„ Bone health and fracture risk among peripubertal and adolescent children – the importance of body weight, adipose tissue distribution and the presence of metabolic abnormalities ”

CONTENTS

PART IX. FINAL REMARKS

LIST OF ABBREVIATIONS

A/G	ratio between android and gynoid fat tissue
A4	androstendion
AF	android fat mass
area/H	bone area/height
BAr	bone area
BA	bone age
BG	blood glucose
BIA	bioelectric impedance analysis
BMAD	bone mineral apparent density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BP	blood preasure
CRP	c-reactive peptide
DHEA-S	dehydroepiandrostendion suphate
DXA	dual-energy x-ray absorptiometry
E2	estradiol
FAI	free androgen index
Fat % (FF %)	percentage fat mass
FFM	fat-free mass
FFMI	fat-free mass index
FM	fat mass
GF	gynoid fat mass
HbA1c	glycated hemoglobin
HDL-C	HDL-cholesterol
IGF1	insulin-like growth factor protein-1

IGFs	insulin-like growth factor proteins
IL	interleukin
LBM	lean body mass
LM	lean mass
LS	lumbar spine
MS	metabolic syndrome
Ob %	percentage of obesity
P	percentile
pQCT	peripheral quantitative computer resonance
AMH	anti mullerian hormone
PBM	peak bone mass
SHBG	sex hormon binding globulin
SD	standart deviation
SMI	skeletal muscle index
SMM	skeleta muscle mass
QUS	quantitative ultrasound
T	testosteron
TBLH	total body less head
TG	triglycerides
UA	uric acid
VF	visceral fat
VF level	visceral fat level
VFI	visceral fat index
WC	waist circumference

PART I. INTRODUCTION AND PREREQUISITES

1. INTRODUCTION

Over the past two decades or so, much new knowledge has been gained in the field of the biology of the growing child's bone. Thus, children's bone health has gradually become a well-recognized and increasingly important medical problem.

The discovery of many of the genetic and molecular mechanisms involved in the regulation of the normal skeletogenesis has made it possible to understand in details the main steps in the processes of bone growth and bone maturation, and in parallel has clarified the nature and pathogenesis of a large number of the skeletal disorders known to date. Additional discoveries have unequivocally proven that bone can no longer be perceived as just an inert connective tissue that performs primarily a structural and supporting role. It is now clear enough that bone is a complex and actively involved in the metabolism system of cells, which has the ability to adapt to the mechanical forces applied to it and can adapt its functions to the specific requirements of the different age periods.

Childhood is the main period of human life during which the growing skeleton accumulates the necessary bone mass and strength. The accumulated mineralized bone tissue during this period is referred to as Peak Bone Mass (PBM). Its accumulation takes place mainly during puberty - most intensively between 11 and 14 years of age for girls and between 13 and 17 years of age for boys. Disturbances in normal bone growth and the inability to achieve adequate bone mass and bone strength during childhood are a serious prerequisite for an increased risk of early-onset osteoporosis and an increased fracture risk in adulthood. Data from several large epidemiological studies published over the past 20-25 years have shown that childhood fractures are a particularly common pediatric problem - about 1/3 of all children break at least once during childhood, and the percentage in boys can reach 50 %.

The increased fracture frequency among overweight and obese children is particularly impressive. The available data unequivocally indicate that adipose tissue is an active

participant in the regulation of bone homeostasis, and obesity is a new and recently recognized potential risk factor for decreased bone strength. The role of adipose tisuue on the regulation of bone metabolism is determined by the reciprocal influences that subcutaneous and visceral fat (VF) mass exert on the processes of bone growth and bone turnover. And if excess weight itself is a mechanical stimulus for increasing bone mass, then VF mass, through the many cytokines and hormone-like substances it produces, predisposes to the development of insulin resistance, dyslipidemia and metabolic abnormalities, which in turn have already been proven to have a negative effect on the bone.

2. PREREQUISITES

Based on the currently available data from the literature published in recent years, the following prerequisites for conducting the present study are formulated:

- We lack sufficient data on the epidemiology of fractures in children and adolescents in Bulgaria, and this is a serious obstacle to the accumulation of additional knowledge about bone health among Bulgarian children.

- Over the past 20 years, obesity among children has become a major health problem not only due to its rapidly increasing incidence, but also in connection with the numerous related co-morbidities and complications. Along with cardiovascular and metabolic abnormalities, however, orthopedic problems related to the mechanical overload of the bone-joint apparatus like valgus and varus deformations of the lower legs, flat feet, epiphysiolysis and aseptic necrosis of the femoral head, etc. have recently become more common among overweight and obese children.

- The increased fracture rates reported in the literature among overweight and obese children is a relatively recently recognized medical problem that requires attention among the Bulgarian pediatric community as well.

- The knowledge about the influence of adipose tissue on bone metabolism is still limited and largely controversial, but recently it is increasingly accepted that adipose tissue can play the role of an independent risk factor for increasing fracture risk, and the presence of

metabolic disorders, in some of the obese children, probably further impairs the proper formation of bone tissue and increases the risk of developing early osteoporosis in adulthood.

PART II. HYPOTHESIS, AIM AND TASKS OF THE STUDY

HYPOTHESIS:

Overweight and obesity in children and adolescents are associated with deviations in proper skeletal structure and with a higher fracture rate. The percentage of VF mass and the presence of metabolic abnormalities are additional risk factors that can compromise bone strength and increase the risk of developing osteoporosis in adulthood.

AIM:

To establish the epidemiological characteristics of fractures among the children from the city of Varna and to study the bone densitometric parameters among overweight and obese girls, determining the influence of body weight, body composition distribution, the amount of VF mass and the presence of metabolic abnormalities on bone maturation processes and the ability to reach optimal bone strength at the end of childhood.

TASKS:

1. **EPIDEMIOLOGY**:

1) To determine the fracture rate among children in the city of Varna and to analyze the distribution of the fractures by gender, age and fracture location.

2) Among children who have sustained fractures, to determine the percentage of those with normal weight, overweight and obesity.

3) To determine the percentage of children with two or more fractures and to make a percentage distribution of fractured children according to the number of fractures

4) To determine the percentage of children with fractures who have additional musculoskeletal complaints and/or other chronic diseases

2. CLINICAL PART:

1) To examine osteodensitometric parameters including whole-body and lumbar spine (LS) bone mineral density (BMD), bone mineral content (BMC) and bone area (BAr)

2) To analyze anthropometric data and body composition distribution, incl. the amount of VF fat mass, and their relationship with bone densitometric parameters

3) To examine basic laboratory biochemical and hormonal markers, establishing the percentage of girls with metabolic syndrome and evaluating the influence of metabolic abnormalities on bone densitometric parameters

4) To determine the levels of vitamin D and to evaluate the calcium-phosphorus metabolism status and their relationship with bone densitometric parameters

PART III. STUDY DESIGN AND RESEARCH METHODS

1. STUDY DESIGN

To fulfill the scientific goals and tasks, the study was conducted in two separate parts:

1.1. EPIDEMIOLOGY:

In the period 2021-2022, a survey was conducted on the territory of 14 secondary schools in the city of Varna, including students aged 16 to 19 years (X-XII grades). The questions in the survey were mainly related to the number and location of the fractures suffered so far, the family history, the presence of other accompanying skeletal or chronic diseases and/or complaints, the possible intake of medications.

1.2. CLINICAL PART:

The clinical part of the study was conducted on the territory of the First Children's Clinic, the Imaging Clinic and the Clinical and Immunological Laboratory of the "St. Marina" - the city of Varna in the period January 2023 - January 2024. 41 girls between the ages of 14 and 17 took part in it.

<u>Inclusion criteria:</u>

1) Age between 14 and 17 years

2) Overweight and/or obesity defined as BMI > 85th percentile for age age

3) Puberty maturation IV or V according to Tanner's classification - menarche must have occurred 1 or more years ago

4) Signed informed consent by the accompanying parent

Exclusion criteria:

1) More than 1 fracture to date

2) Low birth weight (< 3rd percentile for gestational age)

3) Clinical or anamnestic data for the presence of primary or secondary bone disorder

4) Calcium or phosphate disorder: rickets and rickets-like conditions, diseases of the parathyroid glands, etc.

5) Taking medications with an impact on calcium-phosphorus metabolism and bone structure: glucocorticoids, antiepileptics, bisphosphonates, etc.

6) Participation in another clinical trial within the last 6 months

In order to determine the effect of overweight and body distribution (fat mass, BMI, BMTM, muscle mass, etc.) on bone parameters, study participants were categorized according to degree of obesity using the expanded obesity classification in childhood of the American Pediatric Association.

In order to determine the influence of biochemical/metabolic abnormalities on bone parameters, the study participants were divided into new subgroups according to the presence of metabolic syndrome (MS) criteria assessed according to the 2007 International Diabetes Federation (IDF) consensus definition:

1. Waist circumference – > P90 for 10-16 year olds; > 80 cm for 16+ year olds

2. Triglycerides ≥ 1.7 mmol/l

3. HDL-C < 1.03 mmol/l for 10-16 year olds; < 1.29 mmol/l for 16+ year olds

4. Systolic BP > 130/85 mmHg

5. Fasting blood sugar ≥ 5.6 mmol/l

2. RESEARCH METHODS

2.1. HISTORY

Before being invited to participate in the study, all girls underwent a thorough medical history with targeted questions regarding the inclusion and exclusion criteria for participation: perinatal history of term and birth weight, history of previous or concomitant chronic diseases, previous fractures, musculoskeletal disorders or complaints, family history of fractures and early osteoporosis, as well as medical history of taking medications affecting bone turnover (glucocorticosteroids, antiepileptics, anticoagulants, etc.)

2.2. ANTHROPOMETRIC MEASUREMENTS

During the stay at the clinic, the following anthropometric measurements were performed by the admitting or treating physician at the clinic using the same instrumentation and under the same conditions.

1) Height measured by a standard method - with a HARPENDEN wall-mounted meter, without shoes and outerwear, with an accuracy of 0.1 cm.

2) Weight measured according to the standard method with a calibrated digital scale SECA 861 (SECA Ltd, Hamburger, Germany), without shoes and outerwear, to the nearest 0.1 kg.

3) Waist circumference measured with a flexible, inextensible tape measure, to the nearest 1 mm, at the mid-axillary line connecting the 10th rib and the crista iliaca, with the subject standing upright and at the end of a calm expiration.

Body mass index (BMI) was also calculated for all participants using the formula BMI = weight in kg/ height in m2.

US National Institutes of Health Center for Disease Control growth charts with standard deviations for age and sex were used to assess all anthropometric measures (https://www.cdc.gov/growthcharts/clinical_charts.htm)

2.3. PHYSICAL EXAMINATION

During the stay in the clinic, a comprehensive physical examination was carried out by a

pediatric endocrinologist including general condition, fever, body habitus, skin and visible mucous membranes, peripheral lymph nodes, head - shape (marks of past rickets), vision, hearing, sclera (directed looking for blue sclera – a sign of osteogenesis imperfecta); oral cavity - dentition (excluding dentinogenesis imperfecta); neck - mobility, palpation of the thyroid gland; respiratory system - chest shape, deformities, nose, upper and lower respiratory tract - palpation, percussion, auscultation; cardiovascular system with measurement of arterial pressure with a sphygmomanometer according to the Korotkoff method in a sitting position after 5 min of rest, three times in 5 min, using the arithmetic mean of the last 2 measurements separately for sistolic blood preasure (BP) and diastolic BP, pulse rate measured at a. radialis of the right hand after 5 min of rest in a sitting position; abdominal status - palpation, percussion, determination of liver and spleen sizes; musculoskeletal system – muscle tone, skeletal deformities, joint laxity determined according to the Beighton scale. Puberty development - assessed by a pediatric endocrinologist according to Tanner method.

2.4. LABORATORY

All blood samples were taken from peripheral blood, in the morning, after a 12-hour overnight fast, with maximum sparing of the patient, and were examined in the Clinical and Immunological Laboratory of UMBAL "St. Marina" - Varna.

Peripheral blood count (PBC) - hemoglobin (g/L) - automatic colorimetry, erythrocytes (1012/L), leukocytes (109/L), lymphocytes (%), monocytes (%), granulocytes (%), platelets - automatic hardware counting

General biochemical panel: urea (mmol/L) - urease method, creatinine (mcmol/L) - Jaffe kinetic method (Olympus AU400 apparatus), uric acid (mcmol/L), ALT (U/L), AST (U/ L), GGTP (U/L) - IFCC methodology, at a temperature of 37oC, Total protein, albumin, C-reactive protein - immunoturbidimetric method (Olympus AU400 apparatus).

Lipid profile made on an Olympus AU400 apparatus: triglycerides (mmol/L) - GPO-POD enzyme method (phosphoglycerol oxidase-peroxidase), total cholesterol (mmol/L) - CHE-CHOD-POD enzyme method (cholesterol esterase-cholesterol oxidase-peroxidase), with fractions – HDL-cholesterol (immunosuppressive enzyme method CHE-CHOD-POD) , LDL-cholesterol – calculated.

Calcium-phosphorus status performed on an Olympus AU400 device: serum calcium (colorimetric method Arsenazo III), phosphorus (colorimetric method Molybdate UV), magnesium (colorimetric method Xyllidyl blue), parathormone (chemiluminescent immunoassay, device Immulite 2000), alkaline phosphatase (AF) – IFCC methodology, at a temperature of 37oC, 25-hydroxy vitamin D (25OH D) - chemiluminescent immunoassay, Liaison apparatus)

Oral glucose tolerance test (OGTT) according to protocol: fasting blood sample (0' minute) to establish basal values of blood glucose (CG) and serum insulin with intake of a glucose solution in the amount of 1.75 grams of glucose/kg body weight, (maximum 75 grams) and taking blood samples for KG at 30', 60', 120' and for ser. insulin at 30'. CG was examined by the hexokinase method (Olympus AU400 apparatus), serum insulin was examined by chemiluminescent immunoassay (Liaison/Immunlite 2000 apparatus)

Glycated hemoglobin - immunoturbidimetric method (Olympus AU400 apparatus)

Hormonal analysis: thyroid-stimulating hormone (TSH), free thyroxine (FT4) - chemiluminescent immunoassay (Liaison apparatus), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2) - chemiluminescent immunoassay (Liaison apparatus), testosterone (T) – electrochemiluminescent immunoassay (Elexys 2010 device), androstenedione, dehydroepiandrostenedione (DHEA-S), SHBG (sex hormone-binding globulin), AMH (anti-Müllerian hormone)

Urine - a single portion of morning urine for standard chemical testing of pH, protein, sugar, ketobodies, pigments, sediment, etc., as well as calcium, phosphorus and creatinine in urine to determine:

- Calciuria by calcium-creatinine ratio (Ca/Cr in urine)
- Phosphaturia by calculating TmP/GFR - maximal tubular reabsorption of phosphates relative to glomerular filtration rate, for which an internet calculator was used, and the norms are again age-dependent - infants 1.4-3.0 mmol/l, over 1 year 1.2– 2.6 mmol/l, adults 0.6–1.7 mmol/l.

2.5. DENSITOMETRY AND IMAGING TECHNIQUES

DENSITOMETRY

DXA densitometry (dual-energy X-ray absorptiometry) conducted in the Radiography department of the hospital. Densitometric measurements were performed with a Lunar iDXA DXA device (GE Healthcare). Calibration of the apparatus was performed daily using a phantom and according to the manufacturer's instructions.

DXA DENSITOMETRY OF BONE PARAMETERS

1) Whole-body osteodensitometry (total body less head, TBLH) including determination of BAr, BMD and BMC. In addition, whole-body BMC was analyzed automatically by the pediatric software using:

a. The Molgaard/Cole method with determination of percentiles for height for age, percentiles for bone surface for height, and percentiles for BMC for BAr, allowing the DXA results to be assigned to one of the following categories: “small bones," “narrow bones” and “light bones”.

b. The mechanostatic method based on the concept of "the functional musculo-skeletal unit", which makes it possible to differentiate the states of primary bone involvement from primary muscle diseases.

2) Osteodensitometry of lumbar spine (L1-L4) including determination of BAr, BMD and BMC. In order to obtain more accurate volumetric results, the so-called BMAD (bone mineral apparent density) was additionally calculated using the Carter and Katzman method ($BMAD=BMC/BAr^{1.5}$).

3) BMC/LBM (bone mineral content/lean body mass) represents the ratio between BMC and LBM and its values actually reflect BMC after correction for LBM. This ratio is automatically calculated by the DXA software and is part of the instrumented osteometric results. Its inclusion is due to the fact that LBM, and especially muscle mass, as its main component, is a major driver of bone mass accretion and the strongest predictor of whole-body and lumbar BMD. BMC/LBM is measured in percentiles and indicates the extent to which

the bone content is adapted to the muscular forces exerted on the bone. Any decrease in the values of BMC/LBM should be taken as an indicator of the potential presence of bone deficiency.

N.B. All net values are supplemented by results in the form of standard deviations - z-score, i.e. were adjusted for age and sex. Due to the homogeneous nature of the studied group - the absence of gender differences and the same puberty status, it was not necessary to use other methods for additional recalculation of the obtained osteometric data.

DXA DENSITOMETRY OF BODY COMPOSITION

Body composition parameters including percentage of FM and FFM with distribution of adipose tissue - truncal and in the limb area, as well as android and gynoid type plus android/gynoid FM ratios were automatically determined for the whole-body densitometry using built-in software. The lower border of the android region is defined by the horizontal line passing through the upper edge of the iliac crest, while the upper border is located cranially and is a horizontal line spaced at a distance equal to 20% of the distance between the iliac crest and the base of the skull. The gynoid region includes the hips and upper thighs. Its upper border is a horizontal line located caudally from the iliac crest at a distance of 1.5 times the height of the android region, and its lower border is defined as a horizontal line located caudally from the upper border at a distance equal to 2 times the height of the android region. The amount of VF mass is presented in the form of volume (cm3) and mass (grams), calculated using a specific DXA built-in software called Corescan.

In children and adolescents, BMI is not the most accurate indicator reflecting the degree of obesity and body distribution, which is why the FM index (FMI - FM divided by height squared in meters), FFM index (FFMI - FFM divided by height squared in meters) and VF mass index (VFI – VF mass divided by height squared in meters) were also calculated and used in the analysis.

X-RAY OF THE HAND, WRIST AND PHALANGES

X-rays of the hand, wrist and phalanges were carried out in the Radiological department in order to objectify the puberty status and assess the residual growth potential by automatically calculating the bone age (BA) according to Greulich-Pyle method using BoneXpert software.

ABDOMINAL ULTRASOUND

Abdominal ultrasound performed in the Radiological department included kidneys, liver and spleen, was used as a screening for concomitant pathology in order to comply with the exclusion criteria for participation in the study.

2.6. BIOELECTRIC IMPEDANS ANALYSIS

Bioelectrical Impedance Analysis (BIA) used to further determine body composition, performed according to the manufacturer's instructions of the InBody 570 device (Biospace Co., Ltd) at the First Children's Clinic.

Results include data in absolute values (kg) and percentages for body fat content, FFM, incl. skeletal muscle mass (SMM), water content, etc. A segmental analysis was also performed, providing data separately for left arm, right arm, torso, right leg, and left leg, including calculated skeletal muscle mass index (SMI) and obesity percentage (% Ob). In addition, BIA includes so-called metabolic risk indicators - 1) VF level - internal fat level reflecting the amount of VF mass in degrees up to 20. The optimal range is 1-9, where 10 is equal to 100 cm2 VF mass, which is considered for an upper limit of the norm. VF level values of 10-15 are considered risky for the development of metabolic abnormalities, while levels above 15, especially above 20, are associated with a very high metabolic risk. 2) InBody score - the overall score from the bioimpedance analysis measured in percentages that reflect the general metabolic and health status, with lower scores indicating worse body parameters and a greater risk of health problems.

In summary, the parameters studied for FFM included those measured by DXA: 1) absolute amount of FFM in kg and lean mass (LM) in kg, (LM = FFM without bone mass) and those measured by BIA: 2) SMM in kg, 3) SMI in kg/m2, (also known in the literature as ASMI

or ALMI - appendicular skeletal muscle index/appendicular lean mass index), which is calculated by adding the skeletal muscle mass of the four limbs and the result is divided by the height squared in meters, 4) percentage distribution of FFM for trunk, arms and legs, as well as additionally calculated by us 5) FF % - percentage of FFM of body weight, and 6) FFMI

2.7 STATISTICS:

The statistical package Graph Prism version 10.2.2 was used for the medical-statistical processing of the data. for Windows 64-bit. Differences where $p < 0.05$ were considered statistically significant. The following statistical methods were used:

1. Method of statistical grouping of the data - the signs are arranged according to their type in variational, interval, categorical, degree and dynamic statistical rows.

2. Descriptive analysis with determination of measures of central tendency, measures of dispersion of the distribution

3. Correlation analysis - univariate Pearson linear correlation coefficients were determined, where the correlation coefficient r can take values from 0 to -1 for an inversely proportional relationship and from 0 to +1 for a straight one. The strength of correlation between two traits is weak at $r < 0.3$ and strong at $r > 0.7$.

4. T-test for testing the hypothesis that the arithmetic mean values for two or more groups are equal.

5. One-way (ANOVA) analysis of variance to assess continuous variables

6. Linear regression analysis - for a complex evaluation of the independent effect of individual signs on a given continuous variable, a multifactor linear regression analysis was applied.

7. Method of statistical evaluation:

a. Point estimates – to calculate an arithmetic mean value of continuous signs, the formula: $X = [\sum cp.X]/n$ was used. In the case of incorrect distribution, a geometric mean value was used, which represents the median of a given characteristic (a value above and below which half of the individuals are distributed)

b. Interval Estimates - Confidence Probability (Significance) - p. At coefficient p=0.95 (95%), the error of the firs boys 0.05 (5%). Confidence Intervals (CI) – 95% confidence intervals around the point estimate are used, which are interpreted as the probability that this interval contains a true point value 95% of the time.

8. Graphical method – linear and planar graphic images, volumetric diagrams and other diagrams are used

9. Standardization method – normalization was used by calculating the standard deviation index (SDS=X-Xmean/SD)

PART IV. RESULTS:

1. EPIDEMIOLOGICAL PART:

Of all surveys, 415 were completed remotely in the electronic format (Google form), and 2098 in paper format. The survey was correctly filled out by a total of 2513 students, of which 1291 girls - 54% and 1106 boys - 46%, 116 (4.6%) did not indicate their gender.

A total of 612 students, or 24% of all respondents, reported having experienced fractures to date - a total frequency of 13.1/1000. Of them, 337 are boys - 57%, and 257 are girls - 43%, boys/girls ratio 1.38.

The number of boys with fractures represent 30% of all boys, and the number of girls with fractures - 20% of all of them. **(Fig. 1)**

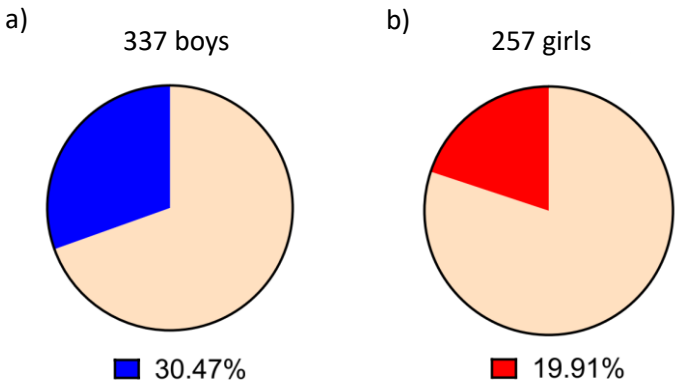


Fig. 1 Percentage of fractures in a) boys; b) girls

Regarding the age distribution in both sexes, two peaks in the frequency of fractures stand out - an earlier and less pronounced one in the pre-pubertal period and a real peak with an increase in the fracture frequency by 3 to 5 times, which occurs in the years of active pubertal growth. More than half of the fractures in boys (57%) occurred between the ages of 12 and 16. **(Fig. 2)** Of all fractures in girls, 50% occur between the ages of 10 and 14. **(Fig. 3)** In both sexes, at the end of pubertal maturation, the fracture frequency decreases sharply and after 15-16 years of age it quickly reaches the levels of preschool age.

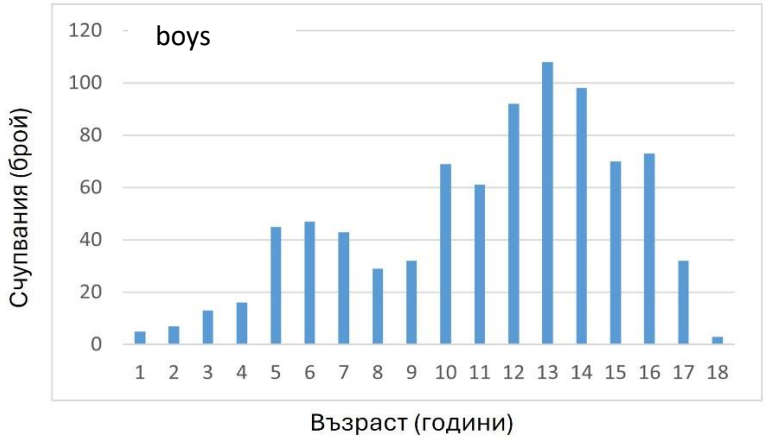


Fig. 2 Distribution of fractures by age in boys

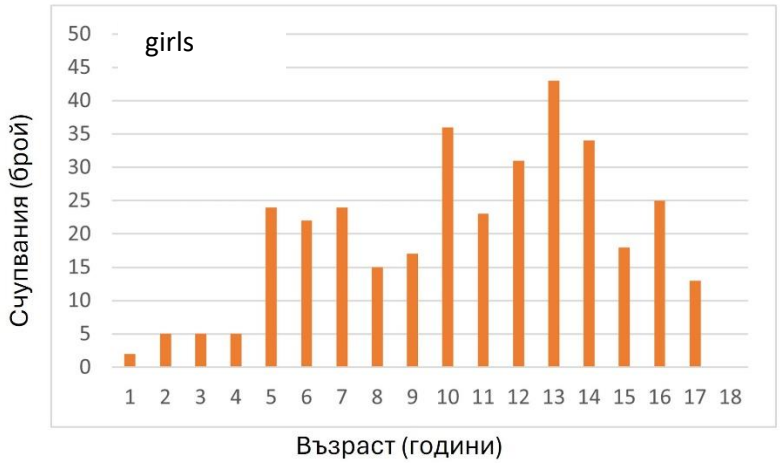


Fig. 3 Distribution of fractures by age in girls

Between the ages of 13 and 18, boys have 3 times more fractures than girls – 179 to 56 fractures respectively.

In both sexes, the most common location of fracture was the upper limb - 72% of fractures in boys and 65% of those in girls. Next is the lower limb - 20% of fractures in boys and 27% in girls, and 8% of fractures in both sexes are in other locations (axial skeleton - clavicle, ribs, skull, etc.). **(Tab. 1)**

Tab. 1 Distribution of fractures by location

Локализация	Момчета	Момичета
ГОРЕН КРАЙНИК	**72%**	**65%**
Ръка	38%	37%
Китка	19%	13%
Пръсти	14%	15%
ДОЛЕН КРАЙНИК	**20%**	**27%**
Бедро/подбедрица	11%	14%
Глезен	4%	8%
Стъпало	3%	3%
Пръсти	2%	2%
АКСИАЛЕН СКЕЛЕТ	**8%**	**8%**

Of the students who experienced fractures, 8% of boys and 17% of girls also reported other musculoskeletal complaints or problems - most often knee pain (33% of boys and 30% of girls), scoliosis (compared to 21% , 16%), back pain (relatively 14%, 18%). However, the same complaints with similar distribution were also reported by 7% of boys and 13% of girls without fractures, therefore it can be assumed that they do not have a direct relationship with the observed fracture rate.

Unfortunately, responses to questions regarding family history of bone and joint disease, osteoporosis, and fractures were incomplete and vaguely worded, thus not amenable to analysis.

In our study, of the boys with fractures, 195 (58%) had only 1 fracture, 86 (26%) had 2 fractures, and 56 (16%) had 3 or more fractures. Of the girls with fractures, 179 (69%) reported only 1 fracture, 51 (20%) reported 2 fractures, and 27 (11%) reported 3 or more fractures. **(Fig. 4)**

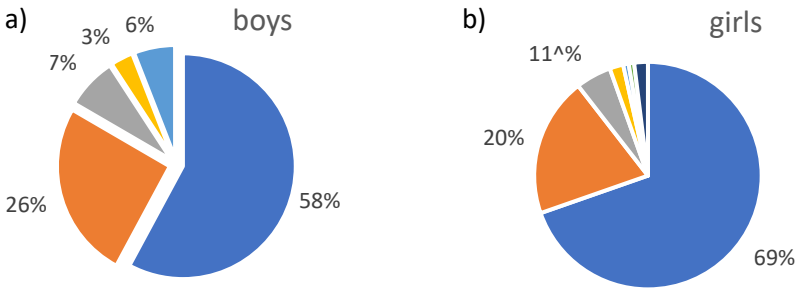


Fig. 4 Distribution of fractures according the number of fractures sustaind in a) boys; b) in girls

Of the girls without fractures, only 9% were overweight or obese. In contrast, girls with current fractures who were overweight/obese were 10% of those with 1 fracture, 15% of those with 2 or more fractures, and 21% of those with 4 or more fractures, respectively. **(Fig. 5)**

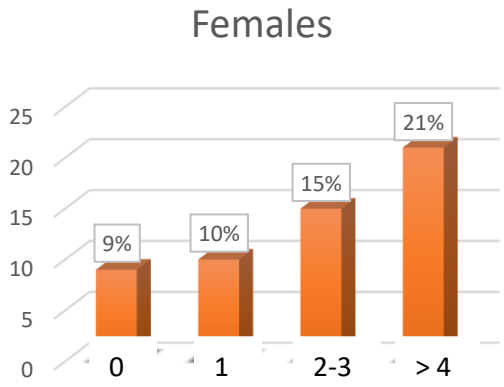


Fig. 5 Distribution of girls with overweight or obesity according to the number of fractures sustained

A similar trend was observed in boys, although not as pronounced, with 16% of boys without fractures being overweight/obese, and 22% of boys with 3 or more fractures being overweight/obese.

