7 ± 1.2	36.7 ± 1.6	<0.001
HOMA-I	6.4 ± 0.3	8.1 ± 0.3	12.1 ± 0.9	<0.001
Ultrasonographic parameters				
RA (mm)	70 ± 2	80 ± 2	110 ± 12	<0.001
CR (mm)	34 ± 1	32 ± 1	23 ± 3	<0.001
RA/CR	2.4 ± 0.1	3.1 ± 0.1	4.8 ± 0.8	<0.001
Anthropometric measurements				
Body weight (kg)	81.8 ± 1.6	88.6 ± 1.8	89.6 ± 3.4	0.027
BMI (kg/m^2)	30.3 ± 0.4	32.5 ± 0.6	34.3 ± 0.9	<0.001
FM (%)	33.8 ± 1.1	33.4 ± 0.8	32.6 ± 2.0	NS
Waist circumference (cm)	99 ± 1	106 ± 1	108 ± 3	<0.001
Hip circumference (cm)	108 ± 1	112 ± 1	112 ± 3	0.004
WHR	0.92 ± 0.01	0.95 ± 0.01	0.96 ± 0.02	0.003
Cytokines + oxidative stress markers				
Adiponectin (μg/ml)	6.9 ± 0.2	6.0 ± 0.1	5.3 ± 0.3	<0.001
CRP (mg/l)	1.61 ± 0.04	1.83 ± 0.05	2.26 ± 0.11	<0.001
IL-6 (pg/ml)	75.0 ± 2.9	83.9 ± 2.3	110.3 ± 3.9	<0.001
TNF-α (pg/ml)	3.1 ± 0.04	3.2 ± 0.1	3.7 ± 0.1	<0.001
8-iso-PGF2α (pg/ml)	161.5 ± 5.4	192.4 ± 5.8	248.8 ± 13.2	<0.001

All values expressed as Mean ± SEM.

BMI, body mass index; CR, cutis-rectis thickness; CRP, C-reactive protein; FM, fat mass; GFR, glomerular filtration rate; HOMA-I, homeostasis model assessment-insulin resistance; IL-6, interleukine-6; 8-iso-PGF2α, 8-iso-prostaglandin F2α; MetS, metabolic syndrome; RA, rectis-aorta thickness; T2D, type 2 diabetes; TNF-α, tumor necrosis factor-α; WHR, waist-to-hip ratio.

[a] p values calculated using analysis of variance (ANOVA).

Table 3 – Carotid intima-media thickness, intra-renal hemodynamic data, of the study participants divided by sex and according to the presence (+) or absence (−) of metabolic syndrome (NCEP-ATP-III [18] and IDF [19] criteria) associated or less to diabetes.

	Univariate analysis				Age-adjusted linear regression analysis			
	MetS−	MetS+	MetS+/T2D	ANOVA, p	MetS−	MetS+	MetS+/T2D	P^a
ATP-III								
IMT (mm)								
M	0.653 ± 0.021	0.735 ± 0.021	0.815 ± 0.026	<0.001	0.646 ± 0.010	0.717 ± 0.011	0.861 ± 0.019	<0.001
F	0.616 ± 0.017	0.725 ± 0.025	0.862 ± 0.048	<0.001	0.610 ± 0.010	0.735 ± 0.012	0.819 ± 0.014	<0.001
RI								
M	0.655 ± 0.005	0.660 ± 0.006	0.710 ± 0.017	<0.001	0.644 ± 0.002	0.652 ± 0.002	0.681 ± 0.003	<0.001
F	0.670 ± 0.004	0.699 ± 0.008	0.707 ± 0.017	<0.001	0.658 ± 0.002	0.678 ± 0.002	0.698 ± 0.002	<0.001
PI								
M	1.150 ± 0.018	1.174 ± 0.025	1.374 ± 0.094	<0.001	1.155 ± 0.007	1.195 ± 0.007	1.294 ± 0.012	<0.001
F	1.190 ± 0.014	1.310 ± 0.035	1.315 ± 0.071	0.003	1.199 ± 0.008	1.282 ± 0.009	1.349 ± 0.010	<0.001
IDF								
IMT (mm)								
M	0.659 ± 0.025	0.714 ± 0.020	0.815 ± 0.026	0.002	0.679 ± 0.014	0.683 ± 0.011	0.756 ± 0.018	0.005
F	0.624 ± 0.022	0.682 ± 0.020	0.862 ± 0.048	<0.001	0.690 ± 0.016	0.712 ± 0.012	0.761 ± 0.014	0.027
RI								
M	0.653 ± 0.006	0.661 ± 0.005	0.710 ± 0.017	<0.001	0.653 ± 0.003	0.653 ± 0.002	0.670 ± 0.004	0.005
F	0.669 ± 0.005	0.689 ± 0.006	0.707 ± 0.017	0.012	0.679 ± 0.004	0.684 ± 0.003	0.695 ± 0.003	0.027
PI								
M	1.141 ± 0.020	1.176 ± 0.021	1.374 ± 0.094	<0.001	1.159 ± 0.011	1.163 ± 0.008	1.219 ± 0.014	0.005
F	1.189 ± 0.019	1.269 ± 0.025	1.315 ± 0.071	0.034	1.227 ± 0.011	1.242 ± 0.008	1.274 ± 0.009	0.0271

