FULL PAPER



Role of calcium/potassium coefficient in calciumregulating hormone activity in pre-obese patients

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^fDepartment of Biophysics, College of Applied Sciences, University Of Anbar, Anbar, Iraq

^gMedical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq

^hMiddle Technical University Baghdad, Baghdad, Iraq Obesity is a heterogeneous group of conditions and syndromes caused by hormonal metabolic disorders and many other factors. The aim of the work is to establish Ca/K coefficients role to gain weight. An assessment of changes in the characteristics of mineral, biochemical, and hormonal metabolism was carried out in 100 practically healthy patients of both sexes living in Grodno, Belarus. The age of the examined patients was 24.3±0.24 for females and 25.5±0.52 for males. The results show a clear, significant, and correlation-dependent imbalance in the elemental status in overweight patients, both men and women, in the age group of patients \geq 18 years, which has an imbalance in hormonal levels (pituitary hormones-TSH, thyroid hormones-T3/T4 free, and adrenal hormones cortisol), metabolites, substrates, and enzymes of blood plasma (glucose, cholesterin, LDL cholesterol, triglycerides, and alanine aminotransferase). The imbalance established in blood serum samples for bioelements (Ca and K), for which there are similar changes in hair samples, suggests that these disorders are associated with metabolic processes in the body, which are expressed in an increase in body weight. The hair Ca/K ratio was greater than 5 in 64% of males with a BMI of 25-29.9. As a result, it is clear that increasing body weight causes a 64% elevation in the activity of hormones regulating calcium, which was not previously considered in the literature. In 92% of females, the increased body weight maximized the activity of calcium-regulating hormones.

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Ali Adeeb Hussein Ali Email: medic_lab2015@yahoo.com Tel.: ++9647818935572 **KEYWORDS**

Bioelements; obesity; BMI; hormone; calcium; potassium.

Introduction

Obesity is a diverse set of conditions and syndromes caused by hormonal metabolic disorders and many other factors [1,2]. The common manifestation of which is the excessive fat accumulation inside the body.

The obesity prevalence has risen rapidly in the recent decades, approaching epidemic proportions, and becoming a significant social and economic burden in modern life [3]. The relevance of additional study in this area is determined by the immense medical and social significance of obesity itself. The pathology of thyroid gland is one of the illnesses for which obesity increases the risk [4,5].

The thyroid gland regulates the metabolism of the whole organism. It can speed it up or slow it down. All other manifestations are a consequence of changes in metabolism and should be logically interconnected [5]. Fat by itself will not go away with the normalization of hormones. It



can go away with the observance of the elementary dietary measures.

Calcium works as the general regulator of the organism [6]. It controls the building of bone tissue and the transmission of nerve impulses. It affects cell membrane permeability, blood coagulation, and provides the immune system protection of the body. The bulk of the calcium transport mechanisms in the body are carried out by bone tissue [7]. In the event that dietary calcium intake is inadequate, calcium is removed from the depot to maintain a normal blood level. The majority of calcium's binds are held by blood plasma proteins (mainly albumin) [8]. There is a lot of debate about the role of calcium in the obesity increase. Ca capillary absorption problems are caused by excess weight and fat [9]. Tin, which has a similar structure to calcium, is swiftly incorporated into the osteogenesis processes in the context of calcium deprivation, leading to various deformations of bone tissue that are nearly impossible to cure, even with the long-term and intense medication [10,11].

In human body, potassium is the most important chemical cation [12]. Nearly all of the body's potassium (more than 98% of it) is found inside the cells [13,14]. 3000-3500 mmol of potassium are found in each cell of an adult. A small percentage of this amount is stored in the bones. About 70% of this amount is found in the muscle tissue. The extracellular area contains 1% to 2% of the potassium of the body [14, 20-25].

Again, acting as a buffer for significant variations in the blood potassium content as potassium intake declines, is intracellular potassium. The kidneys' capacity to store potassium is significantly less effective than that of the kidneys' capacity to store salt, and it occurs relatively slowly in response to a loss in dietary potassium. Consequently, potassium deficiency is a typical clinical issue. The aim of the work was to establish Ca/K coeffients role to gain weight.

