Relationship Between Subcutaneous Adipose Tissue Expression of Leptin and Obesity in Tunisian Patients

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Relation Entre L'expression de La Leptine Au Niveau Du Tissu Adipeux sous-cutané Et L'obésité Chez Des Patients Tunisiens.

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RÉSUMÉ

Prérequis : L'incidence de l'obésité est devenue un vrai problème épidémiologique et de santé publique. C'est la conséquence du déséquilibre entre la consommation et les dépenses énergétiques. La leptine est une hormone sécrétée spécifiquement par le tissu adipeux qui intervient dans le contrôle de la masse grasse en modulant la prise alimentaire et la balance énergétique. Cependant, des niveaux élevés de leptine ont été associés à l'obésité, suggérant un rôle important de cette hormone dans la régulation du poids corporel.

But : Le but de cette étude est d'élucider la relation entre la leptine et l'obésité ainsi que plusieurs variables métaboliques chez des patients tunisiens.

Méthodes : L'analyse de l'expression de la leptine dans le tissu adipeux sous-cutané, par QPCR, a été effectuée chez deux groupes qui ont subi une chirurgie abdominale : un groupe control (n=9) et un groupe d'obèses (n=7).

Résultats : Nos résultats ont montré que l'expression de l'ARNm de la leptine est manifestement augmentée chez les patients obèses ($p < 0.01$). Elle est positivement corrélée avec les paramètres de l'obésité: WC ($r=0, 71$, $p < 0.01$) et BMI ($r=0, 68$, $p < 0.01$). De plus, l'expression de la leptine est aussi corrélée avec l'index de l'insulino-résistance ($r=0, 72$, $p <0.01$).

Conclusions : La présente est une première étude de l'expression de la leptine dans le tissu adipeux sous-cutané associée à l'obésité chez la population tunisienne. Nos résultats confirment le rôle de la leptine dans l'obésité.

Mots-clés

Obésité, leptine, tissu adipeux sous-cutané, désordre métabolique

SUMMARY

Background : The incidence of obesity has dramatically increased in overall the world. It is a consequence of imbalance between energy intake and energy expenditure. Leptin is a fat derived adipokine that has emerged over the past decade as a key hormone in the regulation of food intake and energy expenditure. Elevated leptin levels are found in obese humans, suggesting a role of leptin in regulating body weight and adiposity.

Aim : The aim of this study was to investigate the change of leptin mRNA expression level and its correlation with obesity and several metabolic variables in Tunisian patients.

Methods: Real time quantitative polymerase chain reaction (QPCR) analysis was carried out among two groups who underwent an abdominal surgery: controls (n=9) and obese patients (n=7).

Results: Leptin mRNA expression in subcutaneous adipose tissue was markedly increased in obese patients ($p<0.01$). It was positively correlated with measures of obesity waist circumference (WC) ($r=0, 71$, $p<0.01$) and body mass index (BMI) ($r=0, 68$, $p<0.01$). Interestingly, leptin gene expression was also correlated to insulin resistance index ($r=0, 72$, $p<0.01$).

Conclusion: The present study is the first investigation of leptin regulation in subcutaneous adipose tissue of Tunisian population. Our data showed that leptin levels are higher in obese subjects than in control subjects. This indicates that the subcutaneous adipose plays an important role in impaired adipokine regulation, and consequently in developing metabolic disorder.

Key-words

Obesity, leptin, subcutaneous adipose tissue, metabolic disorder.

Although adipose tissue has long been recognized to have a classical role in energy storage, it is now accepted by the scientific and medical community to be a dynamic endocrine organ contributing to energy homeostasis [1]. Indeed, adipose tissue secretes several inflammatory and immune mediators known as adipokines [2]. The endocrine impacts of adipose tissue emphasize essentially the critical health implications of obesity, particularly abdominal obesity [3-4-5]. Thus, the increase of the obesity epidemic as a critical matter of health concern has prompted research into the mechanisms underlying energy homeostasis and the pathophysiology of obesity.

Dysregulation of adipokine secretion in abdominal obesity [6] shows many health consequences. It is a causal factor associated with the insulin resistance, increased risk of diabetes, hypertension, and cardiovascular disease in the metabolic syndrome [7]. Moreover, obesity was described to be related with certain cancers, such as ovary [8], breast [9], and colon [10].

