



Adipokines as atherothrombotic risk factors in obese subjects: Associations with haemostatic markers and common carotid wall thickness

Q6 É. Csongrádi ^{a,b,*}, M. Káplár ^c, B. Nagy Jr ^a, C.A. Koch ^b, A. Juhász ^{c,1}, L. Bajnok ^d, Z. Varga ^e, I. Seres ^e, Z. Karányi ^e, M.T. Magyar ^a, L. Oláh ^e, A. Facskó ^f, J. Kappelmayr ^a, G. Paragh ^c

^a Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^b Department of Medicine, University of Mississippi Medical Center, Jackson, MS, United States

^c Department of Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^d 1st Department of Medicine, Faculty of Medicine, University of Pécs, Pécs, Hungary

^e Department of Neurology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^f Department of Ophthalmology, Faculty of Medicine, University of Szeged, Szeged, Hungary

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Abstract *Background and aims:* Some crucial associations between obesity-related altered adipokine levels and the main factors of atherosclerotic, atherothrombotic processes are not fully known. We analysed the relationships of classic adipokines, namely leptin, resistin, adiponectin, tumour necrosis factor- α (TNF- α), interleukin 6 (IL-6) with the markers of platelet activation, including mean platelet volume (MPV), platelet surface/soluble P-selectin, platelet-derived microparticles (PMPs), the parameters of coagulation abnormalities and common carotid intima-media thickness (IMT) in obese patients with or without atherosclerotic comorbidities in comparison to age- and sex-matched controls. *Methods and results:* We enrolled 154 obese individuals, including 98 suffering from atherosclerotic concomitant conditions, 56 free of atherosclerotic comorbidities and 62 healthy controls. Plasma levels of leptin, resistin, adiponectin, TNF- α , IL-6, soluble P-selectin, and plasminogen activator inhibitor-1 antigen (PAI-1 ag) were analysed by ELISA. Platelet surface P-selectin and PMPs were measured by flow cytometry. IMT was detected by ultrasonography. Adipokines were closely associated with markers of platelet hyperactivity, hypercoagulability, hypofibrinolysis and IMT. Significant independent associations were found between leptin and platelet count ($p < 0.0001$), MPV ($p = 0.019$), PMPs ($p < 0.0001$), fibrinogen ($p = 0.001$), factor VIII (FVIII) activity ($p = 0.035$); adiponectin and PAI-1 ag ($p = 0.035$); resistin and soluble P-selectin ($p = 0.002$); TNF- α and PAI-1 ag ($p < 0.0001$); and IL-6 and fibrinogen ($p = 0.011$). Finally, leptin ($p = 0.0005$), adiponectin ($p = 0.019$), IL-6 ($p = 0.001$), MPV ($p = 0.0003$), PMP ($p = 0.008$), and FVIII activity ($p = 0.043$) were independent predictors of IMT. *Conclusion:* Overall, we suggest that in obese subjects altered adipokine levels play a key role in common carotid atherosclerosis both directly and through haemostatic parameters.

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* Corresponding author. Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98., Debrecen H-4032, Hungary. Fax: +36 52 417 631.

E-mail address: csongradi69@gmail.com (É. Csongrádi).

¹ Current address: Takeda Development Centre Europe, London, United Kingdom.

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Introduction

Atherosclerotic cardiovascular diseases are identified as leading causes of mortality worldwide [1]. Atherothrombosis as a critical component of cardiovascular events is the acute crisis of atherosclerosis. Obesity is considered one of the major manageable risk factors of atherosclerosis besides hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia and smoking [2]. Obesity is often accompanied by comorbidities predisposing to atherosclerosis, namely, hypertension, T2DM and dyslipidemia. It should be noted that nearly one-third of obese individuals do not suffer from any atherosclerotic concomitant diseases [3].

In abdominal obesity, visceral fat accumulation causes dysregulation of adipose tissue functions, leading to the overexpression of most adipokines including leptin, resistin, TNF- α , IL-6, and hyposecretion of adiponectin [4]. Obesity-associated altered adipokine levels have atherogenic effects exerted via many different ways [5,6]. In obese subjects, the associations between adipokine plasma levels and hypertensive status, alterations in insulin-glucose homeostasis, lipid abnormalities, and chronic low-grade inflammation state as conventional vascular risk factors have been widely demonstrated [7,8]. Adipokines also influence obesity-related alterations of coagulation, fibrinolysis, platelet production and responses [9,10]. However, further aspects remain unexplored regarding the effects of the different adipokines on coagulation abnormalities. Furthermore, to our best knowledge, there are no data on obese subjects about possible associations between altered adipokine plasma levels and elevated levels of platelet surface and soluble P-selectin, and PMPs as sensitive platelet activation markers. These relationships may provide important insight into the additional possible impact of adipokines on accelerated atherosclerosis in obese individuals.