2. CLINICAL PART

1. Distribution of participants

The 41 girls included in the study were between the ages of 14 and 17. Of them, 5 (12.20 %) aged 14, 11 (26.83 %) aged 15, 11 (26.83 %) aged 16 and 14 (34.15 %) aged 17.

1.1. Distribution of the participating females according to the degree of obesity

In the distribution of the participants in the study according to the degree of obesity with overweight (weight between P85 - P95), 8 girls with mild obesity (weight P95 - 120% of P95) and 17 girls with severe obesity (weight 120% of P95 - 140% of P95) 9 girls and with extreme obesity (weight > 140% of P95) 7 girls. (Table 2)

Tab. 2 Distribution of the participating females according to the degree of obesity

Degree of obesity	**BMI in percentiles (number)**	**BMI by age (number)**			
		14 years	***15 years***	***16 years***	***17 years***
Overweight	P85 - P95 **(8)**	23.4 - 27.2 **(2)**	24.1 - 28.1 **(1)**	24.7 - 28.8 **(0)**	25.2 - 29.6 **(5)**
Mild obesity	P95 - 120% P95 **(17)**	27.2 - 32.6 **(0)**	28.1 - 33.7 **(7)**	28.8 - 34.6 **(6)**	29.6 - 35.5 **(4)**
Severe Obesity	120% P95 - 140% P95 **(9)**	32.6 - 38.1 **(2)**	33.7 - 39.3 **(2)**	34.6 - 40.3 **(2)**	35.5 - 41.4 **(3)**
Extreme obesity	> 140% P95 **(7)**	> 38.1 **(1)**	> 39.3 **(1)**	> 40.3 **(3)**	> 41.4 **(2)**

For the purposes of the analysis, the first two categories - girls with overweight and those with mild obesity, were united as group "BMI-1" with a total number of 25 participants, and the last two categories – the girls with severe and extreme obesity, were united in group "BMI-2" - 16 girls in total. There were no significant differences between the two groups in terms of age, puberty status and height, which allowed a correct analysis of the data. **(Tab. 3, Tab. 4)**

Tab. 3 Anthropometric characteristics of the girls from groups BMI-1 and BMI-2

	BMI-1 (number = 25) (SD)	**BMI-2 (number = 16) (SD)**	**Confidential interval (95% CI)**	**P value**
Age (years)	15.88 (1.01)	15.75 (1.13)	-0.130 ± 0.339 (-0.81, 0.56)	.703
Height (cm)	165.3 (6.39)	165.6 (4.84)	0.336 ± 1.869 (-3.44, 4.12)	.858

Weight (κg)	81.73 (10.44)	110.9 (17.86)	29.14 ± 4.410 (20.22, 38.06)	< 0.0001
BMI (kg/m2)	30.06 (3.20)	40.46 (6.25)	10.40 ± 1.479 (7.40, 13.39	< 0.0001
WC (cm)	95.1 (11.77)	110.2 (11.83)	15.13 ± 4.028 (6.94, 23.33)	< 0.001

Tab. 4 FM and FFM in BMI-1 and BMI-2 groups

	BMI-1 (number = 25) (SD)	**BMI-2 (number = 16) (SD)**	**Confidential interval (95% CI)**	**P value**
Total FM (kg)	35.00 (8.82)	55.73 (12.73)	20.73 ± 3.585 (13.44, 28.01)	< 0.0001
Total FM (%)	43.38 (4.63)	51.55 (4.515)	8.177 ± 1.498 (5.15, 11.21)	< 0.0001
VF mass (gr)	721.1 (336.0)	1174 (339.4)	452.8 ± 125.5 (196.5, 709.1)	< 0.01
VF mass (ml)	764.4 (356.1)	1245 (359.6)	480,2 ± 133,0 208,6 to 751,8	< 0.01
VF mass index	0.269 (0.125)	0.423 (0.116)	0.1542 ± 0.0458 (0.0604, 0.2481)	< 0.002
Truncal FM/ Total FM	0.49 (0.039)	0.50 (0.036)	0.0184 ± 0.0122 (-0.0064, 0.0432)	.141
Axial FM/ Total FM	1.02 (0.163)	0.95 (0.145)	-0.063 ± 0.051 (-0.167, 0.040)	.222
Android FM	47.04 (7.432)	58.25 (4.413)	11.20 ± 2.118 (6.92, 15.49)	< 0.0001
Gynoid FM	45.24 (4.435)	52.53 (4.858)	7.293 ± 1.501 (4.255, 10.33)	< 0.0001
A/G ratio	1.038 (0.123)	1.112 (0.075)	0.074 ± 0.035 (0.003, 0.146)	< 0.05
VF level	17.0 (4.41)	23.5 (1.92)	6.467 ± 1.224 (3.974, 8.959)	< 0.0001
InBody score	0.636 (0.095)	0.515 (0.080)	-0.122 ± 0,030 (-0.183, -0.059)	< 0.005
FFM (kg)	43.72 (4.65)	50.10 (6.757)	6.379 ± 1.824	< 0.01

			(2.68, 10.07)	
FFM (%)	56.47 (4.67)	48.45 (4.515)	-8.024 ± 1.517 (-11.10, -4.95)	< 0.0001
FFMI	17.84 (1.64)	20.24 (1.99)	2.396 ± 0.580 (1.22, 3.57)	< 0.001
SMI	7.395 (0.531)	8.320 (0.666)	0.9250 ± 0.2021 (0.5138, 1.336)	< 0.0001
SMM (kg)	27.73 (2.56)	31.01 (3.83)	3.282 ± 1.079 (1.086, 5.477)	< 0.005
FFM legs (gr)	16105 (1810)	18679 (2512)	2574 ± 684.7 (1188, 3960)	< 0.001
FFM arms (gr)	4899 (929.3)	5490 (1206)	590.6 ± 339.6 (-97.00, 1278)	.090

1.2. Distribution of the participating females according to the MS risk factors

In order to determine the influence of metabolic abnormalities on bone parameters, the study participants were divided into two new groups according to the presence of criteria for MS. There were 3 girls without any MS criteria, 17 girls with 1 criterion, 11 girls with 2 criteria, 6 girls with 3 criteria, 4 girls with 4 criteria, and none with 5 criteria. The number of individual positive criteria for MS among all participants were as follows: 1) Waist circumference > P90 for 10-16 years, > 80 cm for 16+ years – 36 girls; 2) SBP ≥ 130 mmHg or SBP ≥ 85 mmHg – 11 girls; 3) Triglycerides (TG) ≥ 1.7 mmol/l – 6 girls; 4) HDL-C < 1.03 mmol/l for 10-16 years; < 1.29 mmol/l for 16+ years – 12 girls; 5) Fasting blood glucose ≥ 5.6 mmol/l – 8 girls. **(Tab. 5)**

Tab. 5 Distribution of the risk factors for MS

years	WC	Systolic BP	Diastolic BP	TG	HDL-C	BG 0'	MS
16	116,0	122	84	1,58	0,83	5,80	3
17	115,0	110	75	1,76	0,83	4,76	3
15	90,0	110	79	1,45	1,28	4,72	1
15	88,0	125	85	0,96	1,16	3,10	2
16	99,0	110	78	1,09	0,87	4,34	2
17	96,0	110	77	0,93	0,97	4,68	2
15	106,0	105	77	0,89	1,14	4,35	1
17	77,0	111	79	0,58	1,38	3,77	0
16	103,0	140	95	0,95	1,48	4,96	2

15	91,0	120	81	1,40	1,23	5,60	2
17	145,0	120	70	1,57	1,31	5,38	1
17	111,0	110	77	1,35	1,34	4,96	1
16	105,0	110	80	1,11	0,97	3,62	2
16	100,0	121	76	0,85	1,89	4,39	1
17	107,0	113	81	0,84	1,31	4,99	1
15	97,0	130	80	0,97	1,50	6,10	3
16	92,0	140	88	2,32	1,27	5,04	4
17	101,5	118	68	0,95	1,48	4,45	1
15	104,0	129	80	1,27	1,22	4,54	1
15	70,0	110	70	0,55	2,05	5,31	0
15	94,0	140	80	2,44	0,89	5,42	3
15	96,0	110	80	1,19	1,18	5,30	1
16	110,0	115	81	0,51	1,10	6,29	3
17	92,5	120	80	1,83	0,95	4,52	3
16	86,0	118	69	0,78	1,03	4,13	1
14	113,0	130	84	1,15	1,32	5,00	2
14	100,5	130	90	2,03	1,10	5,70	4
17	105,0	125	77	1,67	1,09	6,02	2
16	89,0	109	85	0,89	1,29	4,89	2
16	120,0	109	85	1,95	0,99	4,15	4
14	112,0	130	80	1,30	1,05	4,79	2
17	78,0	120	82	1,57	1,20	5,36	1
17	79,0	115	83	0,62	2,59	5,90	1
17	95,0	111	64	0,95	1,53	4,31	1
16	107,0	106	67	0,60	1,32	4,03	1
14	110,0	120	80	0,87	1,29	5,03	1
15	116,0	130	80	0,47	1,48	4,94	2
17	81,0	116	82	0,54	1,46	4,28	1
17	109,0	110	84	1,09	1,75	4,36	1
15	111,0	110	70	1,17	1,09	5,64	2
14	77,0	120	82	0,37	1,62	5,04	0
	36	11		6	12	8	

For the data analysis, girls without any or only with one risk factor for MS were combined into group "MS-1" - a total of 20 girls, and those with 2 or more risk factors for MS were combined into group "MS-2" - 21 girls in total.

No differences were found between the two subgroups in terms of age, pubertal maturation and FM parameters - BMI, WC, Fat %, which allowed a comparative analysis based on MS criteria only. **(Tab. 6, Tab. 7)**

Tab. 6 Anthropometric characteristics of the girls from groups MS-1 and MS-2

	МС-1 (брой = 20) Средна стойност (SD)	**МС-2 (брой = 21) Средна стойност (SD)**	**Средна разлика (95% CI)**	**P value**

Възраст (години)	16.05 (1.099)	15.62 (0.974)	-0.4310 ± 0.3239 (-1.086, 0.2241)	.191
Ръст (см)	163.2 (5.45)	168.0 (5.33)	4,790 ± 1,663 (1.430, 8.151)	< 0.01
Тегло (кг)	87.54 (23.88)	98.40 (13.49)	10.86 ± 6.02 (-1.311, 23.04)	< 0.05
ИТМ (кг/м2)	33.23 (8.31)	34.97 (5.21)	1.742 ± 2.155 (-2.618, 6.101)	.424
Об. корем (см) (ОК)	99.70 (17.74)	103.0 (10.40)	3.300 ± 4.778 (-6.422, 13.02)	.495
% Мастна маса (% ММ)	45.08 (6.50)	47.81 (5.37)	2.725 ± 1.886 (-1.094, 6.544)	.157

Табл. 7 **FM and FFM in MS-1 and MS-2 groups**

	МС-1 (брой = 21) Средна стойност (SD)	**МС-2 (брой = 20) Средна стойност (SD)**	**Mean Difference (95% CI)**	**P value**
Total FM (kg)	40.55 (18.33)	46.41 (10.27)	5.863 ± 4.883 (-4.061, 15.79)	.238
Total FM (%)	45.08 (6.50)	47.81 (5.38)	2.725 ± 1.886 (-1.094, 6.544)	.157
VF mass (gr)	670.2 (323.3)	1059.0 (372.7)	388.8 ± 124.2 (135.2, 642.4)	< 0.01
VF mass (ml)	710.4 (342.7)	1123.0 (395.0)	412.3 ± 131.6 (143.6, 681.1)	< 0.01
VF mass index	0.258 (0.133)	0.372 (0.132)	0.1135 ± 0.04696 (0.0176, 0.2094)	< 0.01
Truncal FM/ Total FM	0.481 (0.0342)	0.505 (0.0387)	0.0240 ± 0.0116 (0.0006, 0.0474)	< 0.05
Axial FM/ Total FM	1.025 (0.157)	0.945 (0.157)	-0.0802 ± 0.0491 (-0.1795, 0.0191)	.110
Android FM	48.67 (9.59)	53.83 (6.33)	5.160 ± 2.569 (-0,0415, 10,36)	.051
Gynoid FM	46.89 (5.95)	49.07 (5.53)	2.180 ± 1.816 (-1.497, 5.857)	.237
A/G ratio	1.034 (0.135)	1.098 (0.074)	0.0640 ± 0.0344 (-0.0057, 0.134)	.071

VF level	18.40 (5.642)	21.00 (3.727)	2.600 ± 1.610 (-0,680, 5.880)	.116
InBody score	0.608 (0.123)	0.565 (0.089)	-0.0429 ± 0.0356 (-0.115, 0.0293)	.235
FFM (kg)	43.86 (5.922)	48.38 (5.989)	4.517 ± 1.908 (0.651, 8.384)	< 0.05
FFM (%)	54.63 (6.541)	52.21 (5.398)	-2.421 ± 1.916 (-6.304, 1.461)	.214
FFMI	18.86 (2.233)	18.63 (2.032)	-0.225 ± 0.6751 (-1.592, 1.142)	.741
SMI	7.694 (0.850)	7.874 (0.658)	0.1799 ± 0.255 (-0,338, 0.698)	.485
SMM (kg)	28.25 (4.084)	29.87 (2.874)	1.624 ± 1.180 (-0.776, 4.024)	.178
FFM legs (gr)	4933.0 (889.1)	5309.0 (1213.0)	375.7 ± 336.2 (-304.9, 1056.0)	.271
FFM arms (gr)	16052 (2321)	18089 (2118)	2038 ± 702.6 (615.1, 3460.0)	< 0.01
FM/FFM arms	0.950 (0.219)	0.966 (0.167)	0.0160 ± 0.0616 (-0,109, 0.1406)	.796
FM/FFM legs	0.875 (0.266)	0.924 (0.260)	0.0495 ± 0.0831 (-0.1187, 0.2177)	.555

2. RESULTS FROM OSTEODENSITOMETRY

From the conducted DXA densitometric studies, the following results were obtained, combined in the tabular form presented below, according to the distribution of the participants, respectively, according to the degree of obesity **(Tab. 8)** and according to the presence of metabolic deviations. **(Tab. 9)**

Tab. 8 Osteodensitometric results for the girls from group BMI-1 and BMI-2

	BMI-1 (number = 25) (SD)	**BMI-2 (number = 16) (SD)**	**Confidential interval**	**P value**

			(95% CI)	
TBLH BMD	1.400 (0.864)	2,133 (0.954)	0.7333 ± 0.293 (0.1395, 1.327)	< 0.05
LS BMD	0.679 (0.786)	0.900 (0.800)	0.221 ± 0.256 (-0.2965, 0.7381)	.393
BMAD	0.339 (0.0217)	0.340 (0.0315)	0.00019 ± 0,0084 (-0.0168, 0.0172)	.982
BMC/LBM	56,19 (25.68)	38,40 (19.93)	-17.79 ± 9.238 (-36.68, 1.104)	.064
BAr/Height	41.13 (24.91)	28.27 (31.65)	-12.86 ± 9.102 (-31.30, 5.585)	.166
BAr arms	344.6 (46.54)	281.6 (76.09)	-62.96 ± 19.32 (-102.1, -23.84)	< 0.005
BAr legs	725.1 (62.39)	725.6 (68.14)	0.48 ± 21.09 (-42.21, 43.17)	.982
BMD legs	1.239 (0.085)	1.307 (0.110)	0.0681 ± 0.0309 (0.0054, 0.1308)	< 0.05
BMD arms	0.855 (0.108)	0.916 (0,092)	0.0611 ± 0,0334 (-0.0065, 0.1287)	.075
BMC arms	290.7 (26.16)	257.4 (70.11)	-33.33 ± 15.47 (-64.64, -2.017)	< 0.05
BMC legs	899.5 (105.3)	950.1 (134.3)	50.59 ± 38.15 (-26.64, 127.8)	.193

Tab. 9 Osteodensitometric results for the girls from group MS-1 and MS-2

	MS-1 (number = 21) (SD)	**MS-2 (number = 20) (SD)**	**Confidential interval (95% CI)**	**P value**
TBLH BMD	1.410 (0.915)	1.940 (0.946)	0.530 ± 0.294 (-0.0655, 1.126)	.080
LS BMD	0.726 (0.870)	0.805 (0.729)	0.07845 ± 0.2528 (-0.433, 0.5902)	.758

BMAD	0.343 (0.024)	0.336 (0.027)	-0.0074 ± 0.0082 (-0.0239, 0.0091)	.372
BMC/LBM	49.18 (24.56)	52.00 (26.60)	2.824 ± 9.201 (-15.99, 21.64)	.761
BAr/Height	42.00 (31.07)	30.65 (24.28)	-11.35 ± 8.902 (-29.39, 6.687)	.210
BAr arms	328.9 (75.63)	313.1 (55.74)	-15.80 ± 21.01 (-58.33, 26.73)	.457
BAr legs	705.9 (54.49)	744.8 (67.63)	38.90 ± 19.42 (-0.4116, 78.21)	.052
BMD legs	1.242 (0.090)	1.287 (0.105)	0.0452 ± 0.031 (-0.0175, 0.1078)	.153
BMD arms	0.849 (0.094)	0.906 (0.110)	0.0566 ± 0.0325 (-0.00912, 0.122)	.089
BMC arms	273.5 (43.60)	283.0 (55.55)	9.510 ± 15.79 (-22.46, 41.48)	.551
BMC legs	878.7 (109.5)	958.3 (115.1)	79.63 ± 35.51 (7.738, 151.5)	< 0.05

2.1. Results for TBLH BMD

DXA measurements showed a significantly higher average TBLH BMD in BMI-2 group - 2.1 SD compared to the BMI-1 group with 1.4 SD ($p < 0.05$), as well as in the MS-2 group of girls with 1.9 SD compared to those from MS-2 group - 1.4 SD, although with slightly lower significance ($p < 0.1$). The mean TBLH BMD for all 41 participants was 1.67 SD (0.1-3.8). **(Fig. 6)**

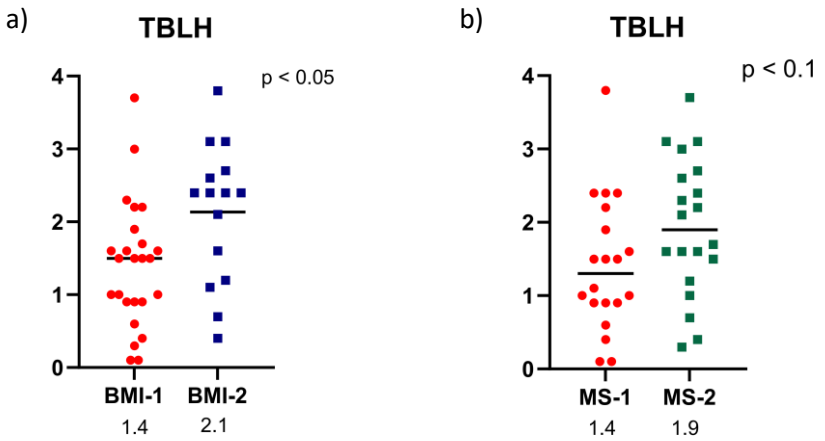


Fig. 6 **Results for TBLH BMD in girls from a) groups BMI-1 and BMI-2; b) groups MS-1 and MS-2**

The data from the conducted osteometric measurements also indicate that in all the girls studied, TBLH BMD increases in direct proportion with increasing body weight and BMI. **(Fig. 7)**

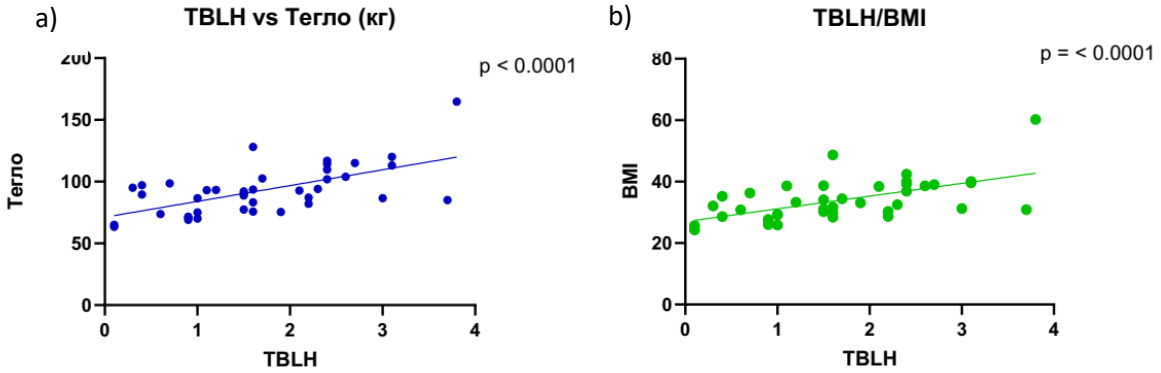


Fig. 7 **Correlations between TBLH BMD and a) body weight; b) BMI**

WC and DXA-determined amount of Fat % showed a significant positive association with c-BMD, $p<0.005$ and $p<0.05$, respectively. A positive correlation was also found between TBLH BMD and BIA data for the percentage of obesity (% Ob), $p < 0.001$, and for the amount of FM in the upper limb region, $p < 0.01$. **(Fig. 8)**

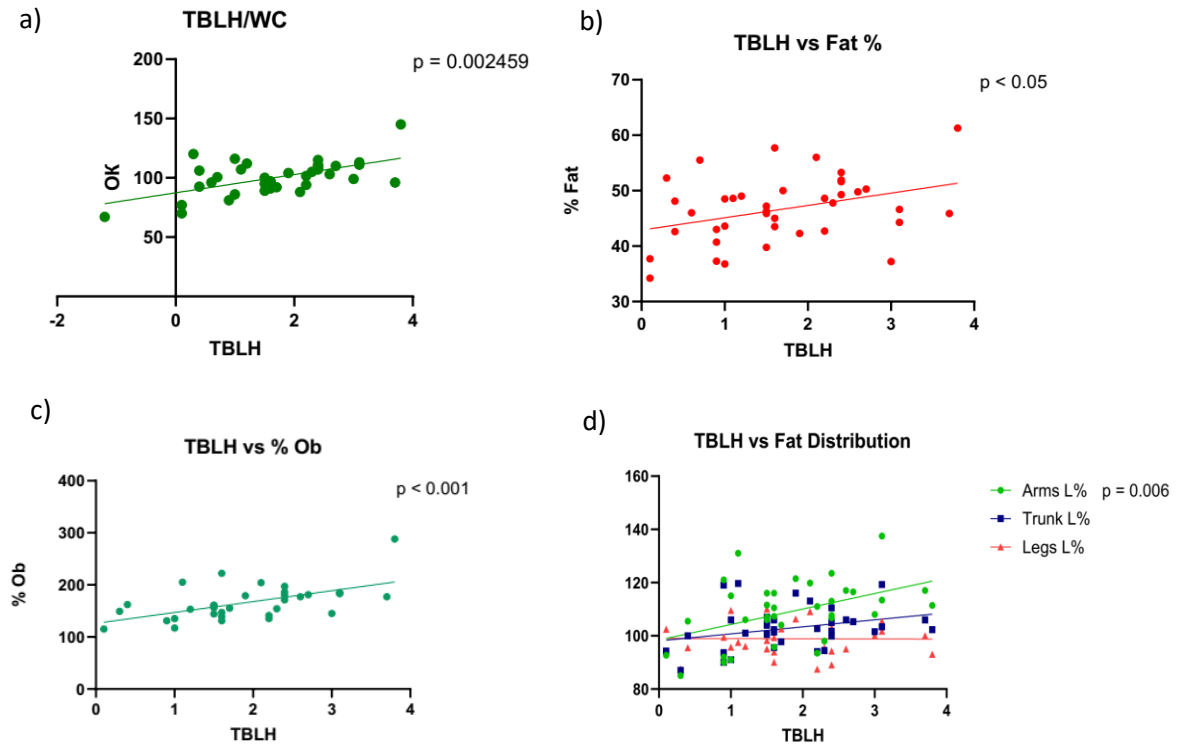


Fig. 8 Correlations between TBLH BMD and a) WC; b) Fat %; c) % Ob; d) the FM distribution

Regarding the type of FM distribution determined by DXA measurements, we find a significant positive correlation only between TBLH BMD and the android type of obesity ($p < 0.005$), incl. A/G ratio ($p < 0.005$), but not between TBLH BMD and gynoid type of FM accumulation ($p > 0.1$). **(Fig. 9)**

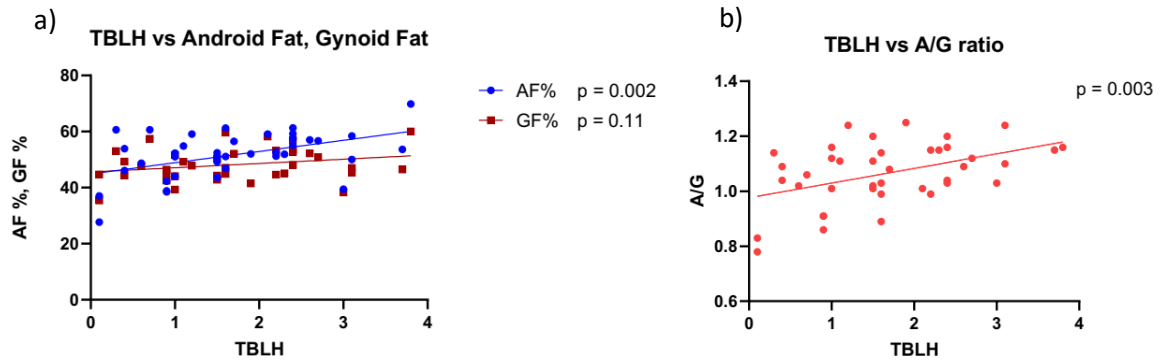


Fig. 9 Correlation between TBLH BMD and a) AF% and GF%; b) A/G ratio

In addition, it was found that the percentage of android FM increased significantly with increasing degree of obesity - from 47.43% in BMI-1 group to 58.25% (+ 10.82%, $p<0.0001$) among girls in BMI-2 group. **(Fig. 10a)** The same trend, although a little less pronounced, is also observed for the gynoid FM – from 45.36% to 52.53% (+ 7.17%, $p < 0.0001$) **(Fig. 10b)**, as

well as for the A/G ratio – from 1.044 to 1.112 (+ 0.076, p < 0.05). **(Fig. 10c) (Tab. 4)** Similar, but with smaller differences, are also the results for increasing FM when dividing the participants according to MS criteria. **(Tab. 7)**

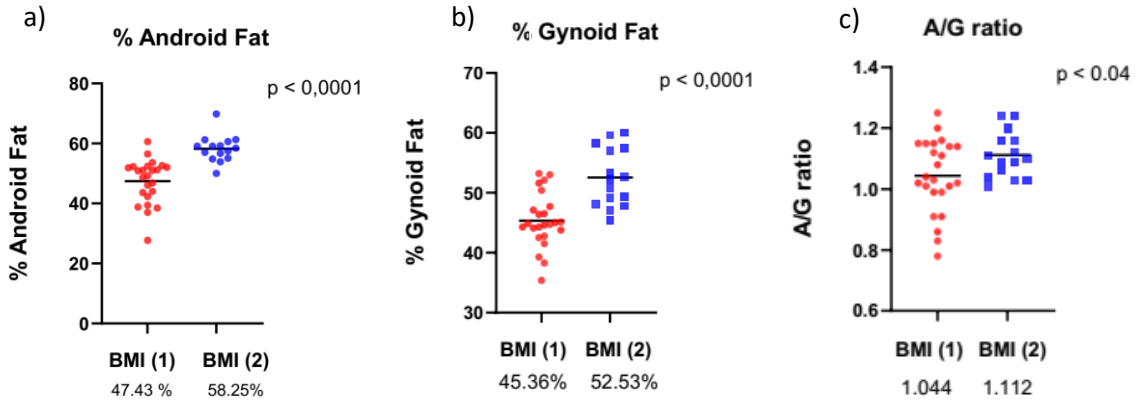


Fig. 10 Differences between girls from groups BMI-1 and BMI-2 in the a) % AF; b) % GF; cA/G ratio

The results for the amount of VF mass measured by DXA follow the same trend – as a percentage of the total FM, the amount of VF mass (VF%) increased from 2.0% to 2.28%, p > 0.1 **(Fig. 11a),** which, however, as an absolute weight (VF mass) was, respectively, on average 721 g of VF mass for the BMI-1 group versus 1174 g of VF mass for the BMI-2 group – a significant difference of + 63%, p<0.01. **(Fig. 11b)** Likewise, VF volume was significantly greater in the highly obese group – mean VF volume in BMI-1 group – 781 ml, mean VF volume in BMI-2 group – 1245 ml, which is a significant difference of +59.4%, p<0.01. **(Fig. 11c) (Tab. 4)**

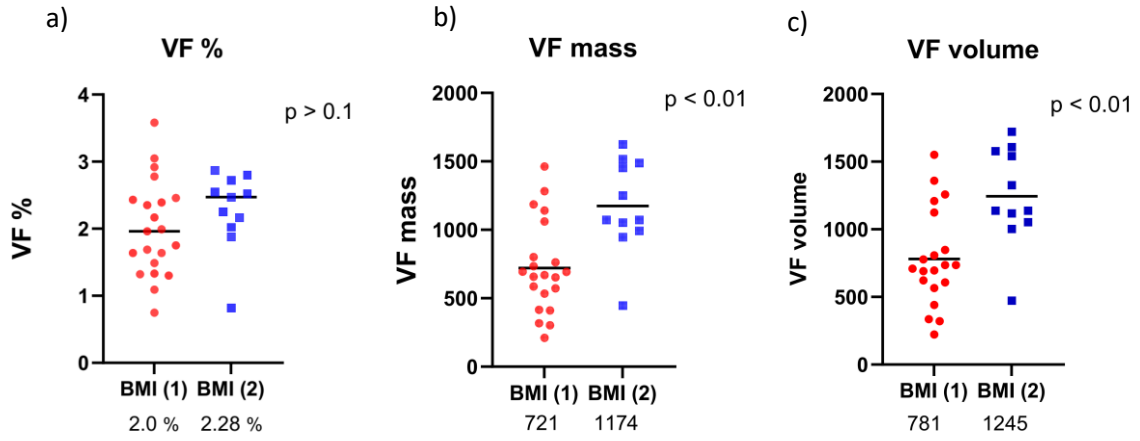


Fig. 11 Differences between girls from groups BMI-1 and BMI-2 according the a) % VF; b) VF mass; c) VF volume