Materials and methods

The blood serum of patients and hair were the materials used for conduction this study. Informed consent was obtained from all patients and participants involved in the study. Likewise, an assessment of changes in the characteristics of mineral, biochemical, and hormonal metabolism was carried out in 100 practically healthy patients of both sexes living in Grodno, Belarus. The examined female patients were in the age range of 24.3±0.24 years old and males were in the age range of 25.5±0.52 years old. All patients were evaluated for body mass index (BMI). BMI (kg/m²) was calculated as follows: BMI (kg/m^2) =weight (kg)/height (m²) [15, 16]. In accordance to the WHO recommendations, the study used the interpretation of BMI 18-24.9 indicators: (normal), 25-29.9 (overweight, pre-obesity). To find out how much calcium and potassium are in hair, the Xray fluorescence analysis (XRF) was done on a CEP-01 or ElvaX device with Elvatech MCA Software and MK-RE-Ob Software.

The spectrum determination of biochemical parameters in the blood (calcium, potassium, , urea, creatinine, cholesterol, highdensity lipoprotein (HDL) cholesterol, lowlipoprotein (LDL) cholesterol, density triglycerides, alanine aminotransferase- ALT, and aspartate aminotransferase-AST) was carried out using the BS-330 analyzer (Mindray, China) and reagents manufactured by Diasense (Belarus). The study of hormones in the blood (thyroxine, triiodothyronine, and pituitary thyroid stimulating hormone/TSH) was carried out by immunochemical analysis on the MiniVidas analyzer (BioMerioux, France) using reagents from BioMerioux, France.

The samples of biological material were obtained in a standard way (using Vacutainer vacuum systems manufactured by Beckton Dickinson, USA). The preparation of blood samples for research was carried out in a standardized way: centrifugation in a Fenox-



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24M centrifuge (China) at 3000 g for 10 minutes. The serum samples were taken into separate systems in which the study was conducted.

Statistical processing of the research material was performed using the SPSS Statistica 22.0 statistical software package. The differences in the compared groups were considered statistically significant at p<0.05.

To assess the features and criteria of the bioelemental status affecting the increase in body mass index, the median values of the calcium and pottasium in the hair of groups of females and males with a body mass index of 18-24.9 and 25-29.9 were calculated, and also the comparative analysis of the concentrations of elements was done in the blood serum.

The results of statistical processing of data on the content of bio elements in hair and blood are presented in Tables 1.1-1.2.

Results and discussion

TABLE 1.1 The quantitative assessment of the availability of bio-elements for a group of females depending on the body mass index according to the elemental analysis of hair and blood

	Blood - n	ng/mL	Hair (mean) - µg/g Ca/k		Ca/k -Hai µg	. ,
Elements	BMI 18-24.9 kg/m ²	BMI 25- 29.9 kg/m²	BMI 18-24.9 kg/m ²	BMI 25- 29.9 kg/m²	BMI 18- 24.9 kg/m ²	BMI 25- 29.9 kg/m ²
Calcium Potassium	51.5±1.7 1513±53.1	58.8±1.33* 1205±65.2	1613.0 89.8	1213,0 72.5	17.9	16.7
* - p < 0.05						

TABLE 1.2 The quantitative assessment of the availability of bio-elements for a group of males, depending on the body mass index according to the elemental analysis of hair

	Blood -	· mg/mL	Hair (mea	an) - μg/g	Ca/k -Hair(mean)- µg/g	
Elements	BMI 18-	BMI 25-	BMI 18-	BMI 25-	BMI 18-	BMI 25-29.9
	24.9 kg/m^2	29.9 kg/m ²	24.9 kg/m ²	29.9 kg/m ²	24.9	kg/m ²
Calcium	50.5±1.1	60.4±2.01*	530.6	338.8	7.9	۲O
Potassium	1422.9±84	1277.3±42*	66.9	57	7.9	5.9
* 0.05						

* - p < 0.05

Analysis of the results showed that the concentration of bio-elements in the blood serum significantly differed in both females and males with various BMI values. We found a higher content of calcium but a lower content of potassium, both in females and in males with overweight compared to patients with the normal BMI values (p = 0.01). In

addition, only the overweight females and males have lower potassium values than males with lower BMI values.

An analysis of the results of a clinical biochemical study and the level of hormones in the blood serum of females and males is provided in Tables 2 and 3.

