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The association of anthropometric parameters with markers of insulin and leptin secretion and resistance in type 2 diabetes mellitus

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Abstract

Aim: *We evaluated the association between anthropometric parameters and markers of insulin and leptin secretion/resistance in patients with type 2 diabetes mellitus (T2DM).* ***Material and methods****: This post-hoc data analysis from a cross-sectional study included 176 T2DM patients. Laboratory tests (serum leptin, soluble form of leptin receptor (sObR), C peptide, glycemic and lipid parameters) and anthropometric parameters were obtained, adiposity indexes (including body adiposity index (BAI), visceral adiposity index (VAI)), indicators of insulin resistance, β-cell function, and leptin resistance (Free Leptin Index, FLI) were calculated.* ***Results****: The body mass index (BMI), diabetes duration, VAI and leptin correlated independently with HOMA-IR, while BMI, diabetes duration and HbA1c with HOMA-B. The total body fat mass (TBFM), C peptide, diabetes duration, BMI and BAI correlated with leptin concentrations, while the first three with FLI. VAI was an indicator of insulin resistance (β=0.166, p=0.003), while BAI of leptin secretion (β=0.260, p=0.010). TBFM strongly associated with leptin resistance and secretion (β=0.037, r=0.688, p<0.0001, and β=0.521, r=0.667, p<0.0001), and BMI correlated weakly with insulin secretion and resistance. While insulin and leptin secretion increased progressively with BMI, leptin and insulin resistance became significant only in case of obesity. The sObR was significantly associated with C peptide concentrations (β=-0.032; p=0.044), but not with HOMA-B or -IR. A strong positive correlation between the C peptide/leptin ratio and non-fat mass /TBFM ratio was noted (r=0.62 [0.52, 0.71], p<0.0001).*

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Conclusions: Parameters of peripheral adiposity correlated better with markers of leptin system, and those of visceral adiposity with markers of insulin secretion/resistance. The sObR correlated independently and negatively with C peptide.

Keywords: *type 2 diabetes; insulin resistance; leptin system; anthropometric parameters*

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Introduction

Obesity is associated with a significant increase in morbidity and mortality by a number of conditions, such as cardiovascular, metabolic, gastro-intestinal, oncologic diseases, etc (1). That is because in addition to being an energy depot, the adipose tissue mediates the regulation of many organs and tissues in an auto- or paracrine fashion, thus playing a significant role in the complex interorgan crosstalk (2). In fact, the adipose tissue functions as an active secretory organ that produces adipokines, including proinflammatory cytokines/chemokines or hormones, but also other signalling molecules like non-coding RNAs, and extracellular vesicles that are involved in metabolism, vascular homeostasis, and others (3).

One of these adipokines is leptin, a polypeptide hormone, secreted in concentrations proportional to body fat mass, that plays an important role in a number of physiological functions like energy homeostasis, immunity and reproduction, with possible implication in other conditions (such as hepatic steatosis, depression etc.) (4-6). Leptin is required to maintain normal body weight, as it lowers food intake and increases energy expenditure (4). There is evidence that leptin suppresses insulin secretion from pancreatic β cells, thus modulating glucose homeostasis and fat deposition, and on the other hand, leptin expression is enhanced by insulin (7, 8). In fact, between β cells and adipose tissue it seems to be a bi-directional feedback (7, 8).

Obese individuals, however, might express high serum leptin concentrations, but fail to properly control food intake and regulate the body energy reserve, thus exhibiting leptin resistance (9). Moreover, high leptin levels that fail to regulate insulin secretion might suggest leptin resistance at the pancreatic β-cell level (7).

The body fat distribution plays an important role in modulating metabolism, because it was shown that the visceral adipose tissue (VAT) is metabolically more active, insulin-resistant and more sensitive to lipolysis than the subcutaneous adipose tissue (SAT) (10). While the correlations of abdominal obesity with insulin resistance is well known, fewer data exist regarding the association of anthropometric parameters with leptin system/leptin resistance in patients with type 2 diabetes mellitus (T2DM). Additionally, the body mass index (BMI), waist circumference and waist-to-hip ratio (WHR) are most commonly used measures of adiposity, while other/newer parameters and indexes are less investigated in these patients.

The aim of our analysis was to investigate the correlations of anthropometric measurements and indexes with leptin system/leptin resistance, and with insulin resistance and pancreatic β cell function in adult individuals with T2DM.

Material and methods

Study participants. This was a post-hoc analysis of data collected in an observational cross-sectional study performed during 2017, that evaluated nonalcoholic fatty liver disease and depression/anxiety in patients with T2DM. Patients were recruited from the Outpatient Diabetes Unit of the Emergency County Clinical Hospi-

tal Târgu Mureș. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș and all subjects signed an informed consent before participating. This analysis evaluated the correlations between anthropometric and metabolic data collected in the study. Details regarding study participants, inclusion/exclusion criteria have been previously published (5).

Medical and demographic data were collected as previously described, and included anthropometric parameters, i.e. weight, height, waist circumference, hip circumference, skinfold thickness at four anatomical sites (biceps, triceps, subscapular and suprailiac), all measured by standard methods (1, 5, 6). Two measurements were averaged for each skinfold.

Laboratory evaluation included serum hormonal and metabolic parameters: leptin, soluble form of leptin receptor (sObR), C peptide, glucose, lipid panel tests, glycated hemoglobin (HbA1c), and other laboratory parameters that are not of particular interest for this analysis. Details regarding specimen collection and processing, as well as laboratory analysis, including assays and technology used, have been described in detail before (5). The serum leptin normal values suggested by the manufacturer were 3.84 (±1.79) ng/ml for males and 7.36 (±3.73) ng/ml for females. We have used these thresholds to define hyperleptinemia.

Calculations. Based on collected raw data, calculations were performed to estimate leptin and insulin resistance, β cell function/insulin secretion and several body adiposity indexes.

