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Spina bifida oculta in skeletal population from Dąbrówki (Poland, Podlaskie Province)

Adrian Wolski[1], *Anna Myszka*[1], *Joanna Wawrzeniuk*[2], *Jacek Tomczyk*[1]

[1] Institute of Biological Sciences, Cardinal Stefan Wyszyński University in Warsaw, Poland
[2] Institute of Archaeology, Cardinal Stefan Wyszyński University in Warsaw, Poland

Abstract: The aim of the study is to evaluate the frequency of spina bifida oculta (SBO) in the early modern population from Dąbrówki (Poland); 26 males, 19 females, 3 adults with unspecified sex, 2 subadult were taken into the analysis. SBO was found in 9 individuals (18%), of whom only one exhibited a complete cleft in the sacrum (2%). In males, SBO was reported in 7 out of 26 skeletons studied (27%). Complete cleft was observed in one individual (4%), partial cleft in 6 individuals (23%). In females, no case of complete cleft was detected (0%), and one case of partial cleft was found (5%). These differences between males and females in the frequency of this skeletal condition were statistically significant.

Due to the lack of uniform methods for SBO analyses, the inability to make interpopulation comparisons, the relatively high prevalence of the SBO phenomenon in ancient and modern populations, and the unclear etiology of the disease, research on SBO should be continued.

Key words: spina bifida, sacral bone, skeletal population.

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Introduction

Spina bifida is a general term that refers to varying levels of pathology involving the spinal cord and nerve root (Melintenda et al. 2003; Mohd-Zin et al. 2017). It is a congenital condition that results from incomplete closure of the neural tube during early embryonic development (Banta et al. 1990; Moore, Persaud 2003). Spina bifida is classified into spina bifida cystica and spina bifida occulta (Mohd-Zin et al. 2017). Spina bifida cystica, which includes meningocele and myelomeningocele, is called "open" spina bifida (McComb 1997). Myelomeningocele, which is the most severe type of spina bifida, involves a sac-like protrusion of the spinal cord and cerebrospinal fluid and an exposure of the nerves and tissues. Meningocele can result in minor disabilities while myelomeningocele may cause moderate to severe disabilities (Copp, Greene 2012).

Spina bifida occulta ("closed" spina bifida) (SBO), represents skin-covered lesions without an exposed cystic mass (Korsvik, Keller 1992). Indicated solely by a bony defect of the vertebral arch, SBO can be located everywhere along the spine but is most frequently observed at the lumbo-sacral junction L5/S1 (80% of the cases), 60% of the cases are associated to spina bifida at level L3-S1 and about 10% at level S2-S5 (Barnes 1994; Kumar, Tubbs 2011; Lee et al. 2011; Saluja 1986, 1988). Non-fusion occurs at segments S4-S5, reaching up to 90% of individuals of European ancestry (Fidas et al. 1988). It is clinically recongised as a natural morphological variation, also referred to as the sacral hiatus (Abera et al. 2021; Henneberg, Henneberg 1999).

SBO does not involve nerve or spinal cord damage and is largely asymptomatic although it might be linked to a recurrent lower back pain, neurological deficits of the feet, and posterior disc herniation (Avrahami et al. 1994; Eubanks, Cheruvu 2009; Sairyo et al. 2006). Asymptomatic spina bifida during childhood can lead to neurological symptoms later in adulthood (Spacca, Buxton 2008). The defect is often indicated by a hairy spot or a dimple in the affected skin area (Kumar, Tubbs 2011).

Despite numerous studies, the causes of the appearance of spina bifida are not fully understood (see Henneberg, Henneberg 1999; Kelty, Henneberg 2022). The etiology of spina bifida is multifactorial, which involves complex polygenic interactions with environment factors (Holmes et al. 1976; Josan et al. 2008). Chromosomal anomalies, maternal obesity, and maternal folate status have been suggested to be linked to neural tube defects (Louie et al. 2008; Northrup, Volcik 2000). Recent studies have identified a series of teratogenic factors that can lead to the appearance of a spinal defect, such as nitrates, lead, valproic acid, oxytetracyclines, some anticonvulsant and antidepressant drugs, pesticides, toxic waste, excessive heat. There are a number of maternal conditions that may contribute to the development of a descendant with spinal dysraphism, such as fever, diabetes, obesity, maternal psychological stress, mother's age, parity (Fornoff et al. 2004).

