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BODY COMPOSITION, BIOCHEMICAL INDICATORS GRIP STRENGTH AND GAIT SPEED IN WOMEN WITH HYPERTHYROIDISM

THESIS SUMMARY

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The defense materials are available at the Scientific Department of MU-Varna and are published on the website of MU "Prof. Dr. Paraskev Stoyanov" - Varna.

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List of abbreviations

ASMI	appendicular skeletal muscle mass index
BIA	bioelectrical impedance analysis
BMI	body mass index
CPK	creatine phosphokinase
DXA	dual-energy X-ray absorptiometry
ESPEN	The European Society of Clinical Nutrition and Metabolism
EWGSOP	European Working Group on Sarcopenia in Older People
FNIH	The Foundation for the National Institutes of Health
FT3	free triiodthyronine
FT4	free thyroxine
HDLc	high-density lipoprotein cholesterol
LDL-c	low-density lipoprotein cholesterol
mRNA	messanger RNA
SAT	subcutaneous adipose tissue
TPO Ab	Anti–thyroid peroxidase antibody
TRAb	TSH receptor antibody
TSH	thyroid stimulating hormone
VAT	visceral adipose tissue
25 (OH) D	25-hydroxy vitamin D

I. Introduction

Hyperthyroidism is a condition characterized by increased production of thyroid hormones and an increase in their level in the bloodstream. The most common causes are Graves' disease, toxic polynodular goiter, and solitary toxic adenoma. Regardless of the cause of hyperthyroidism, it is more common among women than among men (*Meng Z et al., 2015*). The fact that it most often affects the working-age population further necessitates timely diagnosis and adequate therapeutic behavior.

Thyroid hormones are important for the normal functioning of the entire organism, and their excess causes a catabolic state characterized by increased energy expenditure, increased basal metabolism, increased lipolysis, bone matrix degradation, and increased protein metabolism (*Riis AL et al., 2005*). About 80% of those affected exhibit symptoms of asthenodynamia, palpitations, distal tremor, weight loss, and muscle weakness (*Bousquet-Santos K et al., 2006*).

Of interest are the changes in body composition caused by hyperthyroidism and the resulting, in most cases, weight loss. There is increasing emphasis on the optimal ratio of tissues for the health of the individual. Limited and mixed data are available in the literature on changes in body composition in excess of thyroid hormones. This prompted us to study it in newly diagnosed hyperthyroidism, and our results would contribute to the accumulation of information in this area.

Some groups have observed that weight loss and changes in body composition are primarily due to a decrease in adipose tissue, which is the first to increase after reaching euthyroidism (*Greenlund LJ et al, 2008*). Other studies report an initial and more significant impact on lean body mass (mainly muscle), and on body fat to a lesser extent (*Dutta P et al.. 2012*).

Muscles are a target organ for thyroid hormones, as demonstrated by myopathic symptoms occurring in 50–62% of individuals with hyperthyroidism (*Duyff RF et al., 2000*). Both proximal and distal skeletal muscles are affected (*Olson BR et al., 1991*), and objective functional impairment in hyperthyroidism was first assessed in 2015 by Erkol İnal et al. (*Erkol İnal E et al., 2015*).

Grip strength is a test that is gaining popularity in many areas of medicine. It reflects not only the physical capacity of an individual, but is also a marker of their overall health. Decreased grip strength is associated with dangerous falls, longer hospital stays, poorer quality of life, and higher mortality (*Prasitsiriphon O et Pothisiri W, 2018*). It is proposed as a biomarker for determining the risk of disability and deterioration in quality of life with much better predictive value than chronological age. Grip strength testing is increasingly recommended in various areas of medicine for stratifying the risk of cardiovascular mortality, monitoring the effect of therapeutic behavior, and as a predictor of disease outcome (*Leong DP et al., 2015*).

Reduced grip strength is one of the diagnostic components of sarcopenia and age-related frailty. Although it is an easily applicable test, publications reflecting grip strength among hyperthyroid individuals are limited in number.

Another indicator of the functional state of the muscles and physical capacity is gait speed. It is an important indicator of the health status, as slowed walking speed is associated with increased disability, difficulty in performing daily activities, impaired quality of life, as well as increased healthcare costs (*Bano A et al., 2016*). Walking speed is a study recommended by the European Working Group on Sarcopenia (EWGSOP2) in order to objectify the physical performance of those affected, with slowed speed defining sarcopenia as severe (*Cruz-Jentoft AJ et al., 2019*). Studies of quantitative changes, as well as the functional state of the muscles in thyroid dysfunction are limited (*Bano A et al., 2016*). Of interest is the fact whether these changes are identical to sarcopenic changes.

Sarcopenia is a disease that affects various quantitative and qualitative aspects of the musculature and the collection of data on it is in its infancy. The importance of the problem lies in the increased risk of disability, adverse health consequences and mortality. While there is a clear definition of the term "primary sarcopenia", there is a lack of clarity on the question of which diseases can lead to the development of secondary sarcopenia.

A limited number of studies indicate hyperthyroidism as a likely factor for the earlier development of secondary sarcopenia among those affected. The lack of sufficient information in the literature sources about the relationship between these two disease processes stimulates our scientific search in this direction.

II. Aim and objectives

1. Aim

The aim of the scientific study is to assess the relationship of hyperthyroidism in women with body composition, grip strength and walking speed (diagnostic components for sarcopenia), as well as with some biochemical indicators (vitamin D, creatinine, creatine phosphokinase, albumin).

2. Objectives

To achieve this goal, we set the following tasks:

1. To compare anthropometric and biochemical parameters between women with hyperthyroidism and healthy controls.

2. To compare the body composition of the two groups (healthy controls and women with hyperthyroidism).

3. To compare grip strength, appendicular skeletal muscle mass index (ASM/m^2, ASMI) and gait speed in women with hyperthyroidism and healthy controls.

4. To search for a relationship between anthropometric indicators and body composition, as well as between anthropometric indicators and diagnostic criteria for sarcopenia - ASMI, grip strength and gait speed.

5. To search for a relationship between hormonal and biochemical indicators and body composition, as well as between hormonal and biochemical indicators and diagnostic components of sarcopenia - grip strength, appendicular skeletal muscle mass index and gait speed.

6. To establish the risk of developing secondary sarcopenia, to determine its frequency among the studied individuals and the threshold age above which the risk of secondary sarcopenia increases.

7. Among individuals with identified sarcopenia, to search for correlations between anthropometric, hormonal and biochemical indicators, on the one hand, and the diagnostic components for sarcopenia - ASMI, grip strength and gait speed - on the other.

8. Comparison and search for correlations between the studied parameters among women with hyperthyroidism, compared to those with hyperthyroidism and established sarcopenia.

III. Materials and methods

1. Study design

A cross-sectional case-control study was conducted among adult women. The selection and examination of participants took place in outpatient settings, in the period 2020-2022, which coincided with the Coronavirus pandemic.

Patient recruitment began after permission was received from the Research Ethics Committee of MU-Varna on 19.12.19, protocol No. 89.

We studied a total of 90 women, divided into 2 groups - 45 of them affected by hyperthyroidism (group A), and the remaining 45 - without data on thyroid pathology, serving as a control group (group B).

Additionally, for sub-analysis, group A was divided into 2 subgroups - subgroup A1 (women with hyperthyroidism, without sarcopenia), and subgroup A2 (women with hyperthyroidism, with sarcopenia).

2. Criteria for selecting study participants

The selection of participants took place after a strict selection of women who met specific inclusion and exclusion criteria.

2.1. Inclusion criteria:

- female;

- persons over 18 years of age;

- newly diagnosed hyperthyroidism (Graves' disease, single toxic nodule, toxic polynodular goiter) with symptoms lasting no more than 6 months;

- not having undergone therapy for thyroid disease to date;

- controls were similar in gender and age, with no evidence of current or previous thyroid dysfunction with negative thyroid antibodies.

2.2. Exclusion criteria:

Exclusion criteria for participation in the study are: lack of informed consent by the patient; mental disorders, partial or complete immobilization, recent limb surgeries (less than 1 year), recent use (minimum 3 months) of drugs with an effect on the immune or muscular system (antiviral medications, vaccinations, corticosteroids, statins, sex and growth hormones), diseases (acute and chronic viral hepatitis, cirrhosis, tuberculosis, HIV, diabetes mellitus, chronic kidney disease, heart failure, COPD, rheumatoid diseases, anorexia and marasmus, carcinomas, bariatric surgery, Cushing's syndrome, hypogonadism), pregnant women, women who have given birth up to 12 months after giving birth, as well as lactating women.

3. Methods

At the initial visit, a medical examination was performed, including anamnestic data on existing complaints, duration of symptoms, accompanying pathology, medication intake, risk factors, and family history.

3.1. Anthropometric methods

Height (cm) was measured in an upright position with the head and other dorsal surfaces of the body in close contact with the wall using a static height gauge attached to it.

Weight (kg) was measured using a Tanita BC-545N Body Composition Analyzer. The same device was used to determine the various components of body composition using bioimpedance analysis (BIA).

Body mass index (BMI) was calculated based on the following formula:

BMI (kg/m²) = weight (kg) /height² (m²).

A non-stretchable centimeter with an accuracy of 0.1 cm was used for waist and hip circumferences (cm). The measurement was performed at the end of expiration, midway between the lower edge of the 10th rib and the upper border of the iliac bone.

3.2. Physical methods

3.2.1. Muscle strength assessment

To determine muscle strength, we measured grip strength (in kilograms) using a portable ergonomic hand-held dynamometer, model E19.5503. The patient was positioned in a sitting position with the shoulder adducted, the forearm flexed at the elbow joint at 90 degrees, and the forearm and wrist in a neutral position. The subject was instructed to squeeze the device with maximum force 3 times for each hand with a 30-second rest between attempts. The measured maximum absolute value was taken as an indicator (Figure 1).

Figure 1. Measuring grip strength

3.2.2. Physical capacity assessment

To assess physical capacity, according to the recommendations of EWGSOP2, we used walking speed (m/s), measuring the time it took to walk a 4-meter distance with a normal gait.

3.3. Body composition assessment

To assess body composition, we used a portable device available in outpatient settings - a bioelectrical impedance analysis device Tanita BC-545N.

The study was performed according to ESPEN recommendations - in the morning on an empty stomach, without drinking fluids and coffee, without previous physical exertion. The patient is in an upright position with bare feet placed on the lower 2 electrodes and arms bent at 90 degrees at the elbow joint, covering the other 2 electrodes tightly with fingers and palms. The measurement was performed outside the menstrual bleeding period of women (Figure 2).

Figure 2. Body composition measurement

3.4. Laboratory diagnostics

All hematological tests were performed in the morning, after a 12-hour overnight fast. Blood was taken from the cubital vein of each patient, with subsequent centrifugation and separation of serum. Roche Diagnostics laboratory systems were used, with a Cobas Pro- apparatus for biochemical parameters and a Cobac 6000- for thyroid parameters.

Biochemical indicators:

- creatinine, determined by the Jaffe kinetic method, reference values 58.00 – 96.00 mkmol/L;

- creatine phosphokinase- measured by spectrophotometry; reference values for women < 170.0 U/L;

- total protein- spectrophotometry/colorimetry, reference values 60.00 – 83.00 g/L;

- albumin- spectrophotometry/colorimetry, reference values 35.00 – 53.00 g/L;

- ASAT - IFCC method, reference values < 42.00 U/L

- ALAT - IFCC method, reference values < 42.00 U/L

- total cholesterol (TC) - enzymatic method, reference values 3.5 - 5.2 mmol/L

- LDL-cholesterol - enzymatic method, reference values < 3.34 mmol/L

- HDL-cholesterol - enzymatic method, reference values > 1.55 mmol/L

- triglycerides (TG) 0.60 - 2.20 mmol/L

- blood glucose - hexokinase method, reference values 3.3 - 5.6 mmol/L;

- 25-hydroxyvitamin D (25(OH)D) - chemiluminescent immunoassay; reference values 20.6 - 50.00 ng/mL.

Hormonal parameters:

A chemiluminescent immunoassay was used to study thyroid parameters. The following were tested:

- thyroid-stimulating hormone (TSH): reference values 0.35 – 4.59 mUI/L;

- free triiodothyronine (FT3): reference values 3.10 – 6.80 pmol/L;

- free thyroxine (FT4): reference values 12.00 – 22.00 pmol/L;

- thyroid peroxidase antibodies (TPO Ab): reference values <34.0 Iu/mL;

- thyrotropin Receptor Antibodies (TRAb): reference values (U/mL) <1 negative; 1.1-1.9 borderline; >2.0 positive.

3.5. Statistical Methods

Data were processed using IBM SPSS Statistics Version 26.0 for Windows 10.

3.5.1. Method for statistical grouping of data

The main stage of statistical research is the statistical grouping of data. This serves as a basis that provides an opportunity to penetrate the structure of the studied phenomenon and to reveal connections, dependencies and influences. As a result of correctly conducted grouping of data, empirical distributions of one-dimensional, two-dimensional or multidimensional nature are obtained.

3.5.2. Descriptive methods

We used descriptive methods to assess quantitative variables. Depending on the purpose of the study, we applied different descriptive methods, such as using averages and medians to identify central tendencies, using standard deviations to identify differences between units, and using deviations from standard distributions (coefficient of skewness, coefficient of kurtosis).

3.5.3. Statistical hypothesis testing

To test statistical hypotheses, we have formulated a basic (null) hypothesis and an alternative hypothesis. The hypothesis test goes through the following algorithm, which is also followed in the dissertation:

- definition of the null hypothesis H_0 and the alternative hypothesis H_1;

- selection of a significance level α (risk of error) or “p-value”;

- selection of an appropriate statistical criterion and theoretical distribution directly related to the formulated null hypothesis;

- The choice between the null and alternative hypotheses is reduced to a comparison of the adopted reference significance level (risk of error α/ “p-value”) and the calculated threshold significance level. This method was applied in the dissertation when testing statistical hypotheses. Statistically significant results are considered to be those with a “p-value” <0.05, and statistically insignificant - “p-value” >0.05.

- If the significance level calculated based on the sample data is less than the significance level accepted as the norm, the null hypothesis is rejected and the alternative hypothesis is accepted, and if the significance level calculated is greater than the significance level accepted as the norm, the main hypothesis is accepted.

To test statistical hypotheses, we used T-test for dependent and independent variables with Levene's test for equality of variances.

3.5.4. Correlation analysis

Correlation analysis is a statistical method that measures the strength and direction of the correlation between two or more phenomena. The main measure of the closeness of the relationship is the correlation coefficient r. Its value is interpreted according to the scale presented below (table 1).

Value of the correlation coefficient r	Interpretation of the strength of the correlation
0	No relationship
0-0,3	Weak relationship
0,3-0,5	Moderate relationship
0,5-0,7	Significant relationship
0,7-0,9	Strong relationship
0,9-1	Very strong relationship
1	Functional relationship

Table 1. Determining the strength of correlation based on the coefficient “r”

When the correlation coefficient r has a positive value, it can be argued that the relationship between the phenomena is direct. When the correlation coefficient r has a negative sign, it is argued that the relationship is inverse.

It is essential to assess whether the obtained correlation coefficient is statistically significant. The decision is reduced to a comparison of the adopted reference level of significance (risk of error α) and the calculated threshold level of significance (Significance). This method was applied in the present study when checking the statistical significance of the obtained correlation coefficient r.

If the calculated level of significance (Sig) based on sample data is less than the accepted norm level of significance (α/p-value), it is assumed that the obtained correlation coefficient is statistically significant and reliable. If the calculated level of significance (Sig) is greater than the accepted norm level of significance (α/p-value), it is assumed that the obtained correlation coefficient is not statistically significant.

Correlation analysis is developed based on interval scales, that is, for quantitative variables. Logically, the analysis in the dissertation is based on absolute values, and in this case the Pearson parametric coefficient is appropriate.

3.5.4. Method for determining threshold values

To determine the age threshold values for the occurrence of secondary sarcopenia among women affected by hyperthyroidism, we used ROC curve analysis.

3.5.5. Graphical and tabular images

To display the results of the descriptive methods, we used a graphical representation of the empirical distribution through: histogram, structural pie and line diagrams. Scatter/dot diagrams were used to illustrate the correlation dependencies.

IV. Results

1. Results for task 1: Comparison of anthropometric and biochemical parameters between women with hyperthyroidism and healthy controls

1.1. Anthropometric indicators

90 women (45 with hyperthyroidism and 45 healthy controls) aged between 24 and 77 years were studied. The mean age of the patients with hyperthyroidism was 54.98 years (± 15.26), and that of the control group was 54.36 years (± 14.57) (p 0.862). The asymmetry coefficient, which is relevant in the distribution of units, has a value in the entire sample of patients of (-0.118), which indicates moderate asymmetry with a right-skewed distribution curve /shown in the histogram below/ and comparability of the two groups in terms of age (Figure 3).

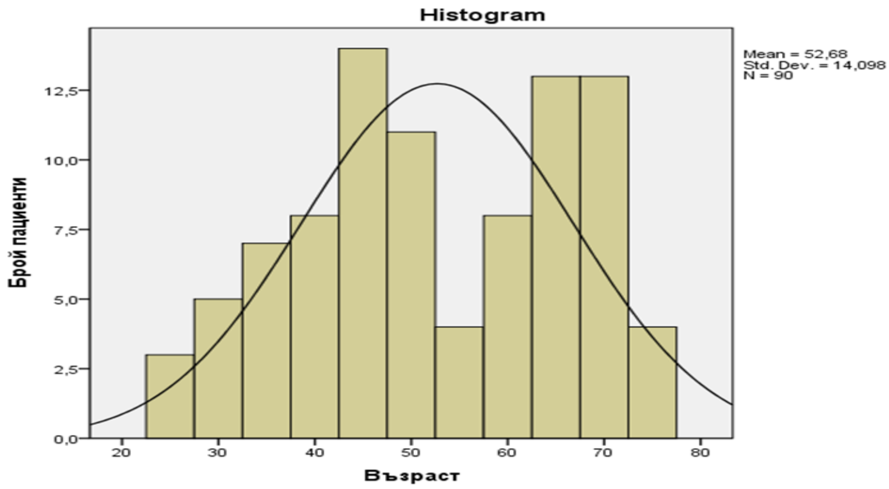


Figure 3. Distribution of patients by age in the total sample

The results of the anthropometric indicators (height, weight, BMI, waist, hip, waist/hip ratio) of the two groups are presented in Table 2.

Indicator	*Group*	*N*	*Mean*	*Std. Deviation*	*p-value*
Age (years)	A	45	54,98	15,26	0,862
	B	45	54,36	14,57	
Height (cm)	A	45	163,07	7,475	0,777
	B	45	163,49	6,598	
Weight (kg)	A	45	69,07	17,688	0,242
	B	45	73,31	16,421	
BMI (kg/m^2)	A	45	26,1489	6,27189	0,300
	B	45	27,5067	6,08394	
Waist (cm)	A	45	86,31	13,237	0,750
	B	45	87,20	13,166	
Hip (cm)	A	45	103,56	13,255	0,406
	B	45	105,82	12,505	
Waist/Hip	A	45	0,8297	0,06810	0,322
	B	45	0,8149	0,07291	

Table 2. Average values of anthropometric indicators of the two groups and statistical testing of hypotheses for the significance of the difference between the two groups (hyper- and euthyroid women).

The waist circumference of women in both groups exceeded the threshold of ≥ 80 cm, defined as a criterion for diagnosing metabolic syndrome in women, while the waist/hip ratio was normal – below 0.85. The majority of patients in both groups had a normal BMI (BMI 18.5-24.9 kg/m^2), with group A accounting for 46.63% of cases, while group B accounting for 42.11% of the included individuals. The proportion of overweight women was similar for both groups (group A – 28.95%; group B – 31.11%), and underweight or BMI < 18.5 kg/m^2 were observed only in the hyperthyroidism group (4.44%). The distribution of BMI among individuals in groups A and B is presented in Figure 4.

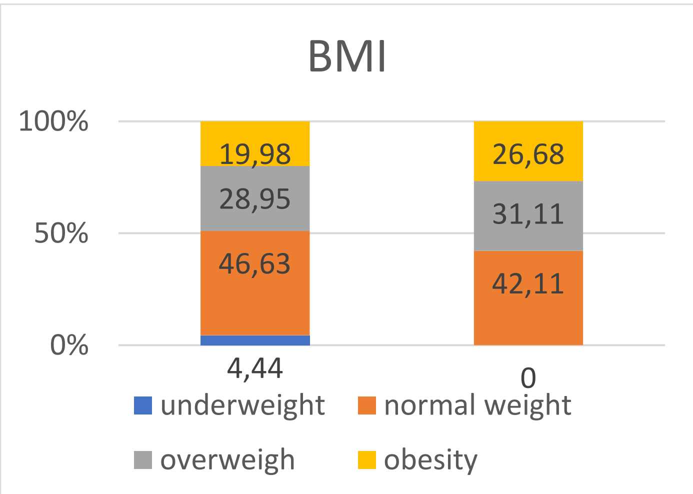


Figure 4. Distribution of individuals according to BMI, expressed in %

1.2. Laboratory parameters

1.2.1. Thyroid parameters

The values of the studied thyroid parameters reflect the sample of women included in the study - those with hyperthyroidism, mainly with autoimmune genesis and healthy controls. A necessary condition for selecting the control group B was the absence of thyroid disease, including a negative immunological profile (TPO Ab, TRAb).

