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Circadian Rhythm of Blood Leptin Level in Obese and Non-Obese People

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A B S T R A C T

Leptin, an adipose tissue hormone, has circadian variations in its secretion. Aims of this study were to show how circadian rhythm depends on fat tissue distribution in obese and non-obese subjects. The research was carried out on 70 subjects (37 men and 33 women) with an average body mass index (BMI) of 25.22 kg/m^2. Concentration of leptin in blood was measured at 8.30 a.m., 12.30 p.m. and 6.30 p.m. Basal leptin level correlated strongly with all isolated regions of subcutaneous fat tissue in women and obese subjects. Circadian changes of blood leptin level in non-obese people are more significant than these changes in obese people. Differences in circadian pattern of leptin secretion between obese and non-obese subjects were probably caused by enlarged volume of subcutaneous fat tissue in obese people. Lean subjects have subcutaneous fat in physiological range which allows influence of some hormones (insulin or cortizol) or food intake on leptin secretion.

Key words: *leptin, circadian rhythm, obese, non-obese*

Introduction

Leptin is a peptide hormone produced in white adipose tissue that acts by activating specific leptin receptors in hypothalamus. Leptin receptors are found in many areas of the brain, including the hypothalamus, cerebellum, cortex, hippocampus, thalamus, chorioid plexus, and brain capillary endothelium[1]. Beside the

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central nervous system, leptin receptors are also found in liver, kidneys, pancreas, lungs and skeletal muscles[2]. Activation of hypothalamic receptors results in decreased food intake and increased energy consumption[3]. In the arcuate nuclei of hypothalamus leptin inhibits production of neuropeptide Y, thus reducing the appetite[4,5]. Basal leptin values are higher in women than in men, they are increased during pregnancy and they correlate with the share of body fat and with the body mass index (BMI)[6,7]. Different regions of body fat tissue do not seem to have the same influence on the leptin blood level. The abdominal adipose tissue seems to have the major effect[8].

Leptin concentration in blood shows circadian variations with the lowest values at noon and the highest values after midnight[9]. Possible interpretations of this daily profile include insulin influence on changing the blood leptin level[10] and increased glomerular filtration in the morning[11] that might reduce the leptin blood concentration. It is still unclear how these circadian changes influence the leptin role in weight control. Another interesting aspect is whether all parts of the adipose tissue have equal influence on the leptin circadian variations. In this study we investigated the influence of obesity degree on circadian rhythm of leptin secretion.

Subjects and Methods

Subjects

The research was carried out on 70 healthy volunteers (37 men and 33 women) who gave an informed consent to participate in this study. For the whole group of 70, the BMI was 24.88±3.87 kg/m^2 (in range between 17.57 and 33.33). Among them, 37 subjects had BMI lower than 25 kg/m^2 (22.06±2.08) while 33 subjects had BMI above 25 kg/m^2 (28.23±2.59).

Anthropometric measurements

All subjects gave consent to anthropometric and ultrasound measurements. Circumferences of the upper arm and forearm, waist, hips, upper and lower leg were measured in the middle of each body part. The ranges between the front and the back wall of thorax and abdomen were also measured. The thickness of subcutaneous fat tissue was measured by ultrasound with a linear probe made by Shimadzu company at frequency of 7.5 MHz at 16 points in body that were determined in advance. List of these points, as well as procedure of ultrasound measurement was fully described in previous paper[12]. Subcutaneous fat tissue in the regions of the forearm, upper arm, upper leg and lower leg that are approximately circles in diameter was calculated as area difference of two concentric circles. On each measuring point the thickness of subcutaneous fat tissue was measured both from the front and the back side and the mean value was put in the formula. The trunk cross-section resembles oval more than circle, so we needed to change the formula for calculating the area of subcutaneous fat tissue in that cross-section. Average thickness of thoracic subcutaneous fat tissue was calculated as the mean of values obtained on two measuring points (the middle of the line connecting the end of the large chest muscle and sternoclavicular joint). Average thickness of abdominal subcutaneous fat tissue was calculated as the mean of values obtained on six measuring points. Since the thickness of subcutaneous fat tissue in regions around the joints is significantly different it was decided to observe the length of 10 cm on every limb to maintain the objectivity of research. In this way we managed to obtain the volume of subcutaneous fat tissue that is comparable with other body regions[13].