The Homeostasis Model Assessment (HOMA) calculator version 2.2.3 was used to estimate insulin resistance (HOMA-IR) and pancreatic β cell function (HOMA-B), based on serum C peptide and glucose levels (11). Leptin resistance was estimated by the Free Leptin Index (FLI), as the ratio of serum leptin to sObR concentrations (12).

Total, visceral and peripheral body adiposity was assessed by several indexes. The BMI was calculated with the standard formula as body weight divided by height^2 (kg/m^2), while the total body fat mass (TBFM) by body weight x percent body fat (%BF)/100. The %BF was computed by using the Durnin & Womersley equation that estimated body density based on Σ4SF (the sum of four skinfolds) and then the Siri equation that estimated %BF based on body density (13, 14). The total non-fat mass (NFM) was calculated by the difference between body weight and TBFM, and the %NFM as NFM/body weight x 100. The resting energy expenditure (REE) was calculated with the Harris Benedict formula (15). The WHR and the waist-to-height ratio (WHtR) were also calculated.

The body adiposity index (BAI), suggested to be a more reliable indicator of body adiposity, was defined as (hip circumference)/(height$^{1.5}$ − 18) (16). As an additional estimate of central adiposity and visceral adiposity function, the visceral adiposity index (VAI) was calculated using two gender-specific equations (17):

VAI = (waist/ 36.58 + (1.89xBMI)) x (TG/0.81) x (1.52/HDL cholesterol) (for females),

VAI = (waist/ 39.68 + (1.88xBMI)) x (TG/1.03) x (1.31/HDL cholesterol) (for males), where triglyceride and HDL cholesterol levels are expressed in mmol/l, for which conversions were performed from mg/dl. Previously described age-specific threshold values were used to identify adipose tissue dysfunction (ATD) (18).

Statistical analysis. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and those non-normally distributed were presented as median (min-max). The normality of data was checked with the Kolmogorov-Smirnov goodness-of-fit test and the Grubbs test was employed to identify outliers. The comparison between means or medians was performed by the Student t-test, Mann-Whitney, ANOVA or Kruskal-Wallis tests,

as appropriate, and the Dunn or Tukey multiple comparison post-test was employed to identify significant differences between groups. The relationships between variables were tested by the Pearson's and Spearman's tests, respectively and data presented as r (95%CI). The z score was calculated to test the differences between two independent correlation coefficients based on formula 2.8.5 from Cohen and Cohen (19, 20). Several variables suggested by bivariate analysis and by clinical knowledge were included in multivariate regression analyses to identify those independently associated with the outcome.

The tests were two-tailed and statistical significance was set at $p<0.05$. GraphPad InStat3 was used for statistical analysis.

Results

We have analyzed data from 176 T2DM subjects. Based on the gender-specific thresholds mentioned above we have identified patients with hyperleptinemia and analyzed the metabolic and anthropometric parameters accordingly (supplementary table 1 available on-line: DOI:10.2478/rrlm-2020-0028). T2DM patients with hyperleptinemia (62.5%) had slightly longer duration of T2DM, lower sObR and higher FLI values (suggesting more important leptin resistance) and higher REE (supplementary table 1 available on-line: DOI:10.2478/rrlm-2020-0028). With the exception of NFM, WHR and VAI, patients with hyperleptinemia had significantly higher values for all anthropometrical parameters (mainly indicators of total and peripheral adiposity) (supplementary table 1 available on-line: DOI:10.2478/rrlm-2020-0028).

Gender-based baseline characteristics are presented in supplementary table 2. T2DM female patients had slightly longer duration of diabetes and higher HDL cholesterol concentrations, but no other differences were noticed with regard to glucose or lipid metabolism. Although the BMI was similar, female T2DM patients had higher TBFM, %BF, Σ4SF, BAI, and lower NFM, %NFM than males (supplementary table 2). Despite having more TBFM than males, there were no significant differences in the insulin secretion/resistance between genders (supplementary table 2).

a. Markers of insulin and leptin secretion/resistance in relationship to parameters of total body adiposity (TBFM and BMI)

In order to investigate the relationship between insulin and leptin markers with anthropometric parameters, the TBFM was divided in quartiles (Q1-4) (IQR: 25.42, 31.63, 36.96 and 65.89 kg), and the BMI separated in the following categories: <27, 27-29.9, 30-34.9 and ≥35 kg/m^2, respectively.

The C peptide concentrations increased slightly with higher TBFM (2.47±1.15 vs 3.10±1.47 vs 2.87±1.43 vs 3.47±1.55 ng/ml, $p<0.01$), but with values being significantly different only for Q1 vs Q4 ($p<0.01$). A similar trend was observed in relationship with the BMI (1.92±0.97 vs 2.75±1.22 vs 2.99±1.41 vs 3.58±1.56 ng/ml, $p<0.001$), and the C peptide values were significantly different for BMI<27 vs ≥30 and 35 kg/m^2 ($p<0.05$ and <0.001, respectively). However, the HOMA-B values increased with higher BMI, but not with TBFM (figure 1a and 1b). At the same time, HOMA-IR increased with BMI and with TBFM (figure 1c and 1d). Mean HOMA-IR values >2.5 (which indicate significant insulin resistance) were seen in case of BMI≥30 kg/m^2. Significantly lower HOMA-IR values were observed for lowest vs highest TBFM quartiles.

With increasing TBFM, the serum leptin levels raised more abruptly (figure 2a). A similar trend was observed with higher BMI (figure 2b). The sObR levels decreased with higher BMI (27.52±11.43 vs 24.24±8.55 vs 18.95±4.88 vs 16.28±3.44 ng/ml, $p<0.0001$) and higher TBFM (25.99±9.96 vs 21.88±6.61 vs 17.51±3.38 vs 16.03±3.52 ng/ml, $p<0.0001$). The FLI however increased with higher BMI, but only over 30 kg/m^2,

while the increase was progressive from the lowest to the highest TBFM quartile (figure 2c and 2d).

b. Markers of insulin and leptin secretion/ resistance in relationship to parameters of peripheral body adiposity

The median value for BAI (rather an indicator of peripheral body adiposity) in our sample population was 33.74. We have compared the markers of interest for BAI below and above the median value. There were no differences with regard to metabolic control between the two groups (data not shown).