In addition, significantly higher values for VFI were also found in girls from groups BMI-2 with VFI 0.42 and BMI-1 with VFI 0.27 ($p < 0.002$). **(Fig. 12a) (Tab. 4)** Similar results for the VF were found when the participants were distributed according to MS criteria. **(Tab. 7)**

Metabolic BIA indices showed a similar trend - VF level 17.0 for BMI-1 and 23.5 for BMI-2 respectively ($p=0.05$) **(Fig. 12b)**, as well as a significantly lower InBody score in girls with severe and extreme obesity from BMI-2 group, compared to overweight and mildly obese girls from BMI-1 group, 51% vs. 64%, respectively ($p<0.01$).). **(Fig. 12c)**

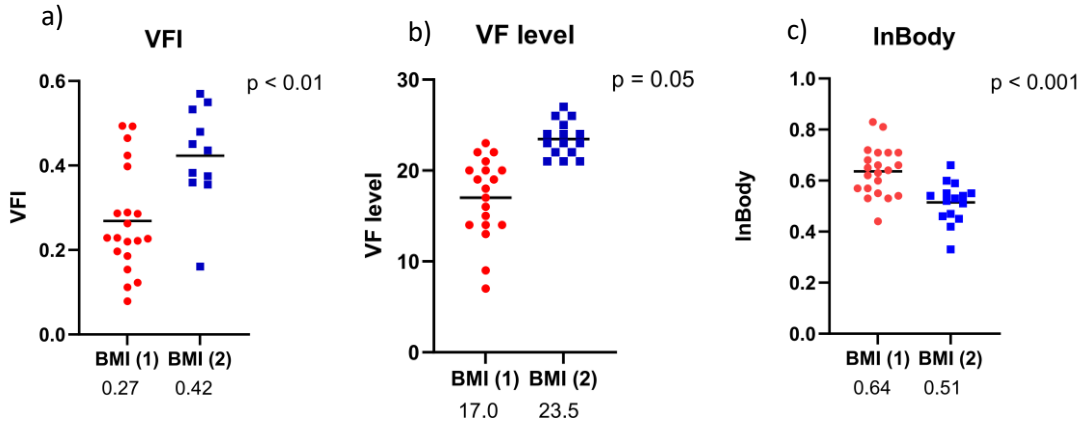


Fig. 12 Differences between girls from groups BMI-1 and BMI-2 according the a) VFI; b) VF level; c) InBody score

Between the groups of girls divided according to MS criteria, the differences for VF level and InBody were not significant. **(Tab. 7)** In all the girls of the study, all three indicators showed a strong positive correlation with each other ($p<0.001$), therefore we can assume that all three indicators equally well reflect the presence of BMD and the relationship with the subsequently analyzed osteometric measurements. **(Fig. 13)**

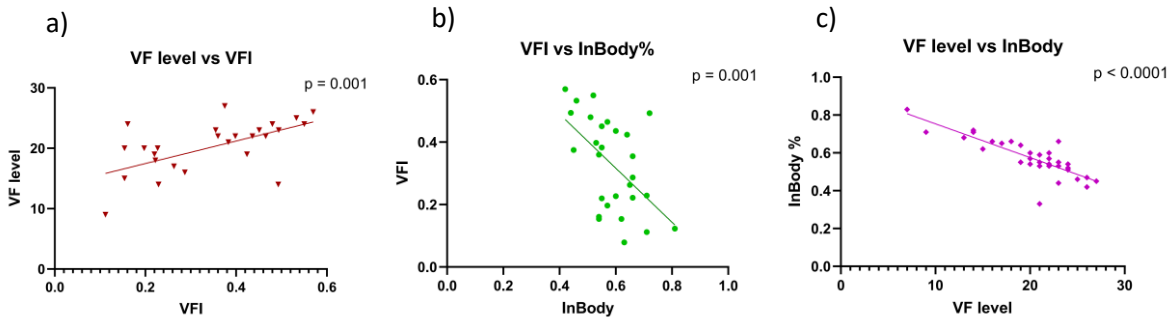


Fig. 13 Correlations between a) VF level and VFI; b) VFI and InBody score; c) VF level and InBody score

With regard to the osteometric results, in the general analysis including all participants in the study, a moderately expressed but positive relationship was found between TBLH BMD and VF quantity indicators - with VF%, p=0.01, with VF mass and volume, p=0.02 and with VFI, p=0.004. **(Fig. 14)**

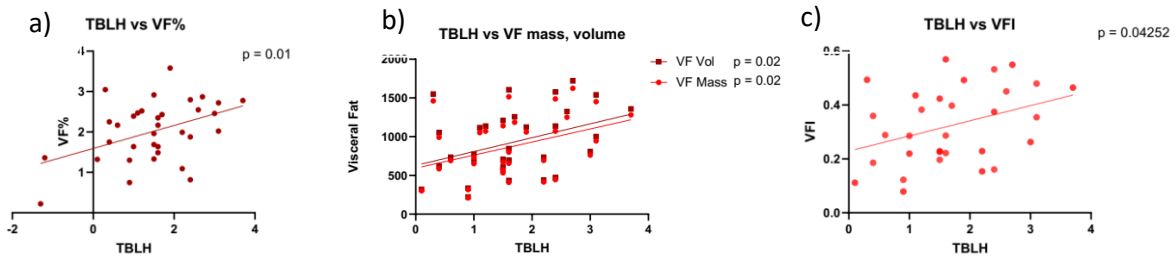


Fig. 14 Correlations between TBLH BMD and a) VF %; b) VF mass and volume; c) VFI

A similar trend was found in the correlation between TBLH BMD and metabolic indicators - VF level and InBody assessment by BIA. (Fig. 15)

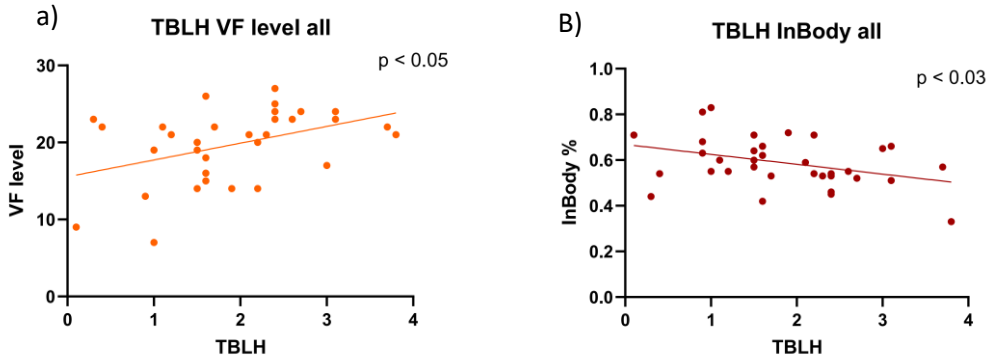
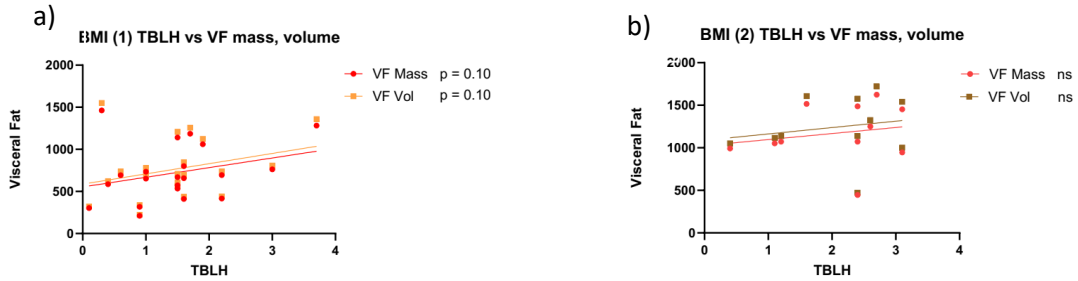


Fig. 15 Correlations between TBLH BMD and a) VF level; b) InBody score

The results for the relationship between TBLH BMD and BMI divided by groups according to the degree of obesity showed a decrease in the positive correlation with the progress of obesity, and in the subgroup of extremely obese girls the same relationship was negative. **(Fig. 16)**



c)

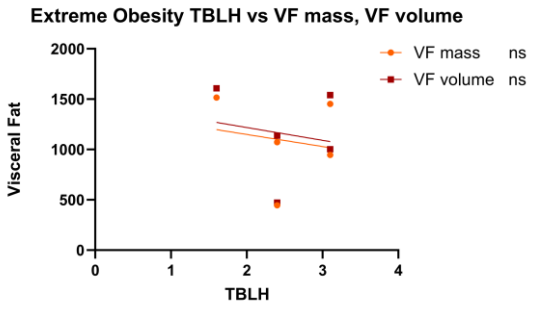
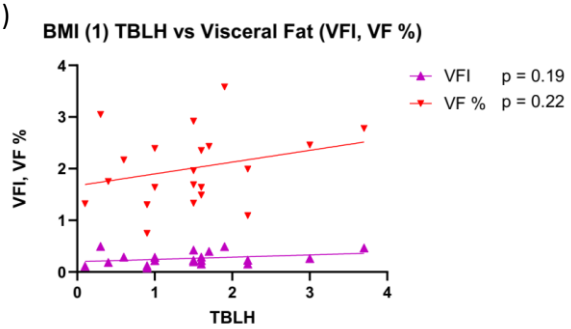


Fig. 16 Correlations between TBLH BMD and VF in group: a) BMI-1; b) BMI-2; c) of girls with extreme obesity

Similar results were also obtained regarding the correlations between TBLH BMD and VF% and VFI - although they do not reach significance, with the progression of obesity, the positive correlations decrease and practically disappear. **(Fig. 17)**

a)



b)

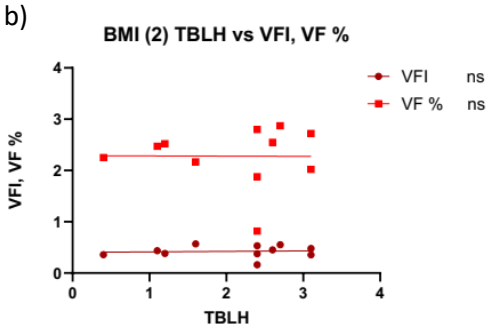
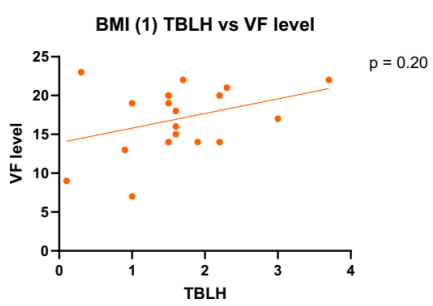


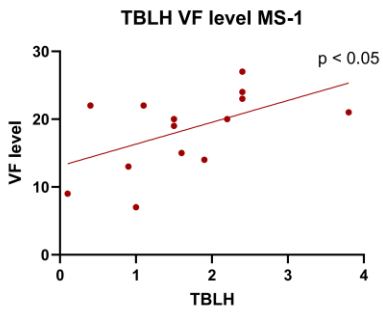
Fig. 17 Correlations between TBLH BMD and VF% and VFI in group a) BMI-1; b) BMI-2

Similarly, it can be seen that as the degree of obesity increases, the correlation between TBLH BMD and metabolic risk indices – VF level **(Fig. 18)** and InBody score derived from BIA – weakens. **(Fig. 19)**

a)



b)



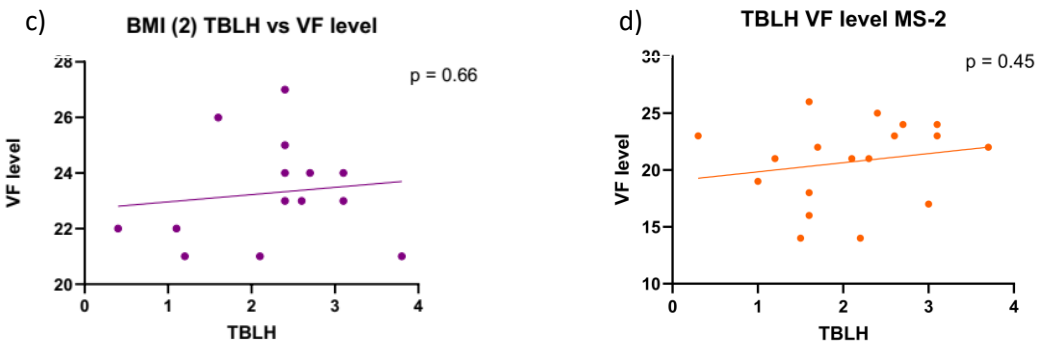


Fig. 18 Correlations between TBLH BMD and VF level in group: a) BMI-1; b) BMI-2; c) MS-1; d) MS-2

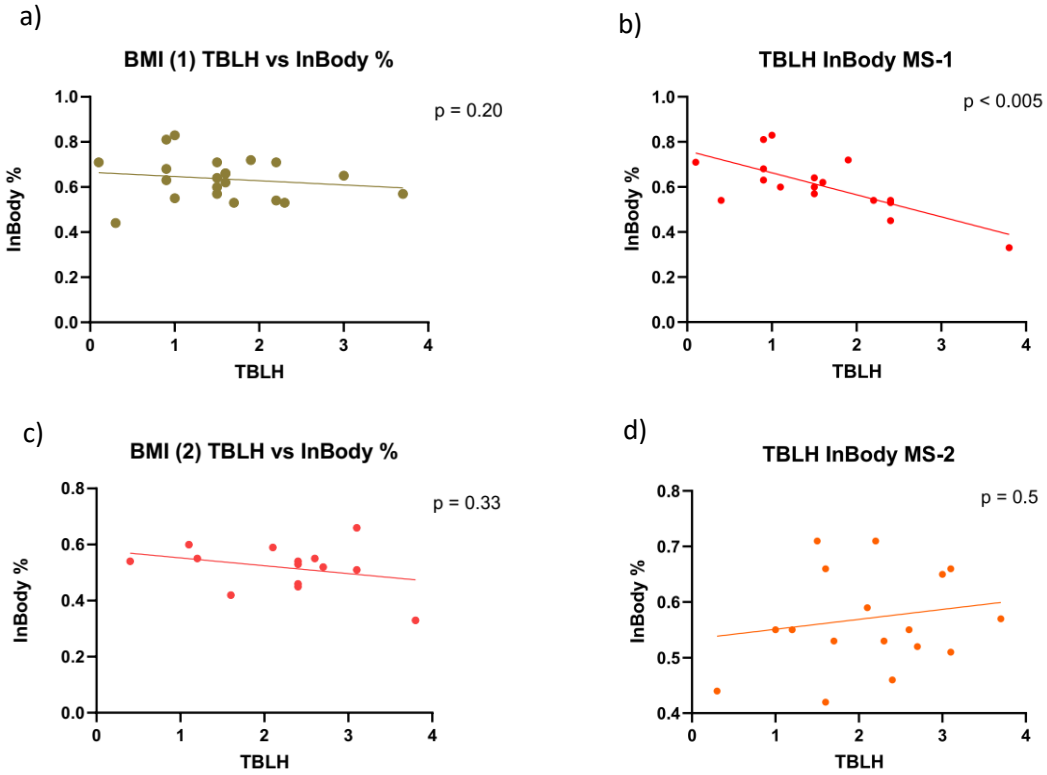


Fig. 19 Correlations between TBLH BMD and InBody in group: a) BMI-1; b) BMI-2; c) MS-1; d) MS-2

The additional analysis of the distribution of the girls according to the "metabolic" principle showed that the correlations between CT-BMD and the amount of BMI, as well as the metabolic BIA parameters established for the general group of participants, were preserved. **(Fig. 20)**

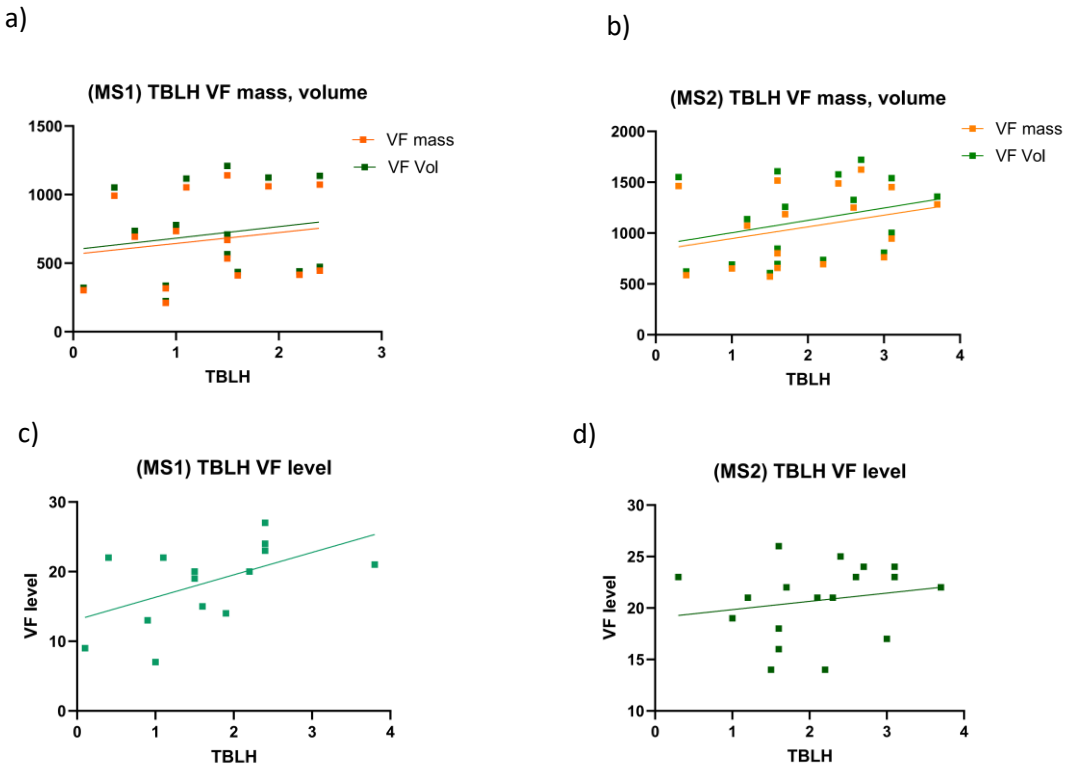


Fig. 20 Correlations between TBLH BMD and VF mass and volume in group: a) BMI-1; b) BMI-2; c) MS-1; d) MS-2

THE INFLUENCE OF FAT-FREE MASS (LBM) ON BONE

In contrast to the results obtained for TBLH BMD without correction for weight and for LBM, in parallel with an increase in the degree of obesity in the girls studied by us, a well-defined tendency to "decrease" of TBLH BMD when corrected for FFM (BMC/LBM) – average value for BMC/LBM for BMI-1 group P56.2, for BMI-2 group P38.4, (p=0.06). When distributed according to the criteria for MS, the same trend is observed, although less pronounced, with almost equal values for BMC/LBM in the two groups – P49.2 for the MS-1 group and P52.0 for the MS-2 group. **(Fig. 21)**

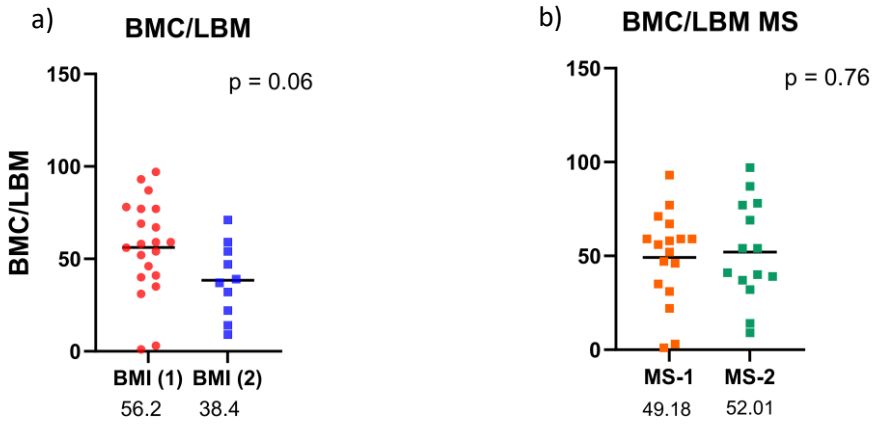


Fig. 21 **BMC/LBM a) in BMI groups; b) in MS groups**

While showing an almost absent correlation between BMC/LBM and body weight (p=0.72) and a weak negative association with BMI (p=0.01), our data revealed a strong negative correlation between BMC/LBM and BIA-derived adiposity (Obesity %). **(Fig. 22)**

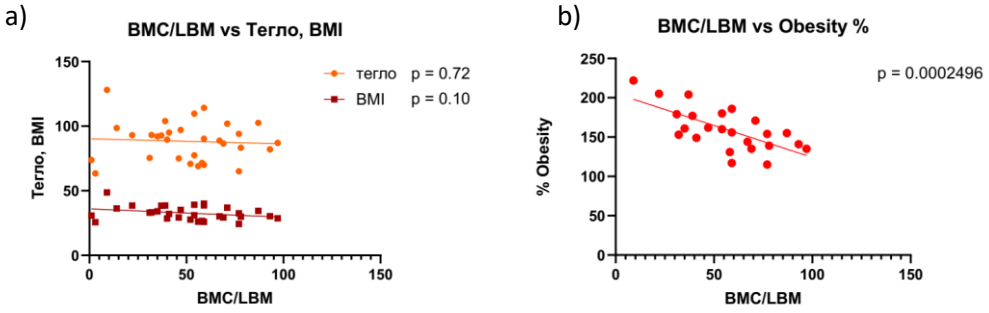


Fig. 22 **Correlation between BMC/LBM and a) body weight and BMI; b) Obesity %**

The degree of obesity (Ob%) shows by what percentage the weight deviates from the ideal weight, with acceptable limits ranging from -10% to +10%, i.e. 90-110% is considered a

normal result. The average degree of obesity for all girls included in our study was 166% (from 115% to 288%), and the difference in the average levels between the BMI-1 and BMI-2 groups was 32%, the average value for the BMI-1 group was 146 % and for BMI-2 group – 193 %. **(Fig. 23)**

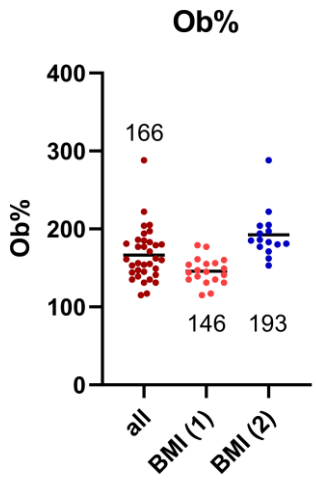


Fig. 23 Percentage obesity Ob%) in all girls and according to BMI

We find similar data in the analysis of the BMC/LBM and the indicators for the amount of FM. It can be seen that, regardless of the research method - DXA or BIA, the increase in the absolute amount of FM and the Fat % also shows an inverse proportional relationship with BMC/LBM. Again, as found in the TBLH BMD results, while in the overweight and mildly obese group of girls there was still some positive correlation between FM and bone mass, in the highly and extremely obese girls the negative effect of FM on bone is already clearly stated. **(Fig. 24, 25)**

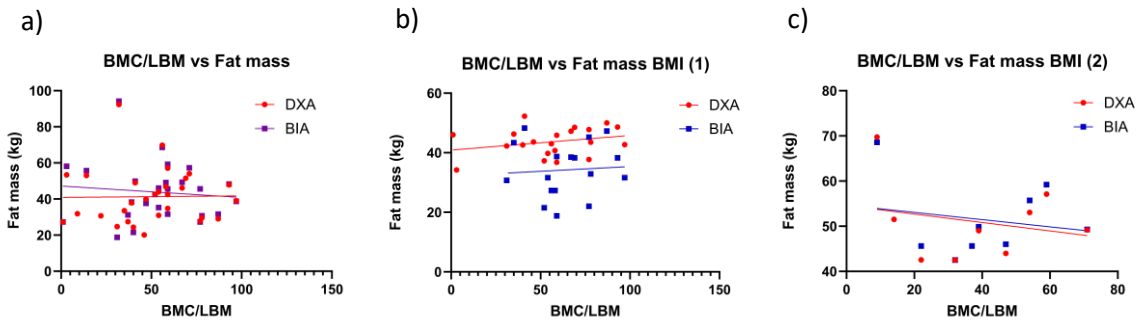


Fig. 24 Correlations between BMC/LBM and FM (kg) a) all participants; b) BMI-1; c) BMI-2

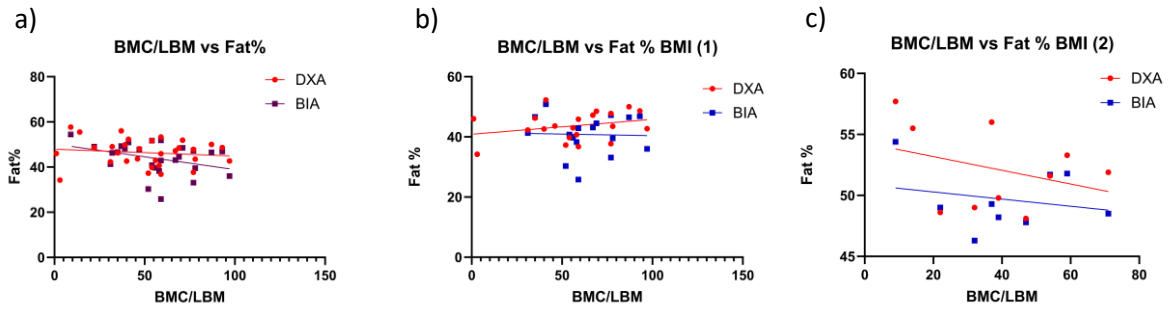


Fig. 25 **Correlations between BMC/LBM and Fat % in a) all participants; b) BMI-1; c) BMI-2**

The lower BMC/LBM values were also observed when the amount of VF was taken into account. The girls we studied showed a progressive decrease in BMC/LBM as well as with increasing mass (VF mass, r=0.11) and volume (VF volume, r=0.11) of VF, where correlations did not reach significance, as well as with percent (VF%, p<0.1) and the VF index (VFI, p<0.05), where the correlations were significantly significant. **(Fig. 26)**

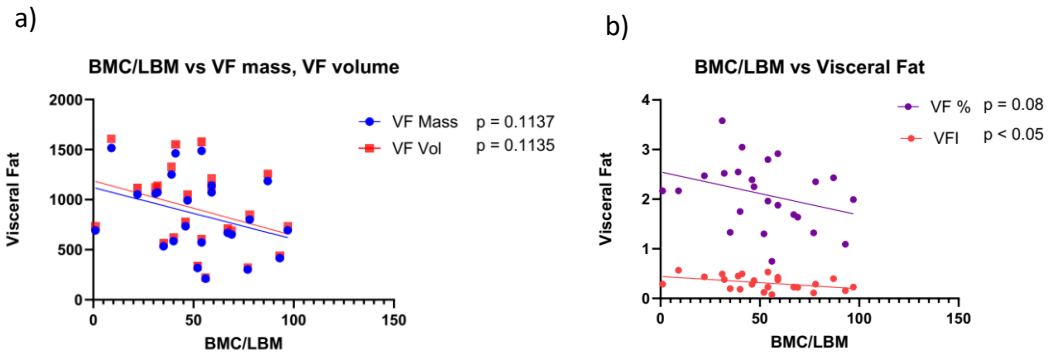


Fig. 26 **Correlations between BMC/LBM and a) VF mass and volume; b) VF% and VFI**

In the same negative way, BIA indicators of metabolic abnormalities also affect BMC/LBM - higher level of VF level, p<0.1, and lower overall InBody score, p = 0.03, correlate with lower bone mass. **(Fig. 27)**

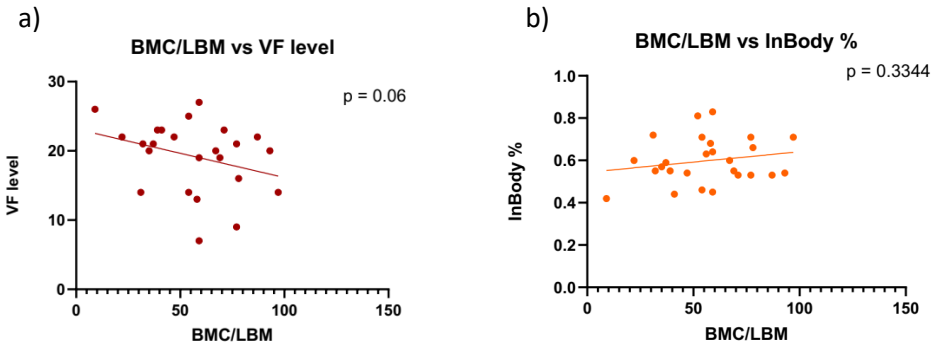


Fig. 27 Correlations between BMC/LBM and a) VF level; b) InBody score

There is no relationship between BMc/LBM and android and gynoid type of obesity, but there is a well-represented negative correlation between BMC/LBM and FM distribution in the trunk (p=0.001) and lower limbs (p<0.001), where statistical significance is reached. **(Fig. 28)**