Leptin level measurements

Blood samples were taken from all subjects at 8.30 a.m., 12.30 p.m. and 6.30 p.m., every time before the meal. The blood leptin level was measured with polyclonal antibody radioimmunoassay by Linco Reasearch, Inc. (HL-81K). The assay sensitivity was 0.5 ng/ml. Antibodies were marked with ^{125}I that has specific activity of 135 μCi/μg. Standard values for non-obese people (BMI 18–25 kg/m^2) were 2–5.6 ng/ml for men and 3.7–11.1 ng/ml for women.

Statistics

The data were classified according to obesity degree as well as to blood taking time. Student t-test for dependent samples was used to test the significance of difference between morning, noon and evening leptin concentration in blood in all groups of data. For comparing the correlations among the examined indexes the Pearson's method of linear regression was used. In tables it was presented as value (r) and probability (p) of linear correlation between two examined indexes.

Results

In 33 obese subjects (BMI > 25 kg/m^2) morning leptin concentration rose proportionally with the increase of BMI, whereas in 37 non-obese subjects (BMI up to 25 kg/m^2) values of leptin level did not correlate with BMI (Figure 1).

Table 1 shows correlation (r) between the volume of total subcutaneous fat tissue as well as the volume of isolated regions of subcutaneous fat tissue as independent variable and blood leptin level in the morning (basal level) as dependent variable in regard to sex and obesity degree. It is evident that basal leptin level strongly correlates with subcutaneous fat in all regions, except for the forearm and the lower leg in men and the forearm in non-obese people.

Table 2 shows differences of leptin concentrations in the morning, at noon and in the evening. Using t-test for dependent samples the differences between morning, noon and evening leptin levels were checked. There were significant differences between morning and evening

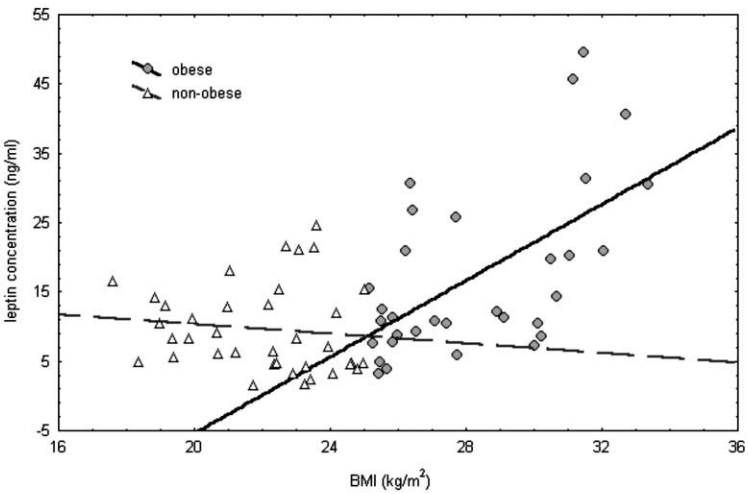

Fig. 1. Correlation between body mass index (BMI) and morning leptin concentration in obese and non-obese subjects.