The Cpeptide values were no significantly different between the two groups (3.06 (0.33-7.06) vs 2.62 (0.29-5.96) ng/ml, p: 0.052), nor were the HOMA-IR values (2.62 (0.44-6.06) vs 2.23 (0.43-5.18), p: 0.105). However, the HOMA-B values were higher in the high BAI group (79.85±35.0 vs 67.50±32.1%, p<0.05) (supplementary figure 1a).

Higher BAI values were associated with higher serum leptin levels (11.35 (4.3-49.8) vs 3.9 (0.9-44.9) ng/ml, p<0.0001), lower sObR concentrations (16.6 (11.2-39.9) vs 21.0 (12.1-65.7) ng/ml, p<0.0001), and higher FLI (0.74 (0.19-3.98) vs 0.18 (0.01-2.08), p<0.0001) (supplementary figure 1a).

c. Markers of insulin and leptin secretion/ resistance in relationship to parameters of visceral body adiposity

VAI and WHtR were used as indicators of visceral adiposity. The VAI was analyzed according

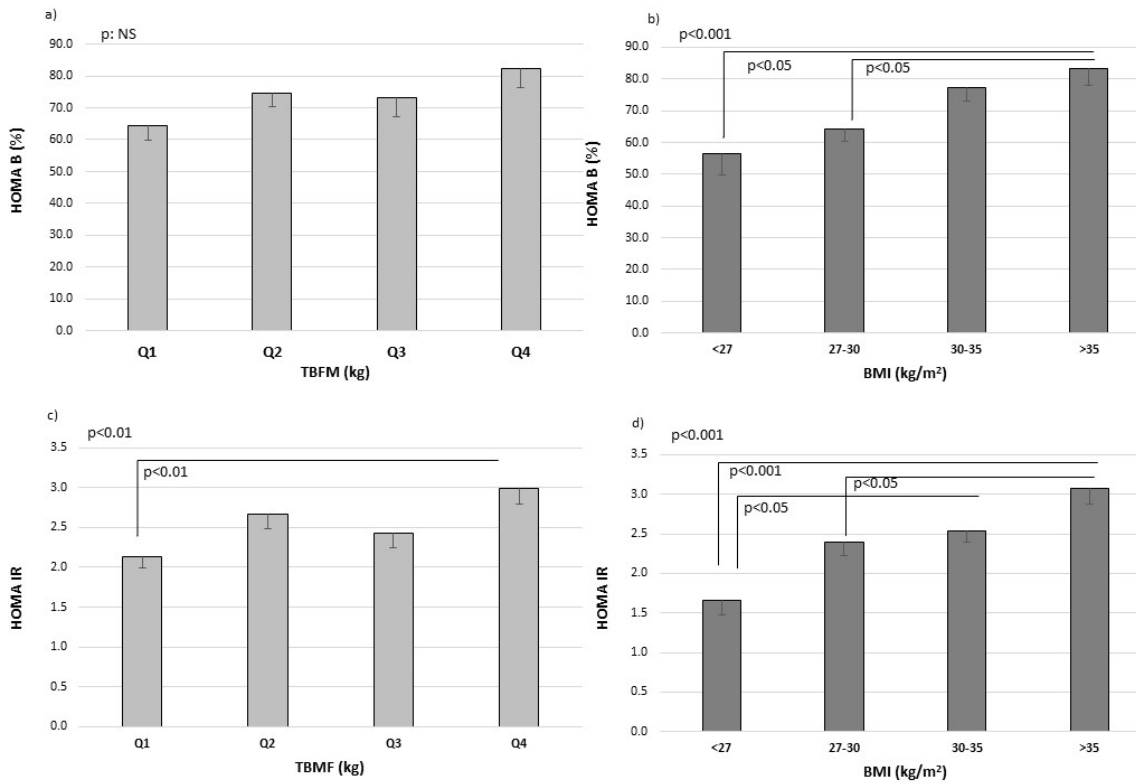

Fig. 1. Insulin secretion (HOMA B) in relation with a) TBFM quartiles and b) BMI intervals, and markers of insulin resistance (HOMA IR) in relation with c) TBFM quartiles and d) BMI intervals (BMI: body mass index; TBFM: total body fat mass; data are means ± SE).

to previously published gender-specific thresholds, as mentioned.

T2DM subjects with increased VAI values had higher Cpeptide concentrations (3.32±1.6 vs 2.66±1.2 ng/ml, p<0.01) and higher HOMA-IR values (2.84±1.37 vs 2.29±1.06), p<0.01), but the HOMA-B was similar between groups (78.79±35.5 vs 69.20±32.3%, p: 0.064) (supplementary figure 1b).

There were no significant differences between groups with regard to FLI (0.50 (0.05-2.32) vs 0.31 (0.01-3.98), p: 0.053) or serum leptin levels (9.1 (1.4-26.8) vs 6.6 (0.9-49.8) ng/ml, p: 0.116) (supplementary figure 1b), but the sObR concentrations were slightly lower (17.8 (11.2-40.8) vs 20.1 (11.8-65.7) ng/ml, p<0.05).