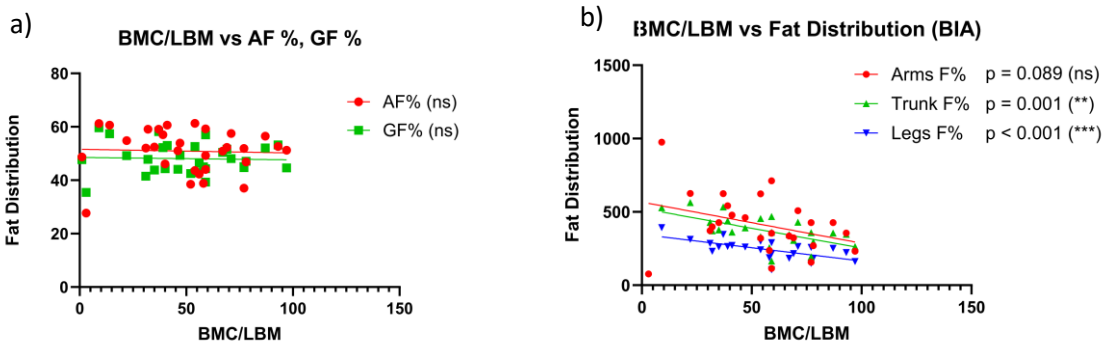


Fig. 28 Correlations between BMC/LBM and a) AF% and GF%; b) FM body distribution

A slightly higher ratio of fat mass/lean mass (FM/LM) was found in the entire group of girls in the area of the upper limbs compared to the lower limbs – 0.96 and 0.90, respectively. Distributed according to the categories of obesity, however, it can be seen that while in the BMI-1 group the LM significantly prevails over the FM (especially for the lower limbs - FM/LM 0.89 for the arms, 0.78 for the legs), then with heavier obese girls of the BMI-2 group, both in the arms and legs, there is a FM/LM ratio above 1 - 1.10 for the lower limbs and 1.06 for the upper limbs. **(Fig. 29)**

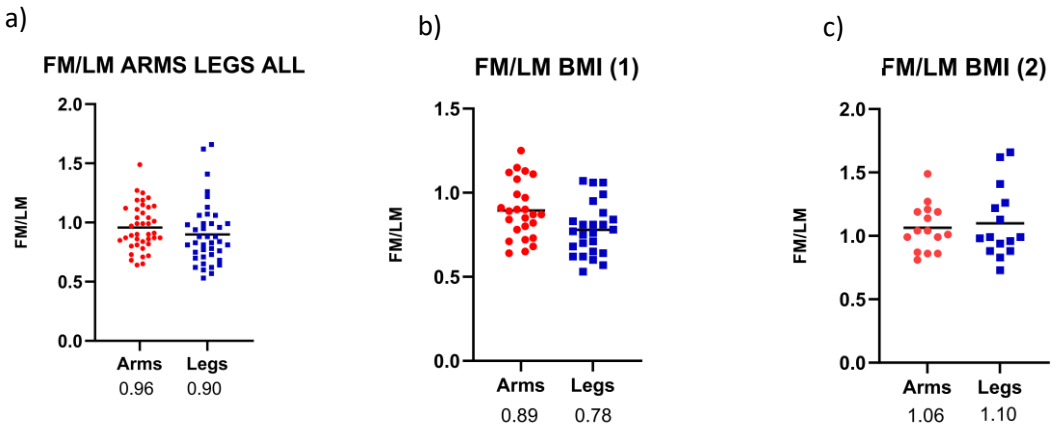


Fig. 29 **FM/LM ratio for arms and legs in a) all participants; b) group BMI-1; c) group BMI-2**

For both upper and lower limbs, the differences found in FM/LM ratio between BMI-1 girls and BMI-2 girls showed statistical significance, p=0.005 and p < 0.0001, respectively. **(Fig. 30)**

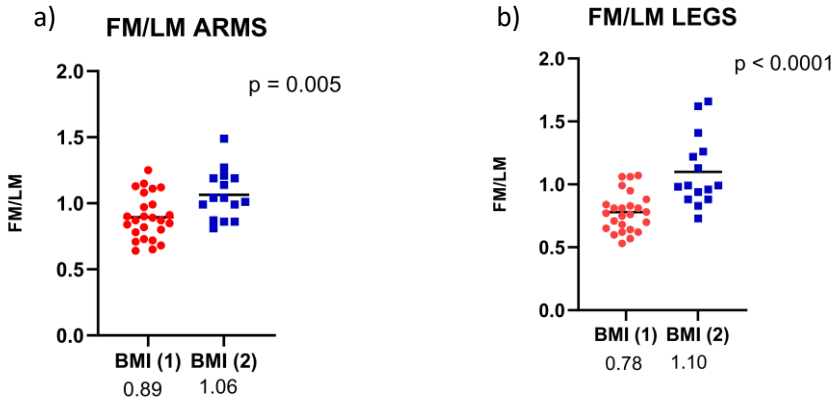


Fig. 30 **Differemces in FM/LM ratio between group BMI-1 and BMI-2 for a) arms; b) legs**

In order to search for a relationship between the ratio of fat/muscle mass and bone osteometric DXA parameters, we compared FM/LM of the upper and lower extremities with their respective BMD, BMC and BAr including all study participants, and subsequently we analyzed the same relationships separately for each group – BMI-1 and BMI-2.

Regarding FM/LM and BMD in the lower limbs, no significant correlations were found, and no differences in BMD values were observed depending on the degree of obesity. **(Fig. 31)**

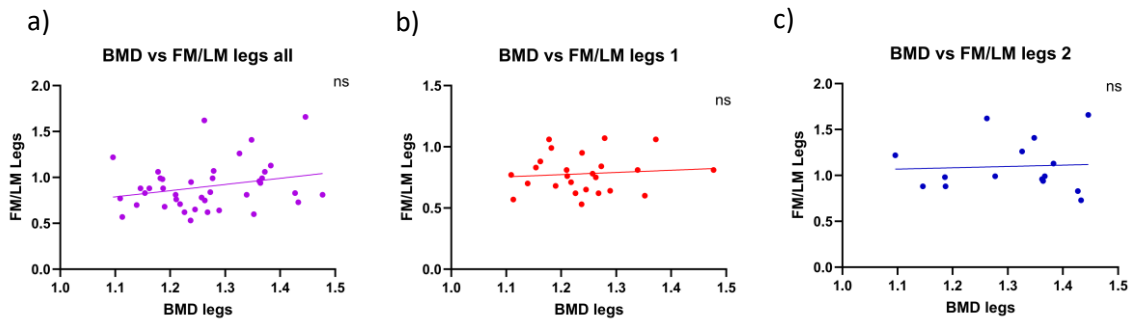


Fig. 31 **Correlations between TBLH BMD and legs FM/LM in a) all participants; b) BMI-1; c) BMI-2**

However, in the analysis of the relationship between FM/LM and BMC, it was found that while in the general group and the BMI-1 group of girls, the increase in FM had a positive effect and led to a certain increase in BMC, on the contrary, in the more obese girls from BMI-2 group, the same correlation already assumes a negative character. **(Fig. 32)** The same correlations are found when comparing FM/LM with BAr - FM appears to contribute to an increase in bone size of the lower limbs in overweight and mildly obese girls - BMI-1 group ($p<0.05$, statistically significant), while with the progression of obesity to the critical degrees characterizing the group of BMI-2 girls, the increase in FM/LM is already associated with a decrease in bone dimensions. **(Fig. 33)**

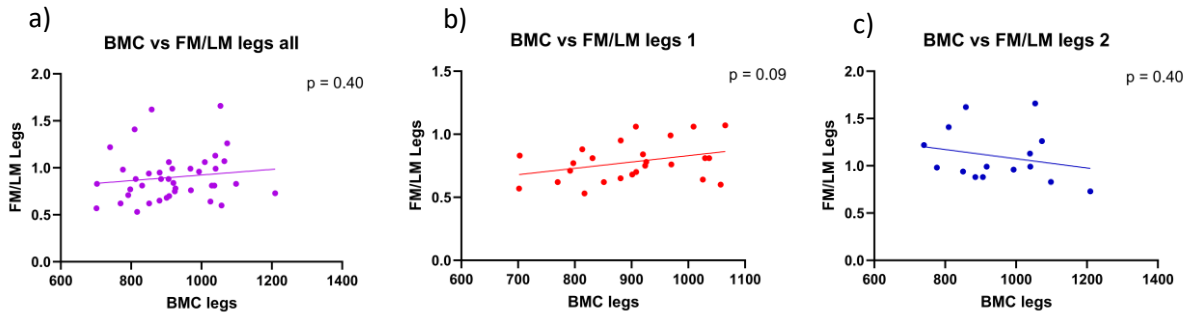


Fig. 32 **Correlations between TBLH BMC and legs FM/LM in a) all participants; b) BMI-1; c) BMI-2**

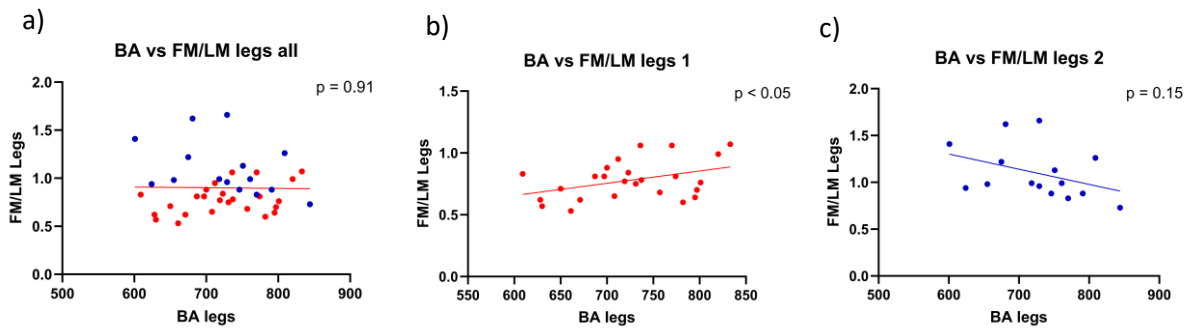


Fig. 33 **Correlations between BAr and legs FM/LM in a) all participants; b) BMI-1; c) BMI-2**

The same analysis conducted for the upper limbs did not find significant correlations between FM/LM values and BMD distribution. **(Fig. 34)**

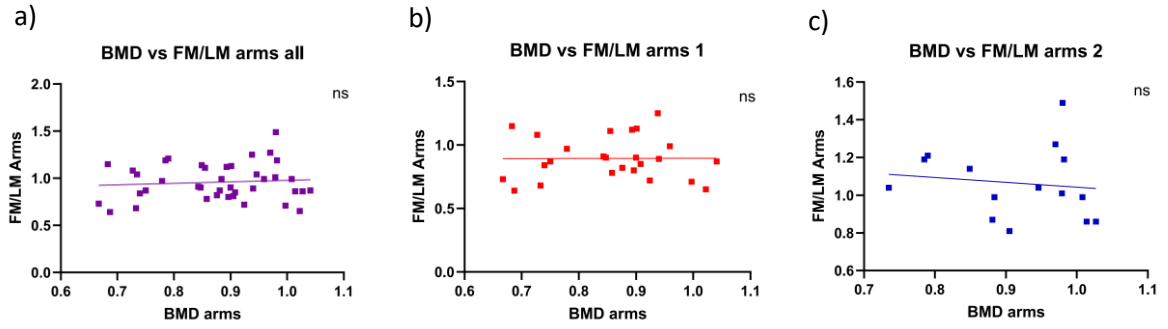
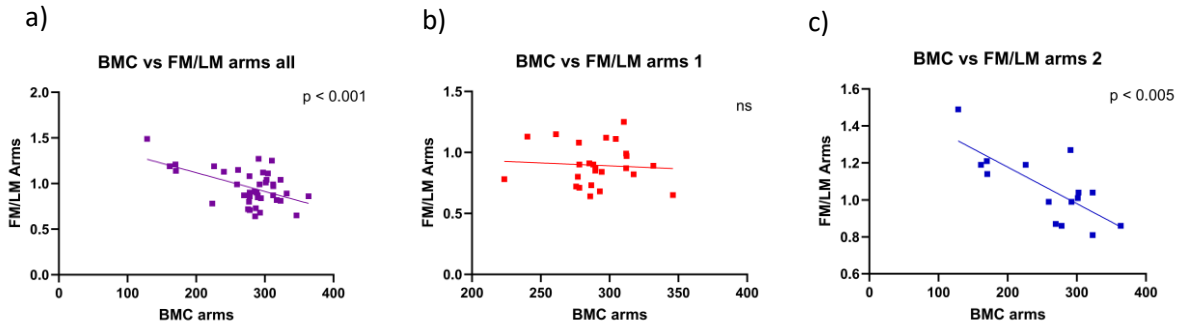
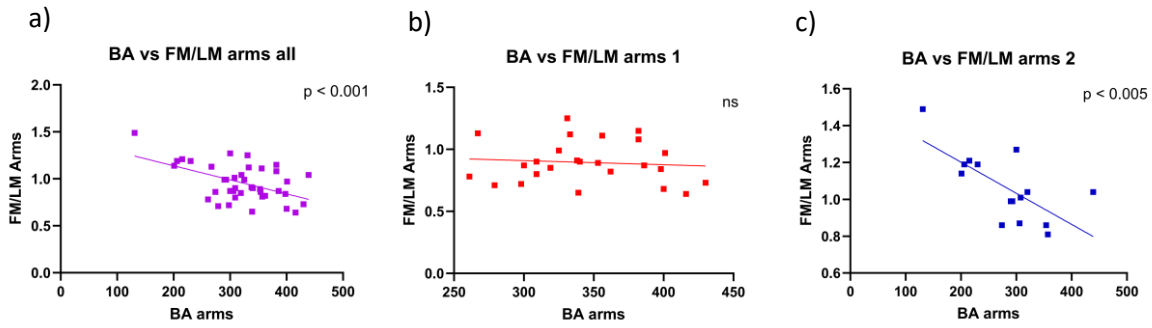


Fig. 34 Correlations between TBLH BMD and arms FM/LM in a) all participants; b) BMI-1; c) BMI-2

On the other hand, in relation to BMC and BAr, the FM/LM ratio was found to play a significant negative role, which was again present in the general group, and especially in the BMI-2 group, where the correlations reached statistical significance from $p < 0.005$. **(Fig. 35, 36)**



Фиг. 36 Correlations between TBLH BMC and arms FM/LM in a) all participants; b) BMI-1; c) BMI-2



Фиг. 37 Correlations between BAr and arms FM/LM in a) all participants; b) BMI-1; c) BMI-2

We also find a strong positive relationship between TBLH BMD and FFM, incl. SMI and SMM. This positive association showed marked significance ($p < 0.0001$) and was observed among all girls participating in the study. **(Fig. 37)**

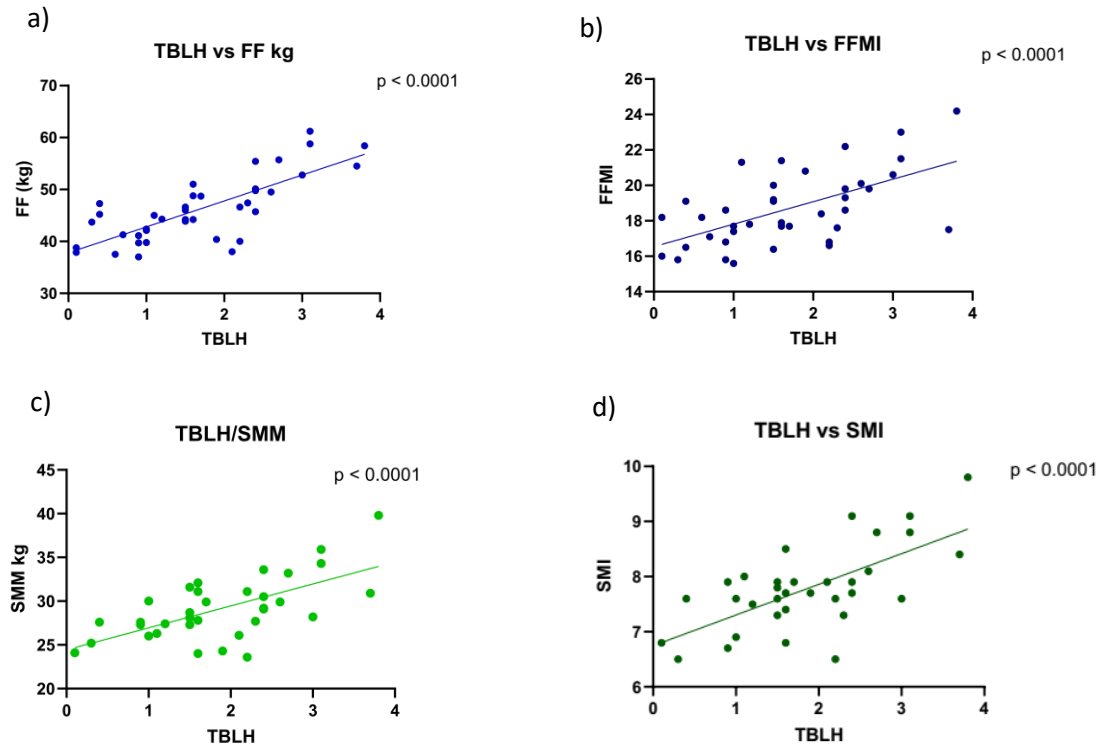
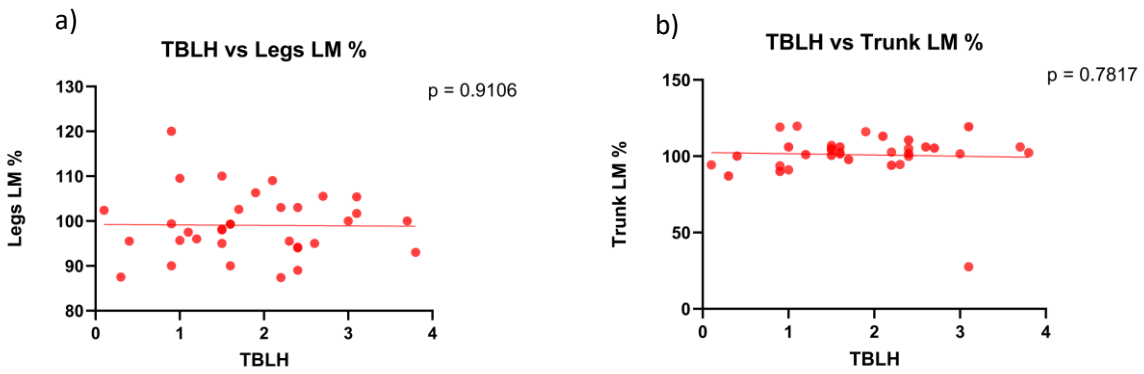


Fig. 38 Correlations between TBLH BMD and a) FFM; b) FFMI; c) SMM; d) SMI

According to their body distribution, LM in the lower limbs (Legs LM%) and trunk (Trunk LM%) did not correlate with the corresponding BMD, but LM in the upper limbs (Arms LM%) had a well-defined positive correlation with BMD, $p < 0.01$. **(Fig. 38)**



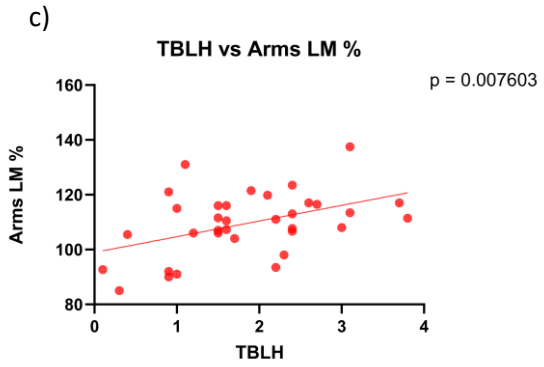


Fig. 38 **Correlations between TBLH BMD and LM in a) legs; b) trunk; c) arms**

OSTEODENSITOMETRIC RESULTS FOR BONE AREA

BAr is an important marker of bone mass in general and is a reliable predictor of bone strength. BAr data obtained from DXA measurements are presented as percentiles of the ratio area/H. For all participants, area/H shows a mean value of P36, with 50% of girls having area/H below P32 (median). The results distributed by groups of degree of obesity indicate that half of the girls with high and extreme obesity (BMI-2 group) have values for area/H even below P10, while when the results are distributed by groups related to the presence of metabolic abnormalities similar significant differences are not found. **(Fig. 39)**

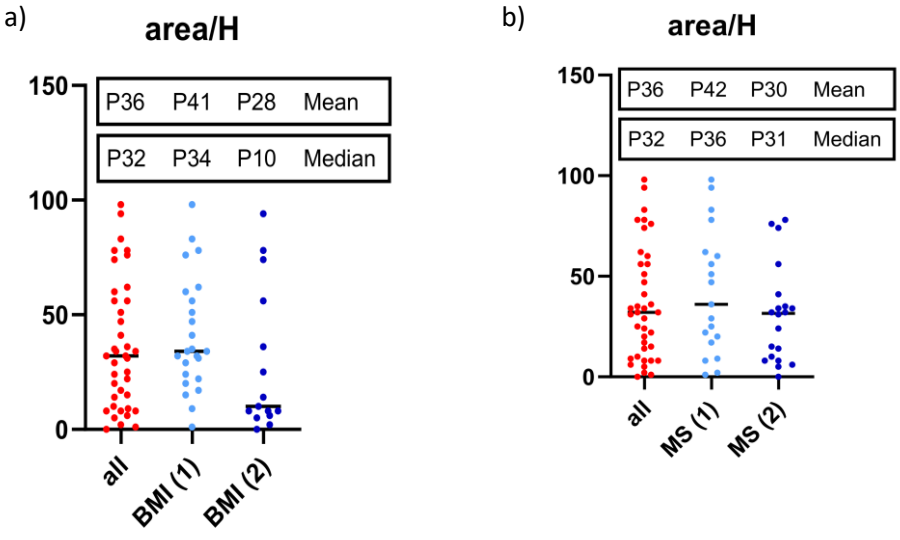


Fig. 40 **Ditribution of area/H a) according BMI; b) according MS risk factors**

The area/H ratio showed a weak negative correlation with weight (p=0.09), BMI (p=0.11) and the degree of obesity (p=0.06), but a negative statistical significance was found with WC, where p < 0.05 was found. **(Fig. 40).**

Similarly, a statistically significant negative influence (p < 0.05) on area/H is also found on the side of adipose tissue - the amount of FM expressed as an absolute value (kg) and as a percentage of total weight (FM %). **(Fig. 41)**

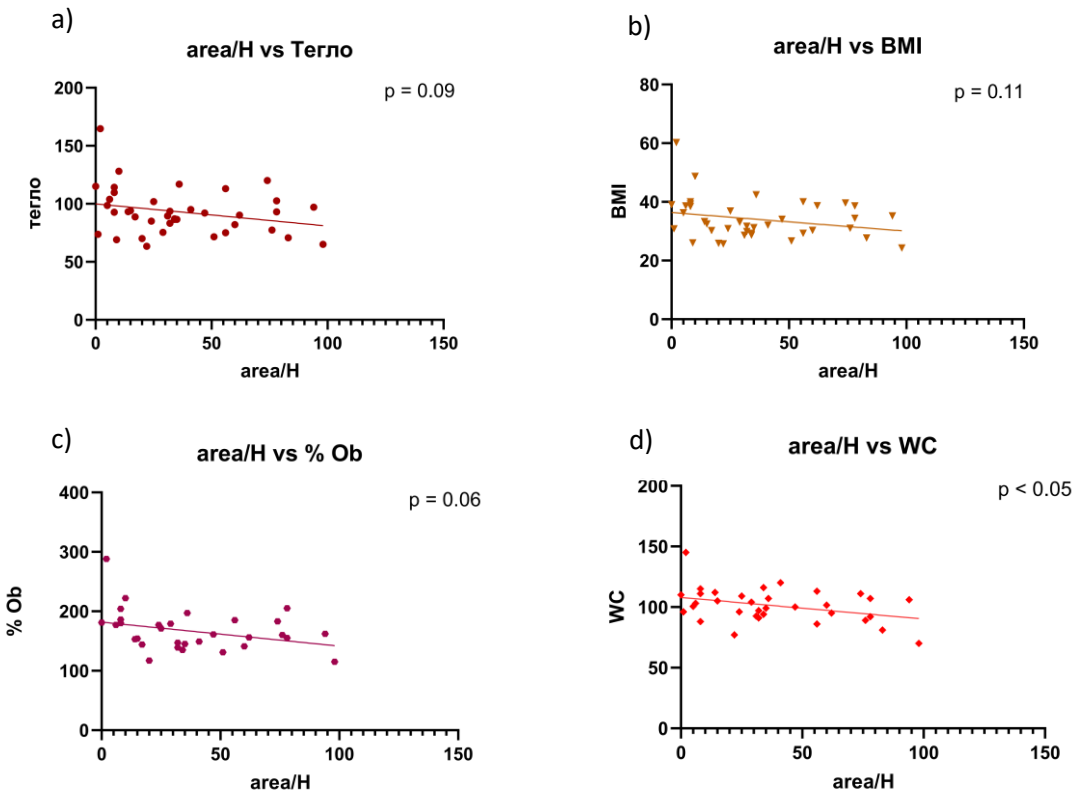


Fig. 40 Correlations between area/H and a) body weight; b) BMI; c) % Ob; d) WC

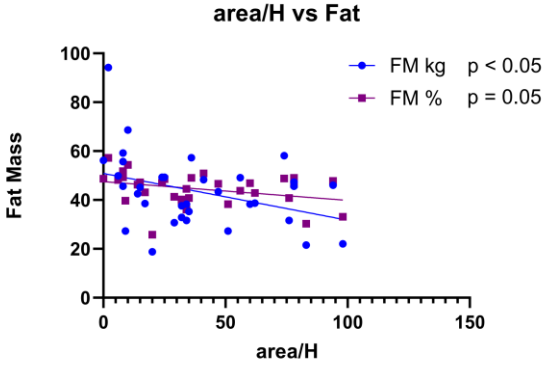


Fig. 41 Correlations between area/H and FM and FM%

A well-expressed significant negative correlation is also found between area/H and the AF% and GF% ($p < 0.05$), and a less significant but again negative relationship is established between area/H and the distribution of fat in the area on the trunk, arms and legs. **(Fig. 42)**

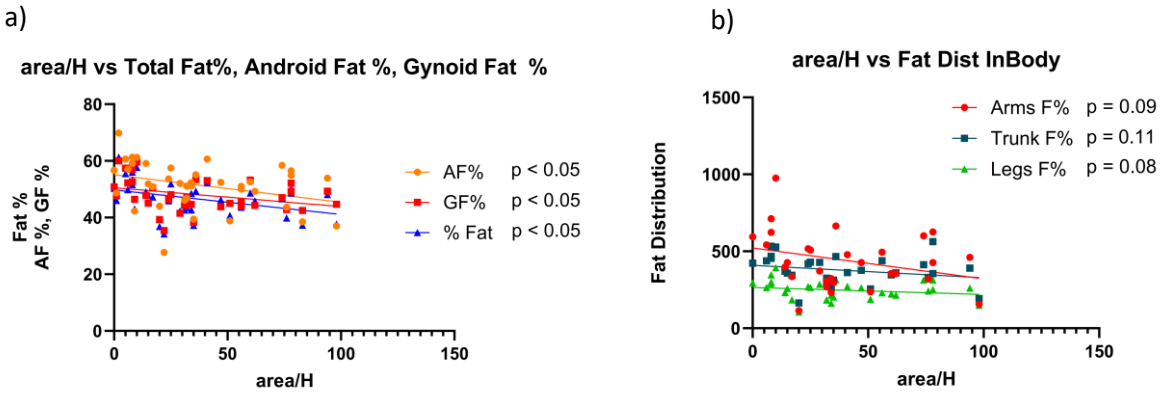


Fig. 42 Correlations between area/H and a) Fat%, AF5 and GF%; b) FM body distribution

Although statistically insignificant but negative correlation is found between area/H and VF mass and volume (p=0.15), VF% (p=0.33) **(Fig. 43)** and the BIA markers for metabolic risk - VF level (p=0.12). **(Fig. 44a)**

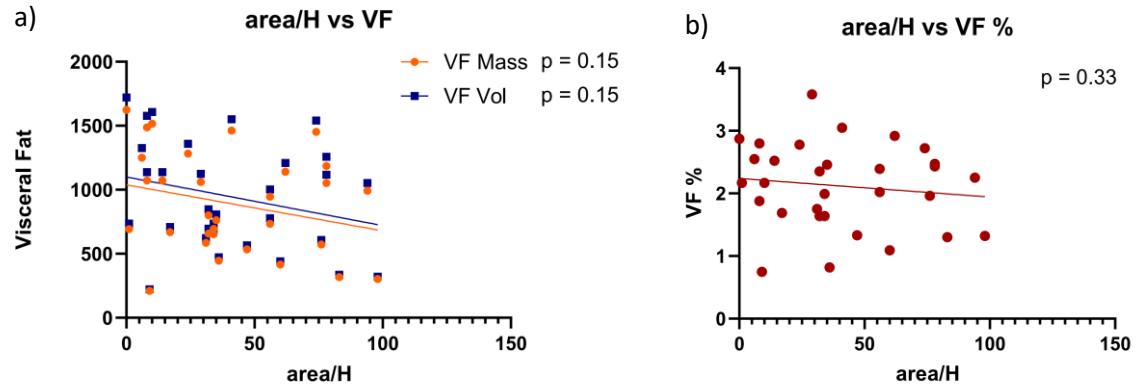


Fig. 43 Correlations between area/H and a) VF mass and volume; b) VF%

The main BIA assessment of health status – InBodyscore, in turn showed a statistically significant positive correlation with area/H ($p < 0.05$). **(Fig. 44b)**