The hormonal tests, expressed in a significantly reduced value of serum TSH and increased levels of FT3 and FT4, characterize the hyperthyroid state of the patients from group A and, as expected, show a statistically significant difference compared to the control group B. The patients from group A are mainly represented by individuals with Graves' disease (n=36), followed by toxic polynodular goiter (n=7) and a single toxic nodule (n=2) - figure 5.

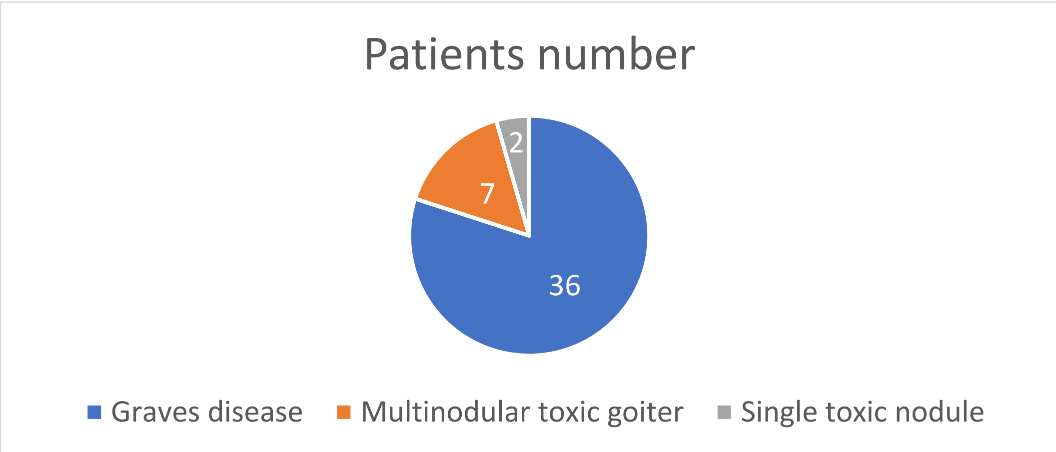


Figure 5. Distribution of patients in group A according to the cause of thyrotoxicosis

According to the thyroid status, 38 of the studied patients from group A had overt hyperthyroidism, and 7 had subclinical hyperthyroidism (2 cases with a single toxic nodule and 5 cases with toxic polynodular goiter).

The mean values of the immunological indicators - TPO Ab and TRAb were significantly higher in hyperthyroid individuals, while in women from group B they were lower and below the reference laboratory values diagnostic for autoimmune disease. The data are reflected in detail in table 3.

Indicator	Group	Mean	Std. Deviation	p- value
TSH (mIU/L)	A	0,1032	0,13294	**0,000**
	B	1,9804	0,91668	
FT3 (pmol/L)	A	10,3507	9,39974	**0,000**
	B	4,2851	0,85573	
FT4 (pmol/L)	A	34,2588	37,17789	**0,003**
	B	16,1598	2,36535	
TPOAb (IU/mL)	A	232,2834	352,06177	**0,000**
	B	12,5697	11,35092	
TRAb (IU/L)	A	5,5213	7,93423	**0,001**
	B	0,9017	0,27490	

Table 3. Thyroid indicators among individuals from group A and group B

1.2.2. Biochemical indicators

We studied a total of 12 biochemical indicators, 8 of which were used to identify individuals with exclusion criteria. The remaining 4 (creatinine, CPK, albumin and vitamin D) were analyzed for research purposes due to the relationship described in the literature with the distribution of body composition and the presence of sarcopenia. They are in our focus for comparison between the two groups of individuals.

Of the target indicators, two stood out with a significant difference in values between the two groups. These are creatine phosphokinase (CPK) - mean value for group A 75.23 ± 35.06 U/L and 98.11 ± 45.13 U/L for group B (p 0.021), as well as vitamin D - mean value for group A 19.30 ± 6.45 ng/mL versus mean 23.56 ± 11.11 ng/mL for group B (p 0.04). Creatinine showed a borderline significant difference (p 0.056) - group A 66.77 ± 14.09 µmol/L; group B 72.76 ± 10.39 µmol/L. The values of all the above-mentioned parameters are lower among individuals affected by hyperthyroidism, compared to euthyroid controls. They do not go beyond the accepted reference limits, with the exception of the vitamin D level. Similar to that in the general population, it is low, corresponding to vitamin D deficiency in group A (average 19.29 ± 6.45 ng/mL).

The individuals diagnosed with vitamin D deficiency and deficiency in the total sample are 31 in total, or expressed as a percentage of 34.4% of all examined. In group A, 20 individuals are vitamin D deficient, i.e. values < 20 ng/mL, with 2 having severe deficiency (< 10 ng/mL). For group B, there were 11 women affected by vitamin D deficiency, one of whom had a severe deficiency.

The vitamin D levels for the entire sample look like this: 2.22% of individuals in group A and 1.11% of women with euthyroidism have severe deficiency. Those with deficiency are 19.98% in group A, and their percentage distribution in group B is 11.15% (Figure 6).

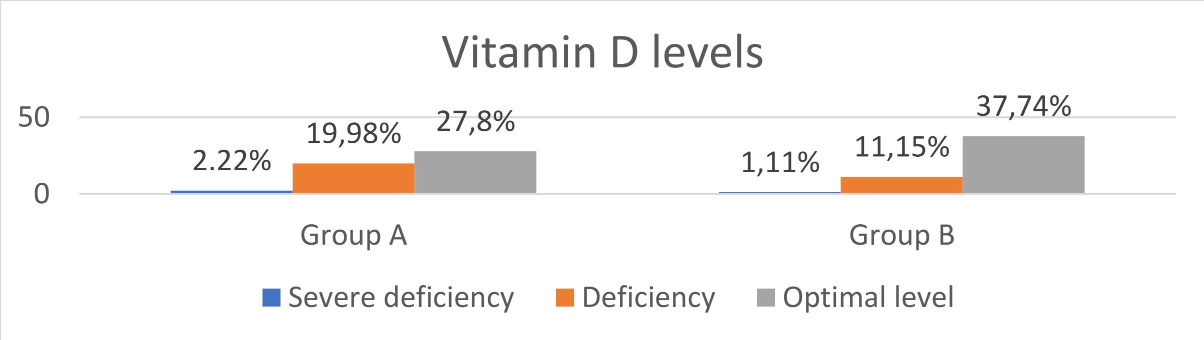


Figure 6. Distribution of patients from group A and group B according to vitamin D levels (the accepted values for severe deficiency are < 10 ng/mL, for insufficiency 10-20 ng/mL, suboptimal level >20-30 ng/mL, and optimal concentrations are >30-50 ng/mL).

Although within reference values, serum levels of total protein and albumin were lower in group A (71.86 ± 3.29 g/L and 44.14 ± 2.96 g/L, respectively) compared to group B (72.54 ± 5.95 g/L and 45.08 ± 5.95 g/L), with the difference not reaching statistical significance. Normal levels of total protein and albumin were an inclusion criterion in the study, therefore no patient in the sample had hypoalbuminemia.

The lipid parameters studied were within the reference range or with a slight deviation in the values, probably due to one of the exclusion criteria – taking statins. However, it is noted that the characteristic of hyperthyroidism is a lower level of total cholesterol, triglycerides and LDL-c. The difference between the levels of LDL-c between the two groups is significant (p 0.006). The values of HDL-c are lower among euthyroid individuals (1.43 ± 0.36), which does not reach statistical significance (p 0.086).

Liver transaminases - AST and ALT - were also examined, and only individuals with normal hepatic status were included in the selection of patients (without deviation in transaminase values and without anamnestic data for liver cirrhosis or viral hepatitis). Both indicators were higher in women with hyperthyroidism (AST 19.92 ± 9.65 U/L; ALT 20.56 ± 10.32 U/L), compared to healthy individuals (AST 17.20 ± 4.46 U/L; ALT 17.78 ± 7.35 U/L).

Glucose was examined to exclude subjects with type 1 or type 2 diabetes mellitus, due to its effect on body composition distribution. Therefore, glucose levels in the total sample were normoglycemic. However, values for patients in group A were slightly higher (5.34 ± 0.51 mmol/L) than controls (5.30 ± 0.57 mmol/L).

The data from the studied biochemical indicators are presented in Table 4.

Indicator	*Group*	*Mean*	*Std. Deviation*	*p- value*
Creatinine (µmol/L)	A	66,7663	14,09038	0,056
	B	72,7635	10,39106	
CPK (U/L)	A	75,2280	35,0554	**0,021**
	B	98,1155	45,13349	
Total protein (g/L)	A	71,8672	3,29038	0,688
	B	72,5406	5,95104	
Albumine (g/L)	A	44,1429	2,96060	0,309
	B	45,0882	2,79709	
Total cholesteron (mmol/l)	A	4,9052	1,00004	0,436
	B	5,2087	1,63228	
Triglycerides (mmol/l)	A	1,3044	0,64671	0,965
	B	1,3126	0,63065	
HDL-c (mmol/l)	A	1,6900	0,55384	0,086
	B	1,4353	0,36734	
LDL-c (mmol/l)	A	2,8052	0,88935	**0,006**
	B	3,5700	1,00536	
ASAT (U/L)	A	19,9174	9,64966	0,204
	B	17,1954	4,45606	
ALAT (U/L)	A	20,5600	10,31670	0,236
	B	17,7800	7,35478	
25 (OH) D (ng/mL)	A	19,2982	6,45188	**0,040**
	B	23,6715	11,10706	
Glucose (mmol/l)	A	5,3481	0,51293	0,700
	B	5,3013	0,57581	

Table 4. Average values of the studied biochemical indicators in the two groups of studied women – group A with hyperthyroidism and group B – healthy controls.2.

2. Results for task 2: comparison of body composition between group A and group B

2.1. Bone mass

Bone mass among women in hyperthyroid state was statistically significantly lower than that of healthy controls - in group A it was 2.23 kg (± 0.31), while in group B it reached 2.40 kg (± 0.35) (p 0.01) (Figure 7).

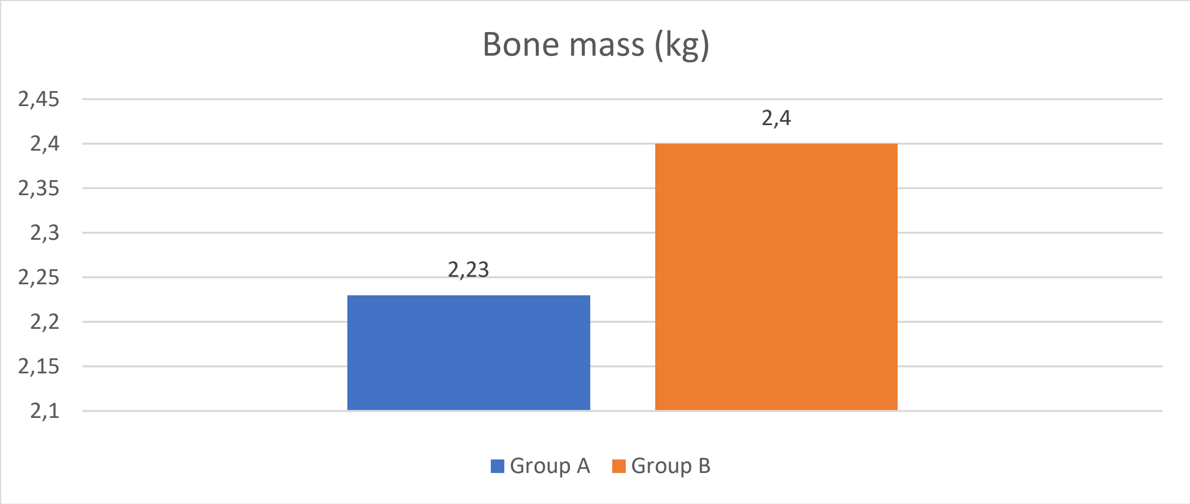


Figure 7. Average bone mass values in group A and group B

According to the reference values presented by the manufacturers of the Tanita analyzer, 20 women in group A had subnormal bone mass, 12 of whom subsequently met the criteria for sarcopenia. In the control group, 13 women had bone mass values lower than the norms for their age, with only one of them also having sarcopenia.

The distribution by age is described in Table 5.

Age (years)	Group A (number of patients)		Group B (number of patients)	
	With sarcopenia	**Without sarcopenia**	**With sarcopenia**	**Without sarcopenia**
18-40	1	3	2	0
41-60	1	3	2	1
61-64	1	2	2	0
>65	5	4	6	0

Table 5. Distribution of women with lower bone mass for age

2.2. Visceral fat

The mean mass of visceral fat among women with hyperthyroidism was 1.04 kg (± 0.44), while in healthy controls it was non-significantly higher - 1.12 kg (± 0.31) (p 0.242) (figure 8).

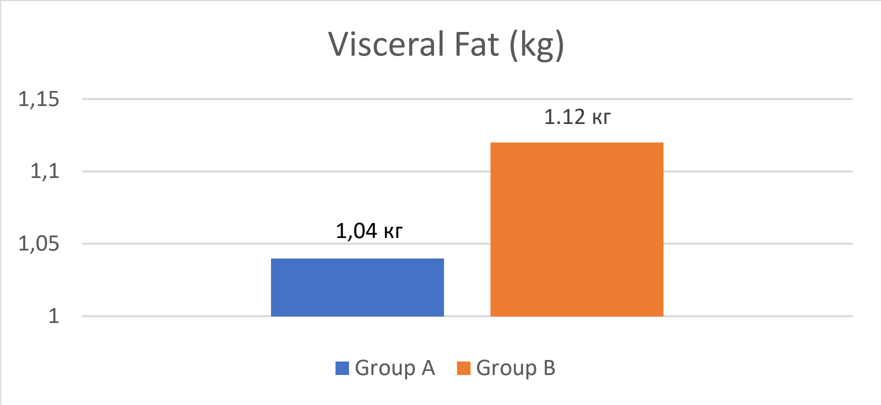


Figure 8. Amount of visceral fat in group A and group B

2.3. Subcutaneous adipose tissue

The percentage of whole-body subcutaneous adipose tissue was lower among women with hyperthyroidism (34.7972 ±6.55%), compared to healthy controls (35.1156 ±10.09%), the difference being non-significant (p 0.861).

The amount of adipose tissue in all four limbs of women in group A was reduced compared to the control group, without this difference reaching statistical significance. In the upper limbs, the lower level of adipose tissue in the right arm compared to the left is striking, while in the legs this relationship is in the opposite direction.

Despite the difference in the amount of adipose tissue in both upper and lower limbs in the two groups studied, this lateralization was not statistically significant (Figure 9, Table 6). Subcutaneous adipose tissue in the central body compartment was approximately the same in the two groups studied (p 0.920).

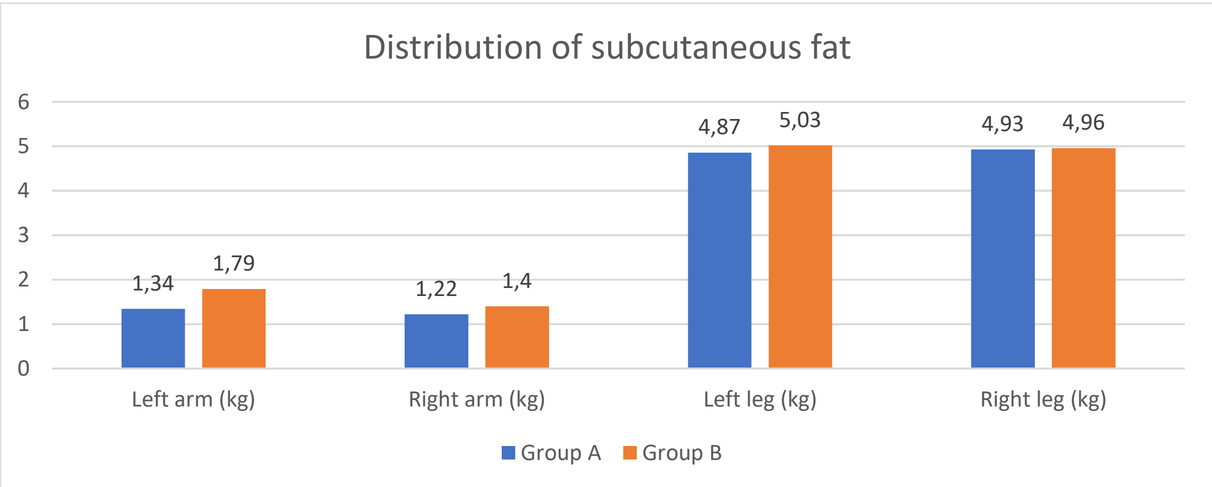


Figure 9. Amount of subcutaneous adipose tissue in the limbs in group A and group B

Indicator	Group	Mean (kg)	Std. Deviation	p- value
Left arm	A	1,3410	0,88998	0,249
	B	1,7868	2,41972	
Right arm	A	1,2183	0,75214	0,318
	B	1,3962	0,92024	
Trunk	A	12,7362	7,54674	0,920
	B	12,5957	5,57765	
Left leg	A	4,8675	2,39221	0,723
	B	5,0272	1,83824	
Right leg	A	4,9314	2,48079	0,943
	B	4,9628	1,54071	

Table 6. Quantitative distribution of subcutaneous adipose tissue by body parts in both groups, expressed in kilograms.

2.4. Muscle tissue

The amount of muscle tissue in the torso of the subjects from group A was 22.91 kg (± 3.70), while in the control group it was higher - 25.83 kg (± 4.18). The amount of muscle tissue in each of the limbs of the patients in a hyperthyroid state was less compared to the euthyroid controls. In all areas, the difference in the musculature of the two groups of subjects reached statistical significance. We also reported lateralization in the amount of muscle tissue - both for the upper and lower limbs. Regardless of the thyroid status of the women, the amount of muscle tissue was greater in the right half compared to the left.

The difference in the amount of muscle tissue between the left and right leg, both in women with hyperthyroidism and in those in a euthyroid state, reached statistical significance, respectively - group A (p 0.035), group B (0.000). And although the difference in the musculature between the left and right arm among patients in group A was more pronounced compared to controls, it did not reach significance (p 0.068). The results are summarized in Table 7 and illustrated in Figure 10.

Indicator (kg)	Group	N	Mean	Std. Deviation	p- value
Muscle tissue left arm	A	45	2,0129	0,46506	**0,000**
	B	45	2,3919	0,46295	
Muscle tissue right arm	A	45	2,0579	0,45131	**0,000**
	B	45	2,3984	0,42646	
Muscle tissue trunk	A	45	22,9112	3,70469	**0,001**
	B	45	25,8364	4,188290	
Muscle tissue left leg	A	45	6,3406	1,09680	**0,000**
	B	45	7,2590	1,17860	
Muscle tissue right leg	A	45	6,4036	1,14148	**0,000**
	B	45	7,3593	1,24926	

Table 7. Distribution of muscle tissue in different body compartments among individuals from group A and group B.