According to the WHtR, subjects were classified as being obese (>54 in females and >58 in males) and non-obese. Obese T2DM patients had higher HbA1c values (6.5 (5.1-12.4) vs 6.0 (5.2-6.8) %, p<0.01), but still in the target range. They also had higher C peptide levels (2.81 (0.29-7.06) vs 1.88 (0.34-3.78) ng/ml, p<0.01) and HOMA-IR (2.49 (0.43-6.06) vs 1.42 (0.46-3.0), p<0.01), but the HOMA-B values were not significantly different between the two groups (74.49±34.3 vs 62.02±28.6%, p: 0.241). Obese individuals presented higher serum leptin concentrations (8.5 (0.9-49.8) vs 3.5 (1.9-6.5) ng/ml, p<0.001), FLI (0.45 (0.01-3.98) vs 0.16 (0.08-0.20), p: 0.0001), and lower sObR values (18.6 (11.2-65.7) vs 25.1 (18.7-40.8) ng/ml, p<0.001).

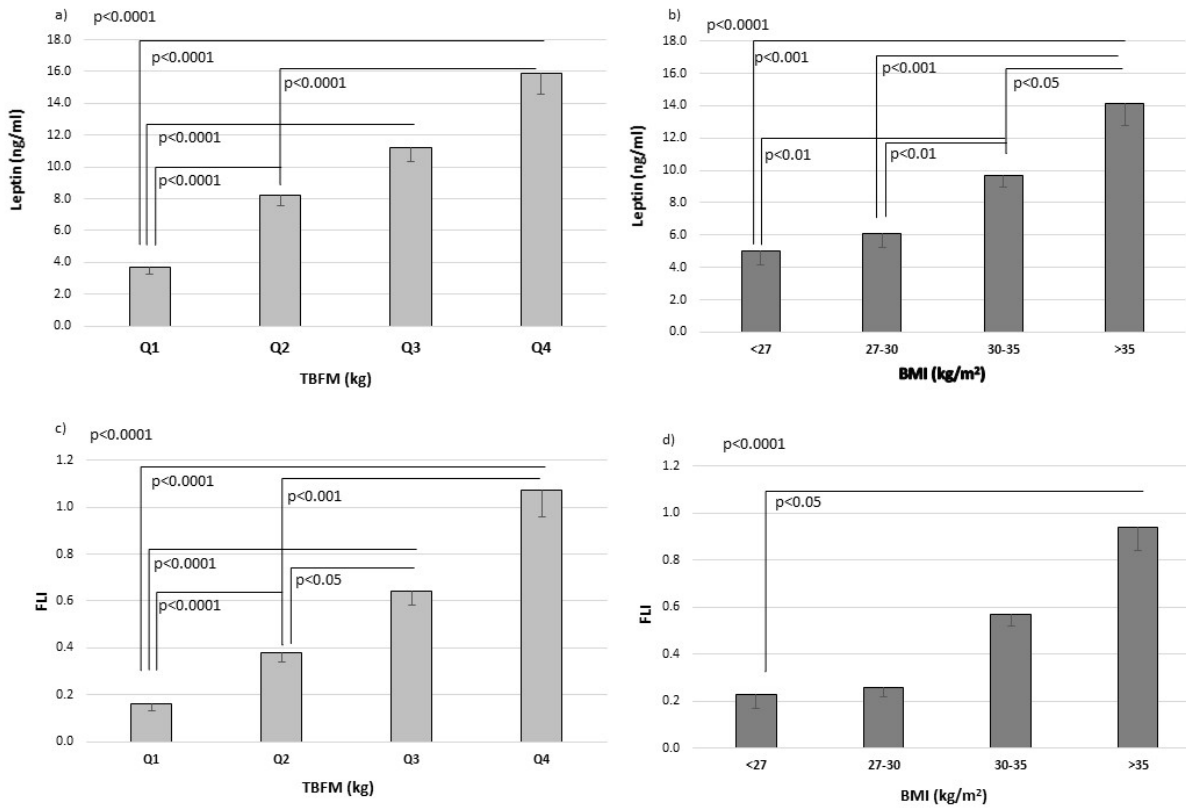

Fig. 2. Leptin secretion in relation with a) TBFM quartiles and b) BMI intervals, and markers of leptin resistance (FLI) in relation with c) TBFM quartiles and d) BMI intervals (BMI: body mass index; TBFM: total body fat mass; FLI: free leptin index; data are means ± SE).

d. Correlations of markers of insulin and leptin secretion/resistance

First, we have analyzed the data performing bivariate correlations between leptin system with markers of insulin secretion and resistance (table 1). Leptin did not correlate with insulin secretion/resistance, but the sObR did, in a negative fashion. Conversely, the FLI presented weaker positive correlations (table 1).

Secondly, we have evaluated the bivariate correlations between anthropometric parameters and markers of insulin and leptin secretion and resistance, respectively.

Serum leptin and FLI presented moderate or strong positive correlations with indicators of total and peripheral adiposity, including BAI, and, among markers of visceral obesity, only with WHtR (table 2). The sObR presented moderate negative correlations with indicators of total and peripheral adiposity (table 2). The correlations of leptin with TBFM and with Σ4SF were stronger for males than for females (r=0.74 (95%CI: 0.59, 0.84) vs r=0.54 (95%CI: 0.39, 0.66), p<0.0001; z score= 2.138, p<0.05, and r=0.82 (95%CI: 0.71, 0.89) vs r=0.51 (95%CI: 0.35, 0.63), p<0.0001; z score=3.618, p<0.001, respectively). The same was true for the correlations between FLI and Σ4SF (r=0.80 (95%CI: 0.68, 0.88) vs r=0.60 (95%CI: 0.47, 0.71), p<0.0001; z score=2.419, p<0.05).

Overall, the markers of insulin secretion and resistance showed weaker correlations with all anthropometric parameters. HOMA-IR and C peptide values presented weak positive relationship with BMI and waist circumferences, while for the rest of parameters, the correlations were negligible (table 2). VAI (as the marker of visceral obesity) correlated only with C peptide and HOMA-IR.