All values expressed as mean ± SEM.
F, females; IMT, intima-media thickness; M, males; MetS, metabolic syndrome; PI, intra-renal pulsatility index; RI, intra-renal resistance index; T2D, type 2 diabetes.
[a] Adjusted p value for age.

MetS+ groups. The ultrasonographic data demonstrated that across the three groups, from the MetS− to the MetS+/T2D, concomitant to a progressive increase of visceral adipose tissue (RA), there was a reduction of subcutaneous adipose tissue (CR). Other measurements demonstrated similar trends between each of the groups (MetS− vs. MetS+ vs. MetS+/T2D). Univariate trends were similar by sex (Table 3) and no different in females or males; trends were replicated in the multivariate linear regression analysis after adjusting for age. There were significant differences in IMT, RI and PI between the MetS− group and MetS+ groups, and from the MetS+ group to the MetS+/T2D groups, using either criteria.

Table 4 presents data in relation to the number of components of ATP-III-MetS in our exploratory analysis. IMT increased significantly across as the number of MetS components increased (0–5 components) and as well as when they were grouped in three class groups (0–1, 2–3, 4–5 components). No significant change was observed with regard

Table 4 – Carotid intima-media thickness, intra-renal hemodynamic data of the study participants divided according to increasing number of determinants of metabolic syndrome (NCEP-ATP-III criteria) [18].

	0	1	2	3	4	5	ANOVA, p
Subjects (N)	25	55	68	59	30	9	
IMT (mm)	0.568 ± 0.030	0.647 ± 0.018	0.688 ± 0.020[a]	0.776 ± 0.024[b,d,e]	0.733 ± 0.027[c]	0.800 ± 0.057[c]	<0.001
RI	0.669 ± 0.005	0.664 ± 0.005	0.675 ± 0.007	0.688 ± 0.007	0.676 ± 0.008	0.678 ± 0.025	NS
PI	1.171 ± 0.018	1.174 ± 0.019	1.239 ± 0.029	1.247 ± 0.028	1.233 ± 0.034	1.302 ± 0.142	NS

	0–1	2–3	4–5	ANOVA, p
Subjects (N)	80	126	40	
IMT (mm)	0.622 ± 0.016	0.729 ± 0.016[f]	0.747 ± 0.024	<0.001
RI	0.666 ± 0.004	0.681 ± 0.005	0.676 ± 0.008	NS
PI	1.173 ± 0.014	1.243 ± 0.020[g]	1.247 ± 0.040	<0.05

All values expressed as mean ± SEM. Bonferroni's t-test: [a] $p < 0.02$ vs. 0; [b] $p < 0.001$ vs. 0; [c] $p < 0.005$ vs. 0; [d] $p < 0.001$ vs. 1; [e] $p < 0.05$ vs. 2; [f] $p < 0.001$ vs. 0–1; [g] $p < 0.05$ vs. 0–1.
IMT, intima-media thickness; MetS, metabolic syndrome; PI, intra-renal pulsatility index; RI, intra-renal resistance index; T2D, type 2 diabetes.