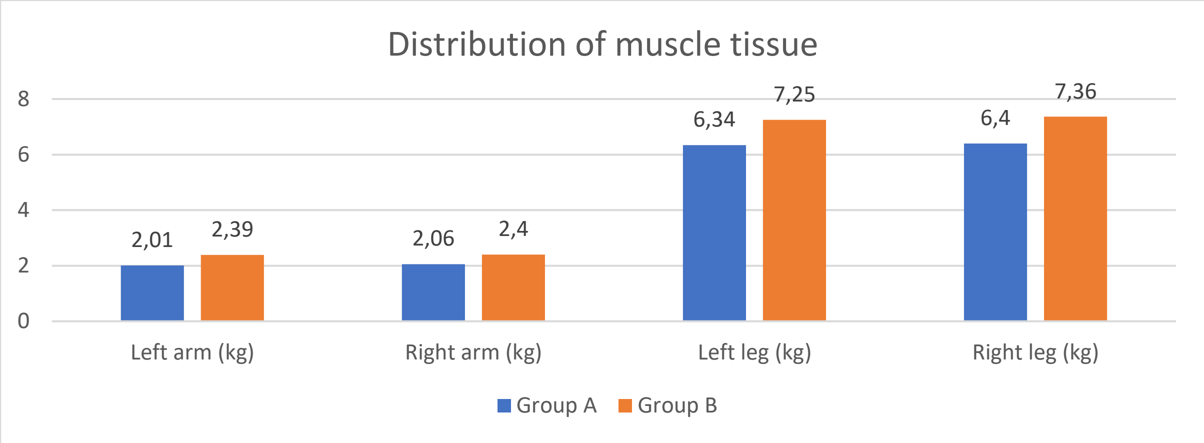
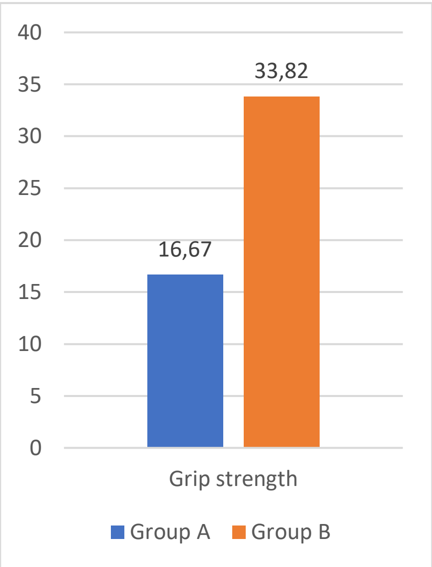


Figure 10. Amount of muscle tissue among the subjects studied, divided by groups

3. Results for task 3: Comparison of grip strength, ASMI (ASM/m²) and walking speed (diagnostic components of sarcopenia) between women with hyperthyroidism and healthy controls

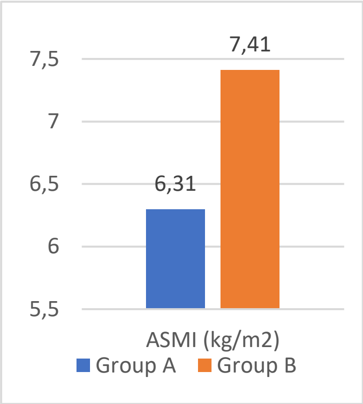
3.1. Grip strength



The decrease in the amount of muscle tissue in the upper limbs in hyperthyroid women is also associated with significantly lower muscle strength and more pronounced muscle weakness compared to healthy women. The results of grip strength show approximately two times lower values in women with hyperthyroidism (16.67±5.96 kg) compared to those in a euthyroid state (33.59±10.5 kg), p 0.000.

Figure 11. Grip strength among women from group A and group B

3.2. ASMI



The amount of muscle tissue in the limbs, expressed as ASMI, was significantly lower in women from group A - 6.31 kg/m² (± 0.99), while in group B it reached a value of 7.41 kg/m² (± 1.24) (p 0.000) (Figure 12).

Figure 12. ASMI- index for muscle tissue in the limbs in hyper- and euthyroid women.

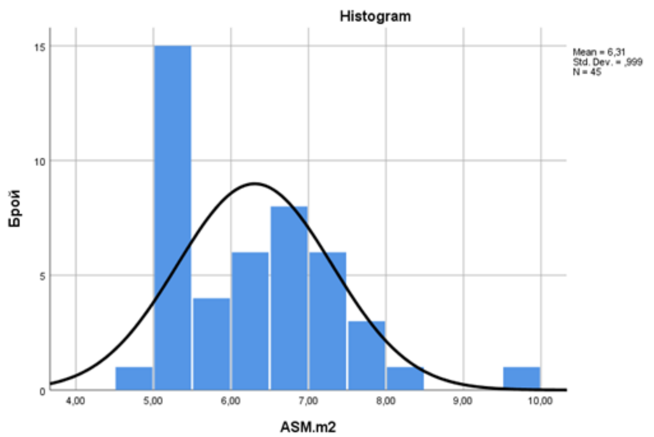


Figure 13. Histogram of ASMI values of patients from group A

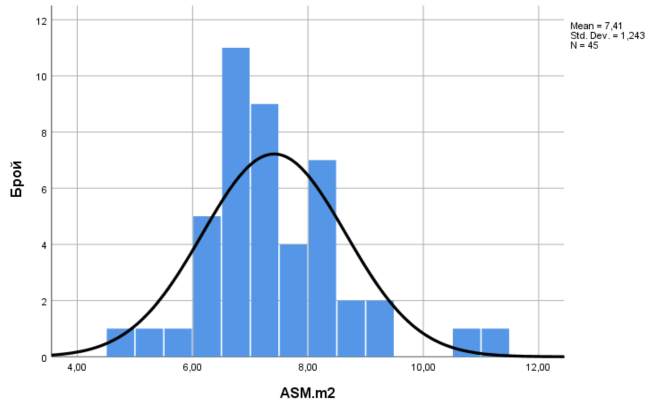
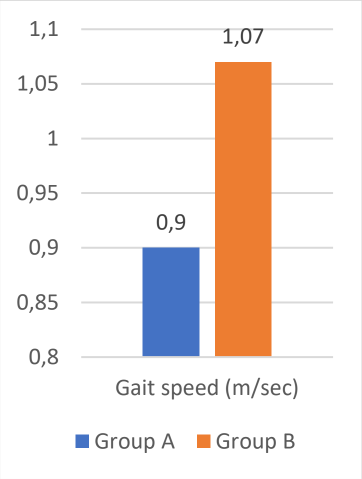


Figure 14. Histogram of ASMI values of patients from group B

The skewness coefficient of group A was 0.863 and that of control group B was 0.965, both approximating a central Gaussian distribution. This speaks of a normal distribution of errors around the mean value and adequacy of the measured quantity.

3.3. Gait speed

The third component in the diagnosis of sarcopenia - gait speed, is not a mandatory criterion for the presence of the disease. Its subthreshold value reflects the impairment of physical



capacity and categorizes sarcopenia as severe. As with the previous ones, the results for this indicator are significantly lower among patients from group A compared to group B, respectively - 0.900 m/s (± 0.19) versus 1.07 m/s (± 0.26), (p 0.001).

Figure 15. Gait speed among women from group A and group B – calculated based on the 4 m walk test.

Data from the diagnostic components for sarcopenia are summarized in Table 8.

Indicator	Group	N	Mean	Std. Deviation	p- value
ASMI (kg/m²)	A	45	6,3061	0,99883	**0,000**
	B	45	7,4124	1,24330	
Grip strength (kg)	A	45	16,67	5,960	**0,000**
	B	45	33,59	10,504	
Gait speed (m/sec)	A	45	0,9005	0,19164	**0,001**
	B	45	1,0764	0,26590	

Table 8. Results of the diagnostic components for sarcopenia (ASMI, grip strength and gait speed) among the studied patient groups

Based on the results of the diagnostic indicators for sarcopenia, the disease was diagnosed in a total of 17 of the subjects, or, expressed in percentage terms, 18.89% of all 90 women included in the study. Of these cases, 16 were women from group A, or 35.56% of women with hyperthyroidism. Among those in a euthyroid state, only one case meeting the criteria for sarcopenia was identified (2.22% of controls) – figures 16 and 17.

Including the results of the gait speed when covering a distance of 4 meters - 3 of these 17 individuals were affected by severe sarcopenia. This represents 3.33% of the entire sample, 6.67% of hyperthyroid individuals or 17.64% of those diagnosed with sarcopenia. All cases with severe form occur only in group A, as this was not found among healthy controls.

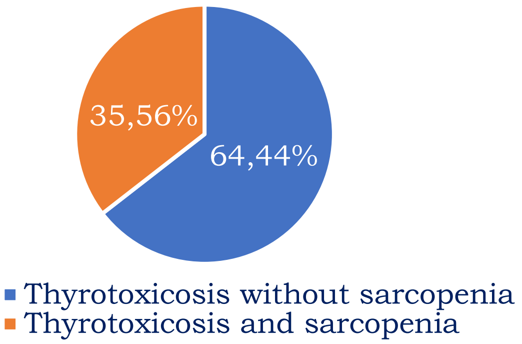


Figure 16. Distribution of sarcopenia among women with hyperthyroidism

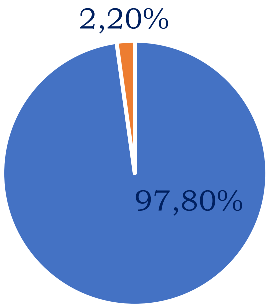


Figure 17.
Distribution of sarcopenia amond healthy controls

4. **Results for task 4: Relationships between anthropometric indicators and body composition, as well as between anthropometric indicators and diagnostic criteria for sarcopenia - ASMI, grip strength and gait speed.**

4.1.Relationships between anthropometric indicators and body composition

4.1.1. Anthropometric indicators and bone mass

The amount of bone tissue shows a positive relationship with height, weight, BMI, waist and hip circumference among the entire sample.

Among women with hyperthyroidism, an additional correlation dependence of the studied indicator was established, namely - a negative moderate relationship with the age of the individuals (p 0.002; r -0.457).

Group		Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/hip
A	p	0,002	0,000	0,000	0,024	0,001	0,001	0,079
	r	-0,457	0,649	0,562	0,335	0,468	0,463	0,270
B	p	0,563	0,000	0,000	0,000	0,000	0,000	0,313
	r	0,089	0,510	0,865	0,727	0,740	0,802	0,154

Table 9. Correlations between anthropometric indicators and bone mass

4.1.2. Anthropometric indicators and visceral adipose tissue

Weight gain, BMI, waist and hip circumferences are associated with an increase in the amount of visceral adipose tissue (VAT), both among the general sample and among women with hyperthyroidism.

Correlations between anthropometric indicators and VAT were not established among the control group.

VAT	Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/hip
p	0,584	0,617	**0,002**	**0,000**	**0,035**	**0,025**	0,921
r	-0,167	-0,153	0,777	0,844	0,587	0,616	-0,031

Table 10. Correlations between anthropometric indicators and the amount of visceral adipose tissue (VAT) in women with hyperthyroidism (group A)

4.1.3. Anthropometric parameters and subcutaneous fat

Weight loss, BMI, waist and hip circumferences were associated with a reduction in subcutaneous fat in each of the four limbs. These relationships were observed both for the entire study population and when dividing patients by thyroid status. The strength of the relationship was similar for both groups of patients.

	Group		Left arm	Right arm	Trunk	Left leg	Right leg
Weight	**A**	p	0,000	0,000	0,000	0,000	0,000
		r	0,734	0,736	0,738	0,664	0,665
	B	p	0,000	0,000	0,000	0,000	0,000
		r	0,831	0,821	0,741	0,758	0,662
BMI	**A**	p	0,000	0,000	0,000	0,000	0,000
		r	0,792	0,790	0,764	0,744	0,745
	B	p	0,000	0,000	0,000	0,000	0,000
		r	0,877	0,865	0,688	0,845	0,727
Hip circumference	**A**	p	0,000	0,000	0,000	0,000	0,000
		r	0,698	0,705	0,685	0,735	0,734
	B	p	0,000	0,000	0,000	0,000	0,000
		r	0,794	0,780	0,721	0,804	0,759
Waist circumference	**A**	p	0,000	0,000	0,000	0,000	0,000
		r	0,769	0,772	0,735	0,750	0,749
	B	p	0,000	0,000	0,000	0,000	0,000
		r	0,815	0,813	0,714	0,784	0,681

Table 11. Significant relationships between anthropometric parameters and the amount of subcutaneous adipose tissue distributed across body parts.

4.1.3. Anthropometric parameters and muscle mass

4.1.3.1. Relationship between age and muscle mass

In healthy controls, no correlations were found between age and muscle mass. In contrast, in the hyperthyroid group, increasing age was associated with a decrease in skeletal muscle mass in each body region. The strength of the relationship was moderate to significant. The significance was borderline in the correlation analysis for the right hand (p 0.054; r -0.289).

In the general population, this relationship was maintained for the lower limbs, but was lost for the upper limbs.

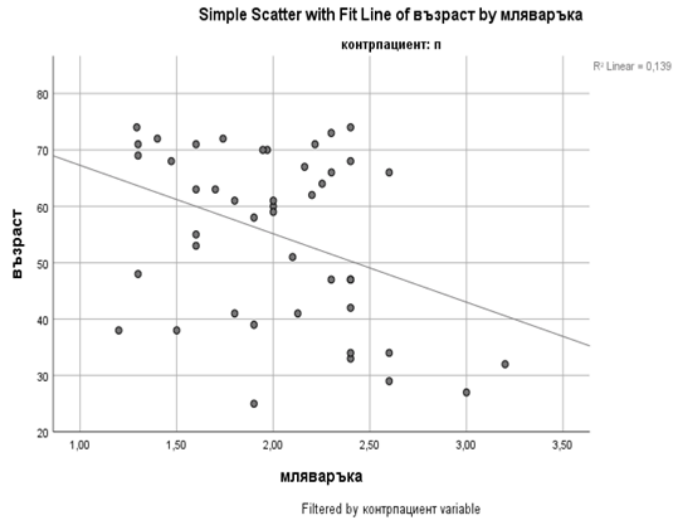


Figure 18. Correlation between age and the amount of muscle mass in the left arm in conditions of hyperthyroidism

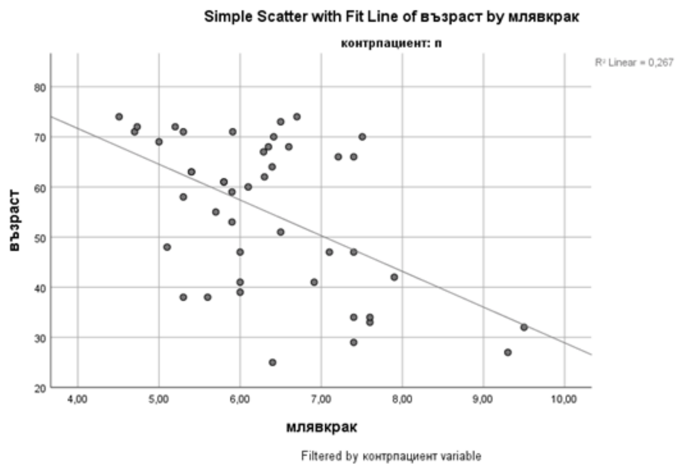


Figure 19. Correlation between age and the amount of muscle mass in the right arm in conditions of hyperthyroidism

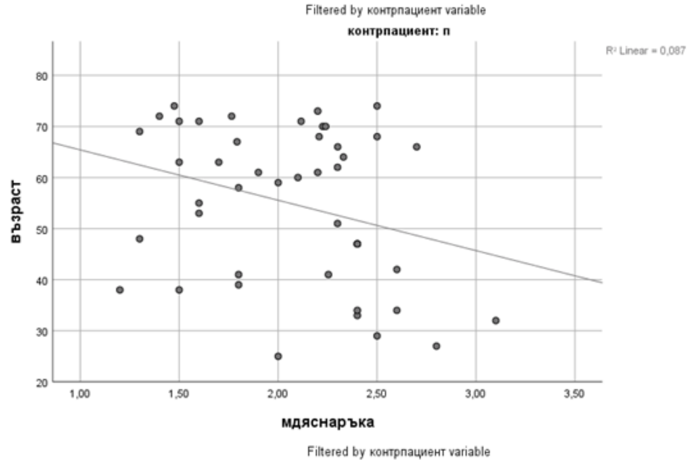


Figure 20. Correlation between age and the amount of muscle mass in the left leg in conditions of hyperthyroidism

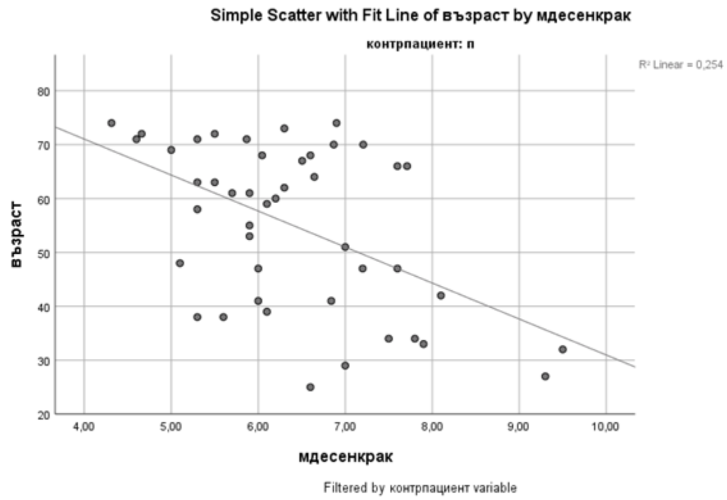


Figure 21. Correlation between age and the amount of muscle mass in the right leg in conditions of hyperthyroidism

4.1.3.2. Relationship between height and amount of muscle tissue

In contrast to the missing relationship between this anthropometric parameter and the distribution of fat tissue, in women from both groups a positive moderate correlation was observed with the amount of muscle tissue in each of the four limbs. These dependencies are also present in the general sample, and due to the larger number of patients, the relationships are strong. No correlations characteristic only for women in a hyperthyroid state stand out.

		Left arm	Right arm	Trunk	Left leg	Right leg
Total number of patients (n=90)	p	0,000	0,000	0,002	0,000	0,000
	r	0,805	0,858	0,423	0,835	0,838
Group A	p	0,000	0,000	0,000	0,001	0,001
	r	0,493	0,527	0,520	0,461	0,471
Group B	p	0,006	0,004	0,004	0,001	0,004
	r	0,401	0,416	0,418	0,462	0,416

Table 12. Relationship between height and muscle tissue distribution

4.1.3.3. Relationship between body weight and amount of muscle tissue

With increasing weight, the amount of muscle tissue also increases in all studied regions, in all studied individuals.

		Left arm	Right arm	Trunk	Left leg	Right leg
Total number of patients (n=90)	p	0,000	0,000	0,011	0,000	0,000
	r	0,401	0,692	0,616	0,572	0,677
Group A	p	0,002	0,012	0,000	0,002	0,002
	r	0,457	0,371	0,550	0,451	0,475
Group B	p	0,000	0,000	0,000	0,000	0,004
	r	0,929	0,891	0,641	0,877	0,864

Table 13. Correlations between weight and amount of muscle tissue

4.1.3.4. Relationship between BMI and the amount of muscle tissue

A decrease in BMI is associated with a decrease in the amount of muscle in each limb, regardless of the individuals' thyroid status.

		Left arm	Right arm	Trunk	Left leg	Right leg
Total number of patients (n=90)	p	0,000	0,000	0,001	0,000	0,010
	r	0,423	0,402	0,398	0,521	0,393
Group A	p	0,005	0,003	0,009	0,050	0,050
	r	0,286	0,387	0,383	0,293	0,293
Group B	p	0,000	0,000	0,000	0,000	0,004
	r	0,823	0,775	0,523	0,755	0,759

Table 14. Correlations between BMI and the amount of muscle tissue

4.1.3.5. Relationship between parameters: waist circumference, hip circumference, and waist/hip ratio and muscle mass.

For both adipose and muscle mass, increases in both waist and hip circumferences were associated with increases in limb muscle mass. No relationships were found between waist/hip ratio and muscle mass in any of the regions studied.

			Left arm	Right arm	Trunk	Left leg	Right leg
Hip circumference	**Total number of patients (n=90)**	p	0,000	0,000	0,011	0,000	0,000
		r	0,401	0,692	0,616	0,572	0,677
	Group A	p	0,003	0,012	0,003	0,008	0,007
		r	0,435	0,373	0,428	0,391	0,393
	Group B	p	0,000	0,000	0,000	0,000	0,000
		r	0,838	0,814	0,511	0,703	0,699
Waist circumference	**Total number of patients (n=90)**	p	0,023	0,009	0,005	0,010	0,008
		r	0,882	0,756	0,801	0,646	0,877
	Group A	p	0,015	0,011	0,002	0,026	0,019
		r	0,361	0,421	0,440	0,333	0,347
	Group B	p	0,000	0,000	0,000	0,000	0,000
		r	0,870	0,808	0,589	0,809	0,808

Table 15. Correlations between waist and hip circumferences, as well as the waist/hip ratio and the amount of muscle tissue

4.2. Relationships between anthropometric indicators and diagnostic criteria for sarcopenia - ASMI, grip strength and walking speed

4.2.1. Relationship with ASMI

With increasing age, the ASMI index decreases, and this relationship is observed only in hyperthyroid individuals (p 0.034; r -0.317).

The ASMI index shows a dependence on anthropometric indicators among all subjects, regardless of thyroid status - with the decrease in weight, waist, hip circumferences and BMI, a decrease in ASMI was observed. Table 22 and figures 27-30 reflect these relationships in the total sample, as well as in the group of hyperthyroid women and the group of euthyroid controls.

		Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/hip
Total number of patients (n=90)	p	0,157	0,682	**0,000**	**0,000**	**0,000**	**0,000**	0,160
	r	-0,150	-0,044	0,570	0,616	0,520	0,551	0,149
Group A	p	**0,034**	0,837	**0,007**	**0,008**	**0,001**	**0,007**	0,115
	r	-0,317	0,032	0,398	0,391	0,460	0,394	0,238
Group B	p	0,133	0,732	**0,000**	**0,000**	**0,000**	**0,000**	0,160
	r	0,233	0,054	0,747	0,848	0,655	0,736	0,367

Table 16. Correlations between ASMI and anthropometric parameters.

