Plasma Leptin in Patients at Intermediate to High Cardiovascular Risk With and Without Type 2 Diabetes Mellitus

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Background: A number of clinical studies have demonstrated that leptin concentrations are related to the metabolic disturbances that constitute the metabolic syndrome (MetS) and to diabetes mellitus (DM). **Aim:** To investigate possible determinants of leptin concentrations in a sample of patients at high cardiovascular (CV) risk carrying two or more features of the MetS and to investigate if any difference exist between at risk patients with or without DM. **Methods:** Serum leptin concentrations were measured in 60 consecutive male patients affected by at least two CV risk factors which belong to the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III) definition of MetS: 30 patients affected by type 2 DM (T2DM) and 30 nondiabetic patients (non-T2DM). Nineteen healthy subjects were included in the study as a control group (HC). **Results:** Leptin was

significantly higher in patients carrying two or more features of the MetS compared with HC ($P = 0.02$). Stratifying MetS patients for DM, we found that leptin level was higher in non-T2DM patients (7.8 ng/ml), intermediate in T2DM (6.2 ng/ml), and lower in HC (4.6 ng/ml). In MetS patients, a positive correlation was found between leptin and waist, triglycerides, and number of MetS criteria. After stratification for T2DM, the correlations were still significant in the non-T2DM but not in the T2DM group. **Conclusions:** In our sample of moderate-to-high-risk patients, leptin level is positively associated with waist circumference and triglycerides but only in non-T2DM patients. Our data suggest that diabetic subjects could modulate leptin production in a different way compared with patients carrying other MetS-related anomalies. *J. Clin. Lab. Anal.* 31:e22031, 2017. © 2016 Wiley Periodicals, Inc.

Key words: cardiovascular risk; diabetes mellitus; leptin; metabolic syndrome; MetS

INTRODUCTION

Leptin is a 146 amino acid long protein member of the helical cytokine family mainly produced by adipocytes (1, 2). Due to this origin, the circulating levels of leptin are highly influenced by fat mass (3).

In physiological conditions, leptin is a beneficial hormone, which works by activating AMP-activated protein kinase and by reducing the activity and expression of lipogenic enzymes, as the malonyl CoA (4). By lowering malonyl CoA, leptin maintains fatty acid oxidation at an appropriate level and prevents lipotoxicity. Moreover, since leptin provide to the central nervous system a signal of energy intake and energy

stores in the body, the hypothalamus can efficiently maintain a stable body weight (5). Nevertheless, obese subjects produce high amount of this hormone but seem to have an inadequate response to it, showing a relative leptin resistance (6–9).

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Several investigations have reported that leptin is involved in atherogenesis and that high leptin levels result associated positively with the risk of coronary heart disease due to its numerous effects: the induction of oxidative stress in endothelial cells, the accumulation of cholesterol esters into foam cells, the increase in blood pressure, and the effects on hemostatic factors (10–16). In addition, leptin interferes with lipoprotein profiles (17).

The metabolic syndrome (MetS) comprises a cluster of cardiometabolic risk factors, such as central obesity, dyslipidemia, glucose intolerance, and hypertension, which increases the morbidity and mortality of cardiovascular (CV) disease and type 2 diabetes mellitus (T2DM) (18, 19). In particular, the visceral obesity, evaluated by mean of waist circumference (WC), is a common feature of MetS (20) and it is included in both the major definitions provided by the International Diabetes Federation (21) and the revised National Cholesterol Education Program (22).

Subjects with the MetS have higher leptin levels compared with individuals without the MetS and leptin might be useful as diagnostic marker of MetS (23–25). To date, it is not clear if relationship between leptin and MetS is mainly mediated by obesity or if high leptin levels could be associated with MetS independently of visceral obesity (23, 26).

The aim of our study was to investigate major determinants of fasting serum leptin concentrations in patients at high CV risk and to test if its levels are significantly different in patients with overt diabetes mellitus (DM).

PATIENTS AND METHODS

Study Protocol

To have a more homogeneous sample, we collected only MetS men, since it has been observed that women, because of the estrogens effect, have threefold higher median concentrations of leptin than men (27).

Sixty consecutive male patients affected by at least two CV risk factors which belongs to the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III) (22) definition of MetS were recruited: 30 patients were affected by type 2 diabetes mellitus (T2DM) and 30 were nondiabetic patients (non-T2DM). Nineteen healthy, young, normal weight subjects (median [min-max] age: 43 (22–56) years; BMI: 23 [20.7–26.6] kg/m²) recruited between blood donors and free from MetS were included in the study as a control group (HC).