Spina bifida has been observed in prehistoric skeletal remains suggesting that this congenital defect has been affecting human populations since the distant past (Kumar, Tubbs 2011; Kelty, Henneberg 2022). Most paleoanthropological studies regarding spina bifida have focused on the sacrum (Kumar, Tubbs 2011). The condition was treated as an instru-

ment for detecting specific features in a population, such as degrees of kinship, biological distances, isolation and endogamy (see Kumar, Tubbs 2011). Some researchers have linked SBO to microevolutionary trends which might suggest a substantial relaxation of natural selection beginning around 1900 changed the mutation/selection balance of modern genetic material, causing an increase of sacral SBO since 1900 (Kelty, Henneberg 2022; Solomon et al. 2009).

Cases of SBO from the archaeological record have been reported although it still raises many interpretative and methodological challenges, such as the lack of a standardized methodology of SBO assessment in skeletal materials, insufficient knowledge about the etiology of the disorder. The purpose of this work is to assess the prevalence of SBO in the early modern population from Dąbrówki. This study has the potential to broaden our knowledge regarding the SBO prevalence in past and modern skeletal populations.

Material and methods

All analysed specimens came from a cemetery located in Dąbrówki (Poland) (Fig. 1). The name „Dąbrówki" appeared for the first time in 1559 (due to the presence of oaks in the local part of Podlasie). The cemetery with skeleton burials was discovered by accident during earthworks in 2018. The cemetery is located on the hill called „Cygańska Brama". Historical sources do not contain any information about the cemetery (Wawrzeniuk 2021a). The burial equipment found in the cemetery allowed for its preliminary dating pointing to the beginning of the 17th century. It cannot be ruled out that in the remaining areas of the cemetery there are burials from both younger and older periods (Wawrzeniuk 2021b).

Fig. 1. The location of the Dąbrówki village (green drop)

Research work has revealed 62 graves most of which contained adults. The few children's burials, who were usually buried with adults (Wawrzeniuk 2021a, 2021b). The samples used in this study are part of the osteological collection belonging to the Institute of Biological Sciences Cardinal Stefan Wyszynski University (Poland).

Standard anthropological methods were applied to determine the age and sex of the individuals. The sex of the individuals was determined using methods previously applied by Phenice (1969) and Buikstra, Ubelaker (1994). This includes visual assessments of pelvic and cranial features. The age-at-death of the individual was evaluated based on changes in the morphology of the pubic symphysis using the Todd's method for changes in pubis symphysis, Brooks and Suchey (1990) standards for changes in the topography of the auricular surface (Buikstra, Ubelaker 1994).

The skeletons of 160 individuals were examined (54 from the 2019 season, 75 from the 2021 season and 31 from the 2022 season). Only 50 of the 160 individuals had complete, undamaged sacral bones, and only these individuals were included in the final study. Table 1 summarises the number of skeletal material from Dąbrówki analyzed in the present study.

Table 1. Number of skeletal material from Dąbrówki

	N
Males	26
Females	19
Adults*	3
Subadults	2
All group	50

N – number of tested individuals; * – unspecified sex; only the division into adults and children was considered, without division into exact age classes).

SBO was analyzed in the study material according to the methods proposed by Buikstra, Ubelaker (1994: 122). Three types of changes were noted: (0) no cleft – all vertebral arches fused, complete; (1) partial cleft – in at least one vertebrae vertebral arch did not fuse with incomplete development or their complete absence; (2) complete cleft – in all sacral vertebrae vertebral arches not fused (incomplete development of vertebral arches or the lack of the vertebral arches) (Fig.2). A word cleft contains any deformity of vertebral arches from discontinuity to the completely lack of these anatomical structures.