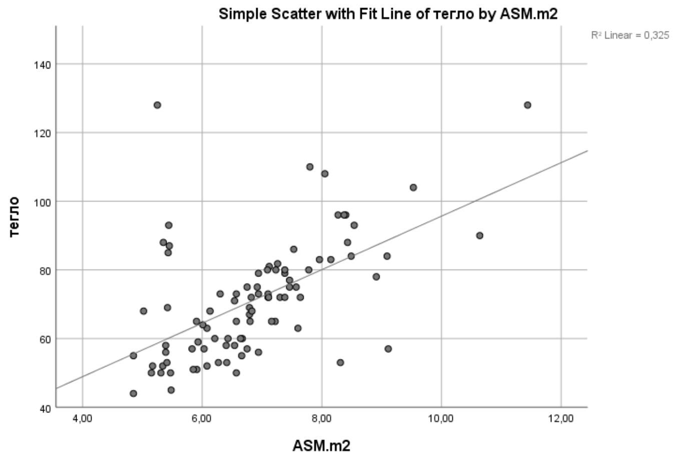


Figure 22. Graphical representation of the relationship between weight and ASMI in the entire study population

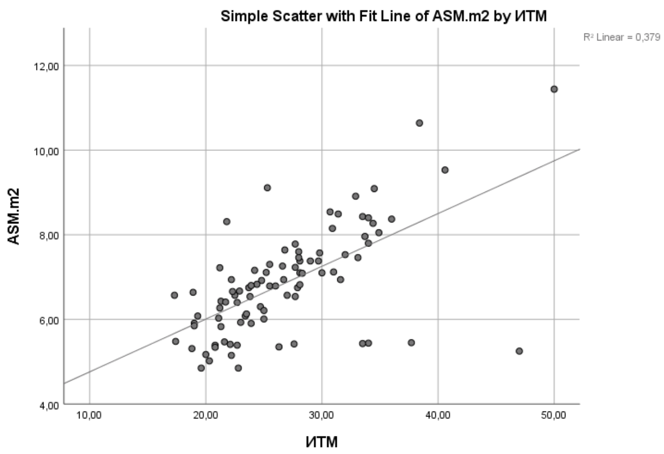


Figure 23. Graphical representation of the relationship between BMI and ASMI across the entire study

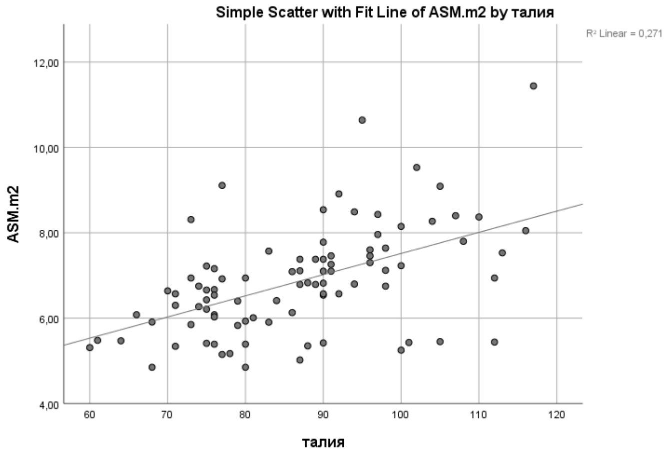


Figure 24. Graphical representation of the relationship between waist circumference and ASMI in the entire study population

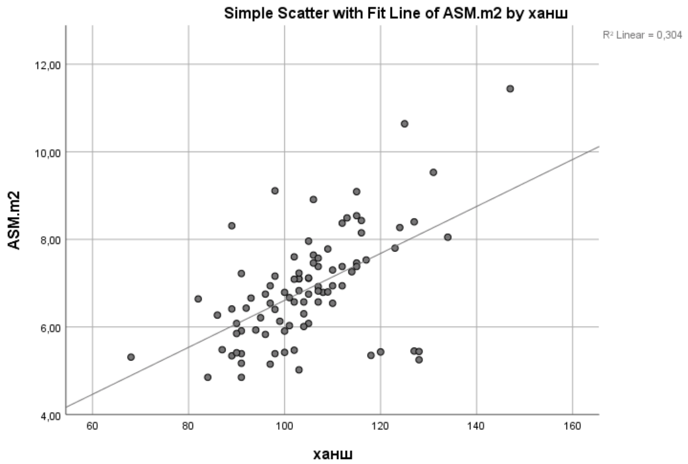


Figure 25. Graphical representation of the relationship between hip circumference and ASMI in the entire study population

4.2.2. Relationship with grip strength

Most of the anthropometric indicators in the target group (group A), as well as among the total sample, show a positive correlation with grip strength, namely - height, weight, BMI, waist and hip circumferences. Only the correlation with age is negative.

Correlations in the control group are established only between grip strength and height (p 0.003; r 0.434), as well as with weight (p 0.032; r 0.320) - on the other hand.

The described results are presented in the table 17 and figures 26-31 presented below.

		Възраст	**ръст**	**тегло**	**ИТМ**	**талия**	**ханш**	**Талия/ханш**
Общо пациенти (n=90)	p	**0,012**	**0,020**	**0,002**	**0,020**	**0,037**	**0,012**	0,993
	r	-0,264	0,245	0,330	0,244	0,220	0,263	0,001
Група А	p	**0,009**	0,186	**0,006**	**0,026**	**0,035**	**0,026**	0,670
	r	-0,386	0,201	0,405	0,331	0,316	0,333	0,065
Група Б	p	0,560	**0,003**	**0,032**	0,242	0,075	0,087	0,309
	r	-0,089	0,434	0,320	0,178	0,268	0,258	0,155

Table 17. Correlations between grip strength and anthropometric indicators

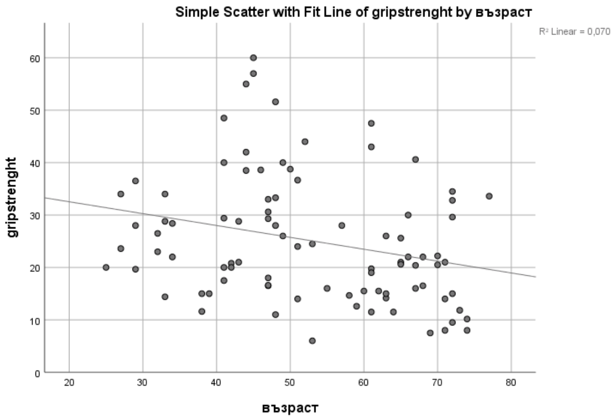


Figure 26. Graphical representation of the relationship between age and grip strength in the entire study population

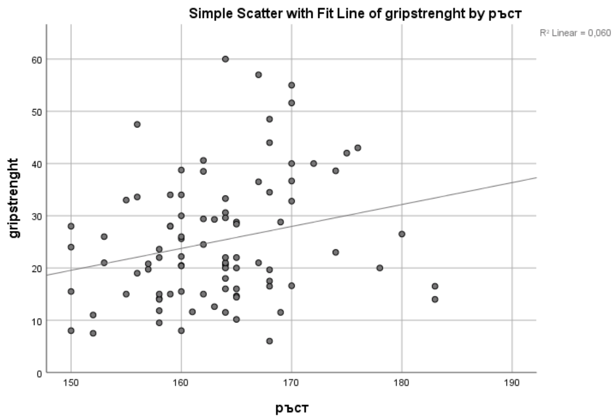


Figure 27. Graphical representation of the relationship between height and grip strength in the entire study population

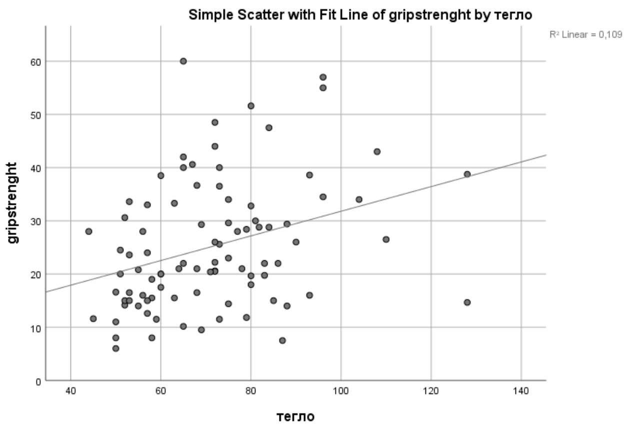


Figure 28. Graphical representation of the relationship between weight and grip strength in the entire study population

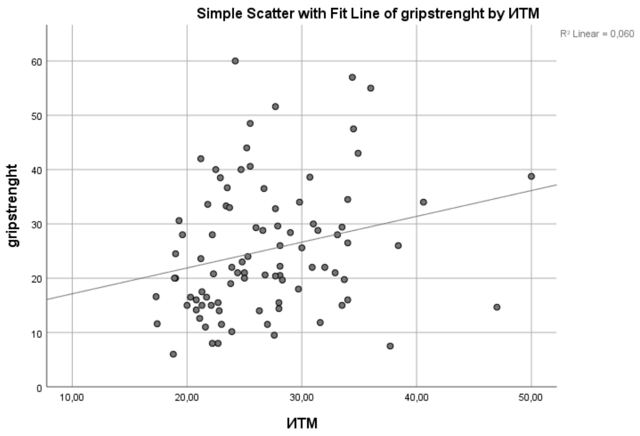


Figure 29. Graphical representation of the relationship between BMI and grip strength in the entire study population

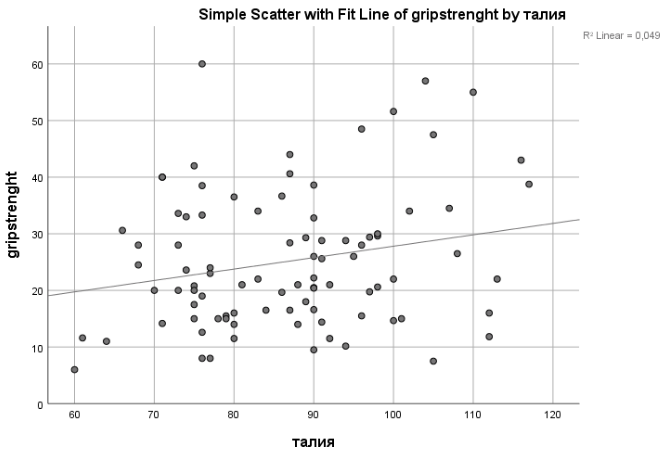


Figure 30. Graphical representation of the relationship between waist circumference and grip strength in the entire study population

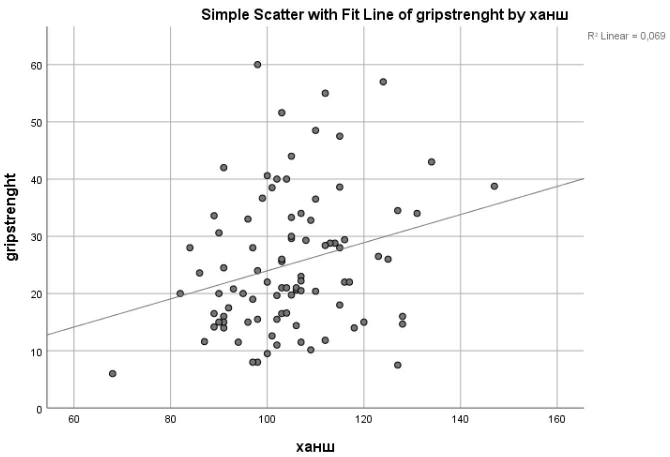


Figure 31. Graphical representation of the relationship between hip circumference and grip strength in the entire study population

4.2.3. Relationship with gait speed

Changes in this indicator of physical capacity demonstrate similar trends for the entire sample (overall and divided into hyperthyroid and euthyroid groups) - with increasing age, gait speed also decreases.

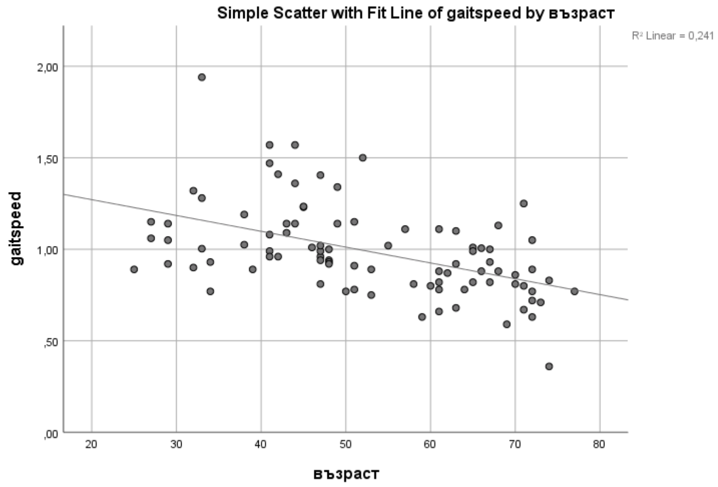


Figure 32. Graphical representation of the relationship between age and walking speed in the entire study population

5. **Results of task 5: To look for a relationship between hormonal and biochemical indicators and body composition, as well as between hormonal and biochemical indicators and the diagnostic components of sarcopenia - grip strength, ASMI and gait speed.**

Correlations with hormonal parameters were examined in the entire sample (n=90), including women across the spectrum from hyper- to euthyroidism (overt hyperthyroidism, subclinical hyperthyroidism, and euthyroid controls).

5.1. Relationship between hormonal and biochemical parameters (creatinine, CPK, albumin and vitamin D) and body composition

5.1.1. Relationship between hormonal parameters and body composition

From the correlation analyses conducted, no relationship was found between some of the thyroid indicators and visceral adipose tissue, as well as subcutaneous adipose tissue and its distribution.

The decrease in TSH from euthyroid to hyperthyroid state is accompanied by a decrease in muscle mass in each of the limbs, as well as a decrease in bone mass.

The increase in the TPO Ab titer is accompanied by a decrease in muscle tissue in the limbs, but not in the body. An increase in the amount of visceral adipose tissue (VAT) was also reported with the increase in TPO Ab, and the relationship was significant (p 007, r 685).

Our data indicate that the TRAb titer is negatively correlated with bone mass and the amount of muscle tissue, but only in the upper limbs and body. A similar correlation is not established for the lower limbs.

Peripheral thyroid hormones (FT3 and FT4) did not show any relationship with any of the body composition parameters.

The above data are presented in Table 18.

			TSH	FT3	FT4	TPO Ab	TRAb
VAT		p	0,252	0,446	0,153	**0,007**	0,993
		r	-0,316	0,213	0,388	0,685	0,103
Subcuteneous Adipose tissue	**Whole body**	p	0,604	0,252	0,572	0,620	0,525
		r	-0,057	0,130	0,153	0,057	-0,194
	Left arm	p	0,665	0,725	0,775	0,656	0,937
		r	0,047	-0,145	-0,078	-0,051	-0,024
	Right arm	p	0,719	0,754	0,621	0,733	0,935
		r	0,039	-0,880	-0,134	-0,039	-0,025
	Trunk	p	0,301	0,575	0,516	0,650	0,626
		r	-0,113	0,157	0,175	0,052	-0,149
	Left leg	p	0,553	0,511	0,978	0,095	0,779
		r	0,065	-0,184	-0,008	-0,190	-0,086
	Right leg	p	0,812	0,603	0,904	0,137	0,879
		r	0,026	-0,146	-0,033	-0,170	-0,047
Bone mass		p	0,005	0,631	0,274	0,066	**0,051**
		r	0,297	-0,135	-0,291	-0,208	-0,238
Muscle tissue	**Left atm**	p	**0,001**	0,899	0,908	**0,008**	**0,034**
		r	0,364	-0,036	0,031	-0,296	-0,257
	Right arm	p	**0,001**	0,850	0,882	**0,001**	**0,034**
		r	0,364	-0,054	-0,040	0,369	-0,258
	Trunk	p	**0,001**	0,452	0,203	**0,001**	**0,036**
		r	0,344	-0,210	-0,336	0,344	-0,255
	Left leg	p	**0,000**	0,602	0,643	**0,000**	0,349
		r	0,380	-0,147	-0,125	0,380	0,283
	Right leg	p	**0,000**	0,707	0,447	**0,000**	0,548
		r	0,398	-0,074	-0,205	0,393	0,184

Table 18. Correlations between thyroid parameters and body composition components

5.1.2. Relationship between biochemical parameters (25(OH)D, creatinine, CPK and albumin) and body composition

None of the target biochemical parameters (25(OH)D, creatinine, CPK and albumin) showed significant correlations with body composition distribution.

		Group		creatinine	CPK	albumin	25(OH)D
VAT		**A**	p	0,543	0,901	0,650	0,263
			r	0,186	0,038	-0,164	-0,335
		B	p	0,654	0,165	0,556	0,434
			r	0,223	0,981	-0,221	-0,429
		n=90	p	0,395	0,079	0,528	0,683
			r	0,496	0,835	-0,202	-0,251
Subcutaneous adipose tissue	**Whole body**	**A**	p	0,302	0,646	0,659	0,243
			r	0,180	0,075	0,095	-0,192
		B	p	0,835	0,514	0,268	0,883
			r	-0,040	0,129	-0,295	0,027
		n=90	p	0,582	0,574	0,695	0,444
			r	0,070	0,107	-0,64	0,561
	Left arm	**A**	p	0,649	0,573	0,778	0,104
			r	0,080	0,092	-0,061	-0,265
		B	p	0,557	0,622	0,203	0,699
			r	0,112	0,097	-0,325	-0,070
		n=90	p	0,264	0.799	0,272	0,157
			r	0,139	-0,031	-0,176	-0,168
	Right arm	**A**	p	0,719	0,644	0,620	0,137
			r	0,063	0,075	0,107	-0,243
		B	p	0,509	0,626	0,143	0,717
			r	0,125	0,096	-0,370	-0,065
		n=90	p	0,554	0,951	0,465	0,458
			r	0,074	0,007	-0,117	-0,088
	Trunk	**A**	p	0,459	0,550	0,585	0,230
			r	0,129	0,097	0,117	-0,197
		B	p	0,692	0,545	0,061	0,639
			r	-0,075	0,119	-0,463	0,085
		n=90	p	0,586	0,383	0,594	0,345
			r	0,068	0,231	-0,086	0,133
	Left leg	**A**	p	0,180	0,550	0,001	0,230
			r	0,232	0,097	0,995	-0,197
		B	p	0,847	0,891	0,253	0,633
			r	-0,037	-0,027	-0,293	-0,086
		n=90	p	0,477	0,719	0,491	0,513
			r	0,089	0,044	-0,111	-0,078
	Right leg	**A**	p	0,197	0,837	-0,019	0,130
			r	0,223	0,034	0,930	-0,247
		B	p	0,828	0,815	0,152	0,779
			r	-0,041	0,046	-0,363	-0,051
		n=90	p	0,431	0,640	0,455	0,516
			r	0,099	0,057	-0,120	-0,077

Table 19. Correlations between some biochemical indicators and adipose tissue distribution

		Group		creatinine	CPK	albumin	25(OH)D
Bone mass		**A**	p	0,542	0,918	0,835	0,157
			r	-0,107	0,017	-0,252	-0,231
		B	p	0,170	0,151	0,892	0,090
			r	0,253	0,274	-0,036	-0,295
		n=90	p	0,346	0,079	0,908	0,683
			r	0,118	0,835	-0,019	-0,251
Muscle tissue	**n=90**	**A**	p	0,799	0,380	0,433	0,679
			r	-0,045	-0,143	-0,132	-0,412
		B	p	0,304	0,370	0,430	0,136
			r	0,191	0,173	-0,221	-0,261
		n=90	p	0,401	0,206	0,340	0,544
			r	0,042	0,121	-0,195	-0,298
	Left arm	**A**	p	0,345	0,380	0,753	0,718
			r	-0,165	-0,143	-0,068	-0,060
		B	p	0,394	0,440	0,583	0,114
			r	0,159	0,149	-0,144	-0,276
		n=90	p	0,229	0,289	0,862	0,329
			r	0,150	0,129	-0,028	-0,116
	Right arm	**A**	p	0,783	0,646	0,856	0,324
			r	-0,048	-0,075	0,039	-0,162
		B	p	0,438	0,101	0,391	0,164
			r	0,127	0,286	-0,222	-0,286
		n=90	p	0,575	0,240	0,969	0,273
			r	0,070	0,143	-0,006	-0,130
	Trunk	**A**	p	0,410	0,336	0,293	0,327
			r	-0,143	-0,156	-0,224	-0,161
		B	p	0,507	0,632	0,890	0,186
			r	0,124	0,334	0,036	-0,298
		n=90	p	0,331	0,412	0,871	0,243
			r	0,122	0,186	-0,026	-0,241
	Left leg	**A**	p	0,205	0,646	0,579	0,324
			r	-0,220	-0,075	-0,119	-0,162
		B	p	0,087	0,256	0,652	0,078
			r	0,312	0,218	-0,118	-0,306
		n=90	p	0,363	0,097	0,784	0,385
			r	0,114	0,202	-0,044	-0,103
	Right leg	**A**	p	0,230	0,262	0,546	0,684
			r	-0,208	-0,182	-0,130	-0,067
		B	p	0,065	0,273	0,842	0,059
			r	0,335	0,210	-0,122	-0,339
		n=90	p	0,331	0,132	0,736	0,318
			r	0,122	0,183	-0,054	-0,119

Table 20. Correlations between some biochemical indicators and the distribution of muscle tissue and bone mass

5.2. Relationship between hormonal and biochemical indicators and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

5.2.1. Relationship between hormonal indicators and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

The correlation analysis showed that the decrease in serum TSH is accompanied by a decrease in ASMI, grip strength and a slowdown in gait speed. The strength of the relationship is significant when it concerns grip strength, while for the other two dependencies it is moderate.