TABLE 1
CORRELATION BETWEEN THE VOLUME OF SUBUCTANEOUS FAT TISSUE AS INDEPENDENT VARIABLE AND BLOOD LEPTIN LEVEL IN THE MORNING AS A DEPENDENT VARIABLE WITH REGARD TO SEX AND OBESITY DEGREE

Subcutaneous fat tissue	Blood leptin level in the morning			
	Female	Male	Non-obese ($\geq$25 kg/m^2)	Obese (>25 kg/m^2)
Upper arm	0.74*	0.69*	0.58*	0.74*
Forearm	0.77*	0.29	0.31	0.69*
Upper leg	0.57*	0.55*	0.64*	0.58*
Lower leg	0.43*	0.29	0.62*	0.46*
Thorax	0.80*	0.75*	0.59*	0.72*
Abdomen	0.84*	0.56*	0.50*	0.74*
Whole body	0.82*	0.75*	0.71*	0.78*

* $p < 0.05$

TABLE 2
DIFFERENCES BETWEEN MORNING, NOON AND EVENING BLOOD LEPTIN CONCENTRATIONS (mg/L) IN ALL SUBJECTS (N=70) (STUDENT T-TEST FOR DEPENDENT SAMPLES)

Leptin levels (X±SD)	Noon (12.49±11.13)		Evening (14.81±12.12)	
	t	p	t	p
Morning (13.16±10.17)	1.68	0.09	3.2	<0.01
Noon (12.49±11.13)			5.3	<0.01

TABLE 3
DIFFERENCES BETWEEN MORNING, NOON AND EVENING BLOOD LEPTIN CONCENTRATIONS (mg/L) IN MALE (N=37) AND FEMALE (N=33) SUBJECTS (STUDENT T-TEST FOR DEPENDENT SAMPLES)

Leptin levels (X±SD)		Males			
		Noon (8.17±5.74)		Evening (9.41±6.42)	
		t	p	t	p
Males	Morning (8.74±6.21)	1.8	0.08	1.8	0.08
	Noon (8.17±5.74)			3.2	<0.01
		Females			
		Noon (17.20±13.54)		Evening (20.70±14.10)	
		t	p	t	p
Females	Morning (17.97±11.47)	1.02	0.31	2.9	<0.01
	Noon (17.20±13.54)		4.5		<0.01

and noon and evening leptin concentrations. The differences between morning and noon concentrations were not statistically significant (p<0.09). When non -obese subjects were observed separately (Table 3), there were significant differences between morning and noon (p< 0.01) and noon and evening values of the leptin concentration in blood (p=0.03). In obese subjects, noon leptin levels were

TABLE 4
DIFFERENCES BETWEEN MORNING, NOON AND EVENING BLOOD LEPTIN CONCENTRATIONS (mg/L) IN OBESE (N=33) AND NON-OBESE (N=37) SUBJECTS (STUDENT T-TEST FOR DEPENDENT SAMPLES)

Leptin levels (X±SD)		Obese subjects			
		Noon (17.55±13.73)		Evening (18.97±14.21)	
		t	p	t	p
Obese subjects	Morning (17.23±12.20)	0.4	0.67	2.1	0.04
	Noon (17.55±13.73)			2.6	0.02
		Non-obese subjects			
		Noon (8.12±5.42)		Evening (11.20±8.65)	
		t	p	t	p
Non-obese subjects	Morning (9.63±6.28)	4.99	<0.01	2.40	0.02
	Noon (8.12±5.42)			4.80	<0.01

not significantly lower than morning values (p=0.78), whereas there was the difference between noon and evening values (p=0.04). When subjects were divided according to sex (Table 4), it was evident that in female subjects there was no significant difference between morning and noon values (p= 0.31).