IMT is considered as a marker of initial asymptomatic atherosclerosis and a strong predictor of future vascular events. Associations of IMT with leptin, adiponectin and IL-6 are known [11–13]. One study reported a significant correlation between resistin and IMT in obese children [14], but there is no evidence of this association in obese adult individuals. The relationship of increased plasma level of TNF- α with abnormal IMT in obesity is under investigation.

The aim of this study was to clarify the associations between plasma levels of different adipokines and haemostatic parameters, and IMT in a large cohort of obese subjects including those with or without comorbid conditions predisposing to atherosclerosis. We also intended to investigate whether the levels of adipokines in obese patients are independent predictors of haemostatic markers and IMT.

Methods

Study population

The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association

and approved by the Ethics Committee of the Faculty of Medicine, University of Debrecen. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m² according to the U. S. National Institute of Health (NIH) and National Heart, Lung and Blood Institute (NHLBI) recommendation (1998). We investigated 98 obese patients suffering from comorbid conditions predisposing them for atherosclerosis (hypertension and/or T2DM and/or dyslipidemia) and 56 age-, sex- and BMI-matched obese subjects free of atherosclerotic concomitant diseases. There were 47 smokers among the obese study participants who had atherosclerotic comorbidities. Obese individuals were enrolled from the Obesity Outpatient Clinic at the Department of Medicine, University of Debrecen. Obese patients with malignancy, impaired liver or renal function, pregnancy, alcohol or drug dependence, infectious diseases, as well as severe symptomatic cardiovascular diseases such as active angina, intermittent claudication and transient ischaemic attack were excluded. Sixty-two age- and sex-matched healthy volunteers were recruited from the staff of the Departments of Medicine and Ophthalmology to serve as a control group. Healthy controls did not suffer from cardiovascular, metabolic and inflammatory diseases, cancer, liver or renal impairment, or alcohol or drug dependence, as observed through medical history, physical examination and routine laboratory tests. All participants gave written informed consent.

Blood drawing and sample preparation

Venous blood samples were taken between 8:00 and 10:00 a.m. after an overnight fast by atraumatic venipuncture into Vacutainer tubes containing 0.105 M sodium citrate (Becton Dickinson, San Jose, CA, USA). Blood sampling and preparation conditions were performed as previously described (15). Plasma samples were kept frozen at -70 °C for ELISA measurements. For flow cytometric analysis, within 2 h of sample collection, 40 μ l whole blood samples were fixed in 1 ml 1% paraformaldehyde and kept at room temperature (RT) for a minimum of 1 h for platelet analysis. Fixed whole blood samples were prepared as we formerly presented (15). To study the amount of PMPs, platelet-poor plasma (PPP) was obtained from whole blood anticoagulated with sodium citrate by using the same protocol we used earlier (15).

Laboratory assays

Plasma levels of leptin, resistin, adiponectin, TNF- α , IL-6, soluble P-selectin and PAI-1 ag were analysed by commercially available ELISA kits (WAK-Chemie Medical GmbH, Bad Soden, Germany for leptin measurement; BioVendor Laboratory Medicine, Inc. Brno, Czech Republic for resistin analysis; R&D Systems, Inc. Minneapolis, MN, USA for adiponectin, TNF- α , IL-6 and soluble P-selectin determinations; and Technozym PAI-1 antigen ELISA kit, Technoclone GmbH, Austria for analysis of PAI-1 ag) according to the manufacturer's instructions. Blood glucose, total cholesterol, high-density lipoprotein cholesterol

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(HDL-C) and triglyceride values were measured on a Hitachi analyser (Roche, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald formula. Haemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography (HPLC) (BioRad, Hercules, CA, USA). Serum insulin concentration was analysed by a commercially available radio-immunoassay kit (MP Biomedicas, Orangeburg, NY, USA). High-sensitivity C-reactive protein (hs-CRP) was assessed by turbidimetric assay on an Integra 800 analyser (Roche Diagnostics). Fibrinogen plasma level was determined by the Clauss method. FVIII activity was measured on an STA Compact instrument (Diagnostica Stago, Asnières, France). Platelet count and MPV were determined by Advia 120 Hematology System (Bayer Diagnostics, Tarrytown, NY, USA). Insulin resistance index was estimated by using the homeostasis model assessment of insulin resistance (HOMA-IR).

Flow cytometric analysis

Fixed platelets were incubated with saturating concentrations of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to GPIX (CD42a) and phycoerythrin (PE)-labelled anti-P-selectin (CD62-PE, Becton Dickinson) for 20 min in the dark at RT to investigate the level of platelet activation. As a control for immunolabeling with anti-CD62 antibody, platelets were incubated with PE-coupled non-immune mouse IgG₁ antibody. 10,000 dual-colour labelled platelet events were acquired on a FACSCalibur flow cytometer by using the CellQuest 3.2 software (Becton Dickinson). Results were expressed as the percentage of double positive platelets.

The number of PMPs was analysed by a standardized method we set earlier (15). The number of PMPs was calculated based on the event count from the bead tube collected for the same time period (30 s). PMPs were gated into a restricted area by forward scatter (FSC) and side scatter (SSC) parameters and then identified by their CD42a positivity.