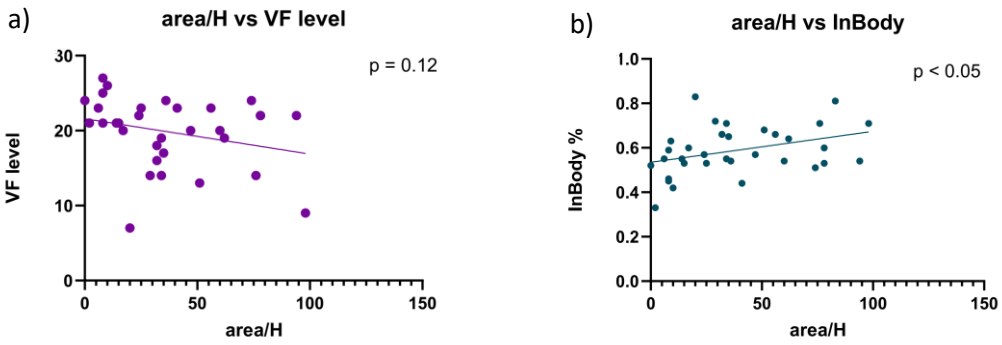


Fig. 44 Correlations between area/H and a) VF level; b) InBody scoreVF level

Between area/H and FFM indices, a positive significant correlations was found only with FFM% (Fat free mass %), p<0.05, while absolute mass (FF) and FFMI as well as the skeletal muscle indices (SMI, SMM) and the distribution of FFM by body segments showed no correlation. **(Fig. 45)**

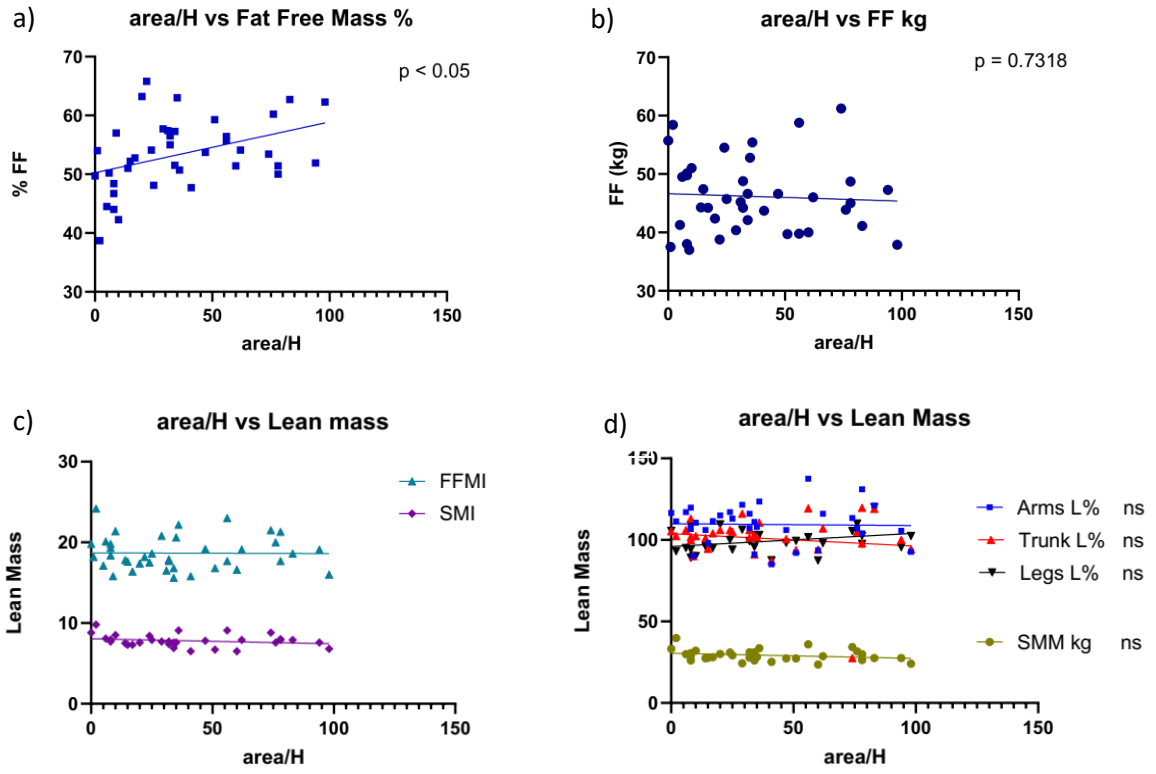


Fig. 45 Correlations between area/H and a) FFM%; b) FFM in kg; c) FFMI and SMI; d) FFM distribution

Regarding the relationships between area/H and BMD measures, it is evident that the lower the total skeletal BAr, the greater is both TBLH and LS BMD. **(Fig. 46)**

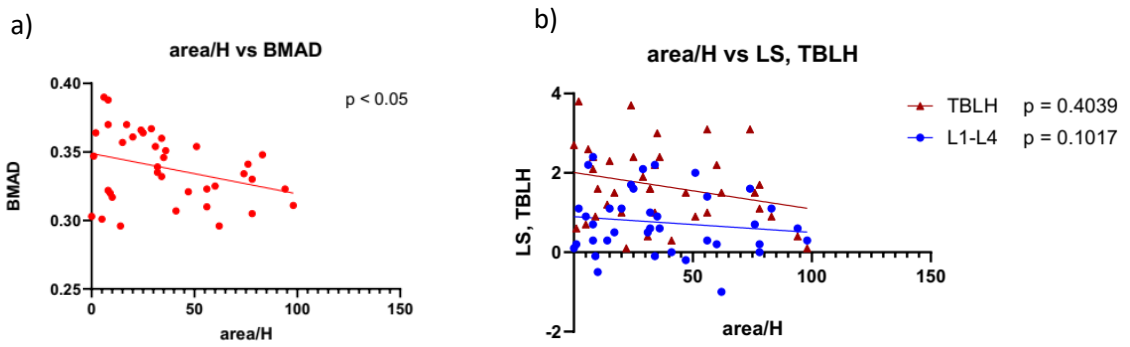


Fig. 46 **Correlations between area/H and a) BMAD; b) LS BMD and TBLH BMD**

The data for BAr in the limbs indicate that in the region of the arms (BAr arms) in the BMI-1 group is significantly higher than that of the girls in the BMI-2 group – 344 cm^2 for BMI-1 and 281 cm^2 for BMI-2. At the same time, the values for BAr in the region of the lower limbs (BAr legs) did not show significant differences between the girls of the two groups. **(Fig. 47)**

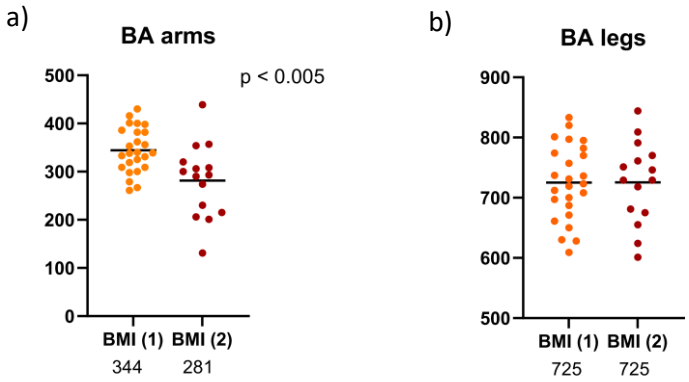


Fig. 47 **Distribution of BAr according BMI in the region of the a) arms; b) legs**

The obtained data on BMD in the arms area did not show significant differences between the girls of the BMI-1 and BMI-2 groups, which, combined with the higher mean BMC found for the girls of the BMI-1 group, means that for them in the arms area more net bone mineral is accumulated.

Conversely, for the lower limbs we find a statistically significant higher BMD for the girls in the group with a higher BMI, $p < 0.05$, which in turn indicates that they have accumulated more bone mineral in the leg area compared to the girls from BMI-1 group. **(Fig. 48)**

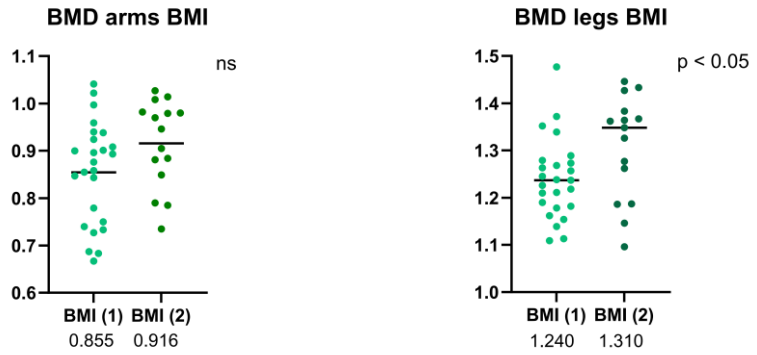


Fig. 48 Distribution of BMC according BMI in the region of the a) arms; b) legs

OSTEODENSITOMETRIC RESULTS FOR LUMBAR SPINE

LS DXA results were obtained from measurements involving the first to fourth lumbar vertebrae (L1-L4) as standard. **(Tab. 8, Tab. 9)**

The mean value of LS BMD for all participants was 0.77 SD. When comparing between the girls from BMI and MS groups, no significant difference was found in the values of LS BMD. **(Fig. 49)**

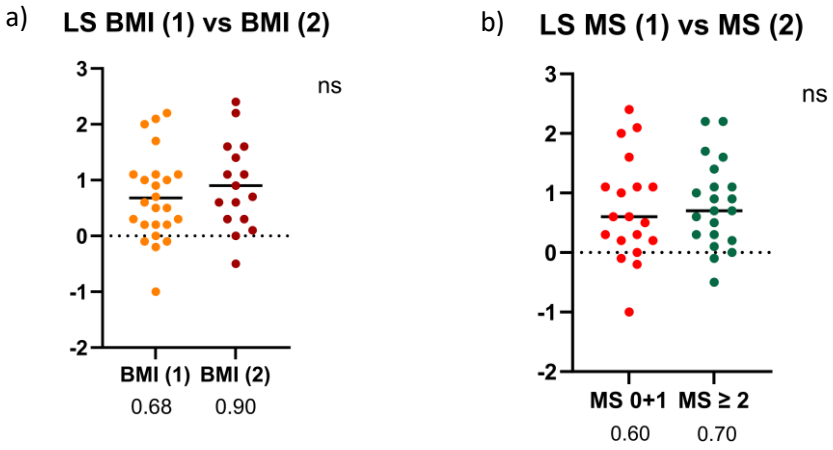


Fig. 49 Distribution of LS BMD according to a) BMI; b) MS risk factors

No association of LS BMD with body weight, BMI, WC, as well as with the Ob % calculated by BIA, was established. **(Fig. 50)**

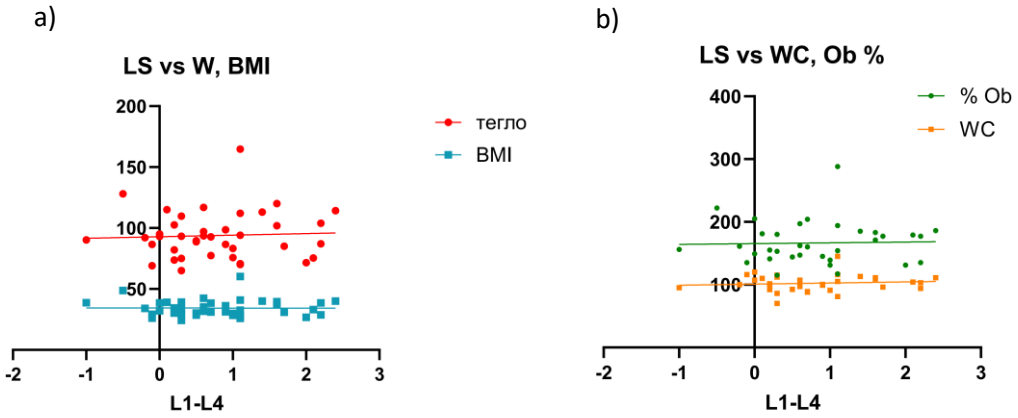
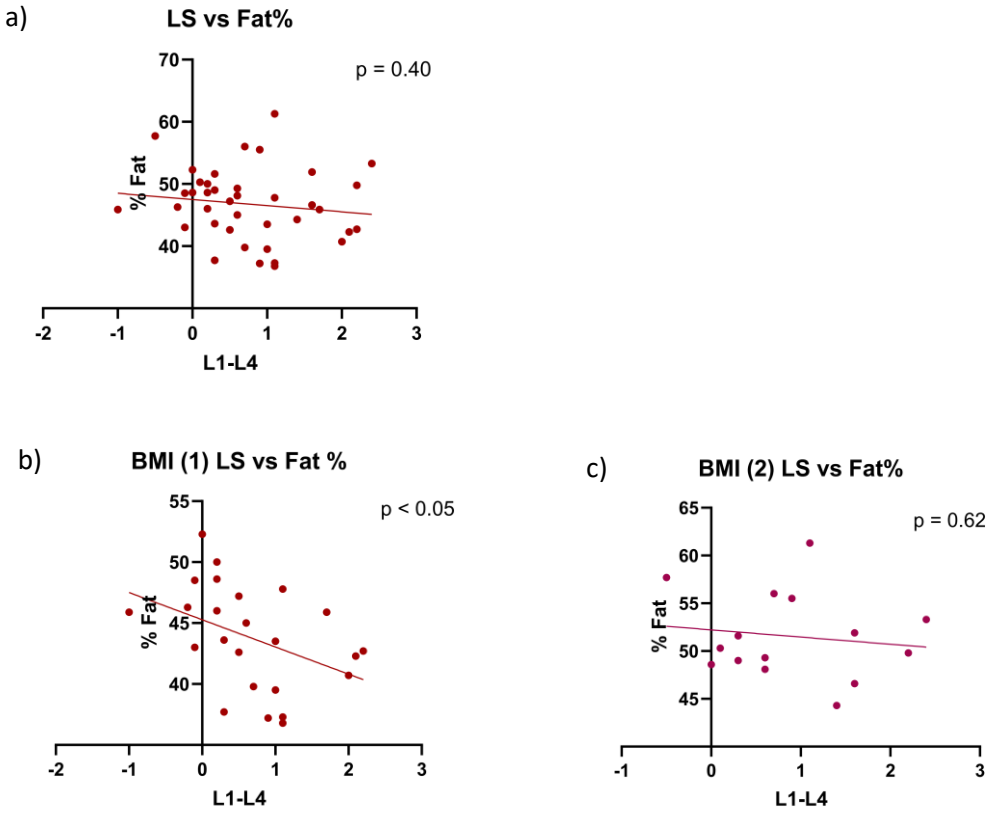


Fig. 50 Correlations between LS BMD and a) body weght and BMI; b) WC and Ob %

However, there is a negative correlation between LS BMD and the Fat %, which is better expressed in the BMI-1 group of girls, where it reaches statistical significance ($p<0.05$), as well as in the girls from group MS-2, also statistically significant ($p<0.01$). **(Fig. 51)**



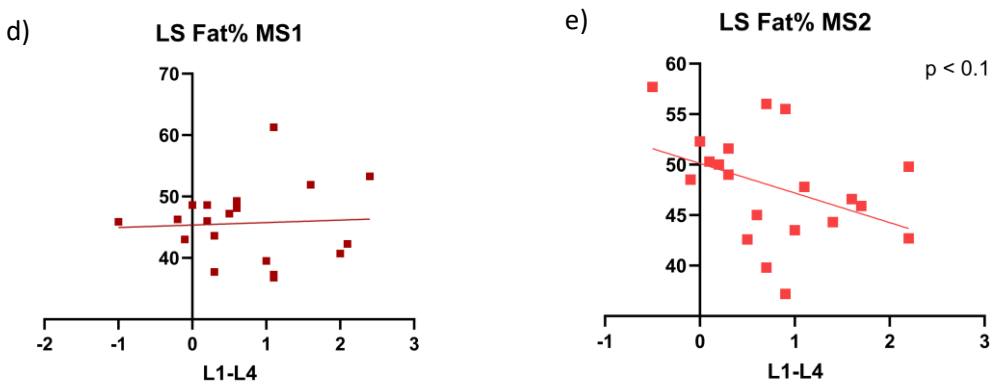


Fig. 51 Correlations between LS BMD and Fat % in a) all participants; b) BMI-1 group; c) BMI-2 group; d) MS-1 group; e) MS-2 group

The correlations between LS BMD and VF parameters - VF volume and mass and VF %, were slightly positive in the whole group and in the BMI-1 group **(Fig. 52, 53)**, and similarly to the results of the TBLH BMD analysis, in the more obese girls from the BMI-2 group and in the girls from the MS-2 group these correlations again disappeared and even acquired a slightly negative character. **(Fig. 53, 54)**

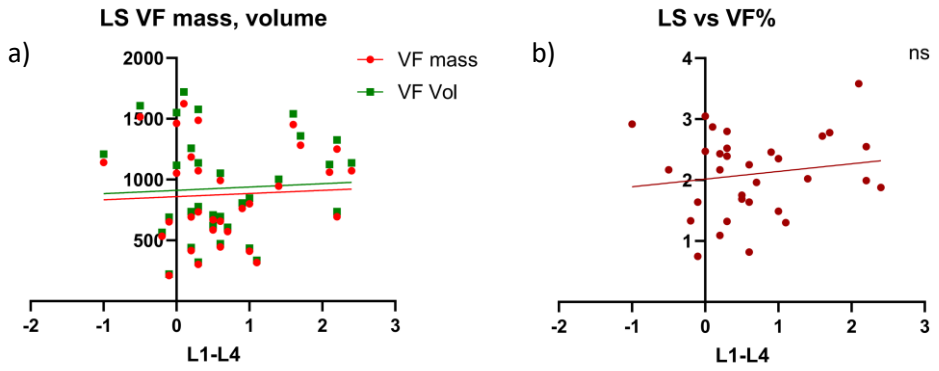


Fig. 52 Correltin between LS BMD and a) VF mass and volume; b) VF %

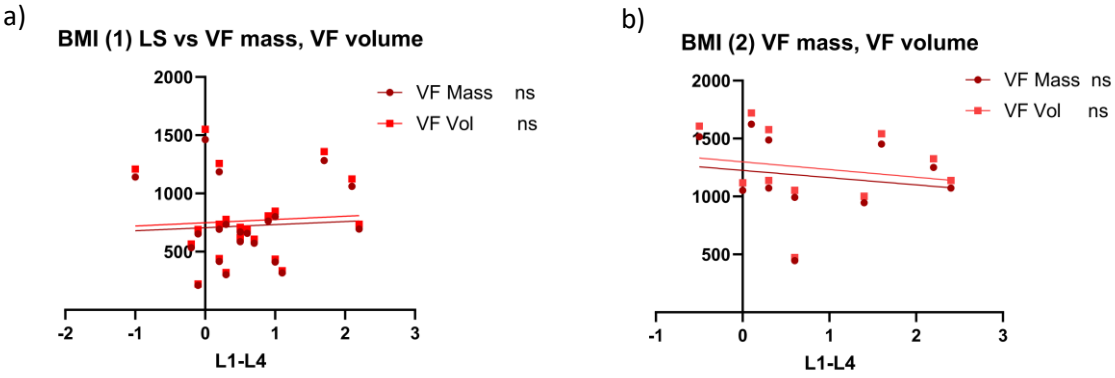


Fig. 53 Correltin between LS BMD and VF mass, volume and VF % in group a) BMI-1; b) BMI-2

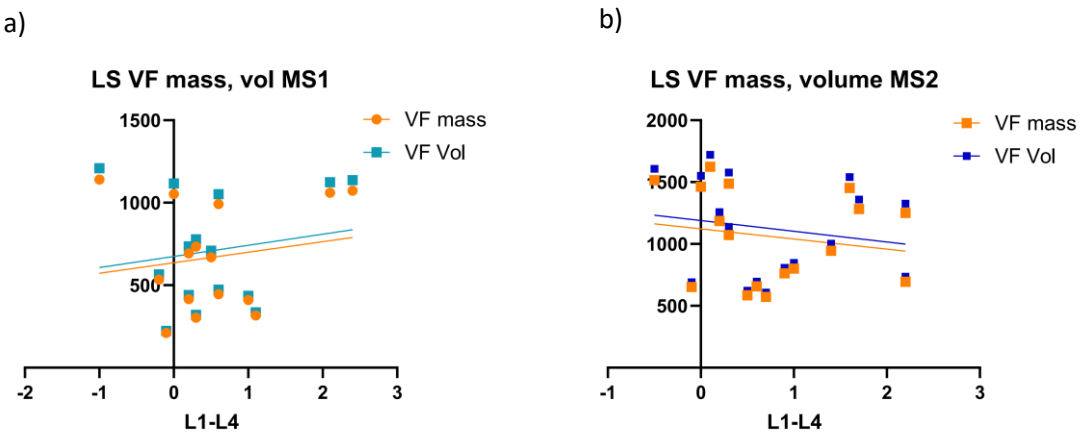


Fig. 54 Correltin between LS BMD and VF mass and volume in group a) MS-1; b) MS-2

Again, contrary to what was found for TBLH BMD, the values of LS BMD in relation to the android and gynoid type of fat distribution show, although moderately expressed, a negative correlation - most strongly represented again in the BMI-1 group with close to significant values, especially for the gynoid type of fat accumulation ($p<0.1$). No significant correlations were found between LS BMD and the A/G ratio, incl. in the separate groups according to the degree of obesity. **(Fig. 55)**

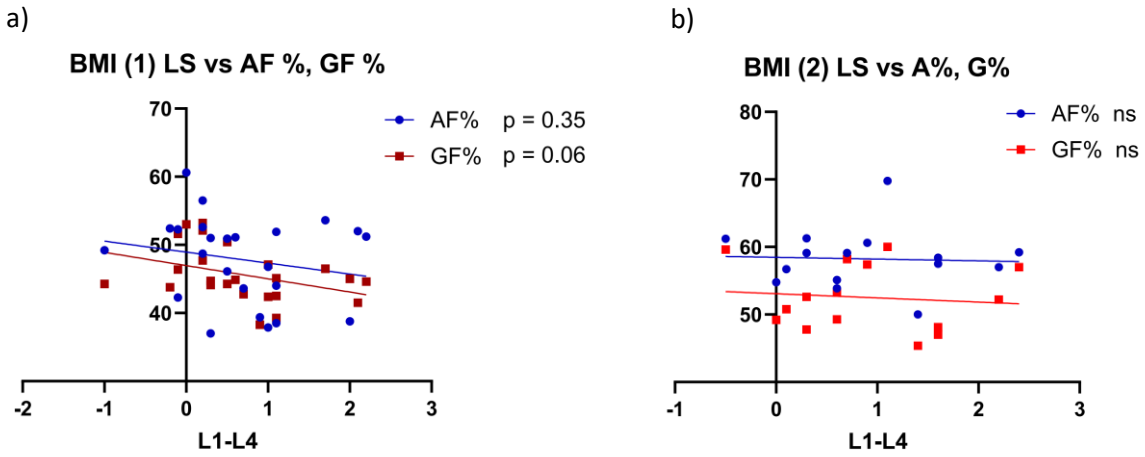
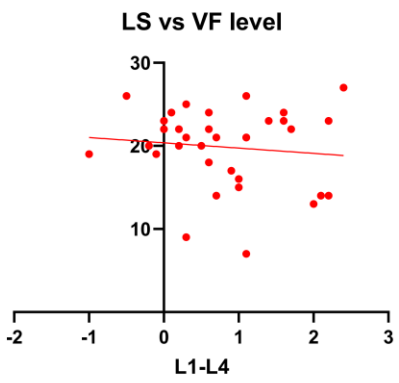


Fig. 55 Correltin between LS BMD and AF% and GF% in group: a) BMI-1; b) BMI-2

However, there was a trend towards an increase in LS BMD in parallel with an improvement in BIA metabolic risk scores, but without statistical significance. **(Fig. 56)**

a)



b)

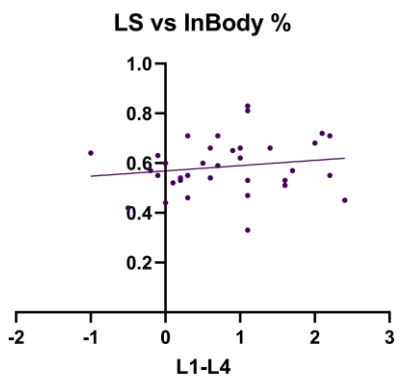


Fig. 56 Correltin between LS BMD and a) VF level; b) InBody score

Both in the overall group and when divided into groups according to BMI and MS criteria, no correlations were found between LS BMD and FFM indices, with the exception of a borderline significantly positive association of LS BMD with FF% in the BMI-1 group (p=0.05). **(Tab. 10, 11, 12, 13, 14)**

Tab. 10 Correltin between LS BMD and FFM in all participants

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,2105	0,1294	0,1026	0,1900	0,1614
95% CI	-0,1171 - 0,4968	-0,1985 - 0,4312	-0,2388 - 0,4214	-0,1529 - 0,4921	-0,1624 - 0,4538
R squared	0,04431	0,01674	0,01052	0,03612	0,02606
P value	0,2046	0,4388	0,5577	0,2742	0,3262
Significance	No	No	No	No	No

Tab. 11 Correltin between LS BMD and FFM in group BMI-1

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,1587	0,3989	0,1397	0,1643	0,1953
95% CI	-0,2712 - 0,5359	-0,01594 - 0,6966	-0,3228 - 0,5483	-0,3000 - 0,5657	-0,2259 - 0,5550
R squared	0,02519	0,1591	0,01952	0,02701	0,03815
P value	0,4695	0,0594	0,5569	0,4887	0,3604
Significance	No	No	No	No	No

Tab. 12 Correltin between LS BMD and FFM in group BMI-2

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,1804	0,1375	-0,009070	0,1883	-0,002688
95% CI	-0,3657 - 0,6341	-0,4032 - 0,6070	-0,5189 - 0,5055	-0,3586 - 0,6389	-0,5142 - 0,5103
R squared	0,03254	0,01890	8,227e-005	0,03545	7,224e-006
P value	0,5200	0,6251	0,9744	0,5016	0,9924
Significance	No	No	No	No	No

Tab. 13 Correltin between LS BMD and FFM in group MS-1

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,1351	-0,07843	-0,01962	0,1209	0,1684
95% CI	-0,3541 - 0,5663	-0,5260 - 0,4032	-0,5104 - 0,4808	-0,3987 - 0,5817	-0,3095 - 0,5784
R squared	0,01826	0,006152	0,0003850	0,01461	0,02837
P value	0,5929	0,7571	0,9425	0,6557	0,4907
Significance	No	No	No	No	No

Tab. 14 Correltin between LS BMD and FFM in group MS-21

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,2841	0,4043	0,2642	0,3097	0,1593
95% CI	-0,1812 - 0,6455	-0,04653 - 0,7183	-0,2159 - 0,6414	-0,1682 - 0,6697	-0,3047 - 0,5622
R squared	0,08072	0,1635	0,06979	0,09590	0,02539
P value	0,2248	0,0770	0,2744	0,1970	0,5022
Significance	No	No	No	No	No

The results for BMAD was similar to those for LS BMD - no correlations with weight, BMI, WC, % obesity **(Fig. 57)**, as well as with FM parameters. **(Fig. 58)**

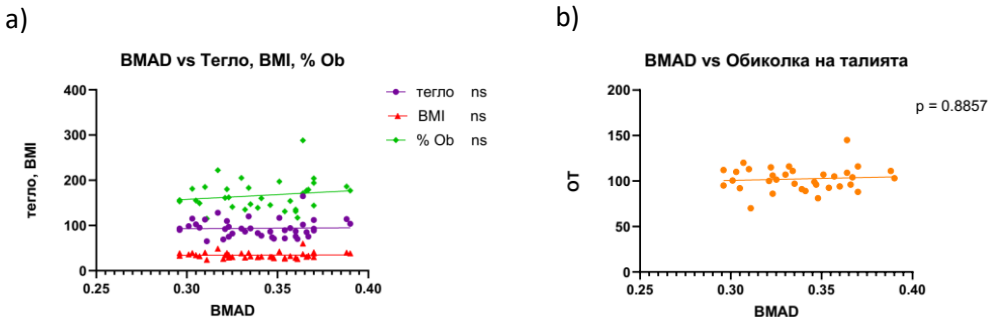


Fig. 57 **Correlations between BMAD and a) body weight, BMI and %Ob; b) WC**

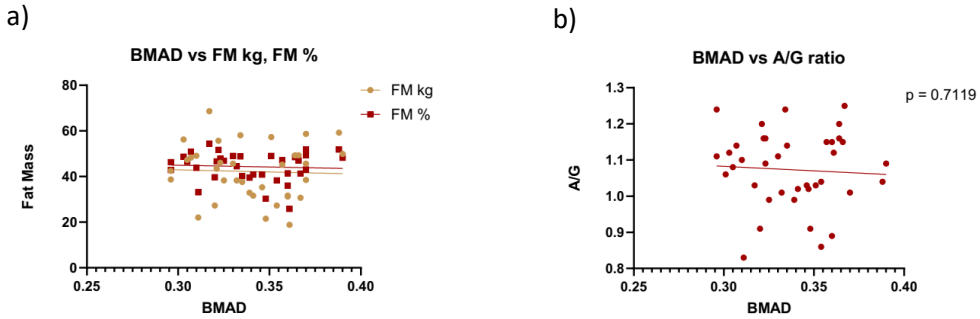


Fig. 58 **Correlations between BMAD and a) FM in kg and FM%; b) A/G ratio**

An important exception is the relationship between BMAD and VF parameters, where a negative correlation was found, although without reaching statistical significance. **(Fig. 59)**

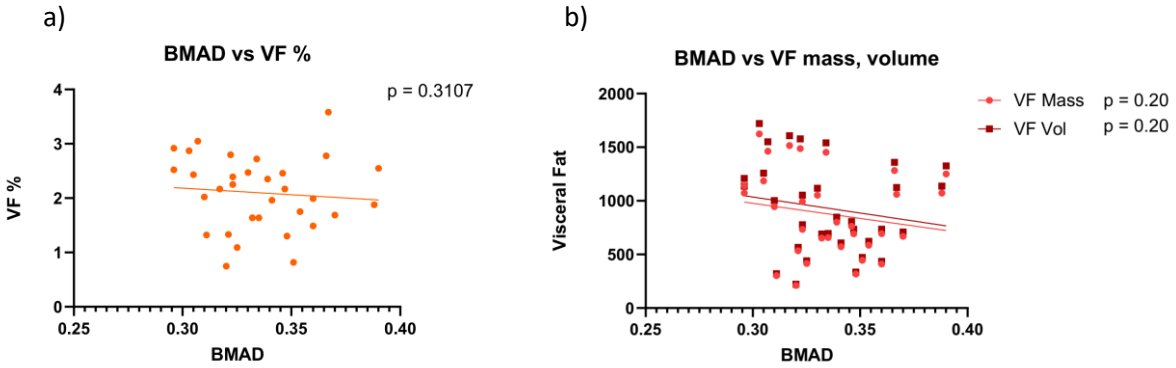


Fig. 59 **Correlations between BMAD and a) VF%; b) VF mass and volume**

The data are similar when dividing the participants into groups according to BMI and MS criteria. **(Tab. 15, 16, 17, 18)** No correlations were also found between BMAD and FFM. **(Tab. 19, 20, 21, 22)**

