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Determination of Obese Protein Concentration in a Biological Sample

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Abstract

Adipose tissue is a metabolically active organ with both endocrine and paracrine functions. It secretes a number of cytokines (adipocytokines) that play critical roles in the development of metabolic diseases and inflammation. Previous studies have shown that the product of an obese protein (leptin), a hormone secreted by adipose tissue, is associated with obesity, type 2 *diabetes mellitus* (T2D), and dyslipidaemia. Moreover, leptin has been identified as a potential and valuable therapeutic molecule for the treatment of glycaemia, dyslipidaemia, and T2D. The aim of this study was to analyse the concentration of obesity protein as an adipocytokine in a population from Sarajevo, Bosnia and Herzegovina. The study included 26 participants: 13 healthy subjects as the control group, and 13 untreated diabetics. Biochemical parameter, such as glucose, glycated haemoglobin, lipid profile, and concentrations of the hormones leptin and insulin were analysed. Biochemical parameters were determined using standard IFCC methods, while leptin and insulin concentrations were analysed using an ELISA assay. The measured concentration of obesity protein in plasma was significantly higher ($p < 0.001$) in diabetics compared to healthy subjects, with females exhibiting higher leptin levels than males in both groups. Significant differences in concentrations of biochemical characteristics between the diabetic and control groups ($p < 0.001$ and $p < 0.05$ respectively) were also observed, with elevated values noted particularly in females. These results suggest that leptin can serve as a biomarker for glucose and lipid regulation in untreated diabetic patients.

Keywords

Obese protein, biomarker, biological sample

1 Introduction

Obesity is currently a significant global health issue and plays a key role in the pathophysiology of serious diseases, such as metabolic syndrome, type 2 *diabetes mellitus* (T2D) and dyslipidemia.[1–5] Adipose tissue is a metabolically active organ with both endocrine and paracrine functions, secreting numerous cytokines (adipocytokines) that are central to the development of metabolic diseases and inflammation-related disorders.[6,7]

Leptin, derived from the Greek word λεπτος leptos (meaning "thin", "light", or "small") is a 16 kDa protein hormone mainly produced by adipose tissue cells (Fig. 1). Its primary role is to regulate energy distribution and consumption within the body. Leptin is a peptide hormone encoded by the obesity gene (*ob* or *LEP* gene), which exerts ifs effects through receptors expressed in both the brain and peripheral cells. While its primary function is the regulation of fat stores, leptin is also involved in other physiological processes, such as many synthesis sites beyond adipose tissue and numerous non-hypothalamic cell types expressing leptin receptors. Many of these additional functions have only recently been fully elucidated.[8,9]

Leptin is secreted from adipose tissue, specifically white adipose tissue, and has been closely linked to both T2D and particularly obesity.[3,5] The key roles of obese protein include regulating appetite, maintaining energy homeostasis, and supporting neurological and endocrine functions (Fig. 2).[10,11]

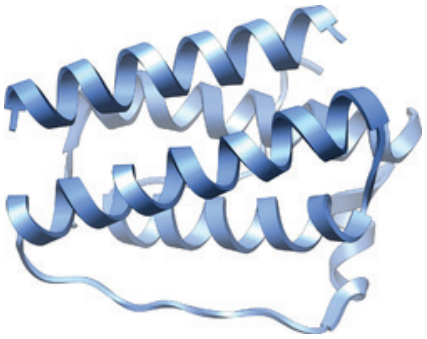

Fig. 1 – Leptin structure
Slika 1 – Struktura leptina

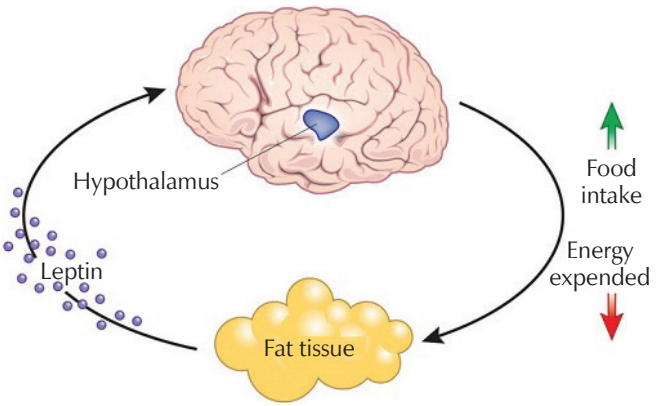

Fig. 2 – Roles of leptin in the body
Slika 2 – Uloge leptina u organizmu

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It also appears to influence various physiological processes, including metabolism, endocrine regulation, and immune response, along with other functions still under investigation.[8,12–15] Reported data have shown that leptin influences the production and secretion of insulin by impairing the function of pancreatic beta cells.[16] Recent studies have shown that leptin holds potential as a therapeutic target for treatment of glycaemia, dyslipidaemia, and T2D.[17–20]

In this study, the concentration of leptin as an adipocytokine hormone in a biological sample was determined.