Common carotid duplex ultrasound examination

Common carotid vessel wall examination was performed at the Ultrasound Laboratory of the Department of Neurology, University of Debrecen. All subjects were examined immediately after blood sampling using a colour-coded HP SONOS 4500 (Hewlett Packard, Andover, MA, USA) carotid duplex machine with a 7.5 MHz linear transducer. Both diameter and area reductions were measured according to the European Carotid Surgery Trial (ECST) method. Online measurements of IMT were performed in both common carotid arteries (CCA) at about 10 mm proximal to the carotid bulb or 20 mm proximal to the flow divider. IMT was measured between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (upper layer of the adventitia) in the far (deeper) artery wall. All measurements were performed on frozen, enlarged images at the end of a

heart cycle (end-diastole), and the transducer was in the mediolateral direction. In each plaque-free common carotid artery segment, three measurements of IMT were performed at 1 mm increments. The mean IMT of the six values in each patient was calculated.

Statistical analysis

Statistical analysis was performed by using SAS™ for Windows™ 9.3 computer software (SAS Institute Inc., Cary, NC, USA). The Kolmogorov–Smirnov test was used for evaluating the normality of data distribution. Data are presented as mean ± standard deviation in parameters with normal distribution, and as median (lower-upper quartiles) in case of non-normal distribution. Non-normally distributed parameters were transformed logarithmically. Differences in various parameters among study groups were tested using one-way ANOVA with post-hoc tests (Newman–Keuls, Tukey). Correlations between continuous variables were assessed by using Pearson's test. A chi-square test was performed to determine the associations of discrete variables. Associations of multivariate parameters were evaluated by multiple regression analysis with the backward stepwise method. With probability value of $p \leq 0.05$ the result was considered statistically significant.

Results

The clinical and laboratory characteristics of all study participants are summarized in Table 1. The adipokine plasma levels measured in the study groups are shown in Fig. 1A–E. No significant differences were observed in the plasma levels of leptin, resistin, adiponectin, TNF- α and IL-6 between the two obese subgroups related to atherosclerotic comorbidities.

We analysed the relationships between adipokine levels and haemostatic markers by Pearson correlation analysis (Table 2). Each of the investigated adipokines showed a significant ($p \leq 0.05$) association with platelet count, MPV, PMP, fibrinogen and PAI-1 ag levels. Leptin, resistin, TNF- α and IL-6 correlated positively, while adiponectin was negatively correlated with these haemostatic parameters. Notably, strong significant ($p \leq 0.001$) and positive univariate correlations of PMPs with leptin ($r = 0.44$), resistin ($r = 0.30$), TNF- α ($r = 0.35$) and IL-6 ($r = 0.33$) were detected, but a negative association with adiponectin ($r = -0.36$) was found. Resistin had significant and positive correlations with platelet surface and soluble P-selectin. Leptin was related significantly and positively with soluble P-selectin. In contrast, a significant inverse correlation was found between adiponectin and soluble P-selectin levels. TNF- α and IL-6 did not show significant associations with P-selectin values. Leptin, resistin and IL-6 demonstrated significant and positive relationships with FVIII activity. Finally, adiponectin and TNF- α had no associations with FVIII activity.

Table 1 Clinical and laboratory characteristics of the study groups.