Discussion

Leptin in blood was obtained in free and bound form. The share of free leptin in blood increases proportionally with the obesity degree[14,15]. We got similar results for the whole group of 70 subjects. However, when we divided them according to BMI into two subgroups of lean and obese subjects, no significant correlation between blood leptin levels and BMI was found in non-obese subjects. These data suggest that the basal leptin value in blood is a relatively good index for the volume of adipose tissue only in obese subjects. It also indicates that blood leptin level does not correlate with body mass index in eutrophic people. There are two explanations for this statement. First, level of free leptin in blood rises significantly when all leptin carriers are tied and second, non-physiological excess of fat tissue changes the pattern of leptin secretion. We used three measurements to get the average diurnal profile of blood leptin concentration. The lowest values were obtained at 12.30 a.m., whereas the evening values were the highest, regardless of the subject's obesity degree. These data are in accordance with studies of Schoeller[16] and Sinha[17]. Beside that, we noticed a difference between non-obese and obese subjects. In the subgroup of non-obese subjects morning values were higher than the noon values, while among the obese subjects the noon values were higher than the morning values. It seems that in obese subjects the nadir of leptin concentration was shifted towards morning. Here are possible explanations. In this study it was pointed out that female subjects had statistically significant higher leptin levels, regardless of the time of day. Both male and female subjects had higher leptin level in the evening, while morning and noon levels were not statistically different. In lean people morning and noon leptin levels were statistically different. They had the lowest peak at noon, while the evening leptin level was increased. In fat people only evening leptin level was statistically increased. These findings suggest that there are no significant differences between male and female subjects in leptin circadian rhythm. There is significant difference only in lean people, no matter what sex they are. More complicated circadian dynamics of leptin secretion in lean subjects is the result of the fact that lean people have smaller amount of fat tissue, so their leptin levels are in the normal range. Thus the leptin level presents dynamics of metabolism, which are connected with metabolism-regulated hormones and with intake of energy and fat in the body. Considering all these facts makes circadian leptin dynamics in lean people more clear. Morning leptin levels are relatively high because of night fast, after breakfast leptin is decreasing, so the lowest value is at noon and the highest values are obtained in the evening. The evening values are connected with numerous factors like hormones, circadian rhythm and dynamics of food intake in the body. The fat subjects have large fat storage which causes higher morning leptin level and because of that there are no great changes of leptin level until evening, when leptin level is statistically increased, possibly because of circadian rhythm of the whole body. For more precise determining of leptin circadian rhythm, additional research should be done which will include study of other circadian hormones. So, the difference in circadian rhythm of the leptin secretion according to obesity degree is that both the volume of subcutaneous fat and leptin secretion are in physiological range in non-obese people. This condition enables influence of other physiological factors on daily dynamics of the leptin level in blood, mostly the influence of insulin[18-20] or cortisol[21]. On the other hand, obese people have much more subcutaneous fat tissue that produces proportionally more leptin. Physiological influence on elevated leptin concentration is of less importance and the consequence is the absence of regular decrease of blood leptin level at noon. So, we can conclude that obese people have different diurnal rhythm of leptin secretion because of their leptin hyperproduction.

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CIRKADIJADNI RITAM RAZINE LEPTINA U KRVI PRETILIH I NEPRETILIH OSOBA

S A Ž E T A K

Leptin, hormon kojeg izlučuje masno tkivo, pokazuje cirkadijadne varijacije koncentracije u krvi. Svrha ovog rada je prikazati utjecaj distribucije potkožnog masnog tkiva na dnevni profil koncentracije leptina u krvi u pretilih i ne-pretilih osoba. Istraživanje je provedeno na 70 osoba (37 muškaraca i 33 žene) s prosječnim indeksom tjelesne mase (BMI) od 25.22 kg/m^2. Koncentracija leptina u krvi je mjerena u 8.30, 12.30 te 18.30 sati. Bazalne (jutarnje) vrijednosti leptina snažno koreliraju s pojedinačnim volumenima potkožnog masnog tkiva u žena i pretilih osoba. Dnevne razlike u vrijednostima razine leptina u krvi su značajnije u mršavih u usporedbi s pretilim ispitanicima. Primijećene razlike u cirkadijadnom ritmu koncentracije leptina između pretilih i ne-pretilih osoba su vjerojatno uvjetovane povećanim volumenom masnog tkiva u pretilih osoba budući da je volumen potkožnog masnog tkiva kod mršavih ispitanika u fiziološkim granicama što dozvoljava utjecaj nekih drugih hormona (inzulin, kortizol) ili unosa hrane na varijabilnost leptinske koncentracije.