Thirdly, in order to better investigate the relationship between insulin secretion and resistance with markers of body adiposity and leptin, we have performed several additional multiple regression analyses, in a stepwise manner, based on the results of the bivariate analyses. The same was done for the leptin system. In model 1 we have included only anthropometrical parameters, markers of total (TBFM, BMI), peripheral (BAI), and visceral adiposity (VAI) as independent variables, while in model 2 metabolic parameters (HbA1c and C peptide – for leptin system, HbA1c and leptin – for insulin system) were added, as well as T2DM duration.

In model 1 of the analyses in which serum leptin concentrations constituted the dependent variable it resulted that TBFM, BMI and BAI were significantly associated with serum leptin concentrations (R^2: 47.98%, p<0.0001). In model 2, leptin levels were significantly influenced additionally by the duration of T2DM, but not by C peptide concentrations or HbA1c (R^2=50.86%, p<0.0001) (table 3a). The strongest correlation was observed for TBFM (table 3a).

The analyses evaluating the factors that influence leptin resistance (with FLI as dependent variable), indicated that in both models TBFM associated strongly with FLI (p<0.001, table 3a), but in model 2, duration of T2DM and C peptide concentrations also contributed significantly to leptin resistance (R^2=53.24%, p<0.0001) (table 3a).

Table 1. Bivariate correlations between markers of insulin secretion/resistance and of the leptin system

C peptide		Insulin system		
		HOMA-B	HOMA-IR	
Leptin system	Leptin	0.13 (-0.02, 0.28)	0.10 (-0.05, 0.25)	0.12 (-0.03, 0.27)
	sObR	-0.32 (-0.45, -0.17)***	-0.38 (-0.50, -0.24)***	-0.28 (-0.41, -0.13)**
	FLI	0.20 (0.05, 0.35)*	0.20 (0.04, 0.34)*	0.19 (0.04, 0.33)#

sObR: soluble form of leptin receptor; FLI: free leptin index; data is r (95% CI); #: p<0.05; *: p<0.01; **: p<0.001; ***: p<0.0001

Table 2. Bivariate correlations between anthropometric parameters with markers of insulin secretion/resistance, and leptin system

Anthropometric parameters		Insulin system			Leptin system		
		C peptide	HOMA-B	HOMA-IR	Leptin	sObR	FLI
TOTAL BODY ADIPOSITY	BMI (kg/m^2)	**0.29** (0.15, 0.43)***	**0.24** (0.10, 0.38)*	**0.30** (0.15, 0.43)***	**0.52** (0.40, 0.62)***	**-0.55** (-0.64,-0.43)***	**0.60** (0.49, 0.69)***
	TBFM (kg)	**0.19** (0.04, 0.33)$^{\#}$	**0.18** (0.03, 0.33)$^{\#}$	**0.18** (0.03, 0.33)$^{\#}$	**0.74** (0.66, 0.80)***	**-0.63** (-0.71,-0.53)***	**0.78** (0.71, 0.83)***
PERIPHERAL ADIPOSITY	Hip circumference (cm)	**0.31** (0.16, 0.44)***	**0.32** (0.18, 0.45)***	**0.29** (0.15, 0.43)***	**0.46** (0.33, 0.57)***	**-0.50** (-0.60,-0.37)***	**0.53** (0.42, 0.64)***
	Σ4SF (mm)	**0.18** (0.03, 0.33)$^{\#}$	0.12 (-0.03, 0.26)	**0.18** (0.03, 0.33)$^{\#}$	**0.70** (0.62, 0.77)***	**-0.59** (-0.69,-0.49)***	**0.74** (0.67, 0.81)***
	BAI	0.13 (-0.02, 0.28)	0.15 (-0.01, 0.29)	0.12 (-0.03, 0.27)	**0.69** (0.60, 0.76)**	**-0.47** (-0.58,-0.34)***	**0.70** (0.61, 0.77)***
VISCERAL ADIPOSITY	Waist circumference (cm)	**0.33** (0.19, 0.46)***	**0.23** (0.08, 0.37)*	**0.35** (0.21, 0.48)***	**0.23** (0.08, 0.37)*	**-0.37** (-0.49 -0.22)***	**0.31** (0.16, 0.44)***
	WHR	0.10 (-0.05, 0.25)	-0.007 (-0.16, 0.15)	0.14 (-0.02, 0.28)	**-0.19** (-0.34, -0.04)*	0.04 (-0.11, 0.20)	**-0.18** (-0.32, -0.03)$^{\#}$
	WHtR	**0.25** (0.10, 0.40)**	**0.19** (0.04, 0.33)$^{\#}$	**0.26** (0.11, 0.40)**	**0.53** (0.41, 0.63)***	**-0.48** (-0.59,-0.35)***	**0.57** (0.46, 0.66)***
	VAI	**0.23** (0.08, 0.37)*	0.11 (-0.04, 0.26)	**0.25** (0.10, 0.39)**	0.09 (-0.06, 0.24)	-0.12 (-0.27, 0.03)	0.10 (-0.05, 0.25)

BMI: body mass index; TBFM: total body fat mass; Σ4SF: sum of the four skinfold thickness; BAI: body adiposity index; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; VAI: visceral adiposity index; sObR: soluble form of leptin receptor; FLI: free leptin index; data is r (95% CI); $^{\#}$: $p<0.05$; *: $p<0.01$; **: $p<0.001$; ***: $p<0.0001$

Table 3. The multiple regression analysis of markers associated with a. leptin secretion and resistance, and b. insulin secretion