	Healthy controls (n = 62)	Obese subjects (n = 154)	
		Without atherosclerotic comorbidity (n = 56)	With atherosclerotic comorbidity (HT and/or T2DM and/or DL) (n = 98)
Age (years)	39.7 ± 10.0	40.4 ± 11.8	40.7 ± 10.7
Gender (female/male)	41/21	36/20	59/39
BMI (kg/m ²) ^{a,b}	22.1 ± 1.96	37.7 ± 8.1	38.5 ± 7.54
Waist circumference (cm) ^{a,b}	78.1 ± 7.75	117.4 ± 20.6	119.5 ± 16.8
Waist-to-hip ratio ^{a,b}	0.86 ± 0.08	0.93 ± 0.09	0.94 ± 0.07
Hypertension (%)	0	0	77.6
Type 2 diabetes mellitus (%)	0	0	31.6
Dyslipidemia (%)	0	0	87.8
Antihypertensive drugs (%)	0	0	64.3
Antidiabetic drugs/insulin (%)	0	0	29.6
Lipid-lowering drugs (%)	0	0	27.6
Systolic BP (mmHg) ^{a,b,c}	115.2 ± 9.75	122.8 ± 9.78	135.1 ± 15.5
Diastolic BP (mmHg) ^{a,b,c}	72.7 ± 7.17	79.3 ± 4.12	83.8 ± 7.19
IMT (mm) ^{a,b,c}	0.46 ± 0.04	0.54 ± 0.11	0.60 ± 0.12
Fasting glucose (mM) ^{b,c}	4.6 (4.3–5.0)	5.0 (4.7–5.3)	5.5 (5.0–7.6)
HbA _{1c} (%) ^{b,c}	5.4 (5.2–5.7)	5.6 (5.2–5.8)	6.1 (5.5–6.9)
Insulin (mU/l) ^{a,b}	13.4 (8.6–16.6)	34.3 (23.5–42.2)	37.6 (26.6–53.3)
HOMA-IR ^{a,b,c}	2.63 (1.84–3.32)	7.57 (4.63–9.45)	9.69 (6.56–13.96)
Triglyceride (mM) ^{b,c}	0.9 (0.6–1.1)	1.2 (0.9–1.4)	1.8 (1.2–2.3)
Total cholesterol (mM) ^{b,c}	4.18 ± 0.49	4.42 ± 0.61	5.49 ± 1.37
LDL-C (mM) ^{b,c}	2.22 ± 0.62	2.61 ± 0.58	3.11 ± 0.95
HDL-C (mM) ^{a,b}	1.75 ± 0.51	1.48 ± 0.39	1.36 ± 0.38
hs-CRP (mg/l) ^{a,b}	0.92 (0.49–1.86)	4.28 (2.57–11.61)	5.4 (2.95–9.64)
Platelet count (G/l) ^{a,b}	242 ± 36.7	291 ± 56.7	303 ± 61.1
MPV (fl) ^{a,b}	8.08 ± 0.41	9.02 ± 0.84	8.93 ± 0.81
Platelet P-selectin (%) ^{a,b}	0.72 (0.39–1.18)	1.18 (0.68–2.02)	1.43 (0.70–2.30)
Soluble P-selectin (ng/ml) ^{a,b}	35.7 (25.1–45.9)	44.3 (38.7–54.9)	48.0 (38.7–61.1)
PMPs (n/μl) ^{a,b}	165 (68–241)	362.5 (236–614.5)	407 (227–777)
Fibrinogen (g/l) ^{a,b}	2.94 ± 0.53	4.13 ± 0.87	4.14 ± 0.92
FVIII activity (%) ^{a,b}	132 (110–156)	196 (140–254)	189 (149–252)
PAI-1 ag (ng/ml) ^{a,b}	36.6 (29.7–45.2)	84.4 (71.3–93.8)	87.4 (74.0–93.9)

In case of a normal distribution, values are presented as mean ± standard deviation.

In case of a non-normal distribution, data are shown as median (lower-upper quartiles).

Differences in parameters among study groups were tested using one-way ANOVA with Newman–Keuls's multiple comparison.

n: case-number; HT: hypertension; T2 DM: type 2 diabetes mellitus; DL: dyslipidemia; BMI: body mass index; BP: blood pressure; IMT: common carotid intima-media thickness; HbA_{1c}: haemoglobin A_{1c}; HOMA-IR: homeostasis model assessment of insulin resistance; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; MPV: mean platelet volume; PMPs: platelet-derived microparticles; FVIII activity: factor VIII activity; PAI-1 ag: plasminogen activator inhibitor-1 antigen.

^a Significant differences between healthy controls and obese subjects without atherosclerotic comorbidity.

^b Significant differences between healthy controls and obese subjects with atherosclerotic comorbidity.

^c Significant differences between the two obese subgroups related to atherosclerotic comorbidity.

The scatter plots of the associations of IMT with adipokine levels are depicted in Fig. 2A–E. Highly significant ($p < 0.0001$) and positive correlations of IMT with leptin ($r = 0.48$), resistin ($r = 0.39$), TNF- α ($r = 0.43$) and IL-6 ($r = 0.45$) were observed. A significant, but negative association was also found between IMT and adiponectin ($r = -0.26$, $p = 0.016$). There were significant and positive relationships between IMT and platelet parameters, as well as markers of hypercoagulation and impaired fibrinolysis (data not shown).

To test whether the investigated adipokines are independently associated with haemostatic parameters, multiple regression analysis was performed. As demonstrated in Table 3A, significant independent associations were retained between leptin and platelet count ($p < 0.0001$), MPV ($p = 0.019$), PMPs ($p < 0.0001$), fibrinogen ($p = 0.001$), FVIII activity ($p = 0.035$); adiponectin and

PAI-1 ag ($p = 0.035$); resistin and soluble P-selectin ($p = 0.002$); TNF- α and PAI-1 ag ($p < 0.0001$); and IL-6 and fibrinogen ($p = 0.011$).

When IMT was considered as a dependent variable, and adipokines, haemostatic markers, age, and gender were entered as independent variables in the model of multiple regression analysis, IMT still maintained independent associations with leptin ($p = 0.0005$), adiponectin ($p = 0.019$), IL-6 ($p = 0.001$), MPV ($p = 0.0003$), PMPs ($p = 0.008$), FVIII activity ($p = 0.043$) and age ($p < 0.0001$) (Table 3B). Leptin to adiponectin ratio (L:A ratio) did not associate independently with IMT ($p = 0.219$).