2 Experimental

2.1 Chemicals

Leptin concentrations were determined using a sandwich ELISA method with a human Leptin ELISA kit (Human Leptin Immunoassay, Billerica, MA, USA) that employs a specific human leptin antibody. All other chemicals used were of analytical grade purity.

2.2 Subjects

The study included 26 participants: 13 healthy individuals (control group), and 13 newly diagnosed T2D patients (as per IDF criteria), aged between 40 and 65 years, from the University Clinical Centre Sarajevo.[21] None of the patients had undergone any therapy (antidiabetic, glucose-lowering or lipid-lowering drugs) or medications that might influence obesity protein concentrations. The control group consisted of healthy individuals who were not taking any medication during the study. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, and each participant provided written consent.

2.3 Sample analysis

2.3.1 Preparation of plasma samples

After overnight fasting, 5 ml of venous blood was collected from each participant. The blood samples were immediately centrifuged at 3000 g, 10 min, at 4 °C, and the resulting plasma samples were stored at −20 °C until further analysis.

2.3.2 Measurements of biochemical parameters

Biochemical parameters were analysed using standard IFCC (*International Federation of Clinical Chemistry and Laboratory Medicine*) protocols. Fasting plasma glucose (FPG), total cholesterol (TC), triacylglycerides (TAG), and high-density lipoprotein cholesterol (HDL-C) levels were measured with a clinical chemistry autoanalyzer VITROS 350 Chemistry System (New York, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated for serum triglyceride concentrations < 400 mg ml^{-1} using the Friedewald formula.

Glycated hemoglobin (HbA1c) was determined in whole blood in EDTA tubes using a standardised boronate-affinity chromatography method.

Serum insulin levels were measured using enzyme immunoassay on an Abbott autoanalyzer (Abbott Laboratories, IL, USA).

2.3.3 Determination of Leptin

Plasma leptin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available human leptin ELISA kit coated with a specific human leptin antibody.

The principle of determining leptin concentrations in plasma followed the manufacturer's instructions. Prior to analysis, quality controls and all plasma samples were diluted to one-third of their original concentration using dilution buffer. A volume of 100 µl of the diluted leptin conjugate was dispensed into each well of the microtiter plate, and incubated at room temperature for 1 h. After completing the protocol steps, leptin concentration of each plasma sample was measured at 450 nm using a Thermo Fisher Scientific ELISA microtiter plate reader (Salzburg, Austria).

According to the manufacturer's guidelines, the lowest detectable leptin level different from the zero standard was 0.2 ng ml^{-1}, and the correlation of the enzyme immunoassay with a commercially available radioimmunoassay was 0.95. The inter-assay and intra-assay reproducibility was determined by the manufacturer by calculating coefficients of variation ranging between 3.10 and 4.50 %, respectively. It is important to note that the measurement of leptin concentrations is not affected by whether the plasma samples were collected after overnight fasting or after a meal.

2.4 Statistical analysis

Statistical analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution were expressed as mean ± standard error mean (SEM). Comparisons between the two groups were performed using the Student's *t*-test, with a *p*-value less than 0.05 considered statistically significant.

3 Results and discussion

The biochemical characteristics of untreated T2D patients and healthy controls are presented in Table 1. Most measured metabolic parameters differed significantly between untreated T2D patients and healthy controls ($p < 0.001$) (Table 1), except for HDL-C levels ($p > 0.05$).

The determined concentration of obese protein in the participants' plasma showed a higher value in untreated diabetics (15.94 ng ml^{-1}) compared to healthy controls (10.97 ng ml^{-1}) (Fig. 3). In addition, the concentration of obese protein was higher in female participants compared

Table 1 – Biochemical characteristics of untreated diabetics and healthy subjects*
Tablica 1 – Biokemijske karakteristike