	Adj. R^2	β (SE)	r	p value
a. Markers of leptin secretion and resistance				
Leptin – constant/ *model 1*				
TBFM	0.479	0.554 (0.079)	0.666	<0.0001
BMI		-0.380 (0.150)	0.499	0.012
BAI		0.278 (0.100)	0.551	0.006
VAI		-0.202 (0.261)	-0.024	0.440
Leptin – constant/ *model 2*				
TBFM	0.508	0.521 (0.080)	0.667	<0.0001
BMI		-0.359 (0.160)	0.498	0.026
BAI		0.260 (0.099)	0.551	0.010
VAI		-0.276 (0.266)	-0.029	0.300
C peptide		0.602 (0.319)	0.176	0.061
T2DM duration		0.242 (0.097)	0.226	0.013
HbA1c		-0.157 (0.379)	-0.095	0.677
FLI – constant/ *model 1*				
TBFM	0.483	0.040 (0.005)	0.686	<0.0001
BMI		-0.014 (0.011)	0.543	0.184
BAI		0.011 (0.007)	0.529	0.120
VAI		-0.010 (0.019)	-0.008	0.583
FLI – constant/ *model 2*				
TBFM	0.532	0.037 (0.005)	0.688	<0.0001
BMI		-0.013 (0.011)	0.542	0.233
BAI		0.010 (0.007)	0.530	0.164
VAI		-0.018 (0.019)	-0.013	0.324
C peptide		0.067 (0.023)	0.251	0.004
T2DM duration		0.020 (0.007)	0.217	0.004
HbA1c		-0.026 (0.027)	-0.119	0.339

HOMA-B) and resistance (HOMA-IR). Coefficients, standard errors, t ratios and R^2 values are presented (adj.: adjusted; TBFM: total body fat mass; BMI: body mass index; BAI: body adiposity index; VAI: visceral adiposity index; WHtR: waist-to-height ratio; HbA1c: glycated hemoglobin; FLI: free leptin index)

Table 3. (continued)

	Adj. R^2	β (SE)	r	p value
b. Markers of insulin secretion and resistance				
HOMA-B – constant/ *model 1*				
TBFM	0.076	0.109 (0.490)	0.219	0.823
BMI		1.963 (0.928)	0.261	0.036
BAI		-0.488 (0.619)	0.141	0.431
VAI		1.390 (1.619)	0.068	0.391
HOMA-B – constant/ *model 2*				
TBFM		-0.239 (0.456)	0.219	0.600
BMI		2.741 (0.798)	0.261	0.0008
BAI		-0.760 (0.514)	0.068	0.141
VAI	0.383	1.829 (1.318)	0.199	0.166
Leptin		0.386 (0.390)	0.160	0.324
T2DM duration		-1.969 (0.484)	-0.231	<0.0001
HbA1c		-16.458 (1.880)	-0.474	<0.0001
HOMA-IR – constant/ *model 1*				
TBFM	0.133	-0.021 (0.017)	0.205	0.210
BMI		0.118 (0.032)	0.309	0.0003
BAI		-0.012 (0.021)	0.160	0.573
VAI		0.170 (0.056)	0.212	0.003
HOMA-IR – constant/ *model 2*				
TBFM	0.186	-0.030 (0.019)	0.205	0.113
BMI		0.111 (0.033)	0.309	0.001
BAI		-0.015 (0.021)	0.160	0.462
VAI		0.166 (0.055)	0.212	0.003
Leptin		0.033 (0.016)	0.172	0.044
T2DM duration		-0.066 (0.020)	-0.251	0.001
HbA1c		-0.079 (0.078)	-0.005	0.314

HOMA-B) and resistance (HOMA-IR). Coefficients, standard errors, t ratios and R^2 values are presented (adj.: adjusted; TBFM: total body fat mass; BMI: body mass index; BAI: body adiposity index; VAI: visceral adiposity index; WHtR: waist-to-height ratio; HbA1c: glycated hemoglobin; FLI: free leptin index)

The multivariate analyses with HOMA-B as dependent variable, indicated an overall small contribution of anthropometric parameters to β cell function (R^2: 7.66%, p: 0.009), with BMI being the only parameter significantly associated with HOMA-B (model 1) (table 3b). When other variables were included in the equation, T2DM duration and HbA1c significantly contributed to HOMA-B in a negative manner, with the strongest correlation being noticed for HbA1c (R^2: 40.85%, p<0.0001) (table 3b).

With HOMA-IR as dependent variable, the analysis indicated that BMI and VAI were the anthropometric variables that positively associated with HOMA-IR in both models (p<0.0001 for both), while in model 2, T2DM duration and leptin were also significant contributors (table 3b).

When sObR was added in the equations, however, its correlations with HOMA-B or HOMA-IR were not significant (data not shown). We further evaluated, though, the sObR relationship with insulin secretion and resistance by multiple regression analyses (supplementary table 3; p<0.0001 for all equations). It resulted that sObR was significantly associated with C peptide concentrations (β =-0.032; t ratio=2.026, p=0.044), but not with HOMA-B or -IR.

e. C peptide/leptin ratio (CLR) and relationship to anthropometry

The CLR decreased steadily with TBFM, while the decrease was significant only for BMI>30 kg/m^2 (figure 3a). In fact, the CLR corelated negatively with TBFM (r=-0.54 (95%CI-0.64, -0.42), p<0.0001) and positively with the NFM (r=0.40 (95%CI: 0.26, 0.52), p<0.0001).The C peptide correlated with NFM (r= 0.24 (95%CI: 0.09, 0.38), p: 0.0012), and so did HOMA-B, but to a smaller extent (r=0.15 (95%CI: -0.0002, 0.30), p: 0.044).

The CLR was significantly lower in the high BAI group (0.25 (0.04-1.02) vs 0.61 (0.06-3.19), p<0.0001) (suppl. figure a), and was not different in the high vs low VAI groups (0.42 (0.04-3.19) vs 0.32 (0.05-2.90), p: 0.644) (suppl. figure b). The same was true for groups defined by the WHtR (0.34 (0.04-3.19) vs 0.60 (0.12-1.05), p: 0.372).

Finally, we have found a strong positive correlation between the CLR and NFM/TBFM ratio (ln transformed values) (figure 3b) (r=0.62 (95%CI: 0.52, 0.71), p<0.0001).