Leptin is considered as a prototype adipocyte-secreted hormone or adipokine. It is a 16 kDa protein, encoded by the obese (OB) gene, produced by mature adipocytes [11]. Leptin is secreted in plasma and its levels are strongly correlated with adipose mass in rodents as well as in humans [12]. Leptin exerts pleiotropic effects; the main effect was to act as a signal from adipose tissue to the brain regarding the quantity of fat tissue stored. It acts via hypothalamic receptors to inhibit feeding and increase thermogenesis [13]. However, alteration in leptin levels has been linked to many human diseases in numerous cross-sectional and prospective studies. Early studies in obese humans showed that, in general, leptin concentrations correlated positively and significantly with adipose tissue depots [14]. Moreover, the high leptin concentrations in animal and human obesity generate a state of leptin resistance, where there is a lack of any evident endogenous hormone effect [15].

Several reports have shown that abdominal obesity is well recognized as an independent risk factor for metabolic disorders associated with the metabolic syndrome, in particular, insulin resistance, increased risk of diabetes, and cardiovascular disease [5]. However, the role of subcutaneous adipose tissue (SAT) subcompartments is not well understood. This study aimed to evaluate by real time QPCR the SAT expression of leptin, a key hormone linked to the development of obesity-associated metabolic complications in control and obese subjects. On other words, we examined the correlation of the leptin mRNA expression with insulin resistance.

MATERIAL AND METHODS

Subject recruitment

We recruited 16 subjects divided into two groups: controls (n=9) and obese (n=7) matched for age and sex and who underwent elective abdominal surgery. The weight, height and waist circumference (WC) were measured using standard methods and the body mass index (BMI) (kg/m2) was calculated. Subjects were classified on the basis of the BMI as obese (BMI>25) or non-obese (BMI <25).

Blood samples were collected from patients after their admission to the hospital for surgery. Subcutaneous adipose tissues biopsies were obtained from patients after an overnight fast undergoing hernia surgery in total anaesthesia. All biopsies were transported to the laboratory within 30 min after removal and were stored at ≥80°C for later RNA extraction.

Blood glucose, insulin, and insulin resistance.

Overnight fasting plasma glycemia and insulinemia were monitored on the morning of blood sample collection. Serum glucose was determined using a glucometer. Serum insulin levels were measured using radioimmunoassay. To estimate insulin resistance, the fasting plasma glucose and fasting serum insulin measures was used to calculate homeostasis model assessment of insulin resistance (HOMA-IR). The HOMA-IR score was determined using the formulae below:

HOMA-IR = [fasting insulin(microunits per milliliter) x fasting glucose/ 22.5], where HOMA indices >2 are defining insulin resistance.

Adipose tissue-RNA extraction and reverse transcription.

Total RNA was extracted using Trizol (Promega, Madison, WI, USA) according to the manufacturer's instructions. Reverse transcription was performed using a reverse transcriptase (RT) kit (Promega, Madison, WI, USA). The obtained cDNA were diluted 20X with double distilled water before PCR amplification.

Real-time QPCR.

Taqman probes for the detection of leptin (Taqman Gene expression Assays) were purchased from Applied Biosystems (Courtaboeuf, France). Direct detection of the PCR product was monitored by measuring the increase in fluorescence generated by the TaqMan probe. The gene-specific PCR products were measured continuously using ABI PRISM 7300 Sequence Detection System (Applied Biosystems) during 40 cycles. The threshold cycle (CT) of each target product was determined and ΔCT between target and endogenous control was calculated. The difference in ΔCT values of two groups (ΔΔCT) was used to calculate the fold increase ($F=2^{-\Delta\Delta CT}$) and to determine the changes in target gene expression.

Statistical analysis

A comparison between two groups was performed using the non parametric Mann-Whitney U–test. The metabolic parameters data are presented as means values ± SD, real time QPCR results are presented as values of median corrected by Dixon test. Spearman's correlation coefficients (r) were used to describe the association between mRNA expression of leptin and other continuous variables of interest. In all cases, differences were considered significant at $p<0.05$. (* : $p<0.05$; **: $p<0.01$; ***: $p<0.001$.)

RESULTS

Physical Measurements and Clinical Characteristics of Study Participants

The physical and biological characteristics of control and obese groups are summarized in Table 1. Metabolic characteristics were evaluated in 9 controls and 7 obese subjects who were

selected based on their age, BMI and WC. The groups exhibited insulin and glucose levels within expected ranges for non-diabetic subjects.