Parameters Parametri	Untreated diabetics Netretirani dijabetičari (n = 13)		Controls Kontrole (n = 13)		p**
	Male Muškarci (n = 9)	Female Žene (n = 4)	Male Muškarci (n = 6)	Female Žene (n = 7)	
glucose / mmol l^{-1}	7.83 ± 0.34	8.90 ± 0.60	5.41 ± 0.14	5.28 ± 0.11	0.001
TC / mmol l^{-1}	5.37 ± 0.31	5.75 ± 0.32	4.40 ± 0.34	4.93 ± 0.46	0.001
HDL-C / mmol l^{-1}	1.19 ± 0.10	1.27 ± 0.11	1.25 ± 0.36	1.21 ± 0.04	0.973
LDL-C / mmol l^{-1}	3.57 ± 0.40	3.17 ± 0.45	2.57 ± 0.38	2.75 ± 0.27	0.001
TAG / mmol l^{-1}	1.92 ± 0.47	1.62 ± 0.29	2.03 ± 0.25	2.12 ± 0.63	0.001
HbA1c / %	6.95 ± 0.21	7.10 ± 0.40	5.83 ± 0.14	5.63 ± 0.20	0.001
insulin / mUl l^{-1}	6.15 ± 1.16	17.60 ± 1.00	8.44 ± 0.61	6.17 ± 0.82	0.010
leptin / ng ml^{-1}	12.66 ± 2.44	23.32 ± 2.52	7.78 ± 1.15	14.68 ± 3.69	0.001
total leptin / ng ml^{-1}	15.94 ± 8.27		10.97 ± 7.18		0.001

* Mean and standard deviation were used for representing data. LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; TAG – triacylglycerol; TC – total cholesterol, HbA1c – glycated haemoglobin. **All analyses were calculated using Student´s t-test.

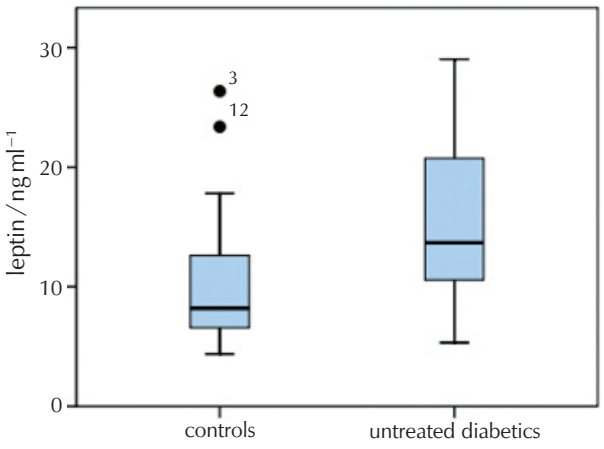

Fig. 3 – Leptin concentrations in healthy controls and untreated diabetics
Slika 3 – Koncentracije leptina kod zdravih kontrolnih osoba i neliječenih dijabetičara

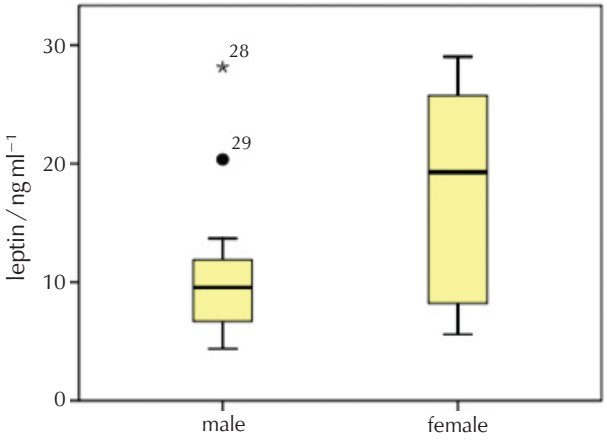

Fig. 4 – Leptin concentrations in males and females among the healthy controls and untreated diabetics
Slika 4 – Koncentracije leptina kod muškaraca i žena zdrave kontrolne skupine i neliječenih dijabetičara

to male participants (23.32 and 14.68 ng ml^{-1} in diabetic patients, and 14.68 and 7.78 ng ml^{-1} for controls, respectively) (Fig. 4). Notably, in both study groups, the circulating obese protein concentrations were twice higher in females than in males.

The findings of this study are similar to those of previous research, which revealed higher concentrations of obese protein in diabetics and female participants, with leptin levels in the blood influenced by the amount of body fat.[22,23]

Today, obesity is increasingly prevalent worldwide, and the costs associated with its treatment constitute a significant portion of healthcare expenditures.[1] The obesity protein (Leptin) is a cytokine hormone synthesised by white adipose tissue, and its structure is similar to pro-inflammatory cytokines found in the body. This adipocytokine acts by binding to leptin receptors (LEPR) located on the surface of various tissues,[9,17] such as neurons, liver, pancreas, heart, and perivascular intestinal tissue. The primary roles of leptin include regulating appetite, body mass, energy homeostasis, metabolism, and immune processes. Factors influencing plasma and serum leptin concentrations are body fat mass index (BMI), hormones, gender, race, and ethnicity, age, and genetic mutations.[3,11,13,24–26] In this study, BMI levels differed significantly between healthy controls and untreated diabetics. However, no significant association was found between BMI values and obese protein concentrations in either study group (data not shown).