Tab. 15 Correlation between BMAD and VF in BMI-1 group

	VF Mass	VF Vol	VFI	VF level	InBody %
r	-0,1214	-0,1210	-0,05200	-0,2765	0,3760
95% CI	-0,5255 - 0,3275	-0,5253 - 0,3278	-0,4731 - 0,3884	-0,6492 - 0,2032	-0,06647 - 0,6949
R squared	0,01473	0,01465	0,002704	0,07647	0,1414
P value	0,6003	0,6013	0,8229	0,2518	0,0930
Significance	No	No	No	No	No

Tab. 16 Correlation between BMAD and VF in BMI-2 group

	VF Mass	VF Vol	VFI	VF level	InBody %
r	-0,2189	-0,2194	-0,2444	0,1709	-0,2453
95% CI	-0,7237 - 0,4386	-0,7240 - 0,4381	-0,7363 - 0,4166	-0,3741 - 0,6282	-0,6730 - 0,3053
R squared	0,04790	0,04815	0,05972	0,02920	0,06016
P value	0,5179	0,5168	0,4689	0,5426	0,3782
Significance	No	No	No	No	No

Tab. 17 Correlation between BMAD and VF in MS-1 group

	VF Mass	VF Vol	VFI	VF level	InBody %
r	0,1281	0,1281	0,1327	0,1399	-0,1692
95% CI	-0,4111 - 0,6009	-0,4112 - 0,6009	-0,4072 - 0,6039	-0,4011 - 0,6085	-0,6010 - 0,3390
R squared	0,01641	0,01640	0,01762	0,01957	0,02862
P value	0,6491	0,6493	0,6372	0,6190	0,5163
Significance	No	No	No	No	No

Tab. 18 **Correlation between BMAD and VF in MS-2 group**

	VF Mass	VF Vol	VFI	VF level	InBody %
r	-0,3403	-0,3403	-0,3244	-0,1863	0,2371
95% CI	-0,7055 - 0,1678	-0,7056 - 0,1678	-0,6964 - 0,1852	-0,5905 - 0,2927	-0,2433 - 0,6241
R squared	0,1158	0,1158	0,1052	0,03471	0,05621
P value	0,1814	0,1814	0,2040	0,4451	0,3284
Significance	No	No	No	No	No

Tab. 19 **Correlation between BMAD and FFM in BMI-1 group**

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,1553	0,3286	0,1617	0,1600	0,2242
95% CI	-0,2745 - 0,5334	-0,09667 - 0,6524	-0,3025 - 0,5639	-0,3041 - 0,5627	-0,1971 - 0,5755
R squared	0,02412	0,1080	0,02614	0,02559	0,05025
P value	0,4792	0,1258	0,4959	0,5005	0,2923
Significance	No	No	No	No	No

Tab. 20 **Correlation between BMAD and FFM in BMI-2 group**

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	-0,02248	-0,2739	-0,04464	-0,01336	0,1306
95% CI	-0,5287 - 0,4955	-0,6894 - 0,2773	-0,5445 - 0,4786	-0,5221 - 0,5023	-0,4091 - 0,6025
R squared	0,0005053	0,07502	0,001993	0,0001786	0,01705
P value	0,9366	0,3232	0,8745	0,9623	0,6428
Significance	No	No	No	No	No

Tab. 21 **Correlation between BMAD and FFM in MS-1 group**

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	0,3166	-0,2865	0,1831	0,3291	0,2976
95% CI	-0,1763 - 0,6826	-0,6645 - 0,2083	-0,3438 - 0,6223	-0,1991 - 0,7091	-0,1811 - 0,6623
R squared	0,1003	0,08207	0,03352	0,1083	0,08856
P value	0,2005	0,2491	0,4973	0,2133	0,2159

Significance	No	No	No	No	No

Tab. 22 Correlation between BMAD and FFM in MS-2 group

	FF (kg)	% FF	SMI	SMM (kg)	FFMI
r	-0,08836	0,2920	-0,01911	-0,1278	-0,05760
95% CI	-0,5109 - 0,3686	-0,1729 - 0,6504	-0,4692 - 0,4389	-0,5501 - 0,3465	-0,4877 - 0,3950
R squared	0,007807	0,08524	0,0003652	0,01634	0,003318
P value	0,7111	0,2116	0,9381	0,6020	0,8094
Significance	No	No	No	No	No

BIOCHEMICAL RESULTS AND THEIR CORRELATION WITH BONE PARAMETERS

1. CARBOHYDRATES AND BONE

The obtained results for HbA1c and the values of BG (0', 30', 60' and 120') and serum insulin (0' and 30') from the OGTT showed the following statistically significant correlations with the main osteometric DXA parameters (TBLH BMD, BMC/LBM, LS BMD, BMAD and area/H). **(Tables 23 – 34)**

- HbA1c is positively correlated with TBLH BMD in the whole group, as well as in BMI-2 and MS-2 groups **(Table 23, 25, 27)**

- HbA1c is negatively correlated with area/H in the MS-1 group **(Table 34, Fig. 60)**

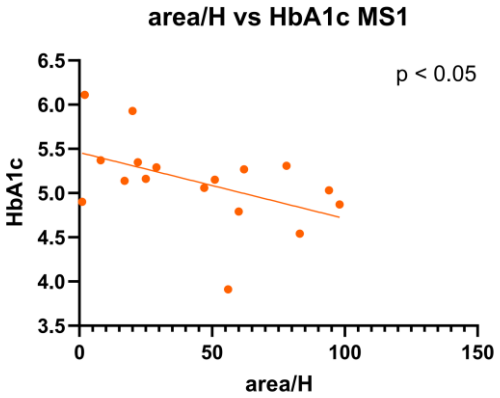


Fig. 60 Correlation between area/H and HbA1c in group MS-1

- fasting serum insulin (0 min) is positively correlated with TBLH BMD in the whole group, as well as in BMI-1 and MS-2 groups. **(Tab. 23, 24, 27)**

- fasting serum insulin (0 min) is positively correlated with LS BMD for the whole group and for the MS-2 group (Tab. 28, 32)

No statistically significant correlations were found between the investigated markers for carbohydrate metabolism and BMAD and BMC/LBM.

Tab. 23 Correlation between TBLH BMD and markers for carbohydrate metabolism in all participants

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,4764	0,05837	0,0006941	0,08522	-0,2156	0,4173	0,2808
R squared	0,2269	0,003407	4,817e-007	0,007262	0,04647	0,1742	0,07887
P value	0,0033	0,7205	0,9966	0,6011	0,1875	0,0102	0,0792
Significance	**	ns	ns	ns	ns	*	ns

Tab. 24 Correlation between TBLH BMD and markers for carbohydrate metabolism in BMI-1 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,3114	-0,03309	-0,2506	-0,08128	-0,2595	0,5379	0,1057
R squared	0,09700	0,001095	0,06281	0,006607	0,06736	0,2893	0,01117
P value	0,1480	0,8752	0,2269	0,6993	0,2207	0,0081	0,6151
Significance	ns	ns	ns	ns	ns	**	ns

Tab. 25 Correlation between TBLH BMD and markers for carbohydrate metabolism in BMI-2 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,6439	0,2356	0,3892	0,2703	-0,1004	-0,09706	0,1801
R squared	0,4146	0,05550	0,1515	0,07306	0,01009	0,009421	0,03245
P value	0,0176	0,3980	0,1516	0,3299	0,7218	0,7413	0,5206
Significance	*	ns	ns	ns	ns	ns	ns

Tab. 26 Correlation between TBLH BMD and markers for carbohydrate metabolism in MS-1 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,4234	-0,1769	-0,06585	0,07941	-0,1774	0,1922	0,1500
R squared	0,1793	0,03128	0,004336	0,006306	0,03149	0,03693	0,02251
P value	0,0800	0,4557	0,7827	0,7393	0,4542	0,4449	0,5278
Significance	ns	ns	ns	ns	ns	ns	ns

Tab. 27 Correlation between TBLH BMD and markers for carbohydrate metabolism in MS-2 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,5578	0,1307	0,2664	0,1754	-0,1955	0,5237	0,2945
R squared	0,3112	0,01709	0,07099	0,03078	0,03821	0,2742	0,08676
P value	0,0161	0,5827	0,2562	0,4594	0,4225	0,0214	0,2074
Significance	*	ns	ns	ns	ns	*	ns

Tab. 28 Correlation between LS BMD and markers for carbohydrate metabolism in all participants

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,2312	0,1700	0,1077	-0,04536	0,08964	0,3616	0,2982
R squared	0,05344	0,02888	0,01160	0,002058	0,008036	0,1307	0,08894
P value	0,1749	0,2944	0,5083	0,7811	0,5873	0,0279	0,0616
Significance	ns	ns	ns	ns	ns	*	ns

Tab. 29 Correlation between LS BMD and markers for carbohydrate metabolism in BMI-1 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,1783	0,1394	0,1949	-0,03138	0,1921	0,3613	0,3476
R squared	0,03180	0,01945	0,03800	0,0009847	0,03690	0,1305	0,1209
P value	0,4272	0,5158	0,3613	0,8843	0,3799	0,0985	0,0960
Significance	ns	ns	ns	ns	ns	ns	ns

Tab. 30 Correlation between LS BMD and markers for carbohydrate metabolism in BMI-2 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,3187	0,2169	-0,1074	-0,07845	-0,05956	0,2382	0,2375
R squared	0,1015	0,04704	0,01153	0,006154	0,003547	0,05673	0,05639
P value	0,2668	0,4198	0,6923	0,7727	0,8266	0,3926	0,3758
Significance	ns	ns	ns	ns	ns	ns	ns

Tab. 31 Correlation between LS BMD and markers for carbohydrate metabolism in MS-1 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,2543	0,01237	0,3001	0,05847	0,1882	0,2192	0,3139
R squared	0,06466	0,0001531	0,09005	0,003418	0,03541	0,04806	0,09856
P value	0,3247	0,9599	0,2120	0,8121	0,4404	0,3979	0,1905
Significance	ns	ns	ns	ns	ns	ns	ns

Tab. 32 Correlation between LS BMD and markers for carbohydrate metabolism in MS-2 group

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	0,2203	0,2862	-0,1151	-0,1381	-0,001426	0,5721	0,3177
R squared	0,04852	0,08191	0,01326	0,01908	2,033e-006	0,3273	0,1010
P value	0,3649	0,2085	0,6192	0,5505	0,9952	0,0084	0,1604
Significance	ns	ns	ns	ns	ns	*	ns

Tab. 33 **Correlation between area/H and markers for carbohydrate metabolism in all participants**

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	-0,3016	-0,1587	-0,03153	-0,05705	-0,1336	-0,1122	0,02158
R squared	0,09097	0,02517	0,0009942	0,003255	0,01785	0,01260	0,0004657
P value	0,0783	0,3347	0,8489	0,7301	0,4240	0,5146	0,8963
Significance	ns	ns	ns	ns	ns	ns	ns

Tab. 34 **Correlation between area/H and markers for carbohydrate metabolism in MS-1 group**

	HbA1c	BG 0 min	BG 30 min	BG 60 min	BG 120 min	Insulin 0	Insulin 30
r	-0,4827	-0,4130	-0,04624	0,04845	-0,1112	-0,1218	-0,01788
R squared	0,2330	0,1706	0,002138	0,002347	0,01236	0,01484	0,0003198
P value	0,0497	0,0788	0,8509	0,8439	0,6505	0,6414	0,9421
Significance	*	ns	ns	ns	ns	ns	ns

LIPIDS AND BONE

The obtained results for lipid metabolism - total cholesterol, TG, HDL- and LDL-cholesterol, showed the following statistically significant correlations with the main osteometric DXA parameters (TBLH BMD, BMC/LBM, LS BMD, BMAD and area/H):

A) with regard to whole-body measurements - TBLH BMD, BMC/LBM and area/H, statistically significant correlations were found only in the girls from group MS-1:

- a negative correlation was found between TG and area/H (r – 0.530, p 0.0196), but also a statistically significant positive relationship with TBLH BMD (r 0.514, p 0.0204)

- total cholesterol is positively correlated with BMC/LBM (r 0.5758, p 0.0150)

B) regarding osteometric parameters for the LS BMD and BMAD, we obtained the following statistically significant results:

- total cholesterol in girls of BMI-2 group is positively correlated with LS BMD (r 0.6529, p 0.0061) and with BMAD (r 0.5940, p 0.0153) **(Tab. 37, 38)**

- HDL-cholesterol is negatively correlated with LS KMP in BMI-1 group (r -0.4543, p 0.0257) and with BMAD in BMI-1 group (r -0.4508, p 0.0270) (Tables 35, 36), and positively correlated with LS BMD in BMI-2 group (r 0.6103, p 0.0121). (Table 37) In addition, HDL-cholesterol showed a very close to significant negative correlation with TBLH BMD as well. **(Fig. 61)**

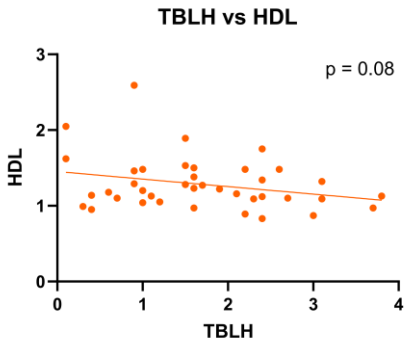


Fig. 61 **Correlations between TBLH BMD and HDL-C**

- LDL-cholesterol is positively correlated in BMI-2 group with LS BMD (r 0.5241, p 0.0372) and with BMAD (r -0.5833, p 0.0177) **(Tab. 37, 38)**

- TG in girls from group MS-1 showed a positive correlation with BMAD (r 0.5608, p 0.0125).

Tab. 35 **Correlations between LS BMD and lipids in group BMI-1**

	T. Chol.	TG	HDL	LDL
r	-0,2554	0,2270	-0,4543	-0,1382
R squared	0,06525	0,05153	0,2064	0,01910
P value	0,2283	0,2861	0,0257	0,5195
Significance	ns	ns	*	ns

Tab. 36 Correlations between BMAD and lipids in group BMI-1

	T. Chol.	TG	HDL	LDL
r	-0,2900	0,1078	-0,4508	-0,1334
R squared	0,08413	0,01161	0,2032	0,01779
P value	0,1692	0,6163	0,0270	0,5344
Significance	ns	ns	*	ns

Tab. 37 Correlations between LS BMD and lipids in group BMI-2

	T. Chol.	TG	HDL	LDL
r	0,6529	0,1738	0,6103	0,5241
R squared	0,4263	0,03021	0,3724	0,2747
P value	0,0061	0,5197	0,0121	0,0372
Significance	**	ns	*	*

Tab. 38 Correlations between BMAD and lipids in group BMI-2

	T. Chol.	TG	HDL	LDL
r	0,5940	-0,07141	0,4050	0,5833
R squared	0,3528	0,005099	0,1640	0,3402
P value	0,0153	0,7927	0,1197	0,0177
Significance	*	ns	ns	*

URIC ACID AND BONE

Uric acid (UA) values showed a significant positive correlation with TBLH BMD **(Fig. 62),** with UA levels rising significantly between girls in groups MS-1 and MS-2 as the number of cardio-metabolic risk factors increased. **(Fig. 63)**

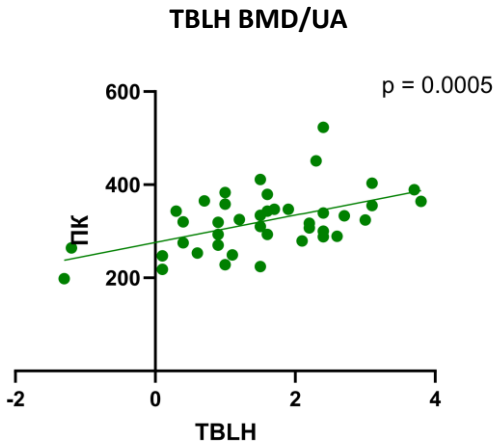


Fig. 62 **Correlation between TBLH BMD and UA**

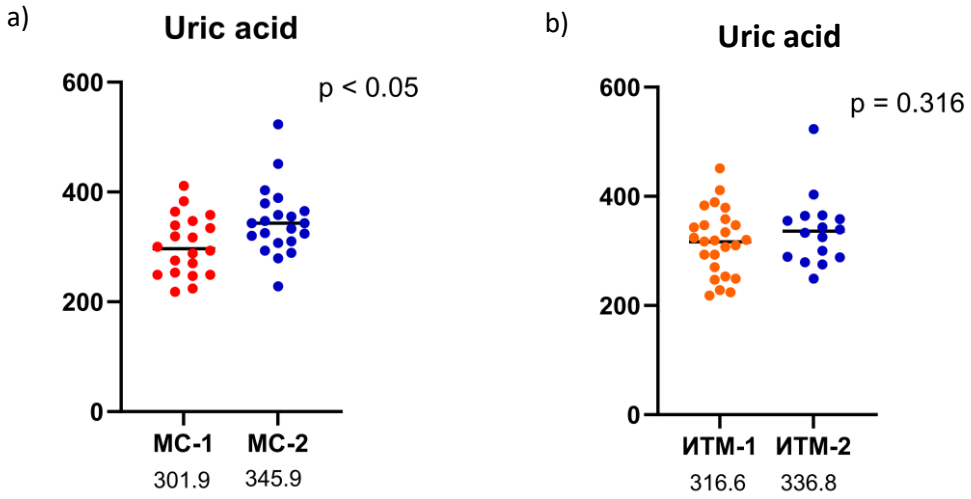


Fig. 63 **UA levels in groups according to a) MS risk factors; b) BMI**

HORMONES AND BONE

The results obtained for the hormones we studied - estradiol, testosterone, DHEA-S, androstenedione, AMH and SHBG, showed the following statistically significant correlations with the main osteometric DXA parameters (TBLH BMD, BMC/LBM, LS BMD, BMAD and area/H) **(Tables 39 – 47)**

- estradiol showed no correlation with any of the osteometric parameters

- testosterone is positively correlated with LS BMD in girls from MS-2 group (r 0.5467, p 0.0284), as well as with BMAD in the general group (r 0.3786, p 0.0298) and the MS-2 group (r 0.6479, p 0.0066)

- DHEA-S is positively correlated with area/H in the MS-2 group (r 0.4723, p 0.0478)

- Androstenedione showed no association with any of the osteometric parameters

- SHBG is positively correlated with BMC/LBM in the MS-1 group (r 0.5288, p 0.352), as well as negatively correlated with TBLH BMD in the general group (r -0.3830, p 0.0232) and TBLH BMD in MS group -2 (r -0.5694, p 0.0170)

- AMH is positively correlated with BMAD in girls from group MS-1 (r 0.4826, p 0.498) and with BMC/LBM in girls from group MS-2 (r 0.6339, p 0.049)

When the results were divided into groups according to the degree of obesity - BMI-1 and BMI-2 groups, no statistically significant correlations were found between the investigated hormone levels and the osteodensitometric measurements.

Tab. 39 Correlations between TBLH BMD and hormone levels in all participants

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,1576	0,2873	-0,01440	0,1754	-0,3830	0,3065
R squared	0,02485	0,08256	0,0002073	0,03078	0,1467	0,09395
P value	0,3585	0,1050	0,9316	0,3210	0,0232	0,0828
Significance	ns	ns	ns	ns	*	ns

Tab. 40 Correlations between TBLH BMD and hormone levels in MS-2 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,2133	0,2093	0,1251	0,2915	-0,5694	0,3427
R squared	0,04552	0,04382	0,01564	0,08498	0,3242	0,1175
P value	0,3805	0,4540	0,6210	0,2918	0,0170	0,2111
Significance	ns	ns	ns	ns	*	ns

Tab. 41 Correlations between LS BMD and hormone levels in MS-2 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,3607	0,5467	0,1559	0,3260	-0,2916	0,4029
R squared	0,1301	0,2989	0,02432	0,1063	0,08504	0,1624
P value	0,1182	0,0284	0,5238	0,2179	0,2403	0,1217
Significance	ns	*	ns	ns	ns	ns

Tab. 42 Correlations between BMAD and hormone levels in all participants

	E2	T	DHEA-S	A4	SHBG	AMH
r	0,02102	0,3786	0,08272	0,1998	-0,1553	0,08616
R squared	0,0004418	0,1433	0,006843	0,03993	0,02412	0,007424
P value	0,9017	0,0298	0,6215	0,2572	0,3730	0,6335
Significance	ns	*	ns	ns	ns	ns

Tab. 43 Correlations between BMAD and hormone levels in MS-1 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,1159	0,09540	0,1968	0,3227	-0,2387	0,4826
R squared	0,01342	0,009102	0,03872	0,1041	0,05697	0,2329
P value	0,6579	0,7157	0,4194	0,1916	0,3562	0,0498
Significance	ns	ns	ns	ns	ns	*

Tab. 44 Correlations between BMAD and hormone levels in MS-2 group

BMAD MC-2	E2	T	DHEA-S	A4	SHBG	AMH
r	0,05020	0,6479	-0,002544	0,05071	-0,1874	-0,06496
R squared	0,05020	0,6479	-0,002544	0,05071	-0,1874	-0,06496
P value	0,8335	0,0066	0,9918	0,8521	0,4566	0,8111
Significance	ns	**	ns	ns	ns	ns

Tab. 45 Correlations between area/H and hormone levels in MS-1 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	0,1957	-0,1220	-0,4793	-0,1299	0,5288	-0,1357
R squared	0,03828	0,01488	0,2297	0,01689	0,2797	0,01841
P value	0,5026	0,6649	0,0516	0,6315	0,0352	0,6164
Significance	ns	ns	ns	ns	*	ns

Tab. 46 Correlations between BMC/LBM and hormone levels in MS-2 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,04178	-0,1693	0,3996	0,5005	-0,2281	0,6339
R squared	0,001746	0,02867	0,1597	0,2505	0,05204	0,4019
P value	0,8922	0,6632	0,1981	0,1406	0,4758	0,0490
Significance	ns	ns	ns	ns	*	ns

Tab. 47 Correlations between area/H and hormone levels in MS-2 group

	E2	T	DHEA-S	A4	SHBG	AMH
r	-0,01378	-0,3179	0,4723	0,09255	0,005632	0,4963
R squared	0,0001899	0,1011	0,2231	0,008565	3,172e-005	0,2463
P value	0,9554	0,2482	0,0478	0,7429	0,9829	0,0599
Significance	ns	ns	*	ns	ns	ns

The investigated hormones showed the following significant differences in their levels when were analyzed between the girls from the different groups:

- Estradiol levels were significantly lower in girls from the MS-2 group compared to those from the MS-1 group **(Fig. 64)**

- Testosterone levels were significantly higher in girls from group BMI-2 compared to those from the BMI-1 group **(Fig. 65)**

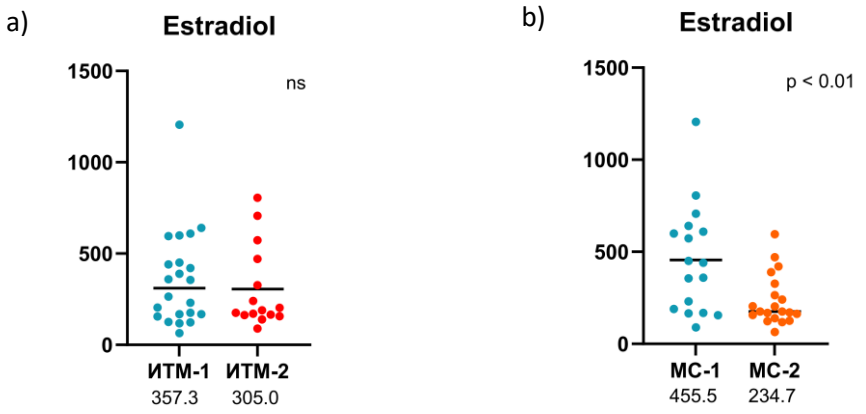


Fig. 64 **Estradiol levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

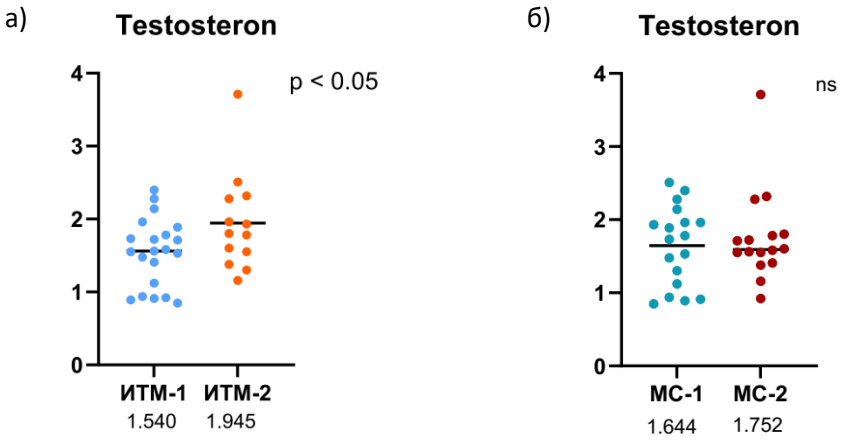


Fig. 65 **Testosterone levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

Levels of DHEA-S, androstenedione, SHBG and AMH did not show significant differences when dividing girls by BMI or MS criteria, with the somewhat exception of SHBG,

where an almost significant decrease in its values was observed in girls from groups BMI-2 and MS-2. **(Fig. 66, 67, 68, 69)**

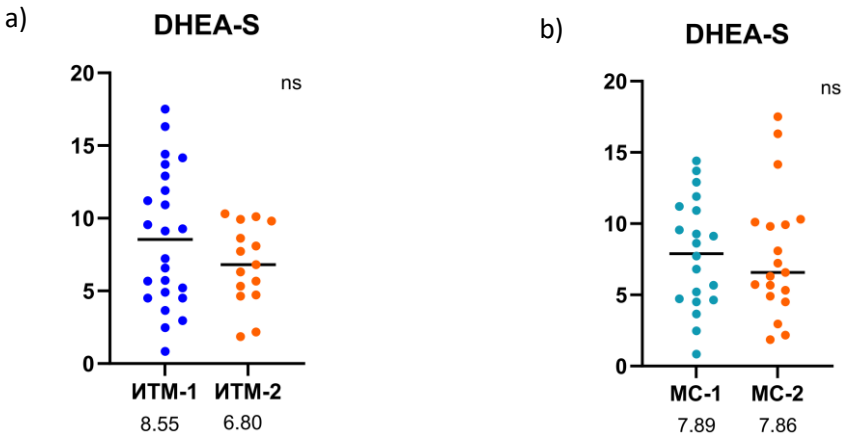


Fig. 66 **DHEA-S levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

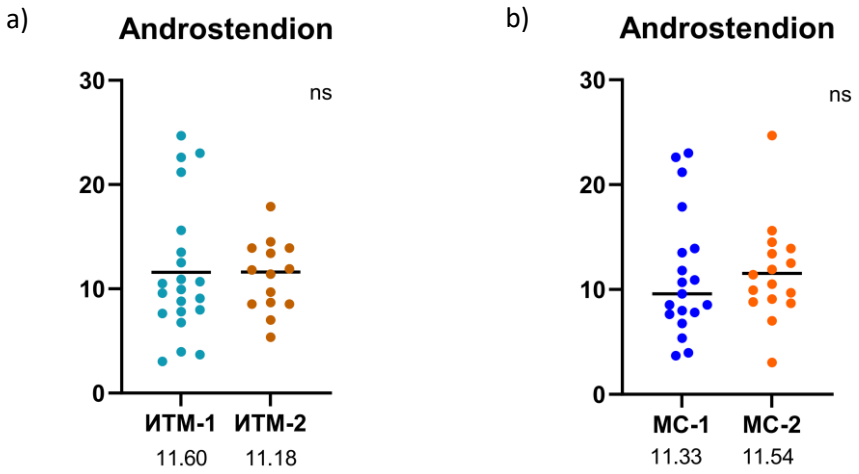


Fig. 67 **Androstendion levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

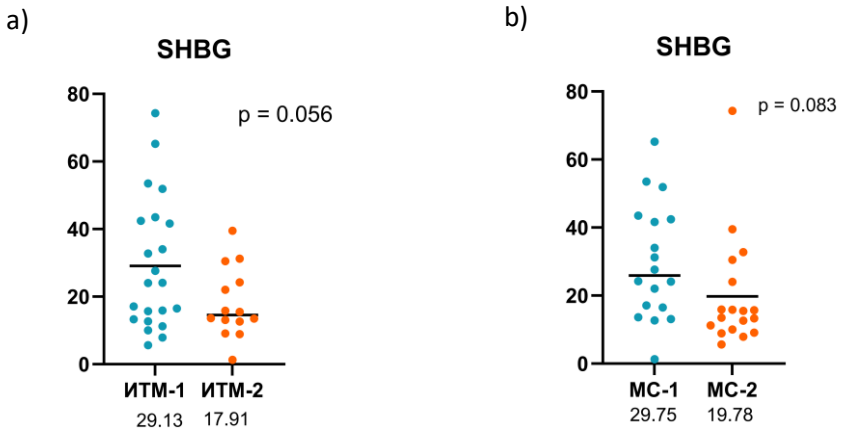


Fig. 68 **SHBG levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

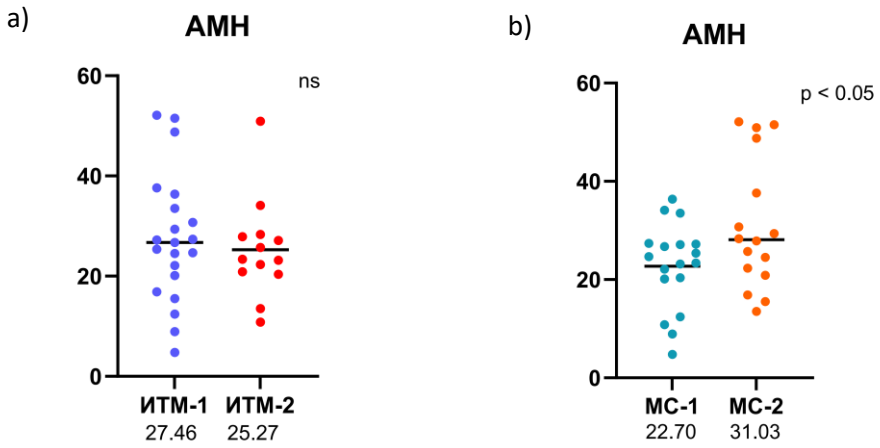


Fig. 69 **AMH levels in girls from groups: a) BMI-1 and BMI-2; b) MS-1 and MS-2**