Table 1 : Physical and metabolic characteristics of the study population (mean ± SD)

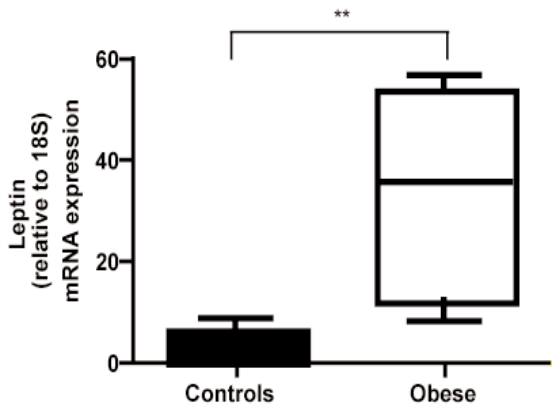
	Controls (n=9)	Obese (n=7)
Anthropometrics		
Age	51.48±2.97	50.33±2.24
BMI	22.67±0.47	31.59± 29
WC	M : 89,69±4,65	M: 108,71±3,68
	F : 77±3,56	F: 117,75±3,89
Glucose homeostasis		
Glucose (mmol/l)	4.94±0.13	5.11±0.37
Insulin (μUI/ml)	2.83±0.07	3.24± 0.18
HOMA-IR	1.37±0.19	1.88±0.26

Data are means ± SD
Abbreviations: WC= Waist circumference, M=male, F=female, BMI= body mass index, HOMA-IR= homeostasis model assessment of insulin resistance.

Subcutaneous adipose expression of leptin is upregulated in obesity

We assessed the mRNA expression of leptin in subcutaneous adipose tissue as can be seen in figure 1. Our data revealed that the obese group showed a significant higher mRNA level of leptin than the control group ($p<0.01$).

Figure 1 : Subcutaneous adipose tissue leptin mRNA expression in control subjects (n=9), obese (n=7). Leptin mRNA levels were measured by QPCR and are expressed relative to 18S mRNA levels. Median values are presented. * $p < 0.05$, ** $p< 0.01$.



Correlation between mRNA expression levels of leptin and metabolic parameters

This increase of endogenous leptin expression is positively correlated with all measures of obesity BMI ($r=0, 68$, $p<0.01$) and WC ($r=0, 71$, $p<0.01$). However, this leptin mRNA expression showed a positive correlation only with HOMA-IR index ($r=0, 72$, $p<0.01$) rather than with fasting glucose ($r =0,5$; $p=0, 2$) or fasting insulin ($r = 0,58$; $p=0, 09$).

DISCUSSION

To our knowledge, this report is the first one which quantifies leptin mRNA changes in subcutaneous adipose tissue of Tunisian patients, who have different genetic, environmental and nutritional backgrounds.

Obesity associated with metabolic disorders is increasing worldwide. Indeed, the increase in body weight as a consequence of genetic, environmental, and nutritional factors plays a significant role in these disorders [16]. Leptin is considered to be the most important molecule in regulating body weight [17]. Yet, since its original description [11], extensive investigations have analyzed leptin gene and protein regulations associated with obesity in many populations of the world [18].

Since leptin mRNA is expressed in the principal site of fat storage [17]; we investigated in this report the steady-state leptin mRNA levels in SAT and the relation to metabolic changes in obesity. Several studies have reported that visceral adipose tissue (VAT) is strongly associated to metabolic indicators [19]. However, few of them addressed the case of SAT. The present study showed that leptin mRNA levels significantly increased in obese subjects compared to controls. Moreover, it was positively correlated with measures of obesity. These results are in line with the previous data describing increase of leptin mRNA expression in SAT of obese subjects [20-21]. Furthermore, on the basis of our finding, we may relate the increase of leptin mRNA expression to high circulating leptin levels in obese. Then, this increase associated with obesity could generate many metabolic dysfunctions, for example a state of leptin resistance [22]. Our result confirms that the subcutaneous adipose stores may be of importance for determining the leptin level. Morethan, it could predict impaired metabolic regulations associated with obesity as do the visceral adiposity [23-24].

Then, we have found that leptin mRNA expression was positively correlated with insulin resistance index in obese. It has been proposed that obesity associated whith elevated leptin levels may directly induce a state of inflammation that might be underlying the development of features of the metabolic syndrome [13], particularly insulin resistance [25]. Indeed, the prevalence of this metabolic disorder constantly increases in connection with the pandemia of obesity and insulin resistance [26]. This may directly support the evidence of this hypothesis in our study. Because of an overlap between signal-transducing pathways of leptin and insulin [27], a common pathogenesis of leptin and insulin resistance has also been suggested. This area remains a significant area of research and would offer a variety of new approaches for novel therapies [28].

In conclusion, this study is the first demonstration of endogenous regulation of leptin expression in the SAT related with obesity in Tunisian subjects. Interestingly, our findings indicate that the SAT seems to act as a molecularly distinct abdominal adipose depot bearing independent metabolic

functions. Nevertheless, leptin should constitute a strong marker for metabolic disorder in Tunisian population. Indeed, the aim of a prospective work is to enlarge our cohort study and to design software with data from some of the most recent publications (including our tunisian study) on obesity, especially those concerning the roles of leptin and other adipokines in the metabolic disturbance. The most notable characteristic of this software is the integration and comparison of the same metabolic parameters from different populations with different genetic and environmental backgrounds.

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