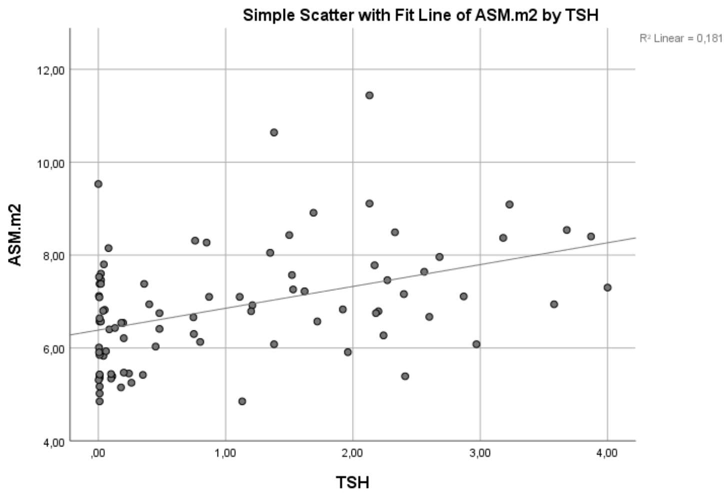


Figure 33. Correlation between TSH and ASMI

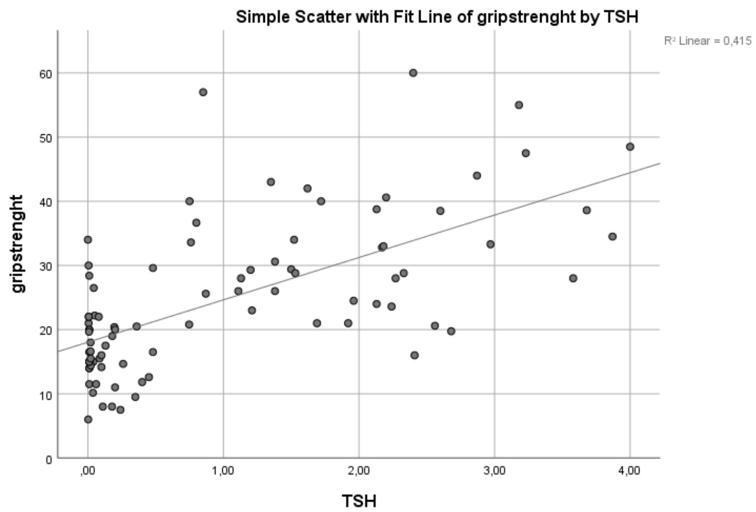


Figure 34. Correlation between TSH and grip strength

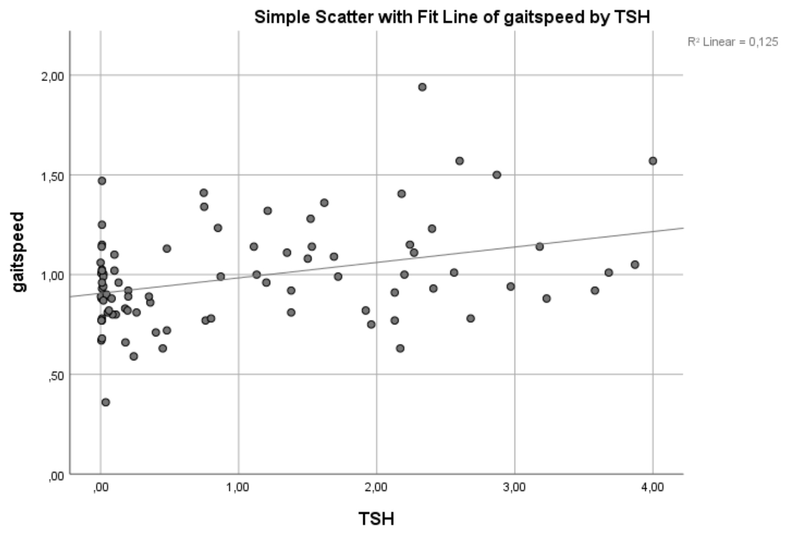


Figure 35. Correlation between TSH and walking speed

Of the peripheral thyroid hormones, correlations were found only with FT3, while FT4 did not correlate with any of the three components of sarcopenia.

An increase in FT3 was accompanied by a decrease in ASMI (p 0.040) and a weakening of grip strength (p 0.015), in both cases the relationship was weak.

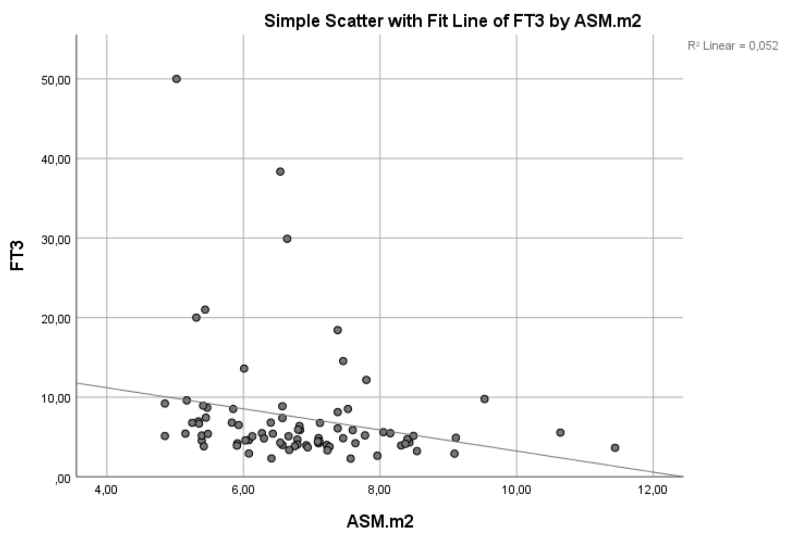


Figure 36. Correlation between FT3 and ASMI

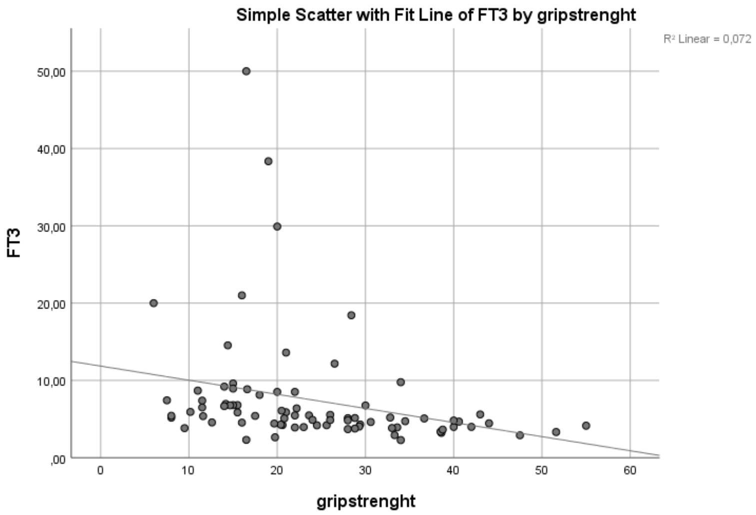


Figure 37. Correlation between FT3 and grip strength

Similar to FT3, autoimmune markers (TRAb and TPO Ab) showed a negative correlation with ASMI and grip strength. Except for the grip strength-TRAb relationship, the other relationships were weak (r -0.349).

		TSH	FT3	FT4	TRAb	TPO Ab
ASMI	**p**	**0,000**	**0,040**	0,153	**0,042**	**0,010**
	r	0,426	-0,228	-0,157	-0,247	-0,289
Grip strength	**p**	**0,000**	**0,015**	0,064	**0,027**	**0,002**
	r	0,644	-0,269	-0,203	-0,268	-0,349
Gait speed	**p**	**0,001**	0,252	0,093	0,242	0,127
	r	0,354	-0,129	-0,184	-0,144	-0,173

Table 21. Relationship between hormonal indicators and diagnostic components of sarcopenia

5.2.2. Relationship between biochemical indicators (25(OH)D, creatinine, CPK and albumin) and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

The increase in creatinine within the reference limits is associated with a decrease in muscle tissue, expressed by ASMI, and this relationship is observed in the general sample (p 0.047; r 0.246) and euthyroid women (p 0.044; r 0.364), but is absent in group A.

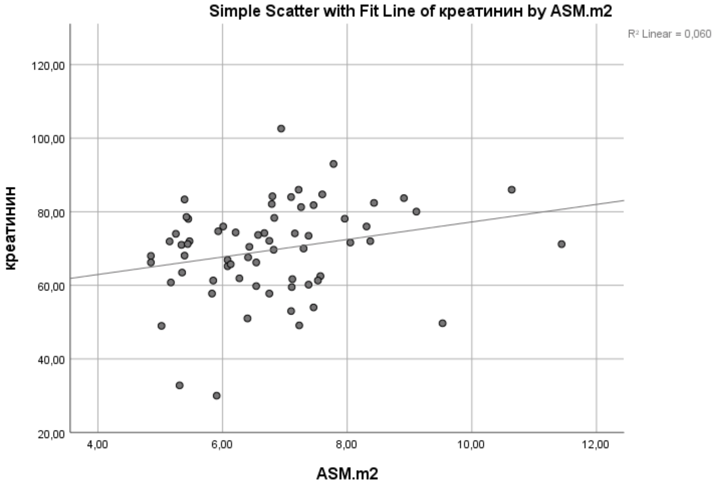


Figure 38. Relationship between creatinine and ASMI in the total sample

No correlations were observed between creatinine levels and grip strength or gait speed.
The decrease in creatine phosphokinase within the reference range was accompanied by a decrease in grip strength in the overall sample (p 0.019; r 0.281). Correlation analysis did not reveal any other relationships with this biochemical parameter, either in the overall sample or by groups.

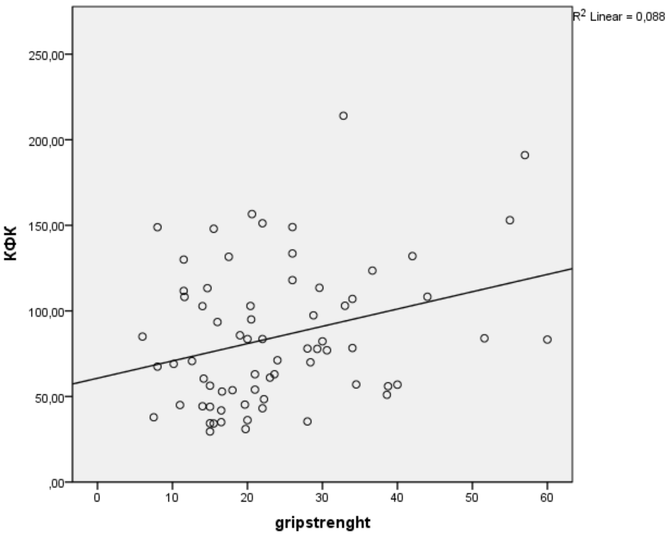


Figure 39. Relationship between CPK and grip strength in the total sample

Albumin value correlated positively but weakly with walking speed in the total sample (p 0.041; r 0.321).

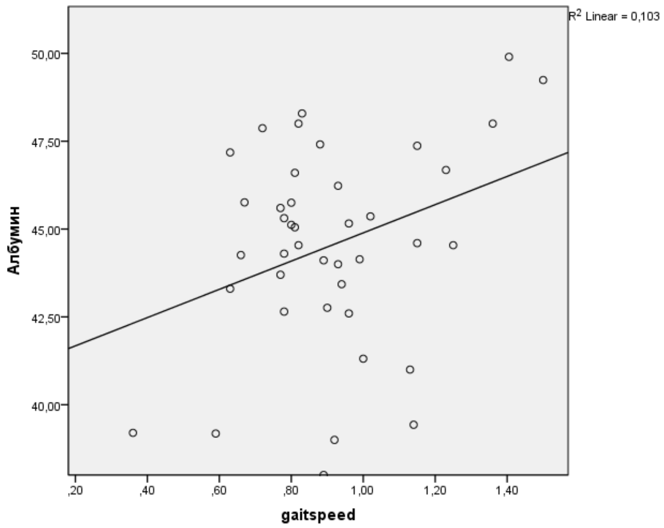


Figure 40. Relationship between serum albumin and walking speed in the total sample

In the study conducted, vitamin D levels did not correlate with any of the above-mentioned indicators, regardless of whether it was the general sample or the individuals distributed into groups.

6. Results of task 6: To establish the risk of developing secondary sarcopenia, to determine its frequency among the studied individuals and the threshold age above which the probability of secondary sarcopenia increases.

Analyzing the results of the studies conducted, it was found that 17 of the women met the diagnostic criteria for sarcopenia, with 16 of them affected by hyperthyroidism and only one woman with sarcopenia from the control group (Figure 41). Therefore, we decided to divide the patients from group A into 2 subgroups: subgroup A1 - hyperthyroid patients without sarcopenia and subgroup A2 - hyperthyroid patients with sarcopenia (meeting the diagnostic criteria after the tests we performed).
Of all women included in the study, 18.87% were affected by sarcopenia, with sarcopenia occurring in 35.52% of patients with hyperthyroidism and only 2.22% of healthy women. The patient from the control group who met the sarcopenic diagnostic criteria was 67 years old, which is probably primary sarcopenia. The mean age of those affected in group A was 58.81 years (± 12.885). Of all 16 women in group A, 5 had subclinical hyperthyroidism and 11 had overt hyperthyroidism. Of the women with subclinical hyperthyroidism and sarcopenia, 1 had a single toxic thyroid nodule and 4 had toxic polynodular goiter.

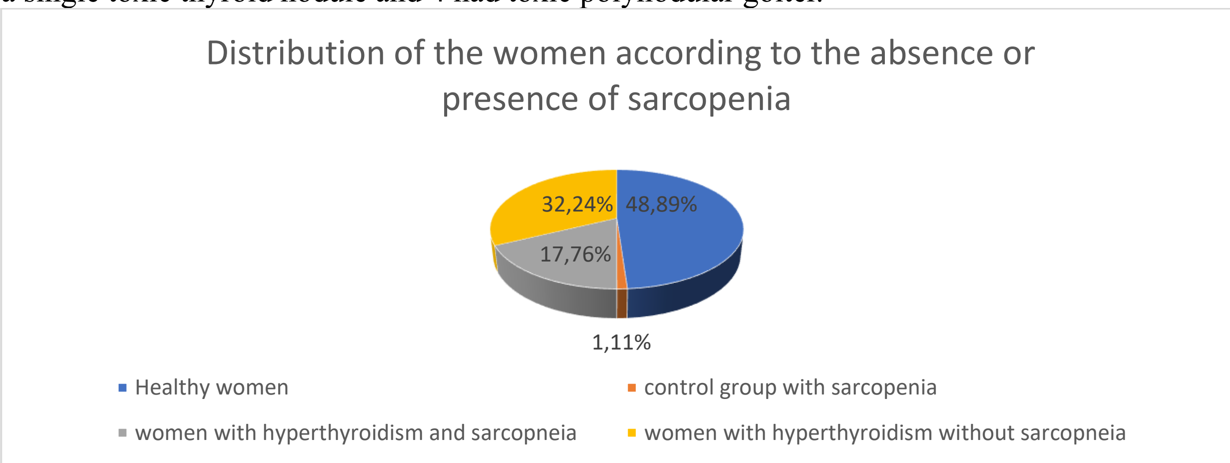


Figure 41. Percentage distribution of women with sarcopenia among the studied individuals

Distributed by age categories, hyperthyroid individuals affected by sarcopenia between 21 and 40 years old are 3, those aged 41-64 years are represented by 7 women, and those over 65 years old are 6. The youngest patient affected by the disease is 38 years old. The data are presented in Figure 42.

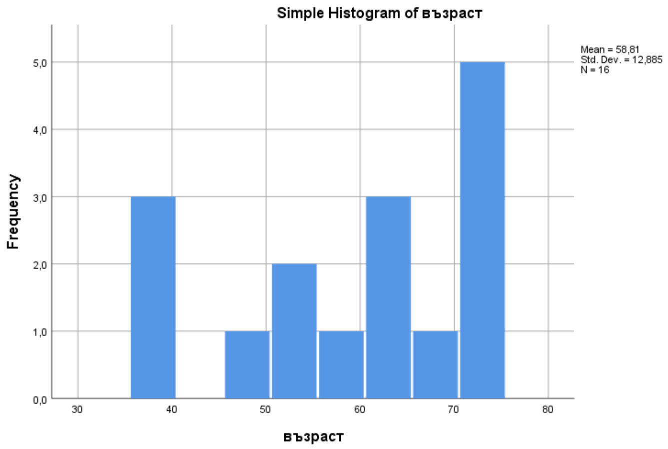


Figure 42. Distribution of patients with sarcopenia from group A by age

There were 3 women affected by severe sarcopenia, all of whom had hyperthyroidism (6.66% of individuals in group A). Their mean age was 68.03 years (± 4.67), with only one woman below the threshold age for primary sarcopenia of 65 years, namely 63 years.

We set ourselves the goal of calculating the threshold age for diagnosing sarcopenia in the available sample. From the statistical model made based on the construction of the ROC curve, the age above which screening for the presence of secondary sarcopenia should be carried out among women with newly diagnosed hyperthyroidism is 54 years (AUC 0.656 (0.519- 0.793)). Or this is 11 years earlier than the age threshold for primary sarcopenia. The model is statistically significant (p 0.042) with a sensitivity of 66.7% and a specificity of 38.9% (Figure 43).

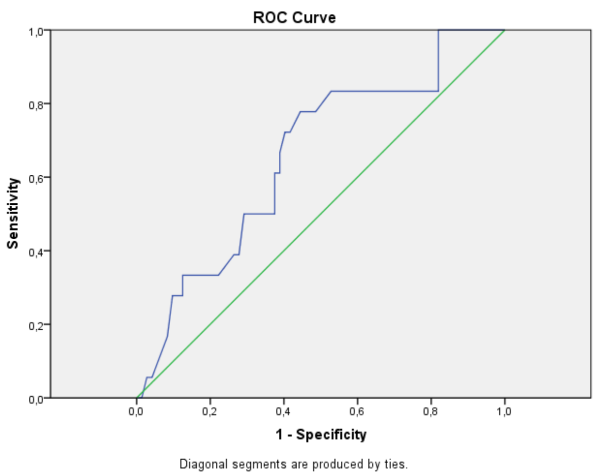


Figure 43. ROC curve for the threshold age for secondary sarcopenia among women affected by hyperthyroidism

7. Results of task 7: to search for correlations between anthropometric, hormonal and biochemical indicators, on the one hand, and the diagnostic components for sarcopenia - ASMI, grip strength and gait speed - on the other, among individuals affected by sarcopenia

7.1. Correlations between anthropometric indicators and ASMI, grip strength and gait speed

The only correlation found in this subanalysis was the significant negative relationship between waist circumference and gait speed (p 0.041; r -0.516) (table 22, figure 44).

		Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/hip
ASMI	**p**	0,076	0,438	0,865	0,801	0,954	0,891	0,680
	r	-0,455	0,208	-0,046	-0,068	-0,016	0,037	-0,112
Grip strength	**p**	0,245	0,498	0,431	0,690	0,480	0,450	0,907
	r	-0,309	0,183	0,212	0,108	0,190	0,203	-0,032
Gait speed	**p**	0,141	0,277	0,197	0,059	**0,041**	0,107	0,379
	r	-0,385	0,290	-0,340	-0,481	**-0,516**	-0,418	-0,236

Table 22. Correlations between anthropometric indicators and ASMI, grip strength and gait speed

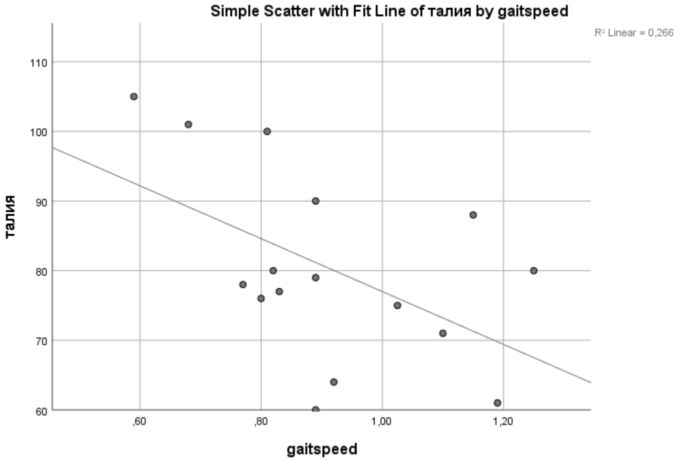


Figure 44. Relationship between waist circumference and gait speed

7.2. Correlations between hormonal parameters and ASMI, grip strength and gait speed

No significant correlations were found between any of the studied hormonal parameters and ASMI, grip strength or gait speed.