CALCIUM AND PHOPHATES

Moderate hypovitaminosis D was found with mean levels of 25(OH) vit D for the whole group in the suboptimal range - 17.77 ng/ml. There is a pronounced drop in 25 (OH) vit D levels parallel to an increase in the degree of obesity - 15.86 ng/ml in the BMI-2 group**. (Fig. 70)**

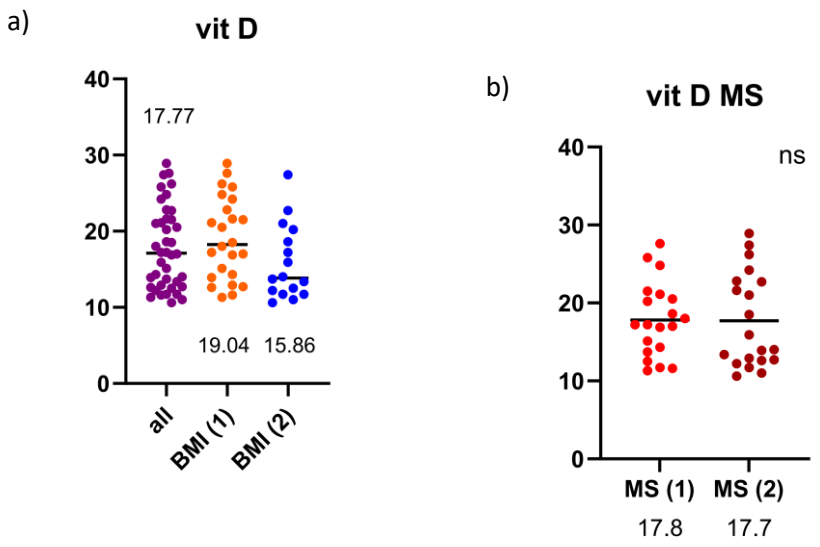


Fig. 70 **25(OH) vit D levels a) in all participants and according to BMI; b) according to MS risk factors**

Levels of 25 (OH) vit D decrease significantly with increasing degree of obesity (% Ob), the amount of FM), VFI and VF level. **(Fig. 71)**

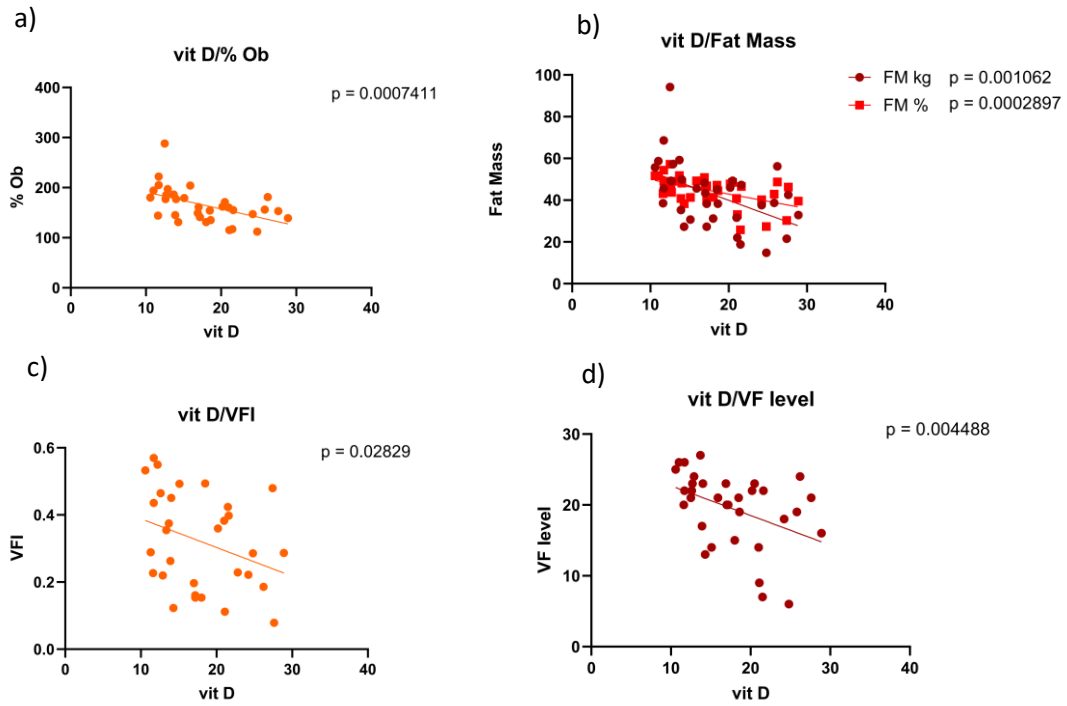


Fig. 71 **Correlations between 25 (OH) vit D levels and: a) % Ob; b) FM; c) VFI; d) VF level**

There are normal levels for serum calcium and inorganic phosphate, as well as normal values for parathyroid hormone. **(Tab. 48)**

Tab. 48 **Results for serum calcium, inorganic phohates, vitamin D and parathormone**

	BMI-1 (N = 25) (SD)	BMI-2 (N = 16) (SD)	Confidential interval (95% CI)	P value
25(OH) vit D	**19.04 (5.41)**	**15.86 (4.90)**	**-3.181 ± 1.682 (-6.587, 0.2245)**	**.066**
Serum Calcium	**2.40 (0.11)**	**2.37 (0.10)**	**-0.0312 ± 0.034 (-0.0998, 0.0365)**	**.354**
Inorganic PO4	**1.25 (0.11)**	**1.197 (0.16)**	**-0.0533 ± 0.0445 (-0.144, 0.037)**	**.239**
Parathormone	**28.07 (11.36)**	**26.16 (14.65)**	**-1.908 ± 4.347 (-10.74, 6.93)**	**.663**

V . DISCUSSION:

1. EPIDEMIOLOGICAL PART

Our epidemiological results are comparable to those previously published in other countries. Data reported in the literature up to date show children's fracture rates ranging from 12.0/1000 to 36.1/1000, meaning that the fracture rate that we found of 13.1/1000 is relatively low**. (Tab. 49)**

Tab. 49 **Fracture rates reported from other European countries and the data from our study**

author	***age***	***period***	***country***	***frequency***
Landin	0-16	1950-1979	Швеция	21,2/1000
Worlock	0-12	1086	UK	16,0/1000
Cooper	0-17	1988-1998	UK	13,3/1000
Moustaki	0-14	1996-1998	Гърция	12,0/1000
Kopjar	0-12	1992-1995	Норвегия	12,8/1000
Tiderius	0-16	1993-1994	Швеция	19,3/1000
Lyons	0-14	1996	Скандинавия	16,0/1000
Lyons	0-14	1996	Южен Уелс	36,1/1000
Brudvik	0-15	1998	Норвегия	24,5/1000

Rennie	0-15	2000	Шотландия	20,2/1000
Hedstrom		1993-2007	Швеция	20,1/1000
Boyadzhiev	0-17	2020-2021	България	13,1/1000

Regarding the gender distribution in our study, the male gender clearly predominates, with boys constituting 57% of children with fractures and girls 43%, a ratio of 1.38. These results are also expected and are in line with the data available so far - everywhere a greater fracture risk is found among the male sex. According to literature, the fracture frequency ratio between the two sexes is between 1.4 and 1.9, and these differences are most pronounced after 12-13 years of age, when boys break 2 to 5 times more often than girls. Our data shows that between the ages of 13 and 18, boys broke 3 times more than girls.

The age distribution curves of fractures among both sexes in our population show a bimodal pattern, which was previously found in many other studies (originally described by Rennie et al.). There is a low fracture incidence in the first 4-5 years of age, a first weaker peak in pre-puberty, with the highest incidence in the pubertal years of active skeletal growth - between 12 and 16 years in boys and between 10 and 14 years in girls.

The data on the distribution of fractures by location are in line with those observed in many other studies earlier – upper extremity fractures are the most common, accounting for about 2/3 of all fractures, while lower extremity fractures account for about 1/4 of the total number of fractures and are mostly characteristic of the younger age of up to 5 years.

Due to the nature of the survey method of data collection, it was difficult to set a precise algorithm for more specific determination of fracture locations. For this reason, it was left to describe the fractures mostly in free text, the answers were more generally categorized as: "arm fractures", "wrist fractures", "foot fractures", etc. The overall incidence of upper extremity fractures (excluding fingers) in our study averaged 53-54% for both sexes (57% for boys, 50% for girls). For comparison, the overall frequency of fractures in the area of the upper limb (without fingers) found by other researchers is similar: Hedstrom – 48%, Worlock – 56%; Rennie – 58%.

Our data regarding the fracture frequency among Bulgarian children is a good basis for tracking possible future changes in these epidemiological characteristics. Until now, especially

in connection with the increase in the percentage of obesity and the change in physical activity among children, including in connection with the recently passed Covid-19 pandemic, in other countries in the last few decades interesting trends for a change in the epidemiology of childhood fractures have been observed. For example, in 1997 Landin et al. found a doubling of fracture rates among children in Sweden for the period from 1950 to 1979 - an increase of about 60% in girls and about 35% in boys. Subsequently, Tiderius published data from 1993-1994, which showed a 9% decrease in the incidence of fractures among Swedish children compared to the period 1950-1979, from 21.2/1000 to 19.3/1000, but also found an increase of distal forearm fractures in girls by 31% over the same period. In Finland, Mayranpaa in 2010, also found an increase in the incidence of fractures by 31% in the forearm and by 39% in the arm. These data are also confirmed by D. Jerrhag, who found an 18% increase in the frequency of wrist and forearm fractures in children under 16 years of age in the region of Skåne, Sweden for the period 1999-2010, and Hedstrom et al. in 2010, who reported a further increase in the number of fractures in Sweden among children and adolescents under 19 years of age - for the research period 1993-2007 the overall fracture incidence increased from 15.1/1000 to 24/1000 – an increase of 59 %. For femoral fractures among children under 18 years of age, a significant increase in incidence was also reported from 0.28/1000 in 2000 to 0.94/1000 in 2010. Interesting data were published in 2022 by Oh et al., who found a significant reduction in trauma and fracture frequency during the Covid-19 pandemic, which was probably due to the imposed isolation and the reduced physical activity.

Among children with fractures, data from the literature show that those with 2 or more fractures are about 25% for boys and 15% for girls. In comparison, our results indicate a significantly higher percentage. Among the children who sustained fractures, 42% of boys and 31% of girls had more than one fracture, making this high-risk group significantly larger than expected.

These data are of great importance as the number of fractures has been shown to be one of the main predictive factors of reduced BMD in adulthood and increased risk of new fractures in future. Back in 2005, Goulding et al. found that children who broke for the first time before the age of 10 had a greater risk of new fractures at a later age.

In 2006 Manias et al found significantly lower BMD in children with 2 or more fractures compared to children with only 1 fracture or no fractures. It turns out that the presence of

previous fractures, the lower bone density and obesity are independent risk factors for future fractures, with obesity alone increasing the risk 1.5-fold, and the reduction in whole-body bone density of 1 SD (6.4%) almost doubling the risk. In another prospective study of a group of girls (100 with fractures and 100 without fractures) followed over a period of 4 years, Goulding et al. found that in the group with prior fractures, 24 girls reported new fractures (total number of 37 fractures), and in the group without prior fractures, only 7 girls had fractured in the past 4 years (total of 8 fractures). The final data indicate that girls with 2 risk factors have a significantly higher risk of new fractures: 1) presence of a previous fracture + low LS BMD - 9.4 times greater risk; 2) previous fracture + overweight - 10.2 times greater risk; 3) previous fracture + low whole-body BMD - 13.0 times greater risk.

Of interest here is the question of whether it is a congenital predisposition to easier bone fragility or the observed increased fracture frequency in these children is more due to factors related to the environment, behavior, etc. Also, is the existing susceptibility to fracture transient or does it really persist into adulthood?

Several large studies have attempted to answer this question, although so far the results are conflicting. For example, in 2009, Pye et al. published results, part of the large-scale international study EPOS (European Prospective Osteoporosis Study), in which 6451 men and 6936 women over the age of 50 were included. Fractures experienced in childhood (between 8 and 18 years of age) were reported by 547 (8.9%) of the men and 313 (4.5%) of the women. The DXA analysis and the history of fractures occurring in adulthood found no differences between those reporting childhood fractures and the rest of the participants, therefore the authors concluded that childhood fractures were not a predisposing factor for future fractures.

On the other hand, Amin et al. in 2013 found an association between the incidence of childhood forearm fractures with increased fracture risk in adulthood among men but not among women. Buttazzoni et al. published in 2013 intriguing results from another prospective study started in 1979-81 including 90 children with fractures and 130 controls (mean age 10 years), in which forearm BMD was measured by the single photon absorptiometry (SPA), a technology that preceded DXA osteometry. After a mean period of 27 years (25-29), 75 of the participants with fractures (about 85% of the original participants) and 84 of the controls (about 65% of the original controls) had new osteometric examinations including, in addition to SPA measurements (with same apparatus used at the beginning), DXA, QUS and pQCT analysis. The final data showed that, compared to controls, participants with childhood

fractures had moderate deficits in osteometric parameters (smaller BMD and smaller bone area) at both baseline measurements and during follow-up. The main conclusion remains the fact that the bone mass deficit in childhood is not compensated at a young age and persists over time, i.e. in some individuals, optimal PBM cannot be reached.

Farr et al., 2014, reported lower BMD and reduced bone strength among young adults with childhood minor trauma distal forearm fractures. Similar data were presented by Kim M. et al., 2022, who found that women with 2 or more fractures in childhood suffered more fractures in adulthood and had significantly lower bone density at age 45 in the femoral neck compared to those who had no childhood fractures.

Regarding the accompanying other musculoskeletal complaints, no differences were found between their frequency and type (most often knee pain, low back pain, scoliosis) between children with fractures and those without fractures, therefore it can be assumed that they are not directly related to fracture risk. Musculoskeletal symptoms and diseases in obese children are a long-recognized problem and rank third after cardiovascular and neurological complications. These are expressed in joint and arthritic changes, frequent lower leg deformities, Blount's disease, epiphysiolysis and aseptic necrosis of the femoral neck.

2. CLINICAL PART

WHOLE-BODY (TBLH) OSTEODENSITOMETRY

The data from the conducted osteodensitometric measurements indicate that the mean TBLH BMD for all 41 participants +1.67 SD (0.1-3.8 SD) is significantly above the average norm for age and sex. In all the girls studied, TBLH BMD was also found to increase in with increasing body weight and BMI. This regularity has been observed in many other studies and is an expected result, at least if we take into account the fact that we are talking about osteometric data obtained by DXA technology, which gives two-dimensional images. DXA results are directly dependent on total body dimensions and especially on body surface area - a characteristic disadvantage of DXA measurements. Therefore, taller and heavier patients have higher TBLH BMD and TBLH BMC DXA values, and it is also known that obese children tend to be taller than their peers.

The mean height in the girls participating in the study is 165.4 cm **(Fig. 72)**

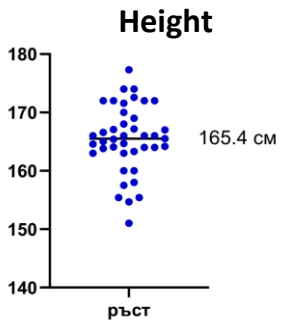


Fig. 72 Mean height of the girls in the study

This average height corresponds to P67 to P78 respectively for girls between the ages of 14-17. Data from radiographs taken in all participants showed an average bone age of 16.8 years and advanced synostosis in the growth plate. In other words, the measured mean height of the female participants, which was around P70 (or only about 0.5 SD above the mean) was very close to their likely final height. Therefore, we can conclude that the established higher values for TBLH BMD are not significantly influenced by the body height factor, but are mainly a consequence of the overweight of the studied girls.

An additional factor in establishing a higher BMD in obese girls is also the well-known tendency for them to enter puberty earlier, a period characterized by a rapid increase in bone mass. In our study, the mean age of menarche was 11.7 years, which corresponds to that found by Tomova et al. in 2009, the mean age of menarche for Bulgarian girls from the city of Sofia was 11.96 years. **(Fig. 73a)**

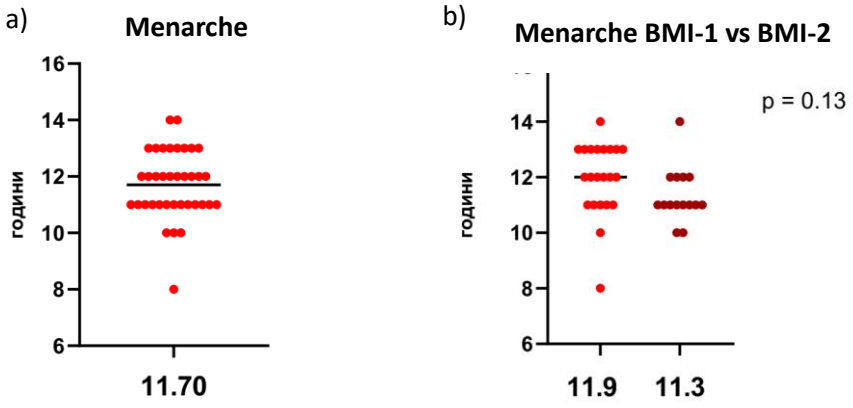


Fig. 73 Mean age of menarche a) in all participants; b) in girls from BMI-1 and BMI-2 groups

Of interest is the fact that in the girls we studied, those with more pronounced obesity from the BMI-2 group, had an mean age of menarche about 6-7 months earlier than the overweight or mildly obese girls from the BMI group -1. **(Fig. 73b)**

The absence of significant deviations in height and pubertal maturation in the girls we studied, compared to the general population, indicates that other reasons should be sought for the found positive correlation between body weight and TBLH BMD. One such explanation is generally accepted that the higher BMD in overweight and obese individuals is a reflection of adaptation of the skeletal system to increased body loads. These observations are part of the so-called "mechanostatic theory" introduced by H. Frost, according to which the bone is restructured according to the magnitude of the static and mechanical forces to which the skeleton is subjected under the influence of body weight. Numerous authors have concluded that in both obese adults and obese children, greater bone mass and BMD are a consequence of the need for greater muscle effort to perform usual daily activities, including maintaining postural tone and locomotion.

These adaptive processes in this case are a typical example of allometric body differences observed in nature. Allometry reflects the differences in the relative growth of a given tissue or body part compared to the growth of the whole body. Positive allometric deviations indicate the better development of a given body region in response to the greater demands imposed by the environment to preserve its anatomical and functional fitness. In this sense, smaller BAr and smaller BMC obtained from DXA measurements in smaller individuals do not mean worse bone structure or poorer bone strength, and vice versa. In this regard, Manzoni et al. as early as 1996 found that in obese children BMC was 46% greater in the area of the lower limbs and only 21% more in the area of the arms compared to children of normal weight.

In recent years, reports have become more frequent in the literature that in overweight and obese individuals, bone parameters recalculated according to body parameters - height, weight, BMI, the amount of FM and FFM, show similar or even lower values compared to those in the normosthenic population. Dimitri et al. in 2010 compared on the basis of DXA measurements of the whole body, lumbar region and radius two groups of children - 52 with obesity (13 without fractures) and 51 with normal weight (13 without fractures), with the DXA results adjusted for age, weight and height. The authors demonstrated that, regardless of the

method of correction, obese children with fractures, compared to children of normal weight and without previous fractures, had a smaller BAr and a lower BMD for all skeletal areas examined - up to a 1.2 SD reduction for whole body, 3.0 SD reduction for lumbar spine and 2.0 SD reduction for radius diaphysis.

Our results show a significant positive correlation between TBLH BMD and all parameters reflecting FM. The influence of FM on bone has long been the subject of many studies, but the data obtained to date are still quite controversial. The results are highly dependent not only on the gender and age of the studied population, but also on the statistical methods used and the need to take into account the many available cofactors.

It remains an open question to what extent FM may have some beneficial effect on growing bone, or whether there are other factors and mechanisms that account for the correlations we found. It is possible, for example, that the better BMD we found is to some extent due to obesity itself and the resulting greater mechanical stress on the skeleton. In a large meta-analysis including 27 studies covering a total of 5985 children aged 2 to 18 years, van Leeuwen et al. confirm the fact that obese children have a higher BMC and BMD compared to children of normal weight. However, in none of the studies included in the cited meta-analysis was correction of the osteometric results in relation to body parameters made. Subsequent studies have shown that after adjusting for body weight, obese children have the same or even lower BMD than normal weight children.

Our results also show that after adjustment for body weight TBLH BMD in the group of more obese girls (BMI-2 group) decreases and the difference with the BMI-1 group already loses significance. **(Fig. 74)**

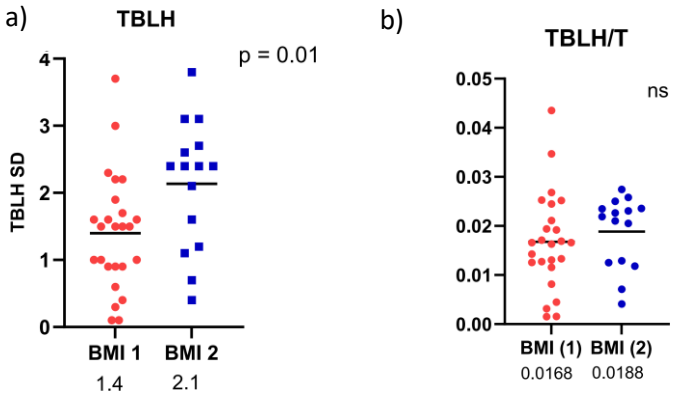


Fig. 74 **TBLH BMD according to BMI a) without body weight adjustment; b) after body weught adjustment**

Therefore, it is possible that the increased bone mass in obese children is not sufficient to absorb the increased skeletal loads caused by the excess weight, and this could be one of the explanations for the increased fracture rate among obese people.

Among others, there are data showing that the influence of FM on bone may change depending on the age period. Thus, for example, there are observations that if in early childhood excessive FM can improve bone strength by increasing FFM, then during the years of puberty additional fat accumulation is associated with no effect or with a negative effect on bone. It is also important to note that there are significant gender differences in the growth of FM. In girls, body fat percentage increases with age and reaches a plateau around age 18, rising gradually from an average of 31% by age 8-9 to around 36-37%, then continues to rise slightly until 25-29 year old. Conversely, in males, body fat percentage initially drops from an average of 27-28% to about 23% between 12 and 15 years of age, stabilizing at approximately 25-26% around age 20. However, studies of obese children suggest that these sex differences in FM rates may disappear as the degree of obesity increases. Despite the greater amount of FM in girls, among healthy children and adolescents with normal weight, it is found that boys have a larger amount of VF mass, which is observed as early as 10-11 years of age.

The positive correlation that we found between TBLH BMD and the AF% and A/G ratio, is probably due more to the increased mechanical load than the obesity itself, but the fact is that with increasing weight there is a tendency to increase the android type of obesity more than the gynoid fat. Android obesity is associated with an increased risk of developing metabolic abnormalities, incl. insulin resistance and carbohydrate metabolism disorders. In confirmation of this, with an increase in the degree of obesity, we found a parallel increase in the amount of VF mass, which between the two groups of participants divided by degree of obesity reached a significant difference of 63% for the absolute mass of VF and up to 60% for the volume of VF. Moreover, with an increase in the amount of VF (VF%, VF mass, VF volume, VFI), a progressive deterioration of the BIA indices for metabolic control (VF level and InBody%) is also established. Contrary to our results, there are also reports in the literature of finding a negative relationship between TBLH BMD and the android type of obesity. For

example, in a study on the relationship between FM and BMD involving children between 6 and 10 years of age, Liang J et al. concluded that body fat has a negative effect on BMD, mainly in children who have an android type of fat distribution, possibly due to greater BMD accumulation.

It is possible, in fact, that the positive influence of FM is valid only up to a certain degree of obesity, after which, due to the additional accumulation of fat and the unlocking of metabolic disorders, this effect is lost and the subsequent influence of FM on the bone becomes negative. P. Dimitri et al. also conclude that if in earlier childhood (prepubertal age) excess FM can improve bone strength by increasing FFM, it subsequently begins to have an independent negative effect.

In fact, if we look at the same correlations between TBLH BMD and BMI, only analyzed in the separate groups of participants we studied - BMI-1 and BMI-2, we will see that if in the group of overweight and mildly obese girls these positive relationships are well expressed and show significance, but for the group with high and extreme obesity, the "beneficial" influence of VF starts to weaken - a significant correlation is no longer established. And even more - if we consider only the subgroup of girls with extreme obesity, despite the really small number of patients in it, we will already find the presence of even a negative relationship between the amount of VF and BMD.

Interestingly, similar data are reported in other studies. In a study involving boys and girls aged 10 to 19 years, Mosca et al. in 2014 found lower mean values of BMC and BMD for all examined skeletal areas only in the group of extremely obese girls (BMI > 99- th percentile) compared to girls with a BMI between the 95th and 99th percentile, although no such differences were found in boys. In 2019 Rokoff et al. published data from a study of peripubertal children (mean age 7.7 years) and found that central obesity had a negative association with BMD only in children with excessive fat accumulation – a percentage of total FM above the 85th percentile. Lopez-Peralta et al. found that, unlike FFM, total and abdominal FM correlated well with TBLH BMD only in normal-weight and overweight children, but not in those with higher degrees of obesity. It seems that gradually with increasing weight and progressive fat accumulation, the influence of FM on BMD begins to decrease and after a certain degree of obesity disappears.

Data on the reciprocal influence of subcutaneous and VF mass on bone metabolism are established in many other studies. In a study of healthy girls and young women (15-25 years of age), Gilsanz et al. in 2009 found that while the subcutaneous FM shows a positive correlation, the VF mass is inversely proportional to all the examined bone parameters in the area of the femur - total BAr, cortical area, bone strength indices. Russell et al., 2010, studied 30 girls (15 obese and 15 normal weight) and found that obese girls with the highest BMD and the highest ratio of visceral to subcutaneous FM had the lowest values of TBLH BMD and LS BMD. In 2013 Junior et al. in a study of 175 obese children found the lowest BMD values among children with the highest percentage of BMI.

THE INFLUENCE OF FREE-FAT MASS ON BONE PARAMETERS

The importance of FFM for the normal development of the underlying bone has been known for several decades. Many childhood muscle diseases (muscular dystrophies, spina bifida, poliomyelitis, etc.) are accompanied by disturbances in normal bone growth. During puberty, the growth of FFM precedes that of bone mass by several months in both sexes. The peak rate of increase in FFM relative to the peak rate of bone mass accretion occurred about 0.36 years earlier in boys and 0.51 years earlier in girls.

For the correct analysis of the results obtained by us, we should note that FFM is influenced first of all by the height of the patient, therefore the ratio LBM/height, automatically obtained by the pediatric DXA software, also occupies an important place in the analysis of body distribution and its relationships with bone parameters. Low values of the LBM/height indicate a potential muscle deficiency. In obesity, as a result of excessive body mass, there is an increase in LBM and especially in the skeletal muscles, as a result of the need to provide sufficient muscle strength to move the body in space. For this reason, overweight and obese children have a high LBM/height ratio. This was also established among the girls we studied - the average value for LBM/height among all the girls studied was P91, and by group it was P88.6 and P94.5, respectively for BMI-1 group and BMI-2 group. **(Fig. 75)**

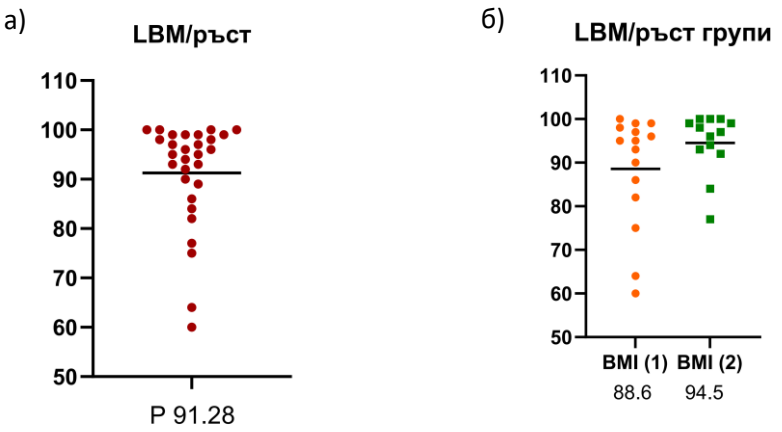


Fig. 75 LBM/height ratio a) in all participants; б) according to BMI

The high values of LBM/height in combination with normal or low values of BMC/LBM, as we found among the girls we studied (mean value P56.2 for BMI-1 group and P38.4 for BMI-2 group), speak of a pronounced bone deficiency, and this was observed in all the participants in the study. Similar are the conclusions made by the groups of Crabtree and P. Dimitri who found that in obese children there is a greater muscle mass relative to height compared to children of normal weight, but also a lower BMC relative to the available muscle mass in the obese, which suggests the presence of primary bone deficiency.