In paleoanthropological studies we used differing classification systems for SBO assessment (see Discussion) what may account for variability in reported frequencies. Therefore, the present study was based on the medical assumption stating that SBO at the level of the vertebrae S3, S4, S5 is treated as morphological variability within normal limits (Kumar, Tubbs 2011); the cleft observed at the height of the above-mentioned vertebrae was treated here as no cleft (see Abera et al. 2021; Kim et al. 2018). This allows for the development of methodological standards for historical populations analyses, which not only are necessary for making inter-populations comparisons, but also allow for the comparison of the obtained results to medical data.

Fig. 2. Normal sacral bone (a), sacrum with partial (b) and complete (c) *spina bifida oculta* (photos by Wolski Adrian)

Statistical significance of the differences in the frequency of the SBO between males and females in the analysed skeletal material was determined using the unilateral test for two components of the structure (a test based on a number of cases and frequencies data). Differences were considered to be statistically significant when $p<0.05$. All analyses were performed using the Statistica 13.3 software.

Results

The bones of 50 individuals from the skeletal cemetery in Dąbrówka were analysed. SBO was recorded in nine individuals, which consists of 18% of the studied population. Only one of them had a complete cleft of the sacrum (2%). In the remaining cases, we were dealing with incomplete SBO (16%).

In females, no case of complete cleft, and one case of partial cleft was recorded (5%). In males, SBO was found in 7 out of 26 examined skeletons (27%), with a complete cleft in one individual (4%), and a partial cleft in 6 individuals (23%). No cases of SBO (0%) were detected in adults whose sex could not be determined. One case of cleft, a partial cleft (50%), was detected in a subadult. Data on the frequency of SBO in the population from Dąbrówki are presented in Table 2.

Table 2. Frequncies (%) of *spina bifida oculta* in the Dąbrówki population

	N/n (% n)		
	Comlete	Partial	All
Males	26/1 (4%)	26/6 (23%)	26/7 (27%)
Females	19/0 (0%)	19/1 (5%)	19/1 (5%)
Adults*	3/0 (0%)	3/0 (0%)	3/0 (0%)
Subadult	2/0 (0%)	2/1 (50%)	2/1 (50%)
All group	50/1 (2%)	50/8 (16%)	50/9 (18%)

N – numer of individuals; n – numer of individuals with *spina bifida oculta*; * – unspecified sex.

The next step of the analysis was to compare the significance of differences in the frequency of SBO in males and females. As the results presented in Table 3 show, males (27%) from Dąbrówki show a significantly higher frequency of SBO compared to females (5%).

Table 3. Significance of the differences in the frequencies (%) of *spina bifida oculta* in males nad females from Dąbrówki

Sex	N/n (% n)	p
Males	26/7 (27%)	0,028
Females	19/1 (5%)	

N – numer of individuals; n – numer of individuals with *spina bifida oculta*; $p \leq 0,05$.

Discussion

In the population from Dąbrówki, spina bifida oculta was recorded in 18% of individuals. Comparing the SBO data in the material from Dąbrówki with other available data from similar historical periods and different regions of Europe, it turns out that the frequency of SBO in the studied material takes intermediate values ranging from 2% (Simalcsik et al. 2011) to 60% (Pliszka 2018).

Although the statistical significance of differences was not assessed here, the observed interpopulation differences and similarities in SBO prevalence may be caused by a number of factors, such as the lack of uniform, standard methods for SBO assessing. For instance, some studies has treated cleft on the height S3-S5 as SBO as a norm and did not take it into consideration (Kim et al. 2018). Similarly, some studies did not provide an information about S3-S5 region treating (e.g., Mays 2006; Silva-Pinto et al. 2010).

Table 4. Frequencies (%) of spina bifida oculta in skeletal populations

Population	Chronology	% (n/N)	Reference
Dąbrówki	XVI	18% (9/50)	Own data
Radom (Poland)	XVII-XIX	60% (44/74)	Pliszka (2018)
Devín-Hrad (Słowacja)	XI-XII	24% (26/109)	Masnicova, Benus (2003)
London	XVIII-XIX	15%	Saluja (1988)
Jassy (Romania)	XVI-XVIII	2% (2/129)	Simalcsik (2011)
Brittany (France)	High Middle Ages	10% (3/30)	Zemirline et al. (2013)

N – numer of individuals; n – numer of individuals with *spina bifida occulta*.