The five criteria proposed by the NCEP/ATP III for MetS definition and used in this study were: elevated

waist circumference (≥ 102 cm in men), hypertriglyceridemia (> 1.7 mmol/l), low HDL cholesterol level (< 1.03 mmol/l in men), high BP (systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg and/or pharmacological treatment), and impaired fasting plasma glucose (≥ 6.1 mmol/l). When three of five of the listed characteristics were present, a diagnosis of MetS was made. According to these criteria, 50 patients were affected by MetS.

DM was defined according to the American Diabetes Association guidelines (28): fasting plasma glucose (FPG) ≥ 126 mg/dl or random plasma glucose ≥ 200 mg/dl or 2-h plasma glucose ≥ 200 mg/dl during an oral glucose tolerance test or HbA1C $\geq 6.5\%$. For the diagnosis of DM, these criteria should be confirmed by repeat testing on a different day. In the non-T2DM group, one subject displayed an HbA1C value of 6.7%. However, this value has not been confirmed by repeat testing.

All T2DM patients were treated with oral antidiabetic drugs: only metformin ($n = 11$) or combined with repaglinide and/or pioglitazone and/or rosiglitazone ($n = 19$).

BMI was calculated as the ratio of the weight in kilograms to the square of the height in meters (kg/m²). Waist circumference was measured with the patient standing at the umbilicus level. BP was measured twice by specially trained nurses in the right brachial artery in the supine position after 5 min rest using a mercury sphygmomanometer. The systolic BP was defined by 'phase I' and the diastolic BP defined by 'phase V' Korotkoff sounds.

The Ethics Committee of the Medical Faculty of Verona University approved the study. Each patient and control gave a written informed consent for participating to the study.

Patients and controls' demographic, clinical, and biochemical data are presented in Table 1.

Laboratory Testing

Blood samples were collected in the morning after an overnight fasting and a resting period of 20 min in vacuum tubes without silica-based clot activators and in vacuum tubes with heparin as anticoagulant (Becton-Dickinson, Oxford, UK). After centrifugation at 1500 g for 10 min at room temperature, serum and plasma were separated, stored in aliquots, and kept frozen at -70°C until measurement.

Serum leptin concentrations were determined by mean of a solid-phase enzyme-linked immunosorbent assay (ELISA) (DRG Instrument GmbH, Marburg, Germany). Intra- and interassay coefficient of variation (CV) for this method comprised between 5.9% and 6.9%, and between 8.6% and 11.5%, respectively.

TABLE 1. Demographic, Clinical, and Biochemical Data of Male Patients Affected by at Least Two Features of Metabolic Syndrome With or Without Type 2 Diabetes

	Non-T2DM	T2DM	<i>P</i> -value	HC
<i>N</i>	30	30		19
Age, years	64.5 (39.0–81.0)	60.0 (44.0–68.0)	0.06	47.0 (22.0–68.0)
Waist circumference, cm	109.0 (94.0–133.0)	115.0 (90.5–130.0)	0.47	–
BMI, kg/m ²	30.1 (23.5–42.9)	32.0 (23.7–39.9)	0.31	23.0 (20.7–26.6)
SBP, mmHg	131.5 (100.0–196.0)	140.0 (110.0–180.0)	0.14	125.0 (110–130)
DBP, mmHg	81.5 (60.5–113.5)	80.0 (70.0–95.0)	0.51	80.0 (70.0–85.0)
Total cholesterol, mg/dl	176.5 (84.0–333.0)	163.0 (103.0–250.0)	0.041	176.0 (122.0–212.0)
HDL, mg/dl	40.0 (20.0–61.0)	48.0 (33.0–72.0)	0.022	51.0 (34.0–85.0)
LDL, mg/dl	106.2 (46.4–211.4)	89.1 (38.8–145.6)	0.006	104.4 (64.0–140.8)
Triglycerides, mg/dl	147.0 (66.0–418.0)	138.0 (45.0–328.0)	0.45	77.0 (52.0–178.0)
Triglycerides/HDL cholesterol	3.6 (1.2–11.0)	2.7 (0.8–9.8)	0.11	1.7 (0.7–5.5)
FPG, mg/dl	106.5 (83.0–143.0)	153.0 (109.0–258.0)	0.001	107.0 (86–123.0)
hs-CRP, mg/l	2.9 (0.5–49.3)	1.5 (0.2–9.0)	0.009	1.2 (0.1–11.7)
HbA1C, %	6.0 (5.2–6.7*)	7.1 (5.8–11)	0.001	5.6 (4.8–6.2)

Values are expressed as median (min–max). Results of measurements between groups were compared by Mann–Whitney test.