From the obtained results, it can be seen that without taking into account the amount of LBM, a positive correlation is established between TBLH -BMD and the central type of fat accumulation (TBLH BMD to the percentage of android FM and TBLH BMD to the A/G ratio), while in analyzing cardio-metabolic risk factors relative to LBM, such correlations with DXA data for the android and gynoid type of obesity are no longer established. Moreover, there is a well-presented negative correlation between BMC/LBM and the distribution of FM in the torso and lower limbs, where even statistical significance is reached.

Similar to our results were obtained by Glass et al., who in girls aged 11-19 years, unlike the results in boys, did not find significant relationships between the A/G ratio and bone parameters, incl. bone strength. They consider that the A/G ratio in obese girls may be strongly influenced by the fact that females normally accumulate more subcutaneous fat the gynoid region during puberty anyway. Thus, the A/G ratio may incorrectly reflect the increase in VF mass in obese adolescents. In other words, behind the higher A/G ratio in obese girls there is also a much higher amount of total subcutaneous FM, which, due to increased mechanical

loading, is known to stimulate osteogenesis in the load-bearing parts of the skeleton. Thus, ultimately, the greater amount of subcutaneous BMI in obese girls is very likely to compensate for the negative influence of BMI on bone, thus erasing or changing the direction of the observed correlations between the amount of fat and bone parameters.

In addition, there are several studies whose data show that there are metabolic differences in the activity of superficially located subcutaneous MT and that located in depth. The deep subcutaneous FM has been found to have regulatory metabolic functions similar to those of the VF, and is better represented in the male sex, which may to some extent explain the well-known gender differences in the risk of developing metabolic abnormalities, especially in the presence of obesity.

Similar to the differences we found in the influence of FM on bone parameters, without and after correction for FFM, have been observed in other studies. For example, in a study of 3082 children with an average age of 9.9 years, E. Clark found a strict positive correlation between FM and TBLH BMD and BAr. However, this correlation decreased significantly after adjusting the resultsfor FFM and patients' height. On the other hand, during the two-year follow-up of the same children, FM remained a very good positive predictor for increase in BAr.

In their longitudinal study among girls, Glass et al. found that after adjustment for FFM, the previously observed positive influence of total and subcutaneous FM on BMD and bone strength at the radius and tibia was lost, and the lack of association between bone parameters and VF before taking FFM into account then indicated negative correlation. In addition, the same team found that the negative effect of VF on bone strength was more pronounced at a younger age - 1 year before reaching peak growth velocity compared to 1 year after.

Khwanchuea et al. found that in adolescent girls, FFM has a positive effect on BMC regardless of the amount of FM, resp. of body weight, while FM itself has a positive effect on bone only among obese girls.

Cristi-Montero et al., 2022, studied 1296 boys and girls aged 10-14 years and found that FFM accounted for about 30% of the negative correlation between FM and BMC, and this relationship was slightly more pronounced in girls (+3%) and in children of normal weight compared to obese adolescents (+13%).

The relationship between FM and the skeletal system appears to depend on several factors, the most important of which are 1) age (early childhood, puberty, adulthood, or senile), 2) degree of obesity (moderate or excessive) and 3) the localization of fat deposits - android or gynoid type, the amount of subcutaneous fat and VF (central fat, truncal fat), the appendicular fat, the skeletal muscle fat or that in the bone marrow, etc.

The difficulties in interpreting the effects of FMT and FFM on bone are best illustrated by the data of Pollock et al., who in a 2007 study of 18-19 year old girls (93 normal weight and 22 overweight) found a negative relationship between percentage FM and indicators of BMD and bone strength in the area of the radius. However, the authors found a positive correlation between FM and BAr and tibial diameter. In overweight girls (average +9 kg), no better pQCT bone indices were found for the examined femur and forearm bones, indicating that the additional loading of the skeleton has no significant advantages in terms of bone structure and strength, incl. the most mechanically loaded bones of the lower limbs. After recalculating the results according to the amount of muscle mass (expressed as MSCA = muscle cross sectional area), overweight girls showed a smaller cortical area and smaller BMC and bone diameter for the tibia, as well as a smaller total BAr for the radius in compared to normal weight girls. The established dependencies are more pronounced in the diaphyseal areas of the examined bones and to a lesser extent in the area of the metaphyses - data showing that FM has a negative effect more in areas made up mainly of cortical bone than in those made up mainly of trabecular bone.

Regarding our results on the relationship between FM/LM ratio and BMC, since there is no difference in mean height between girls in BMI-1 and BMI-2 groups, it can be assumed that the lag in bone sizes in girls from the BMI-2 group should be primarily due to compromising the processes related to the periosteal apposition of newly formed bone, i.e. of disorders in the growth of tubular bones in width (smaller bone diameter).

In general, as has been repeatedly discussed so far, overweight children are taller, have better developed muscle mass and larger bones. These changes are significantly better expressed in the area of the lower legs compared to the forearms - differences that are easily explained by the fact that the lower limbs take the main burden of the body weight, which is why they also have better developed musculature.

Analogous to our data are also found in other studies, which also found that obese children had a greater percentage of FM in the forearm region, where the ratio of fat/muscle

mass reached almost 1:1, while in the lower leg region it was about 0.7:1. Ducher et al. found that a higher fat/muscle mass ratio was negatively correlated with bone strength, and this was independent of body weight.

In our study, in the more obese girls of the BMI-2 group, the FM/LM ratio was greater than 1 for both the lower and upper extremities, indicating that in them FM exceeded muscle mass, which, as shared by others authors, may mean that in severely obese people compensatory skeletal muscle development in the limb region is probably insufficient.

Our data indicate that an increase in the values of the FM/LM ratio correlates strongly with lower bone mass and smaller bone sizes, and these negative correlations are also well represented in the total group of participants, but are entirely due to the statistical significance found among girls from BMI-2 group.

Our results showing a strong positive correlation between TBLH BMD with all indicators of FFM, incl. those for skeletal muscles, are in line with data from two recently published large meta-analysis reviews that summarize the results of similar studies conducted over the past few years. The authors conclude that the increase in bone mass (BMD and BMC) during childhood is directly related to the increase in FFM, and especially muscle mass, a correlation that is observed, according to some, even in infancy. Data indicate that upper limb muscle strength shows a greater relationship with bone parameters than lower limb muscle strength. Deng et al. found that, unlike FFM, total FM showed a weaker positive correlation with BMD, and after adjustment for potential confounding factors, the relationship was abolished for all skeletal sites except the femoral neck. Moreover, the relative amount of FM expressed as a percentage of total body weight was found to be negatively related to bone.

BONE AREA

Skeletal dimensions are directly related to body parameters and are generally expressed in bone volume and BAr. DXA osteometry, as a two-dimensional technology, cannot measure bone volume and only reports BAr, therefore the obtained results should be adjusted mostly to the height of the patient. For this purpose, the pediatric software of the device automatically calculates the ratio between the measured BAr and the patient's height - area/H, which, expressed in percentiles, indirectly shows to what extent the available BAr corresponds to the patient's height for the given age and gender. In other words, BAr is determined on the

one hand by the length of the tubular bones and the height of the vertebral bodies ("long" or "short" bones), and on the other hand by the width of the bone elements ("narrow" or "wide" bones) .

The very low BAr/height levels we found are highly disturbing. Taking into account the fact that the average height of the studied participants is about P70, i.e. these are relatively tall girls, it can be concluded that the low BAr/height values found in them should be due to a smaller bone diameter than to a smaller bone length, i.e. in obese girls there is a marked deficit in the growth of broad bones.

It appears that in obese adolescents the elevated body fat disrupts bone growth primarily by affecting the periosteal apposition by which the bones grow in diameter. The lower values for BAr that we found in the group of girls we studied contrasts with the high levels of BMD found, which in turn indicates that the amount of accumulated BMC is not much greater, but is simply concentrated in a smaller skeleton .

Bone sizes are direct determinants of bone strength and any disturbance in the proper growth of the skeleton, incl. bone diameter, may be key to the additional increase in fracture risk. Therefore, the smaller arm BAr that we found in girls with high and extreme obesity probably plays an important role for their higher frequency of upper extremity fractures (mostly forearm) compared with children with normal weight.

A deficit in BAr was also reported by Skaggs et al. in 2001. In a CT examination of girls with fractures, they found a greater body weight and a smaller cross-sectional area of the radial bone compared to their peers without fractures. Later, in 2006, E. Clark et al., in a study involving more than 6200 children, found that TBLH BMD and Bar adjusted for weight and height were the main risk factors for fractures.

Pollock et al. in 2007 reported a negative correlation between body fat percentage and pQCT measures for BAr in pubertal girls, and Glisanz et al. in 2009 found that BMD was negatively associated with a number of bone parameters, including total BAr, cortical area and indices of bone strength.

OSTEODENSITOMETRY RESULTS OF LUMBAR SPINE

From the obtained data on LS BMD, the first thing that makes a strong impression is the fact that the values of LS BMD, expressed as standard deviations, are significantly lower than those of TBLH BMD - respectively z-score 0.77 against z-score 1.67, a difference of 0.91, although at the same time a very good correlation was found between TBLH BMD and LS BMD values in individual patients. **(Fig. 76)**

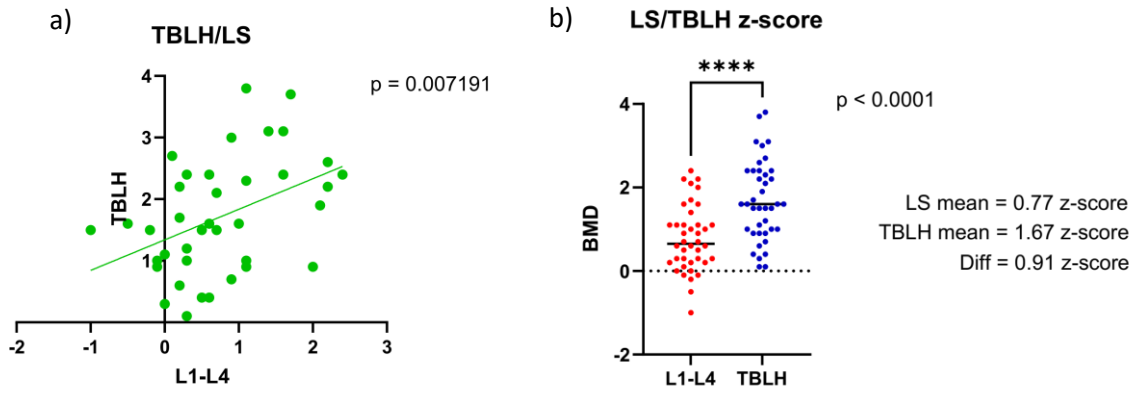


Fig. 76 a) correlations between TBLH BMD and LS BMD; b) comparisson between TBLH BMD SD and LS BMD SD

The absence of any correlation of LS BMD with body weight, BMI, WC and %Ob is in stark contrast to the data from the whole-body analysis (TBLH BMD unadjusted for weight and FFM), where TBLH BMD showed a strong positive influence of all the factors listed above. Obviously, in the spine, the influence of excess body mass has probably a weaker or at least a more specific influence.

Explanations for the observed differences between TBLH BMD and LS BMD data can, of course, be multifaceted. Thus, for example, since the vertebrae are mainly composed of trabecular bone, which is known to be more metabolically active, it can be speculated that the metabolic abnormalities accompanying obesity probably play a negative role in the adaptation of these skeletal compartments to the otherwise stimulating influence of purely mechanical overload. Or simply, the cortical bone is actually the one that is mainly responsible for the adaptation of the skeleton to the increased load, while the trabecular department has more metabolic functions and participates in the maintenance of calcium-phosphorus homeostasis.

In any case, our results for LS BMD mainly show that in obesity, lumbar bone is either not affected to such an extent or simply cannot adequately respond to increased mechanical loads in the same way as the skeleton as a whole.

Of particular interest are the results for a statistically significant negative correlation between LS BMD and the FM percentage, observed only in the less obese girls of the BMI-1 group, as well as only among the girls with more MS risk factors from MS-2 group. These results seem to show again that the influence of obesity probably has a dual nature - higher weight on the one hand can to some extent stimulate the accumulation of greater bone mass, while the appearance and deepening of metabolic disorders with their negative impact on bone can wipe out and shift any "benefits" from being overweight. The tendency we found for an increase in LS BMD in parallel with an improvement in metabolic risk indicators (VF level, InBody score), although not statistically significant, also speaks in favor of this hypothesis.

Furthermore, in our results, LS BMD did not show correlations with the strongest stimulus for osteogenesis – FFM (muscle mass), and a weak positive correlation was found only with the percentage FFM and again only in girls with mild obesity. These results, in our opinion, suggest that the influence of FFM, and in particular skeletal muscle itself, on the axillary skeleton is probably much weaker compared to the pro-osteogenic role that FFM exerts on limb bones. Vertebral bodies, and especially those in the lumbar region, have a limited range of motion and mainly play a supporting role in terms of body integrity (the body core) and maintaining the balance of the body. In contrast, bones in the limb area carry out a much greater range of motion. Through them, not only the movement of the body in space is carried out, but also a wide variety of activities are carried out, most of which, from a physical point of view, are carried out on the basis of the so-called lever mechanism. All of this is related to the application of large muscle forces to the underlying skeleton and in turn requires sufficient bone strength, which should also explain the strong positive relationship between muscle mass and bone mass in the region of the appendicular skeleton (limbs).

Similar results to ours have been reported in other studies, which found that lower spine BMD showed a positive correlation with FFM only in normal-weight or overweight children, but not among obese children. On the other hand, there is evidence that no correlations are found between LS BMD and total and abdominal FM in non-obese children, while a negative one is found only in obese children.

Julian et al., 2021, found no differences in TBLH BMD values between moderately obese (BMI P95-P99) and extremely obese (>P99) girls and boys, but found significantly lower TBS (trabecular bone score) and LS BMD in extremely obese participants. Deviations in TBS values indicate that the apparently reduced LS BMD in the extremely obese is due to changes in the trabecular microarchitecture of the lumbar vertebrae itself. Similar results were reported in studies of young adults, where a negative correlation was found between TBS with BMI, total fat and VF, independent of FFM.

CARBOHYDRATES AND BONE

Our results showed the positive correlation of BMD with fasting HbA1c and serum insulin values, which were best manifested in the girls of the MS-2 group. The influence of glycemia and the presence of insulin resistance on bone turnover have been widely discussed in the literature, especially in studies of adults, including patients with prediabetes and T2DM. There is evidence that insulin exerts an anabolic effect on bone turnover at physiological levels. This also explains the data from studies that found higher values for BMD among type 2 diabetics. Glycemia itself may also have some pro-osteogenic influence, while insulin probably exerts a direct anabolic effect on bone, activating osteoblastic function through a stimulating synergistic effect together with IGF-1 and parathormone and increasing osteocalcin production. Other studies have reported lower LS BMD and lower TBLH BMD in insulin-resistant and prediabetic adolescent girls after adjusting the bone parameters for height and FFM. Our results did not establish statistically significant correlations between bone mass adjusted for FFM and the investigated indices of carbohydrate metabolism.

LIPIDS AND BONE

Our data on correlations between lipid parameters and osteometric bone parameters are complex and multidirectional, making them difficult to interpret. We hypothesize that the effect of excess body mass rather than the initial dyslipidemia associated with obesity is behind most of the results thus obtained. Our findings of a positive association between TG levels and BMD (both whole-body and lumbar) and a negative correlation between HDL-cholesterol and LS BMD have been reported in studies in adults. Han et al. for example, found

in women a significant negative correlation between HDL-cholesterol and BMD of the lumbar spine and BMD of the femoral neck, while the same correlations did not show significance in the men they studied. Furthermore, they found that regardless of gender, TGs were positively correlated with LS BMD and femoral neck BMD, results that are in agreement with our data. In a study of adolescent boys and girls, Wang GX et al. found a negative relationship between HDL-cholesterol levels and BMD only in males. Also interesting are the data reported in 2012 by Lawlor et al. They studied 2305 adolescents, mean age 15.5 years, and found that decreased HDL-cholesterol levels and increased fasting blood glucose and serum insulin were negatively correlated with BMD and BMC values. Adjusted for FM, however, these dependencies disappear. The importance of lipid metabolism disorders and the risk of developing osteoporosis is also confirmed by the data from a study by Abramowicz et al., including 103 obese children, in which the authors found a correlation between fracture risk and elevated serum cholesterol levels in girls.

URIC ACID AND BONE

The significant positive correlation we found between UA and TBLH BMD is an interesting finding that has been reported so far in other studies, mostly in adults. In 2016, Veronese et al. conducted an extensive meta-analysis (19 studies, > 55,800 patients) summarizing the results collected to date. First of all, they concluded that the protective role of UA in relation to bone metabolism should mainly be due to its pronounced antioxidant activity - UA is responsible for 30-50% of the extracellular plasma antioxidant activity. In addition, UA levels were found to increase in parallel with increasing BMI and decreasing 25(OH) vitamin D levels.

One of the few studies in children and adolescents was published in 2020. Pan et al. examined 7,320 boys and girls between the ages of 12 and 19. The results show that there are age and gender differences. A relationship between UA and BMD was found mostly in younger participants - aged 12 to 15 years, and mostly in boys compared to girls. In girls, a U-shaped distribution of the association between UA and BMD was found, indicating that highly elevated UA values may have a negative effect on BMD in females.

Other studies have shown that racial and population differences may also be involved, and body weight is also important. For example, a study by Zhang et al. conducted in China found a negative correlation between UA values in obese men but not in obese women.

HORMONES AND BONE

Regarding the relationship between the hormones we studied and the bone parameters, it is striking that significant correlations are found in the general group and only in the distribution of girls according to MS criteria, but not in their distribution according to the degree of obesity.

In our opinion, the most important results in this part of the study are the significant negative correlation found between SHBG and TBLH BMD and the significant positive correlation between testosterone levels and lumbar BMD indicators – LS BMD and BMAD. A negative association between SHBG levels and BMD has been described in numerous studies to date, most commonly attributed to the activation of bone resorption. More interesting are the data of Zhu et al., who, in a study of postmenopausal women, found that the association between SHBG and BMD has a rather U-shaped shape - i.e. in addition to high SHBG values, subnormal levels can also compromise BMD. Similarly, Xu Ke et al. found a U-shaped association between testosterone levels and BMD in a study of girls aged 12-19 years.

Regarding our results, since the whole-body bone indices reflect more cortical bone status and those of lumbar vertebrae mainly trabecular bone, we believe that it is possible that SHBG exerts its negative influence mainly on the cortical skeleton, while testosterone mainly affects the building of the trabecular skeletal component. However, it is also possible that both observed correlations point in the same direction, given that SHBG and free testosterone levels are directly reciprocally correlated. SHBG is a plasma glycoprotein synthesized in the liver that binds sex hormones and thus regulates their bioavailability. Its levels increase in the prepubertal years, reaching their maximum just before the onset of puberty. SHBG binds with greater affinity to testosterone than to estradiol, and its levels are up to twice as high in women. Estrogens stimulate the production of SHBG, while androgens block it. A decrease in SHBG levels leads to an increase in free testosterone, which is difficult to examine, but can be calculated in the form of the so-called free androgen index -

FAI=100*(Total Testosteron/SHBG). In the girls studied by us, the ratio between SHBG and free testosterone is clearly reciprocal **(Fig. 77a).** The mean FAI value for the girls in the study is 9.1, which is on the uppere norm for the female sex (N 7-10) (Fig. 77b).

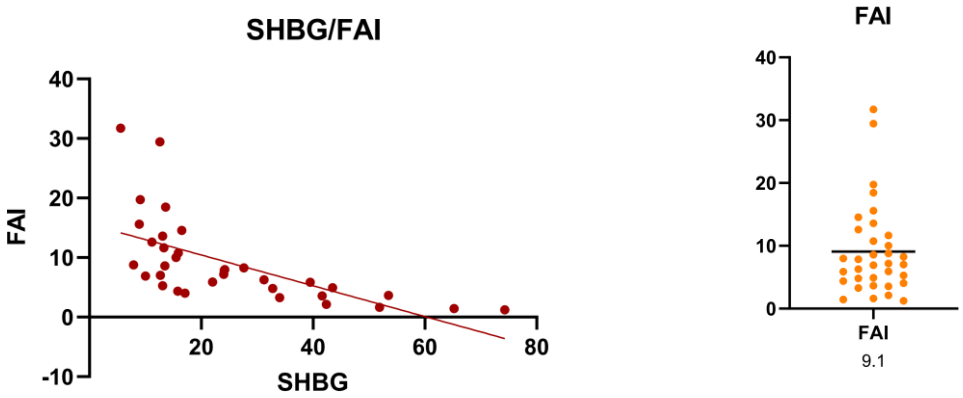


Fig. 77 a) Correlations between FAI and SHBG; b) Distribution of FAI in the study group

SHBG levels have long been found to be decreased in obese adults, for which hyperinsulinemia was initially thought to be primarily responsible, but it has since been found that increases in proinflammatory cytokines in obesity likely play a leading role. A statistically significant decrease in SHBG levels with increasing degree of obesity was also observed among the girls we studied. **(Fig. 78)**

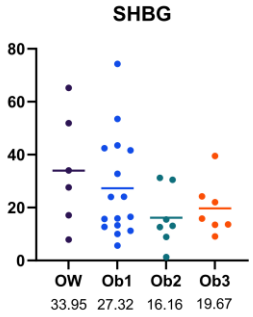


Fig. 78 SHBG levels according to the degree of obesity (OW = overweight; Ob1 – mild obesity; Ob2 – moderate obesity; Ob3 – extreme obesity

Last but not least, the finding of significantly lower estrogen levels in the girls of the MS-2 group compared to those of the MS-1 group is also an important finding - the hypoestrogenemia and the associated activation of osteoclast function, in our opinion, indicates that the girls with pre-existing metabolic complications are likely to be at risk of earlier onset of bone loss and earlier development of osteoporotic changes.

VITAMIN D, CALCIUM AND PHOSPHATES

We found hypovitaminosis D with mean 25(OH) vit D levels of 17.77 ng/ml. For the whole group of studied girls. These hypovitaminosis is moderate but could be considered potentially clinically significant despite the absence of abnormalities in calcium and parathyroid hormone levels. Due to sequestration in the FM, lower levels of 25(OH) vitamin D are a common finding in obese adults and children, and may further impair bone turnover by reducing calcium absorption in the GIT and the development of secondary hyperparathyroidism. Among adults, obesity increases the risk of developing hypovitaminosis D more than threefold, while in childhood, a meta-analysis published in 2020, which included 20 studies and a total of more than 24,600 children and adolescents, found a relative risk for hypovitaminosis D among obese children of 1 .41 (95% CI = 1.26---1.59), ($I2 = 89\%$, $p < 0.01$). Also interesting are the data from another meta-analysis, whose authors concluded that in obese children, the best way to correct hypovitaminosis D and improve insulin resistance is to give high doses of 25 (OH) vitamin D (> 4000U/day).

PART VI. CONCLUSIONS:

1. EPIDEMIOLOGY:

1) The overall fracture rate of 13.1/1000 found in our study is relatively low compared to data from other European countries (12-36/1000, average about 20/1000)

2) The established additional epidemiological characteristics – the distribution of fractures by gender, age, location, etc., are in line with the data from other studies published earlier

3) Among children with fractures, 42% of boys and 31% of girls had more than one fracture, which is higher compared to previously reported data in the literature.

4) In children with more than one fracture, as the number of fractures increased, there was an increase in the percentage of those who were overweight/obese, a trend more pronounced among girls.

5) No differences were found in the type and frequency of the additional musculoskeletal complaints between children without and those with fractures.

1. CLINICAL PART:

1) After adjustment for body weight, the differences in BMD between girls with different degrees of obesity disappeared, indicating that, from a purely mechanical point of view, the absolute bone mass of children with high degrees of obesity is probably insufficient to endure the increased mechanical loads.

2) The positive relationship between whole-body BMD and BMC progressively weakens with an increase in the degree of obesity, and in the subgroup of girls with extreme obesity a negative correlation is established, indicating that the "stimulating" influence of fat mass on the skeleton is present only up to a certain degree of obesity, and subsequently, with the additional accumulation of fat and the possible unlocking of metabolic disturbances, this effect is lost, as the subsequent impact of fat mass on bone accrual becomes negative.

3) The strong positive correlation between whole-body BMD and the fat free mass, including muscle mass as its main component, indicates that fat free mass is the leading stimulus for increasing whole-body BMD, but not the BMD of the spine.

4) BMC adjusted for fat free mass and bone area for height decrease progressively with increasing the degree of obesity and the amount of visceral fat, indicating the direct negative impact of visceral fat on bone tissue.

5) In highly obese girls, the amount of fat mass in the arms exceeds that of the muscles, and correlates negatively with BMI and bone area, indicating that in overweight people compensatory skeletal muscle development in the limbs is probably insufficient to build an adequately strong underlying bone.

6) In girls with severe and extreme obesity, a greatly reduced whole-body bone area and arms' bone area is found. The normal height of the studied girls suggests a deficiency in the growth of the diameter of the bones, which is probably one of the main causes for the increased rate of forearm fractures amongst obese individuals.

7) Unlike whole-body BMD, lumbar spine BMD values do not show a relationship with weight, BMI, waist circumference and other fat or fat-free mass markers – performing only a whole-body osteodensitometry measurements should be sufficient to determine bone status.

8) Less significant differences in bone parameters were found between the girls divided into groups according to the metabolic changes compared to those found when dividing the participants according to the degree of obesity - probably the role of the fat tissue per se (the mechanical overload, the production of adipokines, etc.) is stronger than the effects of the present metabolic disturbances.

9) The positive association between the whole-body BMD and the fasting HbA1c and serum insulin levels suggests that glycemia may have some pro-osteogenic influence, while insulin itself likely exerts a direct anabolic effect on bone.

10) A positive association between the uric acid levels and the whole-body BMD has been demonstrated in multiple studies in adults (adolescent data are few), which conclussions suggest that the protective role of the uric acid on bone may be due to its marked plasma antioxidant activity.

11) Mild to moderate hypovitaminosis D was found in all study participants with mean levels of 25(OH) vitamin D in suboptimal values - 17.77 ng/ml. The vitamin D levels decreased with the degree of obesity, probably due to the additional incorporation of vitamin D into the adipose tissue.

PART VII. CONTRIBUTIONS:

1) The current research is the first Bulgarian study on the epidemiology of children's fractures and the results are comparable to those previously published by other countries. The data are an excellent basis for future studies in the field of children's bone health in our country and especially for the follow-up of fracture rates among Bulgarian children in the future.

2) The results of the epidemiological analysis confirm that children who have sustained more than 2 fractures are indicated for referral and additional evaluation in order to clarify the presence of an underlying bone pathology.

3) For the first time in our country, a detailed analysis of body parameters and their relationship with metabolic disturbances has been made, proving the leading influence of fat mass and lean body mass on bone growth during adolescence.

4) For the first time in our country, DXA densitometric data for the amount of visceral fat mass are used and the correlations of viscera fat with bone health parameters are analyzed in adolescent children.

5) The results of the present study show that bone deficits worsen with the progression of the degree of obesity, with the need for routine osteodensitometric measurements in obese children, especially among adolescents with severe and extreme obesity.

7) The body composition data collected from the bioelectrical impedance analysis showed that in clinical practice it can be a new efficient and highly informative method for the diagnosis and follow-up of children and adolescents with overweight and obesity

8) The latent vitamin D deficiency found among obese girls warrants further studies and requires the introduction of wider screening for vitamin D status among obese children and adolescents.

PART VIII. PUBLICATIONS

Бояджиев В., Йотова В. Костна биология през периода на детството и влияние на затлъстяването върху растящата детска кост, сп. Педиатрия, бр 1/2011 год, стр 20-23

Boyadzhiev V. , V. Iotova, B. Varbanova. Epidemiology of Childhood Fractures in Bulgaria: A Retrospective Survey. ESPE Abstracts (2022) 95 P1-426

Boyadzhiev V., M. Stoyanova, V. Iotova et al. Osteoporosis-pseudoglioma syndrome (OPPG) in two Bulgarian girls with c.2409_2503+79del mutation in LRP5 gene. 14th International Conference on Osteogenesis Imperfecta, Sheffield, UK, 30 August – 2 September 2022, LM 9

Бояджиев В. Х-свързан хипофосфатемичен рахит, сп. „Педиатрия плюс“, бр 9/2023 година II, стр. 30-38

Бояджиев В. „Влияние на телесното тегло и мастната тъкан върху костната здравина и фрактурния риск при деца“, сп. Педиатрия, бр 2/2024 год, стр 7-10

Boyadzhiev V., V. Iotova, B. Varbanova. Bone growth deficits correlated with visceral fat mass volume in adolescent girls with severe and extreme obesity. ESPE Poster (2024) P3-283

PART IX. FINAL REMARKS

Over the past 20 years, childhood and adolescent obesity has become a major health problem not only due to its rapidly increasing incidence, but also due to the numerous associated comorbidities and complications. The number of obese children is expected to reach 250 million by 2030.

Conditions until recently almost unknown in pediatrics, such as insulin resistance, impaired glucose tolerance and arterial hypertension, are now increasingly common even at a younger age. Along with cardiovascular and metabolic complications, among overweight children, orthopedic problems related to the mechanical overload of the bone-joint apparatus - valgus and varus deformations of the lower legs, flat feet, epiphysiolysis and aseptic necrosis of the femoral head - have recently become more frequent.

The increased fracture rate among overweight and obese children is a relatively recently recognized medical problem. The main factors responsible for this increased fracture risk include the worsened indicators of BMD and reduced BAr, body excess weight itself and altered body composition - the percentage of muscle and fat mass and their distribution, especially the amount of VF, the metabolic status, the improper diet, the reduced physical activity, the balance disorders, the presence of concomitant complications and etc.

The complex relationship betweenFM and the skeletal system appears to depend on several factors, the most important of which are 1) age (early childhood, puberty, adulthood, or senile), 2) degree of obesity (moderate or excessive), and 3) the localization of fat deposits - android or gynoid type, the amount of subcutaneous FM and VF, the fat content in the muscles and bone marrow, etc.