Unhealthy lifestyle choices, such as reduced physical activity, sedentary behaviour, and overeating, contribute to elevated leptin concentrations. Current research data consistently show that women exhibit higher circulating leptin levels than men. This study confirmed this trend, with female participants in both groups having significantly higher leptin levels (Fig. 4), twice higher than those of the male participants. Gender differences in obese protein concentrations may result from the influence of sex hormones and differences in fat mass distribution.[3,18,23]

The concentrations of biochemical characteristics, including glucose, glycated haemoglobin, lipid profile (total cholesterol, high-density cholesterol lipoprotein, low-density cholesterol lipoprotein, and triacylglycerol), and the hormone insulin were significantly different between untreated diabetics and controls ($p < 0.001$ and $p < 0.05$, respectively), and also showed significant differences between male and female participants (Table 1).

Leptin and insulin play major roles in metabolism, food intake, and energy distribution and consumption. Previous studies have shown that obesity protein reduces insulin synthesis and secretion,[15,24,27] while it also decreases hepatic glucose production, thereby contributing to reduced glucose concentrations, improving sensitivity to insulin and reducing glucagon levels.[9,10,20] Insulin, on the other hand, plays a role in increasing the production and secretion of leptin in adipose tissue. However, the mechanisms that underlie these metabolic pathways and predisposition to obesity remain unclear.[18,19]

Understanding the association of circulating leptin and insulin levels with obesity, and their roles in the pathophysiology of T2D is essential. The obesity protein together with insulin can be used as a combined therapy in the treatment of diabetes and disorders caused by insulin resistance.[8,12,19]

4 Conclusion

This study determined the concentration of obesity protein in plasma and identified a significant increase in leptin concentrations in newly diagnosed and untreated type 2 diabetes patients compared to healthy controls. In addition, gender differences in leptin concentrations were observed in both study groups. These findings indicate that obesity protein could serve as a biomarker for glucose and lipid control in untreated and newly diagnosed diabetics. Future studies involving a larger number of participants are required to better understand the influence of leptin on glucose and insulin homeostasis, and to explore its potential in the development and application of leptin-based therapies for the treatment of diabetes and its complications.

List of abbreviations

EDTA – ethylenediaminetetraacetic acid
ELISA – enzyme-linked immune absorbent assay
FPG – fasting plasma glucose
HbA1c – glycated haemoglobin
HDL-C – high-density lipoprotein cholesterol
IFCC – International Federation of Clinical Chemistry
LDL-C – low-density lipoprotein cholesterol
LEP – leptin
LEPR – leptin receptor
NGSP – National Glycohaemoglobin Standardization Program
ob – obese
SEM – standard error mean
SPSS – Statistical Package for the Social Sciences
T2D – Type 2 *diabetes mellitus*
TAG – triacylglyceride
TC – total cholesterol

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SAŽETAK

Određivanje koncentracije proteina pretilosti u biološkom uzorku

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Masno tkivo metabolički je aktivan organ s endokrinom i parakrinom funkcijom, a luči niz citokina (adipocitokina) koji imaju važnu ulogu u razvoju metaboličkih bolesti i upala. Prethodne studije pokazale su da je proizvod pretilog proteina (leptina) i hormona koji se izlučuje iz masnog tkiva povezan s pretilošću, dijabetes melitusom tipa 2 (T2D) i dislipidemijom. Također, rezultati su pokazali da je leptin potencijalna i vrijedna molekula u terapijskom liječenju glikemije, dislipidemije i T2D. Cilj rada bio je analizirati koncentraciju proteina pretilosti kao adipocitokina u populaciji iz Sarajeva, Bosna i Hercegovina. Uključeno je bilo 26 sudionika: 13 zdravih osoba kao kontrolne skupine i 13 neliječenih dijabetičara. Analizirani su biokemijski parametri kao što su glukoza, glikirani hemoglobin, lipidni profil te koncentracije hormona leptina i inzulina. Biokemijski parametri određivani su standardnim IFCC metodama, a koncentracije leptina i inzulina ELISA testom. Izmjerena koncentracija proteina pretilosti u plazmi bila je znatno povišena ($p < 0{,}001$) kod dijabetičara u odnosu na zdrave ispitanike, a žene su imale više koncentracije leptina u odnosu na muškarce u obje ispitivane skupine. Razlike u koncentracijama biokemijskih karakteristika bile su značajne između pacijenata i kontrolne skupine ($p < 0{,}001$, odnosno $p < 0{,}05$), a također su bile znatno povišene u žena. Dobiveni rezultati ukazuju na to da se leptin može upotrebljavati kao biomarker kontrole glukoze i lipida u neliječenih dijabetičara.

Ključne riječi
Protein pretilosti, biomarker, biološki uzorak

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