*In the non-T2DM group one subject displayed an HbA1C value of 6.7%. However, this value has not been confirmed by repeat testing.

Bold values represent statistically significant differences.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HC, healthy controls; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FPG, fasting plasma glucose; hs-CRP, high-sensitive C-reactive protein.

Total cholesterol, HDL, triglycerides, and FPG were measured on the Modular System P (Roche Diagnostics GmbH, Mannheim, Germany). Total imprecision, as expressed by the CV, comprised between 3% and 6% for all the measured analytes.

LDL cholesterol was calculated from the values for triglycerides, total cholesterol, and HDL cholesterol according to the Friedewald formula: LDL = total cholesterol – (HDL + triglycerides/5) (29).

High-sensitive C-reactive protein (hs-CRP) was measured by the reference nephelometric assays on a Behring Nephelometer (BN, Dade Behring GmbH, Marburg, Germany).

Statistical Analysis

Values were reported as median and min–max range. Results of measurements and relative values distributions between groups were compared by Kruskal–Wallis or Mann–Whitney tests. The Spearman coefficient was used to study correlation between continuous variables. The level of statistical significance was set at $P < 0.05$. All calculations were performed either with SPSS (version 11.5) statistical package (GraphPad Software Inc., San Diego, CA) and GraphPad Prism 5.0.

RESULTS

Leptin was significantly higher in patients carrying two or more features of the MetS ($n = 60$) compared with controls ($n = 19$) (6.7 vs. 4.6 ng/ml, $P = 0.02$) (Fig. 1a).

In patients carrying only two features of the MetS ($n = 10$), leptin concentration resulted significantly lower compared with patient affected by MetS ($n = 50$) (4.3 vs. 7.6 ng/ml, $P = 0.01$).

Stratifying MetS patients for diabetes status, we found that leptin level was higher in non-T2DM patients (median [min–max]: 7.8 [3.4–18.3] ng/ml), intermediate in T2DM (6.2 [2.1–13.3] ng/ml), and lower in healthy controls (4.6 [3.6–10.7] ng/ml), even if difference between T2DM and the other groups did not reach statistical significance (T2DM vs. non-T2DM $P = 0.07$; T2DM vs. HC $P = 0.052$; non-T2DM vs. HC $P = 0.003$; see Figure 1b).

In MetS patients, a positive correlation was found between leptin and waist ($r = 0.34$; $P = 0.007$), triglycerides ($r = 0.34$; $P = 0.009$), and number of MetS criteria ($r = 0.38$, $P = 0.003$) but not with BMI, HDL, fasting plasma glucose, HbA1C, Hs-CRP, and systolic and diastolic blood pressure. After stratification for T2DM, the correlations were still significant in the non-T2DM but not in the T2DM group (see Figs 2 and 3).

DISCUSSION

In accordance with previously published studies (23–25), also in our sample, leptin concentration was significantly higher in the whole group of 60 patients carrying features of MetS compared to healthy controls.

In example, Ukkola et al. (30) have demonstrated that in male patients, metabolic abnormalities are associated with high leptin concentrations and that leptin levels are predictors of MetS. Indeed, similarly

Fig. 1. Fasting plasma leptin concentrations in healthy controls (HC) compared with patients carrying two or more features of the MetS (a) and compared with patients carrying two or more features of the MetS stratified for diabetes status (b).

Fig. 2. Spearman correlation between fasting plasma leptin concentrations, waist, and triglycerides values in T2DM and non-T2DM patients.

to the latter study, we found a positive correlation between leptin and the number of metabolic abnormalities which characterize the ATPIII definition of MetS especially in non-T2DM patients.

The increase in leptin concentrations in MetS patients compared with HC ($P = 0.003$) could be justified by the pathogenetic link between leptin and each of MetS features (i.e., hypertension, obesity, dyslipidemia) (9). Accordingly, in MetS non-T2DM group, but not in the T2DM group, leptin resulted significantly positively correlated with waist ($r = 0.52$) and

triglycerides ($r = 0.37$) values. Moreover, it has been previously demonstrated that leptin mediates the increase in blood pressure in obese patients (31).