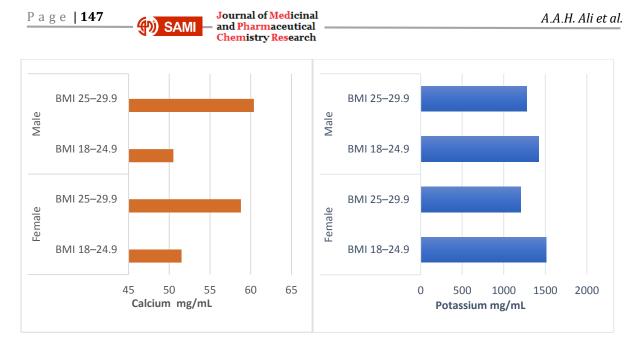


FIGURE 1 Serum calcium and potassium concentrations for males and females with a BMI of 18-24.9 and BMI of 25-29.9

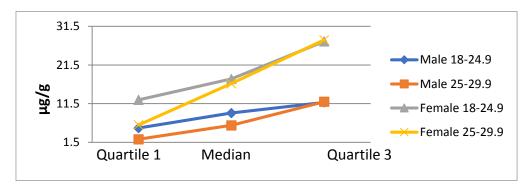


FIGURE 2 Hair calcium/potassium ratio for males and females with a BMI of 18–24.9 and BMI of 25–29

TABLE 2 The concentration of biochemical parameters in blood serum in females and males in the age category of \ge 18 years (clinical biochemical analysis)

	Females		-	· · · · ·	Male		
Indicator	BMI 18- 24.9 kg/m ²	BMI 25- 29.9 kg/m ²	P- value	Indicator	BMI 18- 24.9 kg/m ²	BMI 25- 29.9 kg/m ²	P- value
Total protein	63.4±2.3	71.9±3.5	=0.05	Total protein	74.3±3.2	77.4±4.1	=0.55
Total bilirubin	7.36±0.6 4	9.36±0.61	=0.02	Total bilirubin	11.44±1.18	12.44±1.18	=0.63
Glucose	4.48±0.1 1	5.29±0.21	=0.0.0 1	Glucose	4.63±0.13	5.79±0.18	0.004
Urea	6.11±0.3 8	6.03±0.2	0.85	Urea	6.41±0.41	6.83±0.31	=0.71
Creatinine	74.43±1. 5	77.88±1.3	=0.05	Creatinine	93.69±3.53	97.96±4.73	=0.06
Cholesterol	4.18±0.3	6.38±0.23	=0.001	Cholesterol	4.22±0.28	6.62±0.64	0.001
Cholesterol- HDL	0.59±0.1 9	0.78±0.21	=0.50	Cholesterol- HDL	0.42 ± 0.14	0.62±0.12	0.28
Cholesterol- LDL	2.99±0.4 9	3.69±0.45	=0.29	Cholesterol- LDL	2.09±0.34	4.04±0.53	0.003
Triglycerides	1.43±0.1 6	1.91±0.18	=0.01	Triglycerides	1.22±0.28	2.07±0.48	0.01
ALT	19.7±2.6	27.7±1.5	=0.01	ALT	24.67±3.17	29.67±2.17	0.03

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				Chemistry			
AST	21.6±2.1	24.96±1.6	=0.20	AST	23.9±1.21	24.9±1.36	0.81
Reference va	alues (populati	on of resident	s of the Gro	odno region,	associate profess	sor, O.E. Kuznet	sov, and
]	MSC Adeeb	Hussein Ali)			
Total	l protein			6	5-85 g/l		
Total bilirubin			3.4-20.4 μmol/l				
Glucose			4.1-6.1 mmol/l				
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Glucose	4.1-6.1 mmol/l
Urea	2.5-8.3 mmol/l
Creatinine	Males: 62-115 mmol/l
Creatinine	Females: 53 - 97 mmol/l
Cholesterol	3.5-5.2 mmol/l
Chalastaral UDI	Males: 0.72-1.63 mmol/l
Cholesterol Cholesterol-HDL Cholesterol-LDL	Females: 0.86-2.28 mmol/l
Cholesterol-LDL	1.71–3.5 mmol/l
Triglycerides	<1.7 mmol/l
ALT	5-42 Units/l
AST	2-32 Units/l

TABLE 3 The level of hormones in the blood serum in females and males in the age category of ≥ 18