Discussion

Obesity, a major, modifiable risk factor for cardiovascular disease, is associated with an accelerated atherosclerotic,

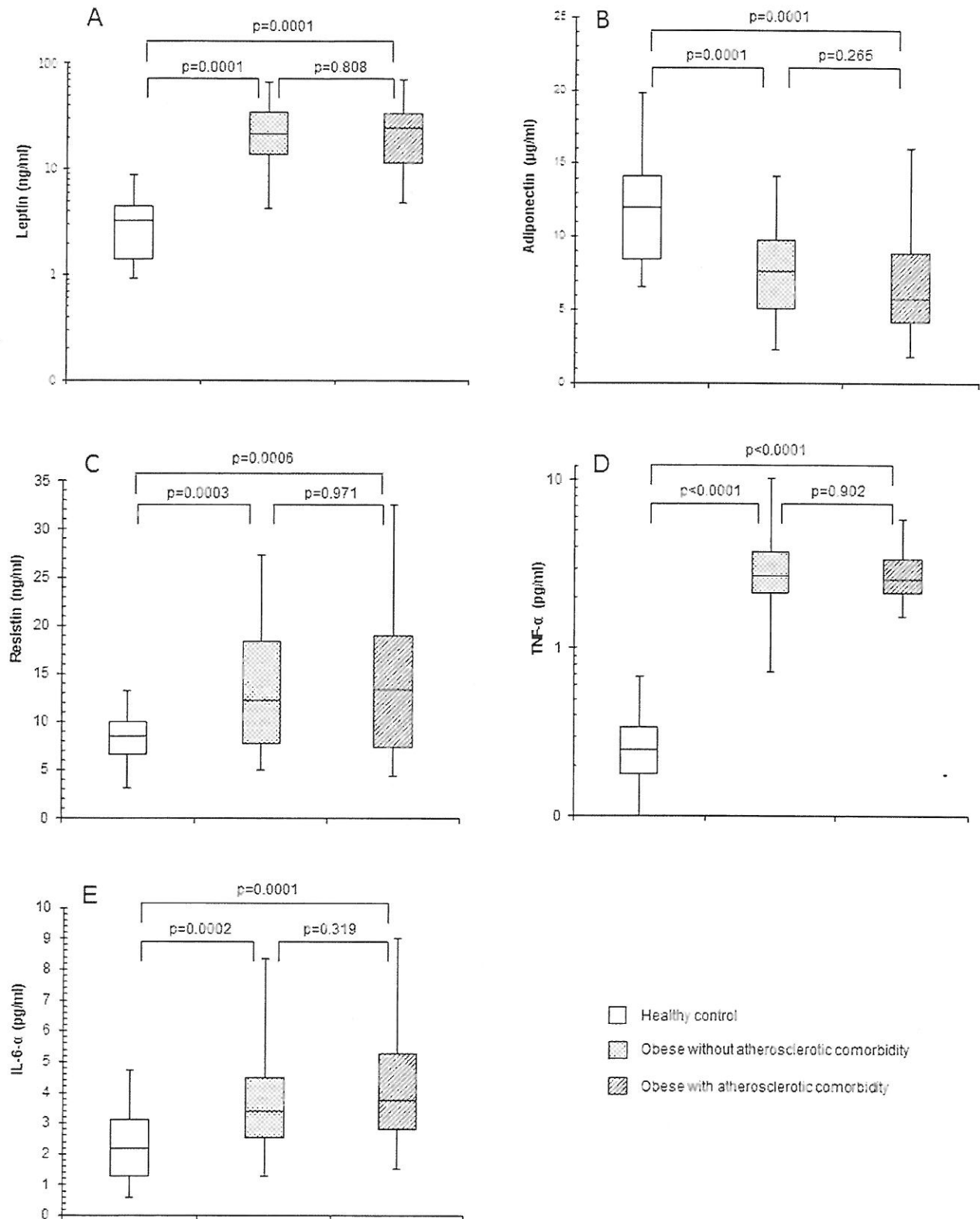


Figure 1 Comparison of leptin (A), adiponectin (B), resistin (C), TNF- α (D) and IL-6 (E) plasma levels among study groups. Differences in adipokine levels among study groups were tested using one-way ANOVA with Tukey post-hoc test. TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6.

Table 2 Correlation coefficients (Pearson's r) between adipokines and haemostatic markers in the whole investigated population.