When we analyzed separately patients with and without T2DM only, non-T2DM had significantly higher leptin concentration as compared with HC whereas leptin level in T2DM was intermediate; moreover, plasma leptin levels were not correlated either to fasting plasma glucose or glycated hemoglobin. All these data suggest that T2DM within the MetS could connote a different condition about leptin “homeostasis”.

Fig. 3. Relationship between fasting plasma leptin concentrations and number of MetS criteria in T2DM and non-T2DM patients.

Other studies have reported that serum leptin levels can be even lower in both type 1 (32) and type 2 (33) diabetics than in controls. Our control group was matched for gender but not for age and BMI, two characteristics that can influence leptin level, whereas the non-T2DM and T2DM groups were quite homogeneous making the comparison more reliable. Thus, the trend toward higher leptin level in non-T2DM patients compared with T2DM requests an in-deep analysis.

Even if we did not observe significant differences in BMI and waist between these groups, many other studies have indicated that adipose tissue distribution could be one of the culprits: already in the 1980s, it was observed that leptin levels were positively correlated with the amount of body fat (34) and BMI (35).

However, some MetS patients may have normal BMI (36) and subjects with similar BMI may display a marked variability in plasma leptin levels (37) making difficult to observe the association.

In particular, recent studies, evaluating samples of subcutaneous and visceral fat by elective surgery, found that especially subcutaneous fat expresses higher amount of leptin compared with visceral fat both in T2DM (38, 39) and obese subjects (40).

Instead, in our sample, leptin correlates with waist and not with BMI, at least in non-T2DM patients, suggesting that leptin level could be more directly associated with central/visceral than with total/subcutaneous body fat. Unfortunately, we do not have direct measure of subcutaneous and visceral fat and waist circumference measures not only visceral but also abdominal subcutaneous fat, one of the preferential site of leptin production.

Also, Abdelgadir and others have hypothesized that the decrease in amount of leptin in diabetics could be a consequence of differences in fat distribution (33, 41), however, diabetics usually have increased visceral fat and less subcutaneous fat (42). Another possible explanation for lower leptin level in diabetics could be that, since the leptin metabolism is interrelated with

insulin secretion and action (43), insulin synthesis suppression characteristic of the advanced T2DM lead also to the suppression of leptin production (35, 44, 45), thus matching the initial increase and late decrease in plasma insulin concentrations of T2DM (46).

Thus, our data in T2DM cannot support the “fat hypothesis” since neither waist nor BMI correlate with leptin in T2DM patients and suggest that a diabetes-specific mechanism could interfere with leptin production and or metabolism. Unfortunately, we do not have data on insulin to verify if the trend observed for leptin in T2DM patients follow the distribution in insulin concentrations.

With regard to adipokines, it has been recently demonstrated that adiponectin is more strongly associated with T2DM risk than leptin (47).

In addition, we cannot exclude an antidiabetic drugs effect on leptin levels, even if, in other samples, no difference was observed in leptin levels between diabetic subjects treated with sulfonylurea and those treated with diet alone (33), and it was reported that leptin do not change following treatment with rosiglitazone and pioglitazone (48). We have to underline that none of our T2DM patients was under insulin therapy, which can directly influence leptin secretion (35).

Then, we have demonstrated a correlation between fasting leptin and triglycerides values. Accordingly, it has been demonstrated that leptin influences the rate of synthesis and degradation of lipids (49, 50). Leptin exerts both a direct action, by inhibiting in adipocytes the synthesis of acetyl-CoA carboxylase, an enzyme essential in the conversion of carbohydrates to long-chain fatty acids, and an indirect effect on lipid metabolism by means of the reduction in the lipogenic effects of insulin (49, 50).

Our results are in accordance with that of Dubey et al. (51), which suggests a role of leptin resistance in the pathogenesis of dyslipidemia. Contrarily, Ostlund et al. (52) did not find a significant relationship between leptin and lipids.

Contrary to previously published studies (27, 53), we did not find a correlation between leptin values and hs-CRP. However, Morteza et al. (54), by investigating leptin and hs-CRP concentrations in both newly diagnosed and long-standing T2DM patients, reported that there was no significant correlation between leptin and CRP in patients with long-standing diabetes.

In conclusion, in a sample of patients at moderate to high CV risk, carrying at least two features of MetS, leptin level is positively associated with waist and triglycerides but only in non-T2DM patients. Our data suggest that diabetes per se could influence leptin production/metabolism in a different way compared with other MetS-related anomalies. A leptin modulating effect of oral antidiabetic drugs cannot be completely excluded.

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