Researchers used to take different segments of the spine into analyses (Hussien et al. 2009; Saluja 1988; Zemirline et al. 2013). There are also studies that did not report any information regarding SBO assessment methods (e.g., Estebaranz-Sánchez et al. 2018). All of this might have contributed to an over- and/or underestimation of the results, and inability to make reliable comparisons between populations. Without clear, unified methods of SBO assessment, it is very difficult to correctly assess and interpret both similarities and differences in SBO prevalence.

Skeletal groups come from different chronological periods as well as different regions of the world. Therefore, their structure might have been influenced by different environmental stress factors and exhibit different genetic endowment adapted to different environmental (socio-economic and biological) conditions (Albrecht et al. 2006). It could be a reason of the observed differences in SBO frequencies. Several studies have found evidence that SBO is associated with a maternal folate (vitamin B9) and folic acid deficiency during the embryonic period (Armstrong et al. 2013; Au et al. 2010; Bentley et al. 2006; Manso, Matos 2023; Melintenda et al. 2023; Pepe et al. 1999). Although the subject of diet in past human populations is still opened, a recent study by Mutlu and colleagues (2020) showed that a Mediterranean Byzantine population, which had access to folate-rich food products, had a lower prevalence of SBO than compared to Anatolian populations. Therefore, it could be assumed that the differences in diet could have influenced the observed interpopulation differences/similarities in SBO prevalence. Proving this hypothesis requires further in-depth research on this research area. Kely and Henneberg (2022) showed an increase of SBO frequency after 1980 despite the introduction of folate supplementation, indicating microevolutionary increase and secular trend in SBO development. This suggests that SBO potentially does not follow the same etiological and embryonic trajectory as spina bifida cistica and has a separate cause. Solving this problem requires further research.

In the population from Dąbrówki SBO was reported more often in males than in females (5%) (Table 3). Similar results were obtained in populations from Iasi (Romania) (5% males, 2% females) (Simalcsik et al. 2011), Devín-Hrad (Slovakia) (males 26%, females 19%) (Masnicova, Benus 2003). In compared populations SBO was more frequently

observed in males, and in some populations the condition was not recorded in female group (Shabana et al. 2014; Groza et al. 2013; Saluja 1988; Simalcsik et al. 2011; Masnicova, Benus 2003; Henneberg, Henneberg 1999; Lee 2022; Sarry, Banna 2006). Most studies do not present data on the significance of the difference in the occurrence of SBO between sexes, which makes it difficult to draw final conclusions about the occurrence of the differences. According to the clinical literature, SBO is less common in men than in women, although, some studies show opposite results, indicating SBO more common in men (Eubanks, Cheruvu 2009; Vannier et al. 1981). The reason of such results is not clear yet.

Many factors may have influenced the results obtained in this study as well as other studies (e.g., regarding the higher incidence in males), such as the specificity of the skeletal material (incompleteness, bone damage, small sample size). There are also difficulties in sex determination in skeletal material. Damaged basic diagnostic bony traits, incomplete skeletal material which is often insufficient knowledge about the biology of an examined skeletal group and an influence of environmental stress on skeletal traits used in sex determination make sex determination not always reliable. Moreover, there are some medical studies showing a male excess in cases with lower spinal lesions, and a female excess in upper spinal lesions (Mariman et al. 1992; Seller 1987). These studies, as well as damage or absence of vertebrae of the upper and middle spine, and therefore the analyses of SBO within the sacrum only, may result in a higher frequency of the disorder in males in the studies of past skeletal populations. Determining the reason for these discrepancies raises the need for further research.