	Age (years)	Leptin (ng/ml)	Resistin (ng/ml)	Adiponectin (μ g/ml)	TNF- α (pg/ml)	IL-6 (pg/ml)
Age (years)		0.013	0.18	0.14	0.03	0.11
Platelet parameters						
Platelet count (G/l)	0.02	0.52**	0.25**	-0.22*	0.28**	0.33**
MPV (fl)	0.11	0.39**	0.27*	-0.29*	0.37**	0.34**
Platelet P-selectin (%)	0.06	0.20	0.28*	-0.19	0.18	0.18
Soluble P-selectin (ng/ml)	0.04	0.23*	0.31**	-0.27*	0.17	0.10
PMPs (n/ μ l)	0.22**	0.44***	0.30**	-0.36***	0.35***	0.33**
Hypercoagulation markers						
Fibrinogen (g/l)	0.15*	0.58***	0.28**	-0.24*	0.39***	0.50***
FVIII activity (%)	0.11	0.35**	0.23*	-0.13	0.19	0.31**
Hypofibrinolysis marker						
PAI-1 ag (ng/ml)	0.09	0.45***	0.26**	-0.34**	0.64***	0.30**

Stars denoting varying level of significance: *p = 0.05–0.01, **p = 0.01–0.001, ***p < 0.001.

TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6; MPV: mean platelet volume; PMPs: platelet-derived microparticles; FVIII activity: factor VIII activity; PAI-1 ag: plasminogen activator inhibitor-1 antigen.

atherothrombotic process, resulting in increased cardiovascular morbidity and mortality. There is increasing evidence (largely from experimental studies) that obesity-associated altered adipokine levels are closely involved in the pathogenesis of atherosclerotic vascular diseases [5–10]. However, in humans, the impact of adipokines as a predictive marker and/or player in cardiovascular diseases are less clear than in experimental models, and still remain controversial [16]. Furthermore, in obesity, there are some crucial effects of adipokines on the main factors of atherosclerotic, atherothrombotic processes that are not fully understood, and are still being studied intensively.

In evaluating the alterations of adipokine plasma levels regarding the obesity-associated atherosclerotic concomitant diseases, it was ascertainable that in obese patients, existence of comorbid conditions known to predispose to atherosclerosis does not result in further significant changes in adipokine levels.

The current study analysed the relationships between adipokines and haemostatic markers, and IMT, trying to provide relevant information about the role of adipokines in the pathogenesis of atherogenesis in obese subjects.

Platelet count and MPV are widely available platelet parameters in clinical practice. Previous data are contradictory regarding the association between platelet count and cardiovascular outcomes. Thaulow et al. published earlier that coronary heart disease mortality is associated with increased platelet concentrations [17]. Moreover, higher platelet counts correlated with adverse clinical outcomes in patients with acute coronary syndrome [18]. In contrast, other population-based studies reported that platelet count was not associated with the occurrence of cardiovascular events [19,20]. MPV is a predictive biomarker of cardiovascular risk and prognosis [21]. Larger platelets are more reactive, and have greater haemostatic potential affecting platelet function.

Platelet number and size are mainly determined during megakaryocytopoiesis in the bone marrow. IL-6 plays a crucial role in stimulating megakaryocytopoiesis resulting in increased circulating platelet count and volume [21,22]. TNF- α may also influence thrombopoiesis via inducing *de*

novo synthesis of IL-6 [23]. Similarly, we found a significant positive association of IL-6 and TNF- α with the platelet count and MPV in obesity. Furthermore, in our present study, leptin and resistin also showed significant positive relationships with platelet number and size, whereas a significant negative correlation was detected between adiponectin and the above-mentioned platelet parameters. Leptin associated independently with these platelet indices.

Activated platelets play a central role in the pathogenesis of atherosclerosis and atherothrombotic events [24]. Sensitive markers of platelet activation, surface and soluble P-selectin, and PMPs, seem to be key components in linking vascular risk parameters to atherosclerosis development in obese individuals [15,25]. Although these platelet activation markers and adipokines are both deeply involved in atherogenesis, their associations are still unknown segments of the pathogenesis of atherosclerosis in subjects with obesity. In the present study, for the first time in the literature, we have demonstrated significant positive associations of resistin with platelet surface and soluble P-selectin. Furthermore, we have detected significant positive correlation of leptin with soluble P-selectin, while we have showed a significant negative association between adiponectin and soluble P-selectin. This study highlights the close relationship of all studied classic adipokines to PMPs with high thrombogenic activity. Additionally, based on multiple regression analysis, independent significant associations have been revealed between resistin and soluble P-selectin, and leptin and PMPs. These findings suggest, that the associations of adipokines with platelet activation parameters partially account for their atherogenic effects in obesity.

Fibrinogen, as an important component of the coagulation pathway, was positively associated with leptin, resistin and IL-6 and negatively with adiponectin, similar to others' observations [26–29]. Moreover, we demonstrated a significant positive association between fibrinogen and TNF- α in obese individuals. The cross-sectional significant positive associations of FVIII activity, a further key coagulation parameter, with leptin and IL-6 in this