	Females				Males		
Indicator	BMI 18- 24.9 kg/m²	BMI 25- 29.9 kg/m ²	P- value	Indicator	BMI 18- 24.9 kg/m ²	BMI 25- 29.9 kg/m²	P- value
TSH	2.45±0.15	4.45±0.27	0.0001	TSH	2.01±0.11	4.64±0.31	0.0001
Triiodothyronine (free)-T3	3.45±0.19	3.38±0.23	0.83	Triiodothyronine (free)-T3	3.12±0.33	4.01±.11	0.013
Thyroxine (free)-T4	17.27±0.21	12.41±0.29	0.0001	Thyroxine (free)-T4	15.44±0.17	11.02±0.41	0.0001
Cortisol	262.1±29.8	370.44±84.5	0.23	Cortisol	240.68±13.6	489.1±54.9	0.0006
Vitamin D	28.6±3.3	19.8±2.54	=0.01	Vitamin D	37.5±2.4	26±3.04	=0.01
Reference valu	es (population	of residents of	the Grodr	io region, associate p	orofessor, O.E. H	Kuznetsov, and	l MSC
			Adeeb Hu	ıssein Ali)			
TSH				0.25-4.	0 μU/mL		
Triiodothyr	onine (free)-T3	3		3.10-5.	9 pmol/l		
T 1 ·	(())			0.40.0			

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According to the results of a biochemical study of patients aged over 18 years with a BMI of 25-29.9, the concentration of glucose, cholesterol, and triglycerides increased with increasing BMI (weight gain) in both men and women (p = 0.001, p = 0.004, and p = 0.01). It should be noted that the high-density lipoprotein (HDL) cholesterol and the lowdensity lipoprotein (LDL) cholesterol in females did not have noticeable changes, although there was a tendency to increase them with increasing body mass index. The LDL cholesterol levels in the male half of patients with a BMI of 25 to 29.9 increased significantly to 1.67 mmol/L, compared with 1.22 mmol/L in patients with a BMI of 18-24.9 (p = 0.003).

Any change in the enzymatic activity of hepatocytes was reliably observed among both females and males. When body weight was increased, the level of alanine aminotransferase increased p = 0.01 and p = 0.03, respectively. This fact indicates that an imbalance in calcium and potassium leads not only to an imbalance of elements in the human body but also to the violation of metabolic processes.

The results of studies of the levels of TSH hormones, free thyroxine, free triiodothyronine, and cortisol, in males and females with a BMI of 18-24.5, was within the physiological reference values.

TSH was considered with (BMI 25-29.9, males and females aged \ge 18, p = 0.0001), free



triiodothyronine with (BMI 25-29.9, males and females aged \geq 18, p=0.013), free thyroxine with (BMI 25-29.9, males and females aged \geq 18, p=0.0001), and cortisol with (BMI 25-29.9, males aged \geq 18, p=0.0006).

According to the results of the study, the level of the hormone cortisol, according to the results of the study, was within the physiological norm for these age groups, taking into account gender, but at the same time, its concentration with increasing body weight (BMI = 25-29.9) tended to increase in females and significantly increased among the male population (p = 0.006). Analyzing the correlation component of cortisol with indicators of a biochemical study, a correlation was established with the glucose level in patients with a BMI of 25-29.9 (r = 0.57, p = 0.038).

Because of the role that hormones play in the body, the imbalance of calcium and potassium found in the overweight patients when conducting a preliminary analysis of hair and the results of calcium and potassium concentration in the blood serum, leads to a characteristic change in men and women, both in the direction of decrease and increase. In the bio-elements concentration, it can be assumed that there is the metabolism violation.

In both groups of the overweight patients, a deficiency of vitamin D (a decreasing trend) was identified (p=0.01).Several mechanisms appear to underlie the association between the overweight (obesity) and vitamin D deficiency. First, with increasing body weight, fat-soluble vitamin D is distributed in a larger volume, which leads to a decrease in its concentration in plasma. This determines the decrease in the bioavailability of vitamin D, both ingested and synthesized in the skin. Secondly, it can be assumed that in obesity, because obese patients tend to wear more closed-clothing and spend less time in the sun, the normal synthesis of vitamin D in the skin is decreased under the sunlight impact [17].

In the examined patients with a BMI of 25-29.9, the level of thyroid-stimulating hormone in the blood varied from 0.05 μ U/mL to 13.1 μ U/mL and averaged 4.56±4.04 μ U/mL. Taking into account the function of thyroidstimulating hormone, all examined patients with a BMI of 25–29.9, depending on the TSH level, were divided into groups:

- TSH <0.5 μU/mL. 4 patients (5.7%), TSH level 0.29±0.03 mIU/l.