Although cases of sacral SBO from the archaeological record have been still reported, there is a lack of consensus in interpretation of spina bifida in skeletal materials (Kumar, Tubbs 2011). One of the reasons is the lack of a standardised methodology of SBO assessment in skeletal remains (Kumar, Tubbs 2011), what causes the under- or overestimation of the results. Another reason is insufficient knowledge about the etiology of the disorder, and therefore an influence of the genetic or/and environmental factors on its manifestation. The results derived from past skeletal populations could not be easily related to the results of clinical records for modern populations. SBO could be documented in higher numbers than those suggested by the medical record because of usually minor symptoms the condition is often clinically undetected (Avrahami et al. 1994; Kumar, Tubbs 2011). Given all the above, research on SBO in past skeletal populations should be continued and developed.

Summary and conclusions

The frequency of spina bifida oculta in the population from Dąbrówka was found in nine individuals, which constituted 18% of the studied population. Only one of studied individuals had a complete cleft in the sacrum (2%). In males SBO was reported in 7 of the 26 studied skeletons (27%). Complete cleft was observed in one individual (4%), partial cleft in 6 individuals, of which 23% belonged to males. No case of complete cleft was detected in females (0%), and one case of partial cleft was detected (5%). We found that differences between males and females in the frequency of this skeletal change were statistically significant.

The population from Dąbrówki did not differ in terms of the frequency of SBO from other populations from different historical periods and areas from other parts of the world. Due to a number of limitations, this study does not allow for drawing far-reaching conclusions.

Due to the lack of uniform research methods for SBO assessment, the inability to make interpopulation comparisons and the relatively high prevalence of SBO phenomenon in both ancient and modern populations, and the unclear etiology of this disease, research on SBO should be continued.

Conflict of interests

Authors declared no conflict of interests.

Authors' contribution

WA – carrying out skeletal analyses, preparation and description of the statistical analyses; MA – planning and supervision of the research, setting a goal, substantive supervision, corresponding author; WJ – preparation and description of the archaeological part; TJ – content supervision.

Corresponding author

Dr Anna Myszka, Institute of Biological Sciences, Cardinal Stefan Wyszyński University in Warsaw, Wóycickiego 1/3, building 23, 01-938 Warsaw, Poland, e-mail: a.myszka@uksw.edu.pl

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Dyslipidemia in pre- and post-menopausal women: a study of peri-urban Paundra Kshatriya women

Dipak Kumar Adak[1], *Nitamoni Bharali*[2], *Saptarshi Biswas*[3], *Niloy Kumar Bagchi*[1], *Tapas Kumar Biswas*[1], *Vadlamudi Raghavendra Rao*[4]

[1]Anthropological Survey of India, 27 Jawaharlal Nehru Road, Kolkata, India
[2] Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India
[3] PGT, JIPMER (Jawahar Institute of Post Graduate Medical Education and Research), Pondicherry, India
[4] Visiting Professor, Thalassemia & Sickle Cell Society, Hyderabad, India

Abstract: Dyslipidemia is an important risk factor of cardiovascular disease, whereas menopause is a decrease in ovarian follicular activity at the end of reproductive age of the women, which is significantly influenced by hormonal changes brought on by menopause's impact on serum lipids. This study was undertaken among pre- and post-menopausal Paundra Kshatriya women in Sonarpur, South 24 Parganas, West Bengal, India in order to determine the prevalence of dyslipidemia. 142 adult Paundra Kshatriya women (n pre-menopausal women = 96; n post-menopausal women = 46) were selected from a peri-urban setting of Sonarpur, South 24 Parganas, West Bengal. Anthropometry and arterial pressure data were recorded. Blood samples were collected from study participants who were on 12 hours fasting. 19.79% of pre-menopausal women and 17.39% of post-menopausal women had normal levels of lipids. Prevalence of dyslipidemia was slightly higher (82.7%) among the post-menopausal women compared to pre-menopausal women (80.2%). While high LDL cholesterol emerged as one of the prime causes for dyslipidemia among pre-menopausal women, hypercholesterolemia emerged as one of the prime causes for dyslipidemia among post-menopausal women. Regression analysis revealed a significant impact of 3 factors in pre-menopausal women and impact of 6 factors in post-menopausal women. Though Paundra Kshatriya women maintain a relatively less stressful and high physical activity lifestyle, they exhibited high levels of lipid abnormalities. The peri-urban population is undergoing lifestyle and dietary changes due to a close proximity to the urban centre, Kolkata.