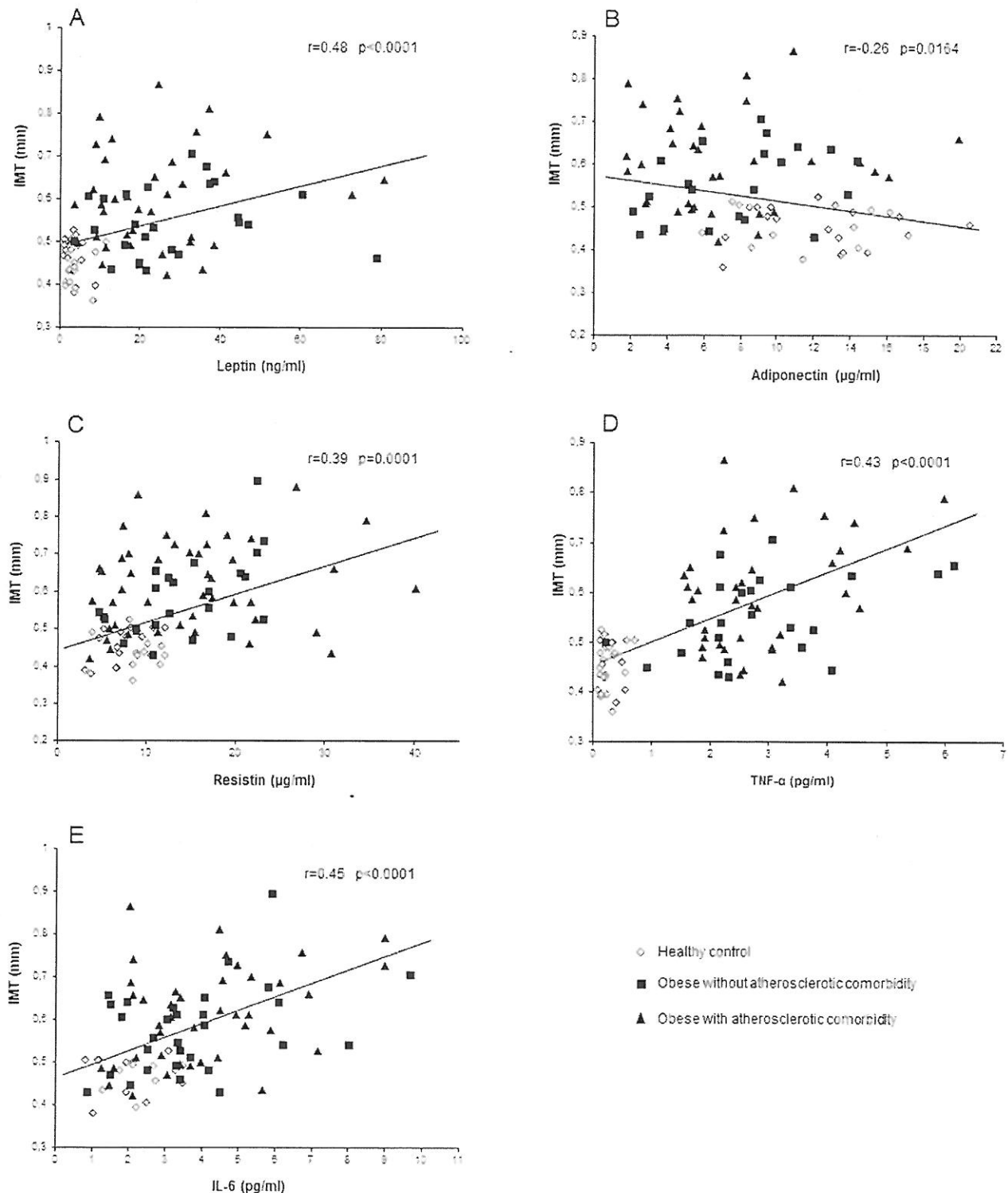


Figure 2 Correlation between IMT and the plasma levels of leptin (A), adiponectin (B), resistin (C), TNF- α (A) and IL-6 (B). IMT: common carotid intima-media thickness. TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6.

study was consistent with previous studies [26,28]. Interestingly, Wannamethee et al. [30] reported that the adiponectin level was positively correlated with FVIII; in our investigation, adiponectin was not found to be associated

with FVIII activity. In addition, we established a significant positive correlation between FVIII activity and resistin in obese subjects. We confirmed previous report that leptin is independently associated with both of these

Table 3 Multiple regression analysis for the association between A: haemostatic markers and adipokines (haemostatic markers as dependent variables); B: IMT and adipokines, and haemostatic parameters (IMT as dependent variable).