- TSH 0.5-2.4 μ U / mL. 22 patients (60.0%), TSH level 1.52±0.03 μ U/mL. Patients with a normal functional state of the thyroid gland.

- TSH 2.5-4.0 $\mu U/mL$ 16 patients (22.8%), TSH level 3.19±0.03 $\mu U/mL$, limiting TSH value.

- TSH> 4.0 μ U/mL, which corresponds to the subclinical hypothyroidism. This group (I) included 8 patients (11.5%). The TSH value was 4.56±0.29 μ U/mL. The level of free thyroxine among the patients in this group was 9.85 - 13.57 μ U/mL (11.71±1.86 μ U/mL).

Thus, in 8 patients, the subclinical hypothyroidism was detected an increase in thyroid-stimulating hormone with a normal level of free thyroxine. Most often, the subclinical hypothyroidism is asymptomatic. In this group (I) of patients, the mean age was 28.5±3 years. The number of the prevailed females was 6 out of 8.

To sort the types of hyperlipoproteinemia, the classification of Fredrickson (1967) [18][19] was used:

-Type I: Hyperchylomicronemia, elevated cholesterol, and triglycerides as normal or slightly elevated levels of pre-β-lipoproteins. Atherosclerosis was not noted.

-Type II: Hyper- β -lipoproteinemia and hypercholesterolemia. The type is divided into 2 subtypes: IIa-increased content of β lipoproteins and cholesterol, normal content of pre- β -lipoproteins and triglycerides, the absence of chylomicrons; IIb - increased content of pre- β -lipoproteins, β -lipoproteins and cholesterol, the increased triglycerides, and the absence of chylomicrons.

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-Type III: The presence of floating β lipoproteins, increased content of β lipoproteins, pre- β -lipoproteins, cholesterol, and triglycerides. There may be a small number of chylomicrons.

-Type IV: Hyper-pre- β -lipoproteinemia. Normal content of β -lipoproteins, the elevated triglycerides, and the elevated or normal cholesterol. Absence of chylomicrons.

-Type V: increased concentration of β lipoproteins, triglycerides, cholesterol, and the presence of chylomicrons.

In group I, the following distribution of hyperlipidemia by groups was established (Fredrickson, 1967):

Type I - 2 patients (25%).

Type IIa - 2 patients (25%).

Type IIb - 3 patients (37.5%).

Type IV - 1 patient (12.5%).

In women, the mean of TSH was higher and amounted to $4.45\pm0.27 \ \mu\text{U/mL}$, and in men - $4.64\pm0.31 \ \mu\text{U/mL}$, compared with patients with BMI = $18-24.9 \ (p = 0.0001)$. Thus, an

odds ratio study (Mantel-Hensel method and logistic regression coefficients) showed that the female gender was linked with an increased risk level of TSH (OR = 1.89, CI = 1.28-2.79) and subclinical hypothyroidism (OR = 3.91 and CI = 2.5-6.10).

As can be seen from the presented data, the subclinical hypothyroidism had a high rank of prognostic significance and increased the risk of developing a high body mass index. Thus, subclinical hypothyroidism, along with wellknown risk factors, may be an additional autonomous sign of the obesity danger.

In the male group, a positive relationship was established between TSH level and BMI (r = 0.12, p = 0.02). This was also detected in the group of examined women (r = 0.20, p = 0.03).

An analysis of smoking data showed that 34.2% of patients had never smoked, 37.3% had smoked in the past, and 28.5% were the current smokers. Analyzing the gender of smoking patients, it was noted that they are mostly men (91.9%; p = 0.01).

TABLE 4 Clinical and laboratory parameters according to smoking

Indicator	Never smoked	Smoked in the past	Smoker
Age	29.4 ± 0.5	26.6 ± 0.5	22.6 ± 0.6*
Male/ Female (%)	52 / 48	90 / 10	91.9 / 8.1
TSH	2.92 ± 0.23	2.21 ± 0.17	2.11 ± 0.11**

* - p = 0.02, between "never smoked" and "have smoked in the past";

** - p = 0.005, between "never smoked" and "smoke"

As can be seen, in patients who have never smoked, the serum TSH levels were higher $(2.9 \pm 0.2 \mu U/mL)$ than in patients who currently smoke $(2.1 \pm 0.1 \mu U/mL)$, (p=0.001). Hormone levels remained within the reference value.