		TSH	FT3	FT4	TRAb	TPO Ab
ASMI	**p**	0,932	0,470	0,762	0,652	0,936
	r	0,024	-0,202	-0,082	-0,139	-0,026
Grip strength	**p**	0,153	0,401	0,760	0,078	0,926
	r	-0,388	-0,234	0,083	-0,506	-0,023
Gait speed	**p**	0,221	0,976	0,387	0,794	0,443
	r	-0,336	-0,008	-0,232	-0,081	-0,216

Table 23. Correlations between thyroid indicators and ASMI, grip strength and gait speed

7.3. Correlations between biochemical parameters (25(OH)D, creatinine, CPK, albumin) and ASMI, grip strength and gait speed

Similar to the results of the analysis with hormonal parameters, no significant relationships were found between some of the biochemical parameters (creatinine, CPK, albumin or 25(OH)D) and ASMI, grip strength and gait speed.

		creatinine	CPK	albumin	25 (OH) D
ASMI	**p**	0,763	0,972	0,990	0,144
	r	0,093	0,010	0,004	0,411
Grip strength	**p**	0,935	0,283	0,176	0,355
	r	0,025	-0,297	0,440	-0,267
Gait speed	**p**	0,483	0,686	0,535	0,901
	r	-0,214	0,114	0,210	0,037

Table 24. Correlations between biochemical parameters (25(OH)D, creatinine, CPK, albumin) and ASMI, grip strength and gait speed.

The cases meeting the criteria for sarcopenia in the study are 16 in group A and 1 in group B. Due to the small number of cases, it is not possible to determine statistically significant correlations. The small number in the subgroup does not provide an opportunity to outline a characteristic relationship.

8. Results of task 8: Comparison and detection of correlations between the studied parameters among women with hyperthyroidism, compared to those with hyperthyroidism and established sarcopenia

Additionally, we decided to compare some of the studied parameters by dividing the patients affected by thyrotoxicosis into 2 subgroups. Subgroup A1 consists of 29 women, none of whom meet the diagnostic criteria for the disease, and subgroup A2 includes 16 women with sarcopenia.

8.1. Anthropometric indicators

Patients affected simultaneously by hyperthyroidism and sarcopenia are older compared to hyperthyroid patients without sarcopenia, but this does not reach statistical significance.

The values of the remaining anthropometric indicators among women from subgroup A2 are lower than those from subgroup A1, but without significance (table 25).

Indicator	Subgroup	Mean	Std. Deviation	p- value
Age (years)	A1	53,00	16,592	0,292
	A2	58,61	12,621	
Height (cm)	A1	163,78	6,104	0,441
	A2	162,00	9,255	
Weight (kg)	A1	70,70	14,723	0,453
	A2	66,61	21,626	
BMI (kg/m^2)	A1	26,5778	5,18669	0,580
	A2	25,5056	7,74198	
Waist circumference (cm)	A1	88,89	11,623	0,110
	A2	82,44	14,849	
Hip circumference (cm)	A1	104,93	10,688	0,402
	A2	101,5	16,511	
Waist/hip	A1	0,8430	0,06484	0,110
	A2	0,8098	0,06981	

Table 25. Average values of individual anthropometric indicators in groups A1 and A2

8.2. Laboratory parameters

8.2.1. Thyroid parameters

None of the hormonal parameters differed significantly between the two groups. The only statistically significant difference was in the level of TPO Ab (p 0.050), with values more than doubled among women with sarcopenia.

Indicator	Subgroup	Mean	Std. Deviation	p-value
TSH ***(mkIU/ml)***	A1	0,1053	0,14838	0,910
	A2	0,1000	0,10816	
FT3 ***(pmol/L)***	A1	9,8544	8,26417	0,683
	A2	11,0806	11,09119	
FT4 ***(pmol/L)***	A1	37,1184	47,27971	0,504
	A2	30,2872	15,17552	
TPOAb ***(IU/mL)***	A1	129,7767	206,84895	**0,058**
	A2	376,9988	458,63955	
TRAb ***(IU/L)***	A1	4,6825	6,65111	0,411
	A2	6,8633	9,74979	

Table 26. Thyroid indicators in subgroup A1 (with thyrotoxicosis) and A2 (with thyrotoxicosis and sarcopenia) and their statistical significance.

8.2.2. Biochemical indicators

Table 27 presents the studied parameters of the biochemical indicators and their mean values. No significant difference in their level was found between the two subgroups.

The levels of creatinine, total protein and albumin were lower in subgroup A1, while among the same women, that of CPK was higher compared to women from subgroup A2.

The analysis of the lipid profile showed higher values of total cholesterol, HDL-c and LDL-c among individuals with thyrotoxicosis and sarcopenia, while the level of triglycerides in them was lower.

The values of liver transaminases were also higher in subgroup A2.

Contrary to expectations, individuals with thyrotoxicosis but without sarcopenia had lower values of vitamin D (18.69 ng/mL ±5.98), falling into the deficiency category.

Indicator	Subgroup	Mean	Std. Deviation	p-value
Creatinine *(mkmol/L)*	A1	66,5990	15,22148	0,935
	A2	66,9893	12,94726	
CPK *(U/L)*	A1	76,7987	34,35307	0,746
	A2	73,1029	36,93747	
Total protein *(g/L)*	A1	71,2269	3,10155	0,321
	A2	72,5608	3,48112	
Albumin *(g/L)*	A1	44,0983	2,39509	0,943
	A2	44,1875	3,54772	
Total cholesterol *(mmol/l)*	A1	4,8587	1,19255	0,795
	A2	4,9547	0,7837	
HDL-c *(mmol/l)*	A1	1,6786	0,41545	0,915
	A2	1,7014	0,68131	
LDL-c *(mmol/l)*	A1	2,7638	0,99197	0,826
	A2	2,8388	0,82857	
Tg *(mmol/l)*	A1	1,3425	0,81922	0,745
	A2	1,2663	0,43606	
ASAT *(U/L)*	A1	18,6375	5,81548	0,363
	A2	21,7457	13,44701	
ALAT *(U/L)*	A1	19,0825	10,04218	0,326
	A2	22,6707	10,70673	
25(OH)D *(ng/mL)*	A1	18,6988	5,97873	0,446
	A2	20,3686	7,33229	
Glucose *(mmol/l)*	A1	5,3520	0,5626	0,954
	A2	5,3428	0,45067	

Table 27. Values of biochemical indicators in the studied subgroups.

8.3. Body composition

8.3.1. Bone mass

The amount of bone mass was significantly lower (p 0.041) among women diagnosed with sarcopenia on the background of thyrotoxicosis (2.11 kg ± 0.33) than in those with thyrotoxicosis alone (2.31 kg ± 0.28). The results are illustrated in Figure 45.

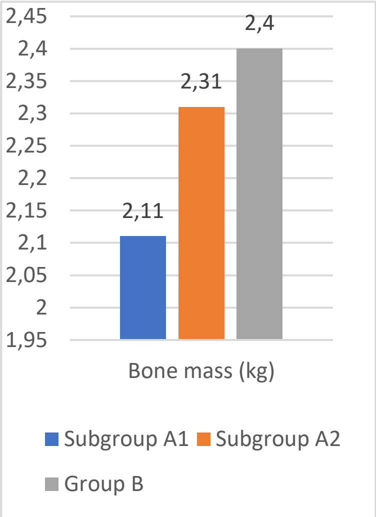


Figure 45. Bone mass was lowest among women suffering from both thyrotoxicosis and sarcopenia, followed by the subgroup with thyrotoxicosis only and highest among the control group.

8.3.2. Visceral adipose tissue

Of interest is the fact that the level of BMD among women from subgroup A2 (1.25 ±0.62 kg) is higher compared to those from subgroup A1 (0.92 ±0.26 kg), but this value does not reach statistical significance (p 0.185).

8.3.3. Subcutaneous adipose tissue

The amount of subcutaneous adipose tissue in each of the four limbs of women with sarcopenia is smaller in value than those in subgroup A1, but in none of the regions does it reach statistical significance.

Among women from subgroup A1, lateralization in the distribution of adipose tissue by limbs is observed, with its amount being slightly greater in the right limbs.

8.3.4. Muscle tissue

Skeletal muscle (SM) in all limbs is significantly less among women with sarcopenia on the background of thyrotoxicosis (p 0.000).

Our analysis shows that individuals with thyrotoxicosis, but without sarcopenia, also have lower muscle mass compared to healthy controls. If patients from subgroup A2 are removed from all studied individuals, it is seen that individuals with thyrotoxicosis continue to have a lower amount of SM than healthy controls.

Although a weak lateralization is reported with a greater increase in SM in the right limbs compared to the left, in none of the groups this result reaches significance.

Indicator	Subgroup	Mean	Std. Deviation	p- value
Muscle tissue left arm (kg)	A1	2,2721	0,35505	**0,000**
	A2	1,6241	0,31655	
Muscle tissue right arm (kg)	A1	2,3320	0,28857	**0,000**
	A2	1,6467	0,31655	
Muscle tissue trunk (kg)	A1	23,7386	3,41698	0,075
	A2	21,6701	3,86619	
Muscle tissue left leg (kg)	A1	6,9587	0,93275	**0,000**
	A2	5,4133	0,51554	
Muscle tissue right leg (kg)	A1	7,0403	0,94655	**0,000**
	A2	5,4486	0,62659	

Table 28. The amount of muscle tissue distributed by body compartments in subgroup A1 and A2.

8.4. Diagnostic components for sarcopenia (ASMI, grip strength, gait speed)

The two indicators on which the diagnosis of “sarcopenia” is based, namely ASMI and grip strength, have significantly lower values in subgroup A2 (p 0.000) compared to A1. However, subgroup A1 (patients with thyrotoxicosis without sarcopenia) shows lower values of the above-mentioned 2 indicators compared to healthy controls. This also applies to gait speed. The gradual deterioration of muscle function is presented in Figure 46.

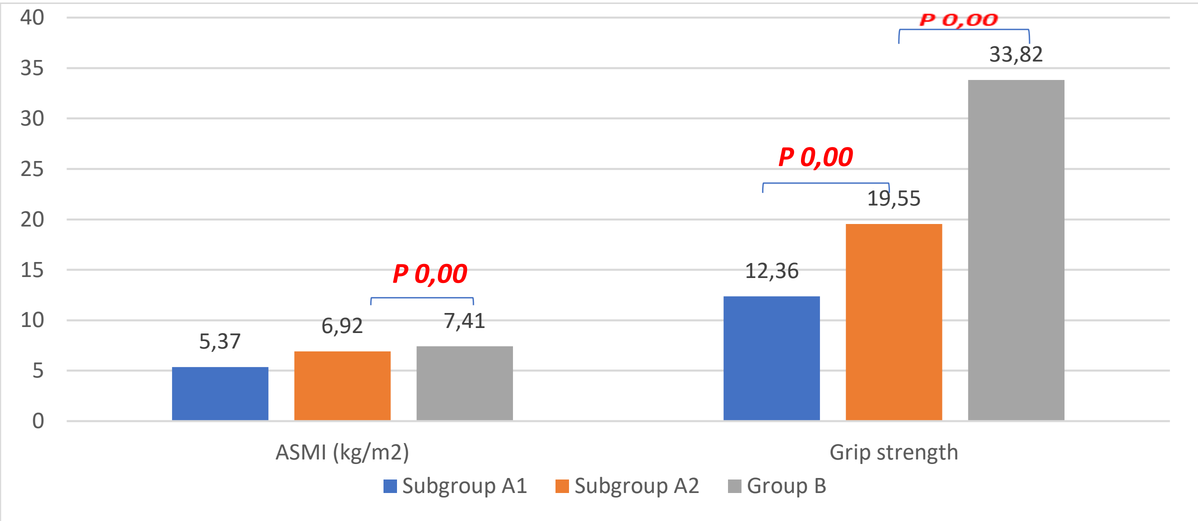


Figure 46. ASMI and grip strength between group A divided by subgroups and group B.

Although slow walking speed is not a mandatory criterion for the diagnosis of sarcopenia, its presence defines it as “severe sarcopenia” because it is a marker of impaired physical capacity.

In total, 3 out of all sarcopenic women in our sample had reduced gait speed. Paradoxically, the average walking speed in subgroup A2 (0.92 ± 0.18 m/s) was faster compared to subgroup A1 (0.88 ± 0.20 m/s), but it was not statistically significant (p 0.496). The values of the considered indicators are presented in table 35 and figure 47.

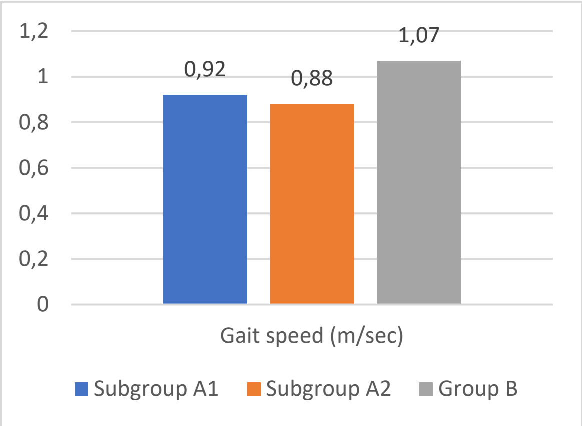


Figure 47. Gait speed in group A, divided into subgroup A1 and subgroup A2 and group B

Indicator	Subgroup	Mean	Std. Deviation	p- value
ASMI (kg/m²)	A1	6,9291	0,79986	**0,000**
	A2	5,3717	0,25056	
Grip strength (kg)	A1	19,55	5,613	**0,000**
	A2	12,36	3,304	
Скорост на ходене (м/сек)	A1	0,8844	0,20233	0,496
	A2	0,9247	0,17724	

Table 29. Data on grip strength, ASMI and gait speed by subgroups

9. Additional results: Correlation analyses of subjects affected by thyrotoxicosis and sarcopenia (subgroup A2)

9.1. Correlations of laboratory parameters in the conditions of hyperthyroidism and sarcopenia

9.1.1. Relationship between thyroid parameters and anthropometric parameters

After the analyses, the only relationship that was found among subjects affected by thyrotoxicosis and sarcopenia was the negative significant correlation between FT3 and hip circumference (p 0.05; r -0.514). Such a relationship was observed only in subjects with hyperthyroidism and sarcopenia.

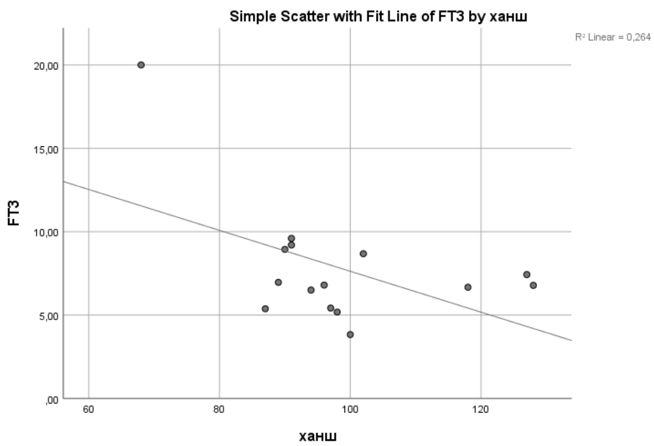


Figure 48. Relationship between FT3 and hip circumference in subgroup A2

9.1.2. Relationship with biochemical indicators (creatinine, CPK, albumin, 25(OH)D)

No significant relationships were established between anthropometric indicators and target biochemical indicators - creatinine, CPK, albumin, 25 (OH) D.

9.2. Correlations between anthropometric indicators and body composition in conditions of hyperthyroidism and sarcopenia

9.2.1. Relationship with bone mass

Among patients in subgroup A2, with the decrease in height and weight, bone mass also decreases, as the relationships are significant.

		Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/hip
Bone mass	**p**	0,628	**0,003**	**0,046**	0,259	0,120	0,078	0,866
	r	-0,131	**0,692**	**0,504**	0,300	0,404	0,453	-0,046

Table 30. Relationships between anthropometric indicators and bone mass

9.2.2. Relationship with visceral fat

The correlation analyses conducted between anthropometric indicators and the amount of visceral fat and in conditions of sarcopenia did not show any dependencies.

9.2.3. Relationship with subcutaneous fat

Weight loss, BMI, waist and hip circumferences are associated with the reduction of subcutaneous fat among individuals from subgroup A2, with strong relationships.

		Age	Height	Weight	BMI	Waist circumference	Hip circumference	Waist/ hip
Subcutaneous fat	**p**	0,077	0,919	**0,000**	**0,000**	**0,000**	**0,000**	0,502
	r	0,454	-0,010	**0,784**	**0,828**	**0,850**	**0,802**	0,181

Table 31. Relationships between anthropometric indicators and subcutaneous adipose tissue

9.2.4. Relationship with muscle tissue

Due to the characteristic of sarcopenia, a decrease in the amount of muscle tissue, we examined in detail the relationships between anthropometric indicators and the distribution of skeletal muscle by body parts among individuals from subgroup A2. With increasing height, the amount of skeletal muscle in the limbs increases, but not in the torso. Muscle tissue in the torso showed a positive significant relationship with weight and BMI.

Aging in women is associated with a decrease in lower limb muscle mass, but not in upper limb muscle mass.

		Age	Height	Weig ht	BMI	Waist circumfere nce	Hip circumfere nce	Waist/ hip
Muscle tissue left arm	**p**	0,578	**0,000**	0,124	0,566	0,552	0,461	0,937
	r	-0,151	**0,805**	0,401	0,155	0,161	0,199	-0,021
Muscle tissue right arm	**p**	0,895	**0,000**	0,265	0,905	0,710	0,619	0,954
	r	-0,036	**0,858**	0,296	0,032	0,101	0,135	-0,016
Muscle tissue trunk	**p**	0,203	0,102	**0,011**	**0,043**	0,170	0,063	0,510
	r	-0,336	0,423	**0,616**	**0,512**	0,360	0,475	-0,178
Muscle tissue left leg	**p**	**0,014**	**0,000**	0,572	0,768	0,737	0,959	0,622
	r	**-0,599**	**0,835**	0,153	-0,080	-0,091	-0,014	-0,119
Muscle tissue right leg	**p**	**0,029**	**0,000**	0,477	0,848	0,884	0,662	0,646
	r	**-0,546**	**0,838**	0,192	-0,052	-0,040	-0,199	-0,125

Table 32. Dependencies between anthropometric indicators and the distribution of SM in subgroup A2

9.3. Correlations between anthropometric indicators and ASMI, grip strength and gait speed in conditions of hyperthyroidism and sarcopenia

No relationships were observed between some of the anthropometric indicators, on the one hand, and ASMI, grip strength or gait speed in conditions of hyperthyroidism and sarcopenia.

9.4. Correlations of laboratory indicators in conditions of hyperthyroidism and sarcopenia

9.4.1. Correlations of biochemical indicators

9.4.1.1. Correlations between biochemical indicators and body composition

Among women from group A2, no relationships were observed between any of the biochemical indicators and the redistribution of body composition.

9.4.1.2. Correlations between biochemical parameters and ASMI, grip strength and gait speed in conditions of hyperthyroidism and sarcopenia

No correlations were found between biochemical parameters and ASMI, grip strength and gait speed in conditions of hyperthyroidism and sarcopenia.

9.4.2. Correlations of thyroid parameters

9.4.2.1. Correlations between thyroid parameters and body composition distribution in conditions of hyperthyroidism and sarcopenia

Women from subgroup A2 did not show any correlations between thyroid parameters and body composition distribution.

9.4.2.2. Correlations between thyroid indicators and ASMI, grip strength and gait speed in conditions of hyperthyroidism and sarcopenia

Similar to the data from the correlation analyses by groups, no relationships were found between thyroid indicators and ASMI, grip strength and gait speed by subgroups.

V. Discussion

1. Discussion of the results of task 1: Comparison of anthropometric and biochemical parameters between women with hyperthyroidism and healthy controls.

1.1. Anthropometric indicators

Hyperthyroidism is a condition characterized by accelerated metabolism and pronounced catabolic manifestations, which is why it is more often associated with weight reduction.