Key words: Lipid profile, Menopause, Peri-urban population, India.

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Introduction

Cardiovascular disease is a condition of lipid metabolism heavily influenced by dyslipidemia. This can involve either an excess or deficiency of lipoproteins, or both. It is thought to be one of the main risk factors for atherosclerotic disease, particularly coronary heart disease. Low HDL cholesterol and/or high triglycerides are two possible components of dyslipidemia (Dyslipidemia: nhp.gov.in/dyslipidemia_mtl). On the other hand, the absence of menstruation at the end of the reproductive age is known as menopause and is caused by a decrease in ovarian follicular activity. The cardiovascular illnesses linked to menopause are significantly influenced by the hormonal changes brought on by menopause's impact on serum lipids (Kanwar et al. 2014). Ovarian function is lost after menopause which results in unfavourable changes in vascular endothelial dysfunction, body fat distribution, coagulation, fibrinolysis, as well as glucose and insulin metabolism (Spencer et al. 1977).

Cardiovascular diseases, which are currently the main cause of female mortality, are becoming more common in menopausal women due to contemporary risk factors, such as smoking, obesity, sedentary lifestyles, hypercholesterolemia, and eating habits, as well as an increase in life expectancy (Solimene 2010). After menopause, the lipid profile alterations that take place are linked to an increased risk of cardiovascular disease. Estrogen deficiency is a crucial component of this process. In addition to maintaining a healthy lipid profile, estrogen alters the tone of the blood vessels by producing more nitrous oxide (Taddec et al. 1996). Numerous recent studies show that an increase in cardiovascular risk during menopause is substantially correlated with the evolution of lipid markers. Menopausal women exhibit higher levels of LDL cholesterol, total cholesterol, and apolipoprotein B than pre-menopausal women do (Bonithon-Kopp et al. 1990; Wu et al. 1990). Menopause may play a role in the adjustment of lipid parameters, as shown by the Framingham study showing that cholesterol levels rose around the time of menopause (Hjortland et al. 1976).

Wang et al. (2016) compared serum lipid levels in pre-menopausal and post-menopausal women and evaluated the relationship between menopause and lipid profiles among the patients in a Beijing hospital. They found that the prevalence rate of dyslipidemia in post-menopausal women was significantly higher compared to pre-menopausal women. The study of Shrestha et al. (2022) in Western Nepal also revealed that prevalence of dyslipidemia was high among post-menopausal women. Various lipids were significantly increased in post-menopausal women compared to pre-menopausal women. A comparative cross-sectional study conducted in Alwar city in Rajasthan, India, revealed a higher prevalence of cardiometabolic risk factors amongst dyslipidemic post-menopausal urban women. Co-existence of dyslipidemia and menopause aggravates the risk condition in post-menopausal women (Parnami and Varma 2021). The risks of atherosclerotic cardiovascular and cerebrovascular diseases in women rapidly increases with age in post-menopausal women. Zhang et al. (2018) found post-menopausal patients were more severely affected compared to pre-menopausal patients in terms of dyslipidemia and systemic inflammation. Jeong and Kim (2022) examined awareness and related factors of dyslipidemia in menopausal women in Korea and

found that prevalence of dyslipidemia in menopausal women was high while their awareness was significantly low.

Although there is a substantial number of studies on dyslipidemia in pre- and post-menopausal women there is no, to our knowledge, a single study conducted in a population living in a peri-urban setting in India. This cross-sectional observational study aimed to determine prevalence of dyslipidemia in a pre- and post-menopausal Paudra Kshatriya women in a peri-urban setting of Sonarpur.