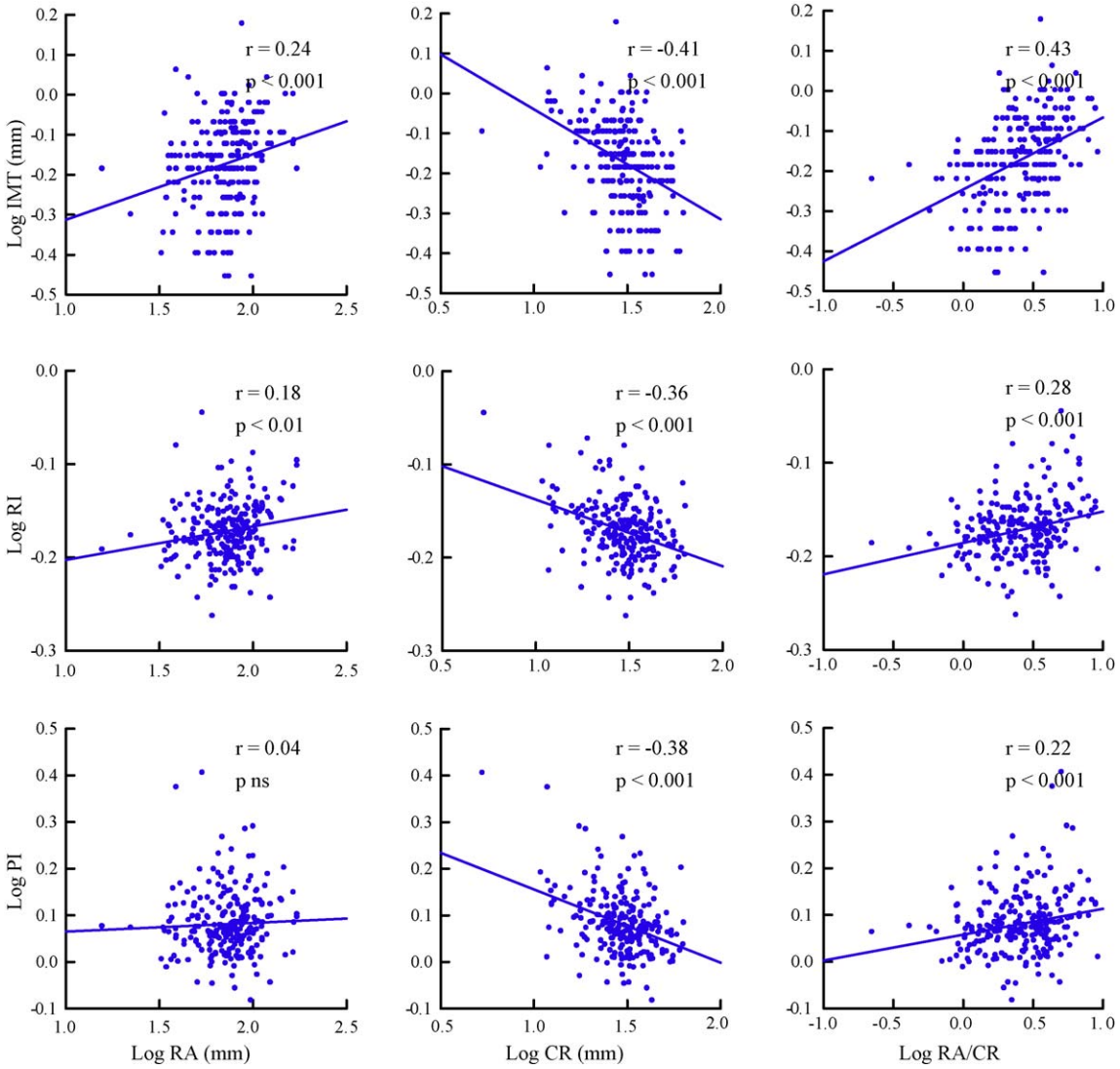

Fig. 1 – Correlations between log-transformed main outcomes of the study and echographic measures of body fat compartments. IMT: carotid intima-media thickness; RI: renal resistance index; PI: renal pulsatility index; CR: cutis-rectis thickness; RA: rectis-aorta thickness.

to the RI, while PI increased significantly only when grouped in three class of number of components of MetS. Fig. 1 represents the relationships between the primary outcomes of IMT, PI and RI with ultrasonographic anthropometric measurements. IMT was significantly associated with RI ($r = 0.25$; $p < 0.001$) and PI ($r = 0.26$; $p < 0.001$). Table 5 reports the independent predictors of IMT, RI and PI.