Although the individuals with hyperthyroidism studied by us had lower weight, BMI, waist and hip circumferences compared to the euthyroid group, we did not report a significant difference between hyper- and euthyroid for any of the listed parameters.

The average weight of women with hyperthyroidism (69.07±17.68 kg) is lower by about 4 kg compared to the control group (73.31±16.421 kg). Similar results were published by Karmisholt's team, but they studied the same individuals and the change in anthropometric indicators in a state of hyperthyroidism and one year after reaching a euthyroid state. The average baseline weight of women before starting therapy in the Karmisholt cohort was 68.9±13.1 kg and increased by an average of 5.2 kg, reaching 74.1±11.2 kg in a state of euthyroidism (Karmisholt J et al., 2021). The average BMI level in the women with hyperthyroidism studied by us is slightly above the upper limit of normal - 26.14±6.27 kg/m², which is explained by the higher baseline weight of the patients, reflecting the widespread prevalence of overweight and obesity in the Bulgarian population. Similar data on BMI in hyperthyroidism (26.39±4.44 kg/m²) were also published by a group of scientists from Pontevedra, Spain (*Ríos-Prego M et al., 2019*).

Underweight - BMI < 18.5 kg/m² was reported only in the group of women with hyperthyroidism (4.44%), which we associate with accelerated catabolic processes in excess of thyroid hormones. For comparison, the prevalence of underweight in the Bulgarian population in healthy women is lower - 2.9% according to data by Borisova et al. (*Borisova A-M et al., 2015*).

Nearly half of the group with hyperthyroidism (48.93%) had a BMI above the norm, of which 28.95% were overweight and 19.98% were obese. Brunova et al. reported similar data in a study of 160 hyperthyroid women - 29.4% had a BMI ≥ 25 kg/m², and 19.3% had a BMI ≥ 30 kg/m² (*Brunova J et al., 2003*). The high percentage of individuals with an elevated BMI supports the data on weight gain or lack of weight reduction in some patients with thyrotoxicosis. This is explained by increased food intake exceeding increased energy expenditure. It is provoked by hyperalimentation due to this condition, combined with a sedentary lifestyle and improper eating habits, which are emerging as a global problem.

The frequency of women with BMI ≥ 25 kg/m² among the healthy controls studied by us is similar to that reported among the Bulgarian population by Borisova et al. Patients from group B with obesity are 26.68% versus 28.3% at the population level, and those with overweight are 31.11% versus 32.5%, respectively (*Borisova A-M et al., 2015*).

Both waist and hip circumferences were insignificantly smaller among women in group A, and with similar data, another group reported statistical significance with euthyroid individuals (p 0.01). The same group of scientists, similar to our results, described a higher ratio between waist and hip circumferences among hyperthyroid individuals, without this difference being significant (p 0.63) (*Cai Z et al., 2022*).

1.2. Laboratory parameters

1.2.1. Thyroid parameters

When comparing thyroid parameters in patients with hyperthyroidism and euthyroid controls, we expectedly reported a statistically significant difference between the two groups of individuals for each parameter.

The most common etiological cause of hyperthyroidism in group A is Graves' disease, with its frequency among this group of studied individuals being 79.92%. Our results fully coincide with Garmendia Madariaga's data for the most common cause of hyperthyroidism, involving between 70% and 80% of cases (*Garmendia Madariaga A et al., 2014*). It is precisely because of this high proportion of autoimmune thyroid disease among the individuals we studied that the difference in TPOAb and TRAb levels compared to euthyroid individuals is also significant.

Of all the women with hyperthyroidism studied, 38 had overt hyperthyroidism (or 84.4% of the individuals in this group), and 7 had subclinical hyperthyroidism (15.6% of the women in group A). The world literature reports that about 8% of patients with subclinical hyperthyroidism progress to overt hyperthyroidism annually, most often those suffering from toxic nodular goiter (*Lee SY et Pearce EN, 2023*).

1.2.2. Biochemical parameters

Although with borderline significance (p 0.056) serum creatinine levels are lower among women with hyperthyroidism. In contrast to our results, most authors report a statistically significant difference in creatinine levels between patients with overt hyperthyroidism and euthyroid individuals (Naguib R et Elkemary E, 2023; Englund Flodström I et al, 2016). In subclinical hyperthyroidism, single studies find a change in serum creatinine. Allam et al. reported significantly lower creatinine values among patients with subclinical hyperthyroidism compared to healthy subjects (Allam MM et al., 2023).

Increased levels of thyroid hormones often lead to a decrease in serum creatinine. The mechanisms involved in this are increased renal blood flow, stimulation of the RAAS, which by increasing blood volume also stimulates glomerular blood flow. The importance of low serum creatinine levels is also due to a decrease in muscle mass in some individuals affected by hyperthyroidism. Increased thyroid hormones increase eGFR by decreasing vascular resistance in the afferent glomerular arterioles (*Naguib R et Elkemary E, 2023*).

The insignificant difference in serum creatinine between the two groups studied by us is probably due to several facts - the small number of individuals studied, the exclusion of patients with abnormal serum creatinine values, as well as the fact that 15.54% of individuals in group A have subclinical hyperthyroidism.

CPK levels among women in group A were significantly lower than in euthyroid subjects (p 0.021). This is supported by the numerous studies that found a similar relationship even in cases of subclinical hyperthyroidism. Some authors suggest its use as a biomarker for sarcopenia and muscle involvement in hyperthyroid subjects (*Wan Nazaimoon WM et al., 2001*).

Serum albumin levels in group A were lower than in group B, but the difference did not reach statistical significance. This is probably due to the inclusion and exclusion criteria, according to which only subjects with values of the studied indicator within the normal range were included in the study. Literature sources report significant hypoalbuminemia only in cases of severe untreated hyperthyroidism (*Evlice M et Aksoz Z, 2017*).

The mean vitamin D level among women with hyperthyroidism was below the threshold for deficiency (19.30 ±6.45 ng/mL) and was significantly lower than in group B (23.67 ±11.1 ng/mL), which is due to the bidirectional relationship between the two conditions. An increased incidence of autoimmune thyroid diseases has been described in vitamin D deficiency and insufficiency, while hyperthyroidism has been implicated as a cause of reduced synthesis and increased clearance of the vitamin (*Babić Leko M et al., 2023*).

Among the women we studied, 34.4% had a vitamin D level ≤ 20 ng/mL, which is comparable to the reported frequency among Europeans - 40% (*Cashman KD et al., 2016*). Our data are very close to those reported by a group of Swedish authors for a subnormal level of the vitamin in 38.4% of the subjects with hyperthyroidism they studied. They also found a more frequent presence of severe deficiency among the same cohort with a frequency of 7.5% versus 0.3% among euthyroid subjects (Planck T et al., 2018). The higher frequency of insufficiency and severe vitamin D deficiency reported by us among women from group A coincides with the majority of data in the literature on the prevalence of these conditions among subjects affected by hyperthyroidism.

Of the lipid parameters, the level of LDL-c deserves attention, without being a target in our study. Its level among women from group A is significantly lower compared to euthyroid individuals (p 0.006). This is due to its increased clearance as a result of the hyperthyroid state, while according to literature data, HDL-c levels are either similar or slightly increased compared to euthyroid individuals (*Duntas LH, 2002*). The remaining biochemical indicators studied by us are aimed at excluding individuals with abnormal results that would reflect on the body composition of the subjects and did not demonstrate differences between the two studied groups.

2. Discussion of the results of task 2: comparison of body composition between group A and group B

2.1. Bone mass

Women with untreated hyperthyroidism have significantly lower bone mass compared to euthyroid controls (p 0.01) and this is not age-related, due to the similar age of the two studied groups. The distribution by age categories again shows a higher number and proportion of patients with low bone mass for a given age among hyperthyroid individuals. Our results are supported by data from numerous sources such as Acotto CG (p=0.001) (*Acotto CG et al., 2002*), Thomas Seppel (p < 0.0001) (*Seppel T et al., 1997*) and others. A similar difference in the amount of bone mass of 0.1 kg between women with hyper- and euthyroidism was reported by Cai et al. (*Cai Z et al., 2022*). In contrast to what has been said so far, the Karmisholt J team did not find a similar difference in bone content (p 0.608) (*Karmisholt J et al., 2021*).

TRα is abundantly expressed in the skeleton, which mediates the action of T3 on bones and the result is increased bone resorption in conditions of hyperthyroidism. The shortened bone remodeling cycle, increased markers of bone resorption and formation, characterize the high bone turnover observed in this hormonal condition. All this increases the risk of developing osteoporosis (*Delitala AP et al., 2020*). This is also supported by a meta-analysis by Vestergaard and Mosekilde based on 25 studies, confirming that untreated hyperthyroidism leads to a decrease in bone mass (*Vestergaard P et Mosekilde L, 2003*).

2.2. Visceral adipose tissue

The amount of visceral adipose tissue was insignificantly higher among euthyroid controls (p=0.242). Our results are supported by the publication of Cai et al., comparing data between women with active thyrotoxicosis and the same women after reaching euthyroidism (p=0.42) regardless of weight gain (*Cai Z et al., 2022*). The smaller amount of visceral MT among group A is due to its high metabolic activity, as the excess of thyroid hormones stimulates lipolysis and thermogenesis in this tissue (*Volke L et Krause K, 2021*).

2.3. Subcutaneous adipose tissue (SAT)

Women affected by hyperthyroidism have a lower percentage of whole-body SAT compared to healthy controls, with the difference of 0.32% in our study being without statistical significance. Similar are the data of Acotto et al., who reported a non-significant difference of 0.8% in the amount of adipose tissue between women with hyperthyroidism and women who achieved and maintained euthyroidism 6 months after treatment (*Acotto CG et al., 2002*). A number of other studies in this direction report similar results, with the closest to ours being the data of an Indian team of scientists for a 0.24% difference between women with hyper- and euthyroidism, without this being statistically significant (*Patil A et al., 2022*).

The percentage of whole-body fat in both groups of our sample is above the obesity threshold of 33%, which is also explained by the higher average BMI in both groups. The observed lateralization in the hands due to a larger amount of fat tissue in the left upper limb is probably due to the fact that all patients participating in the study are right-handed. They use their right hand much more often in performing various activities, which better develops the muscles of the right upper limb, expends more energy and, accordingly, has an absolute and relatively smaller amount of fat tissue in the dominant limbs. On the other hand, activities expressing lateralization for the lower limbs are not so common in everyday life, which is why such a difference in body composition measurement was not recorded.

The lower amount of adipose tissue in women with hyperthyroidism, although not significant, is a result of increased energy expenditure at rest, as well as lipolysis stimulated by hormonal excess (*Silva JE, 2003*). Lahesmaa et al., using [18F]Fluoro-2-deoxy-2-D-glucose in positron emission tomography (PET), found that in conditions of hyperthyroidism, the use of fat as an energy substrate is many times increased, while that of glucose does not show any difference compared to the euthyroid state (*Lahesmaa M et al., 2014*).

2.4. Muscle tissue

The absolute amount of muscle tissue in each of the body regions we studied was significantly less among women with hyperthyroidism compared to euthyroid controls. This is consistent with most literature data indicating a much more severe and earlier involvement of muscle tissue compared to fat tissue by high levels of thyroid hormones (*Acotto CG et al., 2002; Peterson ME et al., 2016*). This is due to the many times stronger effect on proteolysis, which is increased by 12% compared to euthyroid controls, amino acid oxidation is increased by 24%, and the increase in protein synthesis is only 9% higher (*Martin WH 3rd et al., 1991*).

This is supported by data from a team of scientists from Aarhus University Hospital, Denmark, in collaboration with the Mayo Clinic, who used a dilution method with labeled phenylalanine and tyrosine. They reported a significant increase in protein breakdown by 40%, an increase in blood flow in the arms by 35%, without observing a significant difference in protein synthesis between women with hyper- and those with euthyroidism (*Riis AL et al., 2005*).

The decrease in muscle mass observed by us was proportional and statistically significant in each body compartment. Similar to our data, including the pronounced lateralization of the lower extremities, was reported by the Acotto team (*Acotto CG et al., 2002*). The difference in the amount of muscle tissue obtained by us as an absolute value in kilograms between women from group A and group B is respectively: 2.92 kg for torso, 1.86 kg for lower limbs and 0.72 kg for upper limbs. The same parameters in the Acotto cohort are respectively: 1.4 kg for torso, 1.0 kg for lower limbs and 0.4 kg for upper limbs (*Acotto CG et al., 2002*). The higher values in our study are probably due to the higher body weight and BMI values of the patients from both groups.

3. Results for task 3: Comparison of grip strength, ASMI and gait speed (diagnostic components of sarcopenia) between women with hyperthyroidism and healthy controls

3.1. Grip strength

Our results indicate that grip strength is almost two times lower among women with hyperthyroidism compared to euthyroid controls, the difference being statistically significant (p 0.000). Since the two groups are comparable in terms of age, age can be excluded as a determining factor for this result.

Erkol İnal et al. were among the first to study and describe the influence of hyperthyroidism on grip strength in humans, and the results were conclusive - significantly reduced grip strength among those affected by thyroid dysfunction (*Erkol İnal E et al., 2015*). The reduced grip strength in our studied group A is not only lower than that in euthyroid controls, but also below the threshold for age and gender norms, which according to data from Dodds and co-authors should be between 27.5 and 28.7 kg (*Dodds RM et al., 2014*).

Given the already analyzed results of body composition and significant impact on the amount of muscle tissue, it is expected that this will also affect the muscle “quality”, expressed through grip strength. The exact mechanisms linking hyperthyroidism with grip strength have not yet been clarified, and it is believed that the reduced muscle mass as a result of the prevalence of proteolysis is only a small part of the predisposing factors. In support of this opinion are the data from an experiment with rats, where El-Kotb et al. observed that with a reduction in muscle tissue by 20%, a decrease in grip strength by up to 100% was observed. They suggest that contractile function is also affected. In conditions of hyperthyroidism, acetylcholinesterase levels are reduced in the neuromuscular junction, leading to overactivation of the motor end plate with irregular and increased muscle contractions, resulting in impaired muscle function and weakness (*El-Kotb S et al., 2014*).

3.2. ASMI

Studies investigating the amount of muscle mass, reflected by ASMI in women with hyperthyroidism are limited. Our data indicate that the amount of muscle tissue as an absolute value among women from group A is significantly lower compared to group B (p 0.000). The muscle tissue index in the limbs (ASMI) used to assess sarcopenia also takes into account the height of the examined person, as well as giving a more realistic idea of the individual norms of this important indicator.

Hyperthyroidism affects muscle tissue to a much greater extent than fat tissue, which is also reflected in ASMI. The average value of ASMI in group A reaches 6.31 kg/m^2, which is higher than the threshold (<5.5 kg/m^2) for diagnosing sarcopenia. Similar to our results, the team of Brennan et al. described significantly reduced limb muscle mass, approaching but not exceeding the threshold for sarcopenia, in individuals with both overt and subclinical hyperthyroidism. The study followed these individuals during treatment and one year after reaching euthyroidism, and found an improvement in the ASMI parameter studied (*Brennan MD et al., 2006*).

Given that reduced ASMI is associated with a poorer quality of life, increased risk of falls, fractures, and immobilization, as well as early mortality (Lee H et al., 2023), the question arises whether a watchful waiting approach is justified in subclinical hyperthyroidism and whether timely initiation of therapy would not protect the patient from these negative consequences (*Brennan MD et al., 2006*).

Another study found a significant difference in ASMI between women with hyperthyroidism and euthyroid controls only in the age category under 40 years. Above this age, ASMI is lower among those affected by hyperthyroidism, but this difference does not reach statistical significance (*Zhou HY et al., 2023*). This is probably due to the more pronounced metabolic processes occurring in muscle tissue in younger women. A supporting condition for the smaller difference in the age category over 40 years is that age is an independent risk factor for muscle tissue loss even among euthyroid individuals (*de Santana FM et al., 2021*).

3.3. Gait speed

Gait speed is a marker of physical performance, and similar to grip strength, its slowing is associated with immobilization, increased and earlier mortality (*Bano A et al., 2016*). Impaired walking speed is not a mandatory component for the diagnosis of sarcopenia, but its presence defines it as severe.

Hyperthyroidism with the astheno-dynamia that it causes, as well as with the decrease in muscle mass reported by us, leads to significantly slowed gait speed compared to euthyroid controls (p 0.001). The lower value among individuals from group A (0.90± 0.19 m/s) does not cross the threshold for severe sarcopenia in our studied cohort. This is probably due to the average age of the group, which does not exceed 65 years, the relatively short duration of hyperthyroidism (≤ 6 months), as well as additional, as yet unclear factors.

There are few literature sources investigating gait speed in hyperthyroid individuals. Bano et al. reported that subclinical and overt hyperthyroidism slow down walking speed in parallel with the decrease in TSH (*Bano A et al., 2016*). How hyperthyroidism specifically slows down this indicator is not fully understood. The genesis is probably complex and is rooted in a combination of factors - impaired innervation of the muscles, electrolyte changes, impaired muscle volume, and a genetic predisposition is also suspected (Venero C et al., 2006). In contrast to our data, Lee et al. did not find slowed gait speed in women with medicated hyperthyroidism (*Lee JC et al., 2021*).

4. Discussion of the results of task 4: Relationships between anthropometric indicators and body composition, as well as between anthropometric indicators and diagnostic criteria for sarcopenia - ASMI, grip strength and gait speed

4.1. Relationships between anthropometric indicators and body composition

4.1.1. Relationship between anthropometric indicators and bone mass

The observed decrease in bone mass in the total sample, associated with the decrease in height, weight, BMI, waist and hip circumference in parallel with the transition from eu- to subclinical and overt hyperthyroidism, is a characteristic generally valid population relationship, regardless of the thyroid status of women (*Munaisinghe RL et al., 2002*).

Of interest is the fact that in our study a negative correlation relationship was found between age and bone mass only among the hyperthyroid group. The mean age of women in both groups was comparable (p 0.862), 54.98±15.26 years in hyperthyroid women and 54.36±14.57 years in euthyroid women, respectively. In the general population, age is a well-known risk factor for osteoporosis (*Shoback D et al., 2020*). Our data on a negative correlation between age and bone mass in group A indicate an overlap of the negative effect of hyperthyroidism on bone mass and structure to the age factor.

4.1.2. Relationship between anthropometric indicators and the amount of visceral fat

It is generally accepted that visceral fat depot increases in parallel with the increase in weight, BMI, waist and hip circumferences, regardless of the thyroid status of the individuals, which coincides with our results referring to the general sample (*Edston E, 2013*).

Divided by groups, this dependence was more pronounced for group A. It has been shown that excess thyroid hormones lead to a decrease in the amount of visceral fat (*Steinhoff KG et al., 2021*). And although we do not find a statistically significant difference in the amount of visceral fat between women with hyperthyroidism and those in a euthyroid state, most studies report significance (*Steinhoff KG et al., 2021*).

4.1.3. Relationship between anthropometric parameters and the amount of subcutaneous fat

Our data show a positive relationship between weight, BMI, waist and hip circumferences and subcutaneous fat in each of the four limbs. This is valid regardless of the thyroid status of the individuals, with the strength of the relationships being significant to strong. The fact that no correlations specific to hyperthyroid individuals or different strengths of relationships among them stand out supports the generally valid increase in subcutaneous fat in parallel with the increase in the aforementioned anthropometric parameters (*Mouchti S et al., 2023*). Similar to our data, where no correlation is observed between subcutaneous fat and waist/hip ratio, a number of other authors have also reported (*Mouchti S et al., 2023; Barreira TV et al., 2012*).

4.1.4. Relationship between anthropometric parameters and muscle mass

4.1.4.1. Relationship between age and muscle mass

Our data establish a negative moderate to significant relationship between age and muscle mass in each studied body region, valid only in the conditions of hyperthyroidism. Ibad et al. reported similar data to ours, as they found that this correlation was present even in euthyroidism, but with upper limit FT4 (*Ibad HA et al., 2023*). Our studied cohort, similar to the groups studied by other authors, is characterized by a younger age than the reported threshold for significant independent involvement of muscle mass of 65 years (*Wu LC et al., 2021*). This supports the claim that hyperthyroidism is a trigger factor for earlier involvement of muscle mass (*Tufan A et al., 2022*).

4.1.4.2. Relationship between height and muscle mass

As height increases, muscle mass increases in each body region we examined. This relationship is particularly strong when all subjects are analyzed, regardless of thyroid hormone levels, and is maintained in hyperthyroidism, with the strength decreasing as the number of subjects decreases.

Due to the fact that almost all literature sources support this correlation, it is recommended that muscle mass be correlated with the height of the subject when examining muscle mass (*Han DS et al., 2016*).