Materials and methods

Sonarpur is a peri-urban neighbourhood in South 24-Parganas, West Bengal, with excellent bus and rail connections to Kolkata, the State's capital. The largest group of the study area's diverse population is the Paundra Kshatriya or Pod (caste). In terms of total socio-economic development, the the Paundra Kshatriya people are regarded as a disadvantaged group. Only a small number of Paundra Kshatriya families are able to support themselves entirely from the income they generate (Singh 2008). The subsistence economy of the Paundra Kshatriya, who live in this area, is heavily dependent upon a variety of occupations. Males primarily work for themselves (33.8%) and in a variety of white-collar jobs (27.33%). Males work in both skilled (17.95%) and non-skilled (13.85%) occupations in significant numbers. Only a small proportion of females work in non-skilled (4.27%), skilled (2.40%), white collar jobs (3.73%), and self-employment (2.93%) occupations. Most women in this group are housewives (86.13%) (Bagchi and Adak 2012).

This cross-sectional observational study was conducted among adult Paundra Kshatriya women. Exclusion criteria were as follows: i) being unrelated to the Paundra Kshatriya (i.e., belonging to other ethnic lineages) and ii) patients with diabetes who take lipid-lowering medication or who have a history of dyslipidemia. Inclusion criteria were the following: i) pre- and post-menopausal unrelated Paundra Kshatriya women and ii) the women who were without regular intake of diabetic and lipid-lowering drug. A standard questionnaire, developed by the authors of this study, was employed, which included questions about eating habits, physical activity, sex, age, and more. Following the presence of one or more abnormal serum lipid readings, dyslipidemia was defined. Cutoff values were used in accordance with NCEP's (National Cholesterol Education Programme, III, 2001) recommendations, including the following:

1. TG≥150 mg/dl
2. LDL-C≥100 mg/dl
3. HDL-C≤40 mg/dl for men and HDL-C≤50 mg/dl for women
4. TC≥200 mg/dl

This study included 142 adult women aged between 20 and 70 of whom 46 were menopausal and 96 premenopausal. Data on arterial pressure and anthropometry were recorded. Blood samples were taken from unrelated people who had fasted for 12 hours. The samples were examined in the Kolkata laboratory of the Anthropological Survey of India. The Semi-Auto Analyzer, Minitechno, manufactured by Logotech India Pvt. Ltd., was used to perform the analyses.

Anthropometric indices, including body mass index (BMI), waist circumference (WC), and waist-hip ratio (WHR), have been recognized as beneficial screening tools mainly due to an easy access and cost-effectiveness (Kushkestani et al. 2020). We looked at anthropometric

measurements such as (a) height (cm), (b) weight (kg), (c) waist circumference (cm), and (d) hip circumference (cm). While taking the measurements, study participants were dressed in light clothing and were not wearing shoes. Martin's anthropometer rod was used to measure height and a weighing machine equipped with a weighing scale was used to assess weight. The BMI (body mass index) was computed by dividing weight (kg) by height (square metres). A steel tape was used to measure the waist and hip circumference. To determine the relationship between various parameters and dyslipidemia a linear regression was applied. The Venn diagram was created to show how the various dyslipidemia components intersect for pre- and post-menopausal separately. Hypercholesterolemia (serum cholesterol levels of 200 mg/dl) and hyperglyceridemia (serum triglyceride levels of 150 mg/dl) were defined following Joshi et al. (2014). BMI and waist- hip ratios were calculated. T-test and binomial test of equality of proportion were performed to determine whether differences between investigated parameters were statistically significant. The study obtained ethical approval from the Ethics Committee of the Anthropological Survey of India. Once written informed permission had been obtained, all subjects were enrolled.

Results

Mean values of various lipids of pre- and post-menopausal women are shown in Table 1 and Figure 1. Post-menopausal women had greater levels of serum cholesterol, serum triglycerides, LDL (low density lipoprotein) cholesterol, HDL (high density lipoprotein) cholesterol, and VLDL (very low density lipoprotein) cholesterol compared to pre-menopausal. However, t-test results showed statistically significant difference between pre- and post-menopausal women in terms of serum cholesterol ($t=5.03$; $p<0.05$), serum triglyceride ($t=13.88$; $p<0.05$), LDL cholesterol ($t=6.88$; $p<0.05$), HDL cholesterol ($t=8.78$; $p<0.05$) and VLDL cholesterol ($t=11.43$; $p<0.05$).