4. Discussion

The results of this study suggest that MetS, especially when associated with T2D, is characterized by higher values of carotid IMT, an indicator of subclinical atherosclerosis, but also by higher intra-renal resistances as assessed by RI and PI. Body fat distribution, hormonal, inflammatory and oxidative markers reported in the three studied groups are also in agreement with the hypothesis of a natural progression of the MetS that is associated with a progressive vascular impairment. This study's novelty is that we are not aware of other studies investigating these relationships particularly in obese patients managed non-pharmacologically. Therefore, the implications of this study provide further insight into the natural history, progression and pathophysiology of insulin resistance and the MetS.

It has been described that subjects with T2D have the same risk of developing myocardial infarction [28] or stroke [29] as non-diabetic patients who already had cardiovascular events. Previously, an adverse cardiovascular risk profile may antecede the diagnosis of diabetes [30]. Subsequent data obtained from the Nurses' Health Study cohort [5] confirmed impaired cardiovascular risk before the clinical diagnosis of diabetes. Our results demonstrating increased carotid IMT and intra-renal resistances in subjects with newly diagnosed MetS or MetS with T2D are in accordance with our hypothesis that the atherosclerotic process is initiated before the clinical

appearance of diabetes. Furthermore, contrary to carotid IMT results, the association between intra-renal resistances and the number of components of MetS (Table 4) was less significant with respect to grouping subjects according to the presence of MetS and T2D. The latter finding is in agreement with the hypothesis that MetS per se and not its single components are associated with increased risk. Although all the individual components may be individual risk factors impacting renal resistance indices, they also may interact with one another in different ways leading to different effects. However, we caution the validity of these additional analyses as not only were we limited in study power among the six groups (0–5 components), but we would currently be unable to ascertain the effect of the interaction between these components. We hypothesize that with a higher number of subjects, this modelling may have demonstrated stronger associations.

Our study proved that intra-renal RI and PI were correlated with carotid IMT. Studies in subjects with kidney diseases showed an association between renal resistance indices and local renal impairment, demonstrating their ability to predict kidney diseases. Few studies investigated the association between intra-renal hemodynamic indices and carotid IMT exclusively in subjects with hypertension [9,27,31], with chronic kidney diseases [32] or kidney transplanted [33]. A major limitation of these studies, though, was their rather small sample sizes [9,27,32]. However, the association between carotid IMT and intra-renal indices of vascular resistance in hypertensive patients without known organic kidney damage suggests that these indices may have a more complex pathophysiologic significance likely reflecting systemic vascular disease. To the best of our knowledge, our results confirm the association between intra-renal hemodynamic indices and carotid IMT in nonpharmacologically treated subjects with MetS without renal disease, suggesting that increased intra-renal resistances reflect a systemic vascular impairment. Since an increased carotid IMT is known to be associated with impaired endothelial function [34], our results may also lend credence to the hypothesis that endothelial dysfunction may have vasoconstrictor effects [11] and therefore induce elevated intra-renal RI and PI. To the best of our knowledge, there is no data examining the discriminatory differences between intra-renal PI and RI compared to carotid IMT. Whether one technique is preferable or more predictive than the other on incident vascular disease or endothelial dysfunction requires further investigation. Interestingly, the blood concentrations of 8-iso-PGF2α, a molecule considered a marker of oxidative stress [35], correlated significantly with IMT, RI and PI.

Total adiposity (FM%) was not significantly different between the three groups and it was independent and negatively correlated with IMT and intra-renal PI (data reported in Appendix). This apparently contradictory result is explained at least in part by the negative correlation between these variables and CR thickness. In fact, subcutaneous fat is better represented in women than in men and accounts for the higher total fat mass commonly observed in women. Despite the association between waist circumference and visceral fat, it cannot discriminate subcutaneous from visceral fat at the individual level [25]. Therefore, the elevated subcutaneous fat sizes in women may explain their lower cardiovascular risk compared to men, despite the higher BMI and waist circumference observed in females. These considerations may have at least two possible implications: methods to intervene reducing excess body weight likely need to have sex-specific targets, possibly on the basis of a more accurate body fat measurement [36]; and secondly, body circumferences are probably inaccurate measures of body fat distribution in pathophysiology studies that consider limited numbers of subjects of both sexes. Our study's echographic CR and RA thicknesses, as direct measurements of body fat compartments are safe, cost-effective and relatively easy to perform method with good reproducibility, compared to highly accurate but costly and impractical methods such as dual x-ray absorptiometry, nuclear magnetic resonance or computer tomography; furthermore, our results are in agreement with studies that demonstrate that ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases [37,38]. The RA/CR discriminated better than WHR subjects with MetS from those with MetS and T2D and it was positively correlated with the carotid IMT and the intra-renal RI and PI. The results emphasize the role of the balance between visceral and subcutaneous adipose tissue compartments in the pathogenesis of atherosclerosis.