4.1.4.3. Relationship between weight, BMI, waist circumference, hip circumference, waist/hip ratio and the amount of muscle tissue

The results of our analysis support the generally valid data on the increase in the amount of muscle tissue, in parallel with the increase in weight, BMI, waist and hip circumferences. We do not establish a relationship only between the waist/hip ratio and the amount of muscle tissue. These relationships are independent of the thyroid status of the individuals and are confirmed by a number of authors (*Han SS et al., 2010*).

4.2. Relationships between anthropometric indicators and diagnostic criteria for sarcopenia - ASMI, grip strength and gait speed

4.2.1. Relationship with ASMI

Our results report a negative correlation between ASMI, i.e. limb muscle mass, relative to height and age, was only observed in hyperthyroid subjects (p 0.034; r -0.317). This again suggests the reinforcing effect of hyperthyroidism on age as a risk factor for sarcopenia.

The ASMI index shows a dependence on anthropometric indicators among all subjects studied by us, regardless of thyroid status. A decrease in weight, waist and hip circumferences, as well as a decrease in BMI were associated with a decrease in ASMI, both in the entire sample and by groups. These relationships between the indicated anthropometric indicators and ASMI have also been proven by Han and his team (*Han DS et al., 2016*).

4.2.2. Relationship with grip strength

The data obtained by us on a positive correlation between grip strength and anthropometric parameters - height, weight, BMI, waist and hip circumferences and the negative correlation with age in the entire sample are also supported by studies including young healthy individuals (*Doğan G et al., 2023*). Similar studies in healthy individuals in the age group 7-22 years are of interest, where all the above-mentioned dependencies are present, but the correlation between grip strength and age is positive (*Musa TH et al., 2022*). The reason for this is the still unattained peak muscle mass and strength in adolescents.

Regarding waist circumference, there are also results opposite to our results. Studies of individuals with an average age of 76 years establish a negative correlation between grip strength and waist circumference (*Lin MH et al., 2021*). Such a correlation is absent with the other anthropometric parameters.

4.2.3. Relationship with walking speed

Our results are in line with the well-known slowing of walking speed with increasing age, which also applies to healthy individuals. It is associated with involution in the musculoskeletal system - physiologically, as well as influenced by the increase in comorbidities accompanying the aging process (*Kasović M et al., 2021*).

5. Discussion of the results of task 5: relationship between hormonal and biochemical indicators and body composition, as well as between hormonal and biochemical indicators and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

5.1. Relationship between hormonal and biochemical indicators (25(OH)D, creatinine, CPK and albumin) and body composition

Regarding the relationships between hormonal and biochemical indicators (25(OH)D, creatinine, CPK and albumin) and body composition in conditions of hyperthyroidism, we found an extremely limited number of data in the world literature to compare with the results obtained by us.

5.1.1. Relationship between hormonal indicators and body composition

The correlation analyses we conducted did not show a relationship between some of the thyroid parameters and white adipose tissue, which is also due to the lack of significance in its quantity between the two groups of individuals. In contrast, Ittermann et al. described a positive relationship between TSH levels and adipose tissue in people over 50 years of age. Such relationships are absent with the other components of body composition, as well as in younger individuals (*Ittermann T et al., 2021*). The same analysis, but in participants over 60 years of age, found that a decrease in TSH was accompanied by a decrease in muscle and bone mass, which is in line with the results of our analysis.

In contrast, Szlejf et al. found no association between TSH levels and muscle mass in patients with subclinical hyperthyroidism over the age of 50 (*Szlejf C et al., 2020*).

Several other authors also support the association between decreasing TSH levels and reduction in muscle tissue, which supports the view that hyperthyroidism affects muscle catabolism much more than lipolysis (*Peterson ME et al., 2016*).

On the other hand, TPO Ab showed a significant positive association with visceral adipose tissue, which is in line with the results of a team led by Baranowska-Bik (*Baranowska-Bik A et al., 2020*). Leptin is likely at the root of this association, as a significant difference in its levels was found between women in a euthyroid state, with some having positive antibodies and others not. The same team reported a positive correlation between Th17 cells and leptin levels in women with immunological evidence of autoimmune thyroiditis (*Wang S et al., 2013*).

According to our data, the increase in TRAb is accompanied by a decrease in muscle mass mainly in the torso and upper limbs. We did not find a similar relationship for the upper body, described in the literature. The effect of TRAb on some muscle groups is known, and in this case, its role in reducing muscle tissue in the upper body compartment cannot be excluded, as the mechanisms are unknown. It is possible that the relationship is indirect, due to the effect of lower serum TSH, associated with the increase in TRAb, on the amount of muscle mass. An unknown autoimmune mechanism, analogous to the relationship between another autoimmune disease, such as rheumatoid arthritis, with a decrease in muscle mass (*An HJ et al., 2020*), is not excluded.

It is believed that one of the mechanisms for the reduction of muscle tissue in autoimmune diseases is the chronic inflammation they cause. In support of this, Garan Jones' team reported that the presence of an autoimmune disease increases the risk of developing sarcopenia (*Jones G et al., 2020*). In order to clarify these theories, more clinical data in this direction are needed.

5.1.2. Relationship between biochemical indicators (25(OH)D, creatinine, CPK and albumin) and body composition.

Despite the data in the literature on the relationship between each of the biochemical indicators - 25(OH)D, creatinine, CPK and albumin with body composition and the amount of muscle tissue, we did not find such relationships. The reasons are probably in the normal levels, within the reference norms, for creatinine, CPK and albumin in our cohort, as well as probably the small number of patients included in the study.

5.2. Relationship between hormonal and biochemical indicators and diagnostic components of sarcopenia - grip strength, ASMI and gait speed.

5.2.1. Relationship between hormonal parameters and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

In our analysis, the decrease in TSH from normal to levels characteristic of hyperthyroidism was associated with weakening grip strength, a decrease in ASMI and a slowdown in gait speed. The increase in FT3, but not FT4, as well as the antibodies TPO Ab and TRAb, was accompanied by a decrease in grip strength and ASMI.

The data of Chen J et al. confirm the correlations established by us for TSH and FT3, and they also establish another relationship, namely that the increase in FT3 is accompanied by a slowdown in gait speed. They, like us, do not describe the influence of FT4 on some of the mentioned parameters (*Chen J et al., 2023*).

Other authors also confirm the established correlations between TSH and ASMI and grip strength, as well as between FT3 and ASMI. In the study led by Szlejf, the correlation between TSH and ASMI disappears with the transition from overt to subclinical hyperthyroidism (*Szlejf C et al., 2020*). Conversely, Choi et al. did not establish such relationships (*Choi YJ et al., 2015*).

Skeletal muscle is a target organ for the action of thyroid hormones, and hyperthyroidism, affecting it, is a predisposing factor for the development of secondary sarcopenia in some individuals. In addition to the clarified mechanisms, including increased activity of Na+K-ATPase and Ca2+ATPase, it is believed that low TSH and high FT3 levels exhaust muscles through prolonged stimulation of mitochondrial activity. In studies in mice, increased expression of p43 and the mitochondrial T3 receptor on skeletal muscle cells has been found, which enhances oxidative processes and contributes to muscle atrophy (*Casas F et al., 2002*).

5.2.2. Relationship between biochemical indicators (25(OH)D, creatinine, CPK and albumin) and diagnostic components of sarcopenia - grip strength, ASMI and gait speed

Contrary to the numerous sources cited relationship between the development of secondary sarcopenia and reduced vitamin D levels (*Mizuno T et al., 2022*), we do not observe such a relationship, even when the effect of hyperthyroidism is superimposed on this. A probable reason for the lack of such a relationship in our sample is that the studies among the majority of patients coincided with the Coronavirus pandemic, when preventive vitamin D supplementation was promoted among the public.

The creatinine level is a parameter closely related to the state of skeletal muscle. The relationship between high creatinine levels within CKD and a decrease in muscle mass among those affected is known (*Patel SS et al., 2013*). A U-shaped relationship has been established between serum creatinine and the reduced amount of muscle tissue, therefore its low levels are also associated with muscle loss and can serve as a marker for sarcopenia (*Curcio F et al, 2016*). Hyperthyroidism also often leads to a decrease in its serum level and in this aspect, the relationship that is lost for the correlation between serum creatinine and ASMI in group A is due to the small number of subjects studied and creatinine within the reference values. Kusunoki et al. confirmed the relationship between serum creatinine and ASMI and proposed the serum creatinine/cystatin C ratio as a marker linking sarcopenia with increased mortality (*Kusunoki H et al., 2021*).

Chen's team confirmed the results of a decrease in grip strength with a decrease in CPK, but additionally described a positive correlation between CPK and ASMI, which we did not find in our analysis (*Chen Z et al., 2023*). It is assumed that this is due to the inhibition of the enzyme by high levels of thyroid hormones in the CM, as well as the decrease in muscle mass that accompanies hyperthyroidism.

The decrease in serum albumin within the normal range is accompanied by a slowdown in walking speed, and the relationship is weak. This is valid not only for hyperthyroidism conditions. Kobayashi et al. found an inverse relationship between serum albumin and walking speed in people over 75 years of age (*Kobayashi K et al., 2023*). Most available data relate to the relationship between hypoalbuminemia and diagnostic components for sarcopenia, and we did not find an analysis of its levels within the reference range and its association with grip strength, ASMI or walking speed among people with hyperthyroidism.

6. Discussion of the results of task 6: establishing the risk of developing secondary sarcopenia, determining its frequency among the studied individuals and the threshold age above which the probability of secondary sarcopenia increases

Of all the women we studied, sarcopenia was found among 17 of them (16 from group A and 1 from group B), or 18.87% of the total sample and 35.52% of hyperthyroid patients. The frequency in our total sample approaches the upper limit frequency at the global level of about 20% (*Moon JJ et al., 2018*).

Among all those affected by sarcopenia, 64.7% of women with overt hyperthyroidism, 29.4% with subclinical hyperthyroidism, and 5.9% euthyroid. Among 3 of the 17 individuals affected by sarcopenia (6.66%), it is severe, and all affected are individuals with overt hyperthyroidism. Our data are similar to those of Tufan et al., who reported 23.1% sarcopenia associated with overt hyperthyroidism; 15.4% with subclinical hyperthyroidism; and 7.7% severe sarcopenia, and this was only among individuals with overt hyperthyroidism. They also found a higher risk of developing sarcopenia in women with hyperthyroidism compared to healthy controls and men with hyperthyroidism, as well as a younger age of onset (*Tufan Cincin A et al., 2022*). Conversely, Szlejf et al. did not find an increased risk among individuals with subclinical hyperthyroidism (*Szlejf C et al., 2020*). It is noteworthy that the youngest woman affected by sarcopenia in our sample was 38 years old. This motivated us to calculate the threshold age above which sarcopenia should be sought among women with hyperthyroidism, namely 54 years of age. This is 11 years earlier than the age of primary sarcopenia, which supports the claim that hyperthyroidism accelerates and predisposes to its development. A younger age of hyperthyroid individuals affected by sarcopenia was reported by Engin et al. - a mean age of 44.9 years, including men (*Engin E et al., 2021*).

We did not find a threshold age for sarcopenia among women with hyperthyroidism described in the literature with which to compare our results.

7. Discussion of the results of task 7: correlations between anthropometric, hormonal and biochemical indicators, on the one hand, and the diagnostic components of sarcopenia - ASMI, grip strength and gait speed - on the other, among individuals affected by sarcopenia.

The relationship of sarcopenia with age is known, both among women with hyperthyroidism and at the population level (*Ibad HA et al., 2023*). Unlike the literature sources, we did not establish correlations between anthropometric parameters (except waist circumference) and any of the diagnostic components of sarcopenia among patients affected by the disease.

No correlations were established between hormonal and biochemical indicators, on the one hand, and the diagnostic components of sarcopenia - on the other. This is probably due to the fact that 17 individuals were sarcopenic, and this number does not allow for a statistically significant analysis. Unlike us, the team of Tufan et al. reported a negative correlation between T4 level and grip strength and ASMI (*Tufan CA et al., 2022*).

8. Discussion of the subanalysis of the results among women affected by thyrotoxicosis (subgroup A1 and subgroup A2): comparison and detection of correlations between the studied parameters among women with hyperthyroidism, compared to those with hyperthyroidism and established sarcopenia.

Due to the high frequency of sarcopenia among women with hyperthyroidism, we divided group A into two subgroups. Subgroup A1 consists of 29 women, all hyperthyroid without sarcopenia, and subgroup A2 includes 16 women with sarcopenia and hyperthyroidism. Studies investigating a similar combination of sarcopenia and hyperthyroidism are scarce, a total of 2 studies, 1 of which in rats (*Tufan CA et al., 2022; Yoon JA et al., 2020*).

Regarding anthropometric parameters, we did not find a statistically significant difference between the two subgroups, but the age of subgroup A2 is higher. These data are consistent with the results of Tufan, where the age difference is significant when combining the two pathologies (*Tufan CA et al., 2022*). Although it does not reach statistical significance, the almost twice higher level of TPO Ab among subgroup A2 is striking, which cannot rule out a probable autoimmune genesis linking hyperthyroidism with sarcopenia (*An HJ et al., 2020*). And while our subanalysis did not find significant differences between biochemical parameters, a similar study in rats reported significantly lower CPK levels among those affected by sarcopenia and hyperthyroidism simultaneously (*Yoon JA et al., 2020*).

Body composition data show significantly lower bone mass in subgroup A2 (p 0.041), and compared with the data from the correlation analysis, it is associated with lower height and weight in this subgroup. There was also no significant difference between the amount of visceral and subcutaneous fat. The musculature in all limbs, but not in the torso, was significantly smaller in patients with sarcopenia, which is expected, given the fact that the amount of muscle tissue in the limbs (ASMI) is one of the diagnostic criteria for sarcopenia. It is for this reason that the two indicators on which the diagnosis of "sarcopenia" is based, namely ASMI and grip strength, had significantly lower values in subgroup A2 (p 0.000).

Correlation analysis revealed a negative relationship between FT3 and hip circumference (p 0.05; r -0.514) in subgroup A2. Weight loss, BMI, waist and hip circumferences were associated with a decrease in subcutaneous fat tissue among individuals from subgroup A2, and the relationships were strong. We did not find other correlations that would indicate the influence of thyroid or biochemical indicators on some of the diagnostic components of sarcopenia (grip strength, ASMI, gait speed).

VI. Main conclusions

1. Women with hyperthyroidism have lower values of anthropometric indicators compared to euthyroid women - weight, BMI, waist and hip circumferences, without this being significant.

2. Patients with hyperthyroidism have significantly lower values of LDL-c, CPK, 25(OH)D and borderline lower levels of serum creatinine compared to healthy controls.

3. Untreated hyperthyroidism leads to a change in body composition, with significantly lower bone mass and muscle mass in each examined body compartment.

4. Hyperthyroid women have a smaller amount of visceral and subcutaneous fat tissue, compared to euthyroid women, without this being significant.

5. Untreated hyperthyroidism leads to a decrease in grip strength, ASMI and gait speed.

6. In conditions of hyperthyroidism, increasing age is associated with a decrease in the amount of bone and muscle tissue.

7. With increasing age, grip strength weakens and walking speed slows down in all subjects studied, regardless of thyroid status.

8. The decrease in weight, BMI, waist and hip circumferences are associated with a decrease in bone mass, the amount of muscle and subcutaneous fat in all subjects studied, regardless of thyroid status.

9. The decrease in weight, BMI, waist and hip circumferences in the transition from euthyroid state, through subclinical hyperthyroidism to overt hyperthyroidism, are associated with a decrease in all studied parameters of body composition, ASMI and grip strength.

10. In women with hyperthyroidism, the amount of visceral fat tissue decreases with a decrease in weight and BMI.

11. The decrease in TSH from euthyroid to hyperthyroid state is accompanied by a decrease in muscle mass in each of the limbs, as well as a decrease in bone mass.

11. The increase in TPO Ab is accompanied by a decrease in muscle tissue in the limbs, but not in the trunk, as well as a parallel increase in BMT.

12. The titer of TRAb is negatively correlated with bone mass and the amount of muscle tissue in the upper limbs and trunk.

13. The decrease in TSH from euthyroidism to hyperthyroidism is accompanied by a decrease in ASMI, grip strength and a slowdown in gait speed.

14. The increase in FT3, TPO Ab and TRAb are accompanied by a decrease in ASMI and a weakening of grip strength.

15. An increase in creatinine within the reference range is associated with a decrease in muscle tissue, expressed by ASMI, in the general sample.

16. A decrease in creatine phosphokinase within the normal range is accompanied by a decrease in grip strength in the general sample.

17. A decrease in albumin within the reference range is associated with a decrease in gait speed among all women.

18. Hyperthyroidism affects skeletal muscle qualitatively and quantitatively to the extent of developing secondary sarcopenia. Sarcopenia occurs in one third of untreated hyperthyroid women.

19. Hyperthyroidism predisposes to an earlier onset of sarcopenia, with the threshold age above which one should specifically seek is 54 years.

20. Hormonal and biochemical indicators do not differ between hyperthyroid women with sarcopenia compared to hyperthyroid women without sarcopenia.

21. Women with hyperthyroidism and sarcopenia have significantly less bone mass, muscle and subcutaneous fat compared to hyperthyroid patients without sarcopenia.

VII. Final conclusion

In summary, sarcopenia, or age-related loss of skeletal muscle tissue, is a growing clinical problem related to demographic changes, increasing life expectancy, sedentary lifestyles, and other factors. Research in this area is growing exponentially, but is more limited in terms of the search for muscle damage in diseases not related to age.

The present study is one of the few to investigate body composition, muscle mass, and muscle quality in hyperthyroidism. Based on the results obtained, hyperthyroidism emerges as a risk factor for quantitative reduction in muscle and bone mass, as well as qualitative muscle deficiency with reduced grip strength and reduced physical capacity. Untreated hyperthyroidism can be considered as a cause of secondary sarcopenia.

Grip strength stands out as a valuable marker, providing a comprehensive picture of the change in anthropometric indicators, bone, fat, and muscle tissue in the body, as well as the ASMI index. Measuring grip strength is of high informative value and easy to perform, therefore it is appropriate to implement it as part of the routine clinical examination of individuals affected by hyperthyroidism.

VIII. Contributions of the dissertation

For the first time in Bulgaria:

• it is performed an analysis of the body composition of women with newly diagnosed and untreated hyperthyroidism;

• it is investigated the presence of sarcopenia among women with untreated hyperthyroidism;

• it is used grip strength and walking speed as valuable, informative and easy-to-perform studies among hyperthyroid women;

• it is made a simultaneous comparison between anthropometric and biochemical indicators, body composition, grip strength and gait speed in women with newly diagnosed hyperthyroidism;

• it is investigated the relationships between thyroid and biochemical indicators (on the one hand) and body composition, grip strength and gait speed (on the other hand) in women with newly diagnosed hyperthyroidism.

In addition to being a pilot study in Bulgaria, this study is one of the few worldwide that:

• makes a comprehensive assessment of muscle health in women with hyperthyroidism, taking into account both the amount of skeletal muscle as an absolute value and through the ASMI index, as well as its functional state;

• analyzes grip strength and gait speed within the framework of hyperthyroidism;

• identifies hyperthyroidism as a risk factor for the development and earlier onset of sarcopenia.

IX. Scientific publications related to the dissertation

1. Publications

- Tosheva, Gergana M., Siderova, Mira V. Sarcopenia- a new challenge. Journal Endocrinologia. 2021; 1: 3-10. (in Bulg.)
- Tosheva, Gergana M., Siderova, Mira V. Grip strength as a biomarker of adverse health outcomes. Varna medical forum. 2021; 2: 46-54.(in Bulg.)
- Tosheva, Gergana M., Siderova, Mira V. Comparative Analysis of Sarcopenia and its Components (Muscle Strength, Muscle Mass and Physical Performance) Between Women with Newly Diagnosed Hyperthyroidism and Those Undergoing Thyreostatic Drug Therapy. Journal Endocrinologia. 2021; 4: 227-236.(in Bulg.)
- Tosheva, Gergana M., Siderova, Mira V. The role of thyroid hormones on the skeletal muscle and the development of sarcopenia. Varna medical forum. 2022; 2: 124-132.(in Bulg.)

2. Participations in national congresses and symposia

- Tosheva, Gergana M., Siderova, Mira V. Thyrotoxicosis as a predisposing factor for the development of secondary sarcopenia. XII National Congress of Endocrinology, 12-14 October 2023, Plovediv.

3. Participation in international congresses and symposia

- Marinova, G., Siderova, M. Thyrotoxicosis and its association with sarcopenia. 23rd European Congress of Endocrinology , 22-26 May 2021. Abstract book.
- Tosheva G, Siderova, M. Body composition comparison in patients with thyrotoxicosis- prior to and during treatment. Annual Meeting of the European Thyroid Association- Virtual Conference. September 4–7, 2021. Abstract book.
- Tosheva G, Siderova, M. Grip strength in newly diagnosed hyperthyroidism. 45th Annual Meeting of the European Thyroid Association, 9-12 September 2023, Milan, Italy. Abstract book.

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