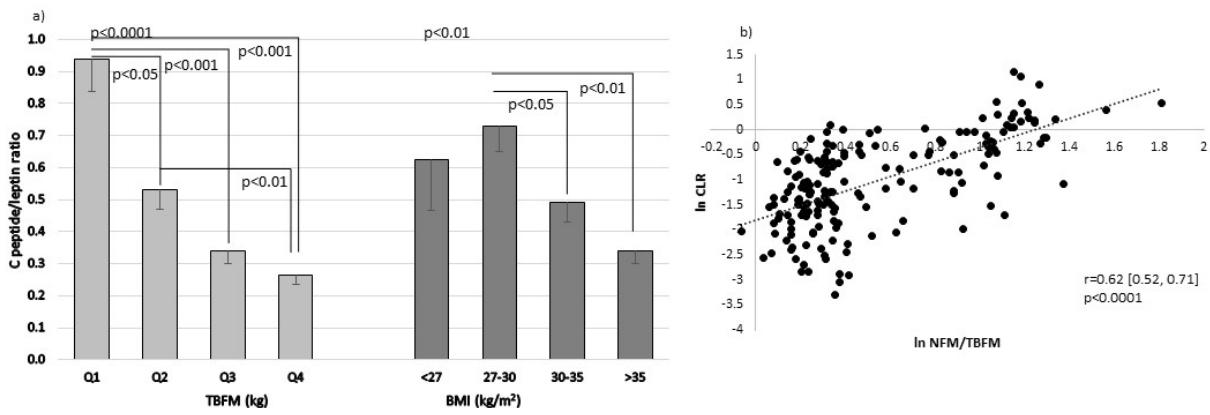

Fig. 3. a) C peptide to leptin ratio (CLR) in relationship with TBFM quartiles and BMI intervals, and b) correlation of (ln transformed) CLR to NFM/TBFM ratio (BMI body mass index; TBFM: total body fat mass; NFM: non-fat mass; data are means ± SE).

Discussion

Our data indicated that even if the BMI was similar between genders, T2DM females had higher total body adiposity and less total non-fat mass than males, with a predominant peripheral distribution of the adiposity. The excess body adiposity did not influence the insulin secretion/resistance, as no gender differences in terms of HOMA-B, HOMA-IR or C peptide levels were observed, and the metabolic control was similar. However, serum leptin concentrations were significantly higher in females, even after adjusting for body weight or total fat mass. We have also noticed that female T2DM patients had lower CLR compared to men, despite similar C peptide concentrations, due to higher leptin levels and the predominant peripheral (subcutaneous) distribution of adiposity. This is in conformity with the published literature showing that women have higher leptin levels than men, which may be related to sexual hormones and/or body fat distribution (men have more visceral fat, whereas women have more subcutaneous fat, which is a major leptin producer) (21, 22). In fact, a previous study in obese women showed differences in leptin secretion rates, explained by increased adipocyte size and leptin gene expression in SAT versus VAT (23). One possible explanation for the differences in tissue-specific leptin expression is the relationship between promoter methylation and the level of leptin expression, although other mechanisms might be involved (24).

Moreover, patients with hyperleptinemia had higher total and peripheral adiposity. Accordingly, markers of peripheral adiposity (BAI, hip circumference and Σ4SF) were mainly correlated with leptin system parameters, while markers of visceral adiposity were correlated mainly with indicators of insulin resistance and secretion. The increased peripheral obesity was associated with an important raise in leptin secretion and a smaller increase in insulin secretion, thus a decrease of CLR.

T2DM patients with high VAI values associated with moderate-severe adipose tissue dysfunction (ATD) as previously defined, had increased markers of insulin resistance (higher HOMA-IR and C peptide levels) (18). The multiple regression analyses in fact indicated that VAI was the only parameter that had a significant contribution to HOMA-IR. Other studies have shown that VAI is a predictor of visceral adiposity associated with insulin resistance and cardiometabolic disturbances (25, 26). A study in a smaller group of T2DM patients, however, has shown strong correlations of VAI with leptin, sObR, FLI and other adipokines, as well as with C peptide and HOMA-IR (26). The study also indicated better correlations of leptin parameters with BAI (than with VAI), while insulin parameters correlated better with VAI (than with BAI) (26). Similarly, our data showed that BAI correlated with leptin, while VAI correlated with C peptide and HOMA-IR, making it rather a marker of insulin resistance. Another study in children and adolescents indicated that VAI is inferior to BMI in terms of association with adipokines (27). In addition, another recent study in healthy adolescents demonstrated that circulating leptin levels are valid predictors of SAT and not VAT (28).

BAI and WHtR are somewhat similar in the sense that they report either hip or waist circumference to height, yet there are differences between the two anthropometric parameters in terms of association with hormonal parameters. Obese T2DM patients, as defined by WHtR, had higher serum C peptide concentrations and HOMA-IR than non-obese individuals (yet the HOMA-B was similar), while T2DM patients with higher BAI had increased HOMA-B, but similar indicators of insulin resistance to those with lower BAI. This again confirms the role of central obesity in increasing insulin resistance.