We acknowledge that increased visceral fat co-exists with insulin resistance and that inflammatory cytokines are overproduced in visceral fat together with other markers not measured in this study [39]. Not surprisingly, our correlations confirm that low circulating levels of adiponectin are a characteristic of the MetS [40], and that increased visceral fat is associated with a shift in the normal balance of the adipokines resulting in a pro-inflammatory state; however, subcutaneous fat is thought to have a protective role from both the metabolic and the cardiovascular point of view, likely because it might be a preferential site of adiponectin production [41]. We cannot exclude that subcutaneous fat has not only a neutral role in terms of metabolic and cardiovascular risk but that it may actively antagonize visceral fat and that the echographic derived ratio RA/CR is an appropriate measurement when the influences of body fat distribution is investigated.

Our study has inherent limitations. The limited number of subjects was not homogeneous as far as age and sex distribution, but we were able to adjust for this in our multivariable analysis. Despite the prevalence of hypertension was similar in the MetS groups, it was slightly lower in the group without MetS, this difference was due to the particular procedure of enrolment of subjects in the study design. However, we feel that such a little difference does not fully explain differences observed in the main outcomes. A study with a higher number of enrolled subjects is probably needed to confirm these preliminary results. The data collected by ultrasonography were measured by an individual clinician that was not blinded to the study hypothesis. This inherently involves diagnostic suspicion bias, and measurement bias. Alternatively, we should have collected multiple measurements by different observers to minimize such bias. However, to what extent this may have affected our results is unknown. In addition, ascertaining independent predictors of our outcome variables including determinants of the number of MetS components require further investigation.

Table 5 – Multiple stepwise linear regression analysis (forward selection).

Variable	β-coefficient	p
Log IMT		
Age	0.004	<0.001
Log IL6	0.001	<0.001
MetS (0 = not, 1 = yes)	0.036	<0.005
T2D (0 = not, 1 = yes)	0.048	<0.01
Log RI		
Age	0.001	<0.001
Log IL6	0.0001	<0.001
Log adiponectin	−0.036	<0.05
Log CR	−0.055	<0.001
Log TNF-α	0.082	<0.05
Log PI		
Age	0.002	<0.001
Log CR	−0.142	<0.001
Log adiponectin	−0.11	<0.001

CR, cutis-rectis thickness; IMT, intima-media thickness; IL-6, interleukin-6; MetS, metabolic syndrome (NCEP-ATP-III criteria); PI, intra-renal pulsatility index; RI, intra-renal resistance index; T2D, type 2 diabetes; TNF-α, tumor necrosis factor-α.

We demonstrated that using the IDF criteria for MetS, the prevalence in each category differed from that observed using the ATP-III criteria. This is an expected result because of the more inclusive nature of the IDF criteria. Although there is debate as to which definition of MetS is more accurate [2], this was outside the scope of the current study. More importantly, though, the outcome data and their trends were no different using these different classification criteria. Other studies have consistently used ATP-III definitions allowing better comparison with our own results [42,43].

The study design was prospective in nature, allowing the investigation of untreated subjects with newly diagnosed MetS or MetS/T2D, thus excluding any pharmacologic influence on hemodynamic intra-renal measurements. Furthermore, ultrasound measurements were performed by the same operator thus excluding inter-observer variability. We have made inherent assumptions regarding the underlying pathophysiology of the MetS, including that there is a natural progression between each stage of disease. However, this has been confirmed in other studies [44], and indeed our data provide further evidence to this fact. In our community, primary care physicians often refer patients with obesity or at high risk for cardiometabolic abnormalities to our clinic, and although does not eliminate referral bias completely, as in a population-based cohort, it minimizes it to a large degree. Hence, our results have good external validity.

In summary, we have demonstrated that newly diagnosed MetS is characterized by increased carotid IMT and intra-renal resistances, implicating that this clinical condition recognizes specific pathogenic mechanisms involving body fat distribution, insulin resistance and inflammation.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

This study was supported in part by the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST funds 60% project 2006).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.diabres.2009.09.015.

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