A							B	
	Platelet count (G/l)	MPV (fl)	Soluble P-selectin (ng/ml)	PMPs (n/μl)	Fibrinogen (g/l)	FVIII activity (%)	PAI-1 ag (ng/ml)	IMT (mm)
Leptin (ng/ml)	$\beta = 24.70^{***}$ CI: 15.07; 34.32	$\beta = 0.026^*$ CI: 0.004; 0.047		$\beta = 0.51^{***}$ CI: 0.34; 0.68	$\beta = 0.40^{**}$ CI: 0.17; 0.63	$\beta = 0.17^*$ CI: 0.012; 0.33		Leptin (ng/ml) $\beta = 0.086^{***}$ CI: 0.050; 0.123
Adiponectin (μg/ml)							$\beta = -1.25^*$ CI: -2.41; -0.091	Adiponectin (μg/ml) $\beta = -0.009^*$ CI: -0.016; -0.002
Resistin (ng/ml)			$\beta = 0.21^{**}$ CI: 0.08; 0.35					IL-6 (pg/ml) $\beta = 0.028^{**}$ CI: 0.014; 0.041
TNF- α (pg/ml)							$\beta = 10.87^{***}$ CI: 7.45; 14.28	MPV (fl) $\beta = -0.830^{***}$ CI: -1.16; -0.50
IL-6 (pg/ml)					$\beta = 0.11^*$ CI: 0.023; 0.19			PMPs (n/μl) $\beta = -0.008^{**}$ CI: -0.013; -0.003
Age (years)				$\beta = -0.02^*$ CI: -0.039; -0.001				FVIII activity (%) $\beta = -0.0006^*$ CI: -0.011; -0.0002
								Age (years) $\beta = 0.008^{***}$ CI: 0.006; 0.009

Stars denoting varying level of significance: * $p = 0.05-0.01$, ** $p = 0.01-0.001$, *** $p < 0.001$.

TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6, MPV: mean platelet volume; PMPs: platelet-derived microparticles; FVIII activity: factor VIII activity; PAI-1 ag: plasminogen activator inhibitor-1 antigen; IMT: common carotid intima-media thickness; β : standard regression coefficient, CI: 95% confidence interval.

hypercoagulation markers [26]. Moreover, we demonstrated an independent relationship between IL-6 and fibrinogen. In agreement with earlier findings, we detected a significant positive correlation of hypofibrinolysis displayed by elevated plasma PAI-1 ag level with leptin [31], TNF- α [32] and IL-6 [33], and a negative association between PAI-1 ag and adiponectin [34]. Furthermore, the current study revealed significant positive association between PAI-1 ag and resistin plasma level. TNF- α is a potent stimulator of the obesity-linked elevation of PAI-1 expression in adipose tissue and may substantially contribute to the increased plasma concentrations of PAI-1 observed in obesity [35]. In the present investigation, TNF- α had an independent relationship with PAI-1 ag in obese individuals. In addition, our study showed, that adiponectin is also an independent predictor of PAI-1 ag. Thus, our data provide further evidence that adipokines are closely associated with markers of hypercoagulability and impaired fibrinolysis.

According to previous studies [11–13], in obesity we found a significant positive association of IMT with leptin, and IL-6, and an inverse correlation with adiponectin. The present study demonstrates that obese adults, similar to obese children [14], have a significant positive relationship between resistin and IMT. Moreover, we showed here a possible influence of the plasma level of TNF- α on the initial phases of atherosclerosis in obese patients. In previous investigations of our research team, platelet surface and soluble P-selectin, and PMPs were significantly and

positively correlated to IMT (15). Corroborating previous data [36], fibrinogen was related significantly and positively to IMT. In the study by Arslan et al., a significant positive association between MPV and IMT was detected in obese adolescents [37]. We have verified this association in obese adults. Additionally, in our study patients, we demonstrated significant positive relationships of platelet count, FVIII activity and PAI-1 ag with IMT. Norata et al. reported that L:A ratio is an independent predictor of IMT in healthy subjects [38]. Our study could not confirm this independent association between L:A ratio and IMT in obese patients. The independent relationships of leptin, adiponectin, IL-6 and age with IMT were evident in our study similarly to previous reports [11–13,39]. Furthermore, our multiple regression analysis also retained MPV, PMPs and FVIII activity as independent predictors of IMT. These results suggest the possibility that disturbances in adipokine levels beside abnormalities in haemostasis may have direct unfavourable influences on the thickening of the arterial wall.

A limitation of our study is that we did not control the timing of the blood draw for measuring adipokine levels in relation to the pre-procedure meal. Accordingly, we cannot rule out if the dietary intake might contribute to the adipokine plasma levels by influencing its associations with the factors of atherosclerotic process. Furthermore, due to the cross-sectional nature of this study, we could not assess the cause-and-effect relationships between adipokines and haemostatic parameters, and IMT. Whether

adipokines play a causal role in the development of atherosclerosis in obese patients needs to be evaluated in upcoming follow-up studies. Further studies are also needed to clarify the role of adipokines in the long-term atherosclerotic cardiovascular outcome in obese individuals.

In conclusion, our study suggests a crucial role of altered adipokine plasma levels in common carotid atherosclerosis, and emphasizes the importance of associations between adipokines and haemostatic parameters in this pathogenetic pathway by which adipokines may significantly influence enhanced atherogenesis and increased cardiovascular risk in obese subjects regardless of manifest accompanying atherosclerotic diseases. Therefore, adipokines might serve as early biomarkers for increased atherothrombotic risk in obese patients. Accurately determining the direct effects of adipokines on atherosclerosis may significantly contribute to the explanation of high cardiovascular risk in obese individuals, and may reveal some promising specific vasculoprotective and therapeutic targets in the near future.

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Disclosure

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