This fact seems unusual, since it was expected that TSH in smokers would be significantly higher due to the fact that the thiocyanate contained in tobacco blocks, the flow of iodine to the gland, and the organization processes. Apparently, in the population rich in iodine, such a pronounced negative outcome of smoking on the thyroid gland is not observed. In addition, when the data interpretation is obtained, it should be borne in mind that non-smokers are older than smokers (p = 0.01). It is known that an increase in the TSH level is more often detected in the elderly. However, among the surveyed, the increase in the TSH level depended on age only in females, and in the group of males, there was no such pattern.

In patients with the elevated glucose levels and a BMI of 25-29.9, the mean of TSH level was higher (2.97 \pm 0.23 μ U/mL) than in patients with a normal level of carbohydrate metabolism and a BMI of 18-24.9 (2.45 \pm 0.09 μ U/mL), (p<0.01). Thus, among patients with the elevated glucose concentration (6 patients), an increase in TSH was more often detected: subclinical hypothyroidism was



detected 1.5 times more often than in patients with normal glucose concentration. The presence of the elevated TSH concentrations among patients with a presumed history of subclinical hypothyroidism was correlated with the elevated glucose levels (OR = 1.93, CI 1.11-1.36, p = 0.026).

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Indicator	r	P-value	
Cholesterol	0.68	p< 0.05	
Triglycerides	0.57	p< 0.05	
LDL cholesterol	0.68	p< 0.05	
Triiodothyronine (free)	-0.39	p< 0.05	
Thyroxine (free)	-0.45	p< 0.05	

TABLE 5 Correlation between thyroid stimulating hormone (TSH) levels and lipid profile

A moderate direct relationship was found between TSH levels and all lipid parameters except HDL cholesterol (p=0.125).

The content of TSH shows a significant correlation with the cholesterol level (p = 0.000521) and triglycerides (p=0.00084).

Thus, since according to the results of a biochemical study of patients \geq 18 years with a body mass index of 25–29.9, it can be reliably stated that the concentration of glucose, cholesterol, and triglycerides increased with a change in body mass index (weight gain) in men and women. Among the male half of the LDL cholesterol significantly subjects, increased with body weight (p = 0.003), and the level of thyroid-stimulating hormone has a direct moderate relationship with all lipid parameters, except for HDL cholesterol (p=0.125) [26-30].

The level of the hormone cortisol was within the physiological norm for these age groups, taking into account gender, but at the same time, its concentration in patients \geq 18 years old with a BMI of 25-29.9 tends to increase among women and significantly increase among the male population (p = 0.006). The correlation of cortisol with indicators of biochemical research is significant with the glucose level in patients with a BMI of 25-29.9 (r= 0.57, p = 0.038) [31-35].

The results show a clear, significant, and correlation-dependent imbalance in the elemental status in overweight patients, both men and women, in the age group of patients ≥ 18 years, which has an imbalance in hormonal levels (pituitary hormones-TSH, thyroid hormones-T3/T4 free, and adrenal hormones cortisol), metabolites, substrates, and enzymes of blood plasma (glucose, cholesterol, LDL cholesterol, triglycerides, and alanine aminotransferase).

The imbalance established in the blood serum samples for bioelements (Ca and K), for which there are similar changes in hair samples, suggests that these disorders are associated with metabolic processes in the body, which are expressed in an increase in the body weight.

Conclusion

The hair Ca/K coefficient can be used to assess the activity of calcium-regulating hormones as a bio-element expression of their effect. The Ca/K ratio was greater than 5 in 64% of males with а BMI of 25-29.9.The hair calcium/potassium ratio was greater than 5 in males and females with a BMI of 25-29.9. This has not been previously covered in the literature. As a result, it is revealed that the increased body weight causes a 64.0% rise in the activity of calcium-regulating hormones in the male group, and in 92% of the females. Calcium content can be seen as a sign of how well bone tissue absorbs calcium and as a violation of the hormonal activity in the endocrine system that controls how calcium is used in the body.

Conflict of interest

The authors declare no conflict of interest.

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The study was performed without external funding.

Conformity with the principles of ethics

The study was approved by the Local Ethics Committee.

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