Moreover, leptin and insulin secretion and resis-

tance were analyzed in relationship to total body fat markers (TBFM and BMI). Data indicated a progressive increase in insulin (C peptide) secretion with higher BMI (about 1.4 times from the lowest to highest BMI) and a steady, more important increase of serum leptin concentrations with higher body adiposity (about 3-4 times). HOMA-B increased slightly with BMI, but not with TBFM, which is in accordance with data from another recent study in a Chinese diabetic population, that showed a better correlation of HOMA-B with BMI than with overall obesity (29). In fact, the multiple regression analyses performed for our data confirmed only BMI (among the anthropometric parameters) as being associated with β cell function, while duration of diabetes and HbA1c had a significant negative impact on HOMA-B. This implies that not only fat mass, but also non-fat mass may affect insulin secretion. We have actually found a weak positive correlation between NFM and insulin secretion. In fact, it was demonstrated that there is a feed-back loop between pancreatic β cells and bones, in which osteocalcin produced in the osteoblasts enhances β-cell insulin secretion, while insulin signals back to bones to increase osteocalcin activity and therefore β-cell function (30). A similar inter-relationship exists between skeletal muscle and pancreatic β cells. The effect of insulin on skeletal muscles is well known. Recent data also indicated that primary myotubes present specific myokines (e.g osteoprotegerin, angiogenin, interleukin 6) and mRNA signatures that could differentially impact β-cell insulin secretion, either directly or indirectly, via α cell/glucagon-like peptide-1 (GLP-1) levels (31, 32). The leptin and insulin resistance increased progressively, but became significantly higher only in case of obesity (BMI>30 kg/m^2). Interestingly, even if the serum leptin concentrations increased steadily with increasing body adiposity (mainly due to increased peripheral SAT), the insulin secretion was not blunted (HOMA-B increased slightly), and this may indeed suggest β-cell leptin resistance. This was also observed in relationship with gender, as females had higher leptin secretion per kilogram TBFM and weight, respectively, however, the insulin secretion was not significantly different compared to males. In fact, the multiple regression analyses confirmed the lack of influence of serum leptin levels on β-cell function (HOMA-B), which may also suggest β-cell leptin resistance and a dysregulated adipo-insular feed-back loop. The adipo-insular dysfunction was earlier suggested to play an important role during the development of T2DM in obese individuals (33). Our data suggest that it might also be significant in patients with longer duration of the disease. Perhaps, the β-cell leptin resistance might develop as an adaptive mechanism, and thus further studies to evaluate the β-cell leptin resistance in patients with T2DM are definitely worthy. Moreover, the adipo-insular dysfunction seems to be bi-directional, as the insulin secretion did not influence the leptin secretion either.

Another interesting finding was the correlation of the soluble form of leptin receptor (but not of leptin) with insulin resistance and secretion. The multivariate analyses confirmed the correlation of C peptide with sObR in a negative manner. A recent Japanese study in patients with T2DM has shown that plasma sObR levels were independently and negatively associated with β cell function (C peptide index and HOMA-B), but not with insulin resistance in patients with T2DM (34). Further research is needed in order to evaluate the mechanisms by which the sObR inhibits insulin secretion, whether independently or in a glucose-dependent manner. Previous work has suggested that the sObR modulate the levels and biologic activity of leptin, and is in fact the major leptin binding protein in the circulating human blood (35, 36).

From a different perspective, it should be mentioned that the hyperinsulinemic-euglycemic

glucose clamp is the gold standard for measuring insulin resistance, but it has limited clinical applicability, while clear criteria for defining leptin resistance have not been yet established. Thus, while waist circumference and BMI are the most frequently used anthropometric measurements in clinical practice, other parameters also bring valuable information, as these two are mainly indicators of insulin resistance, while other markers of total, peripheral and visceral adiposity provide additional insights into the insulin and leptin systems, which are inter-correlated. In this context, the clinically useful surrogate measures of insulin and leptin resistance/secretion might enlarge our understanding about early features of the pathogenesis and/or progression of obesity and T2DM, and might be used as indicators of disease prevention or treatment response. Perhaps, they might as well serve as markers that aid in a better classification and/or phenotyping of diabetes, which would offer the opportunity of a more tailored approach.

A study limitation that should be mentioned is that since this was a post-hoc analysis of data collected in a cross-sectional study not specifically designed to evaluate anthropometric parameters, more sophisticated methods to evaluate anthropometry (such as bioimpedance, DXA a.s.o.) were not employed. However, the measurements and indexes used here are well accepted. Also, perhaps, a larger study population, with equal gender distribution and a non-diabetic group would have been advantageous.

Conclusions

The parameters of peripheral adiposity correlated better with markers of the leptin system, those of visceral adiposity with markers of insulin secretion/resistance, while those of total body adiposity with both, supporting the role of visceral adiposity in promoting mainly insulin resistance, and of peripheral and global (mainly TBFM) adiposity in promoting leptin secretion and resistance. The soluble form of leptin receptor correlated independently and negatively with C peptide.

Abbreviations

VAT - visceral adipose tissue
SAT - subcutaneous adipose tissue
T2DM - type 2 diabetes mellitus
BMI - body mass index
WHR - waist-to-hip ratio
sObR - soluble form of leptin receptor
HbA1c - glycated hemoglobin
HOMA - Homeostasis Model Assessment
TBFM - total body fat mass
REE - resting energy expenditure
WHtR - waist-to-height ratio
NFM - total non-fat mass
BAI - body adiposity index
VAI - visceral adiposity index
HDL - high density lipoprotein
ATD - adipose tissue dysfunction
SD - standard deviation
CLR - C peptide/leptin ratio
DXA - dual-energy x-ray absorptiometry

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Authors' contribution

SC: conceptualization, methodology, formal analysis, investigation, resources, writing – orig-

inal draft preparation, review and editing, visualization, supervision, project administration, funding acquisition
BE: investigation, writing – review and editing
AF: validation, writing – original draft preparation, review and editing.

Conflict of interest

Authors declare that they do not have any conflict of interest related to this work.
SC received payment for lectures from AstraZeneca, Boehringer Ingelheim, Berlin-Chemie Menarini, Eli Lilly, MSD, Novo Nordisk, Sanofi, Servier Pharma, for clinical trial Steering Committee meetings as National Lead Investigator for DECLARE-TIMI58 from TIMI Study Group, consultant fees for Advisory Board from AstraZeneca and support for travel to meetings from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Sanofi, MSD, Novo Nordisk, Worwag Pharma. AF received payment for lectures from AstraZeneca, Eli Lilly, MSD, Novo Nordisk, Sanofi, Servier Pharma, Boehringer Ingelheim and support for travel to meetings from AstraZeneca, Eli Lilly, Sanofi, Novo Nordisk, MSD.

Supplementary material

Supplementary data available in electronic version published on the journal's website: www.rrml.ro

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