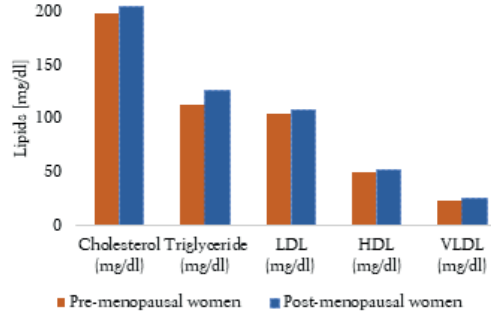

Fig. 1. Mean values of lipids in pre- and post-menopausal women

Table 1. Mean values of lipids in study population

Variables	Pre-menopausal (n=96) Mean (SD)	Post-menopausal (n=46) Mean (SD)
Serum cholesterol (mg/dl)	197.99 (4.64)	205.59 (9.73)
Serum triglyceride (mg/dl)	112.59 (4.03)	127.05 (6.49)
LDL cholesterol (mg/dl)	103.62 (2.50)	108.34 (4.32)
HDL cholesterol (mg/dl)	49.26 (1.50)	52.33 (2.13)
VLDL cholesterol (mg/dl)	22.50 (0.79)	24.77 (1.23)

Table 2 provides general information about study participants based on the presence or absence of dyslipidemia. More than 82 percent of the participants had dyslipidemia. Both pre- and post-menopausal women may attest to this. Additionally, it was discovered that post-menopausal women (82.7%) had a higher prevalence of dyslipidemia compared to pre-menopausal women (80.2%). In the category of subjects with no lipid abnormality, pre-menopausal women showed higher means in the variables like BMI, hip circumference, fasting plasma glucose, serum cholesterol, LDL cholesterol and HDL cholesterol. The rest of the variables showed a reverse trend in this respect. In the category of subjects with any lipid abnormality, post-menopausal women showed higher means almost in all the variables compared to pre-menopausal women excepting in case of BMI and hip circumference. However, t-test results showed statistically significant difference in all the category between women with no lipid abnormality and lipid abnormality among pre-menopausal women, except fasting plasma glucose. Side by side, statistically significant difference is found in most of the category between women with no lipid abnormality and lipid abnormality among post-menopausal women excepting in case of age, hip circumference, serum triglyceride and HDL cholesterol.

Table 2. General characteristics of the subjects on the basis of dyslipidemia

Variables	Pre-menopausal women (n=96)		Post-menopausal women (n=46)	
	Study participants with no lipid abnormality (n=19) Mean (SD)	Study participants with any lipid abnormality (n=77) Mean (SD)	Study participants with no lipid abnormality (n=8) Mean (SD)	Study participants with any lipid abnormality (n=38) Mean (SD)
Age (years)	34.00 (1.44)	30.87 (0.60)	54.25 (2.20)	52.89 (1.08)
BMI	22.63 (0.85)	24.63 (0.46)	22.17 (1.54)	24.21 (0.68)
Waist circumference (cm)	78.65 (2.15)	81.42 (1.28)	79.11 (2.34)	81.71(1.87)
Hip circumference (cm)	91.91 (1.64)	94.78 (0.96)	91.01 (2.63)	90.17 (1.98)
Waist hip ratio	0.85 (0.01)	0.86 (0.01)	0.87 (0.01)	0.91 (0.02)
Systolic blood pressure (mm Hg)	120.89 (3.73)	124.73 (2.28)	148.87 (10.85)	137.24 (3.04)
Diastolic blood pressure (mm Hg)	77.71 (2.30)	82.41 (1.49)	93.87 (4.96)	87.84 (2.26)
Fasting plasma glucose (mg/dl)	89.81(3.35)	88.62 (1.86)	89.63 (2.52)	92.33 (3.04)
Serum cholesterol (mg/dl)	180.51 (6.76)	202.31 (5.45)	176.54 (7.58)	215.18 (5.64)
Serum triglyceride (mg/dl)	92.75 (7.36)	117.22 (4.54)	124.26 (5.43)	127.66 (7.80)
LDL cholesterol (mg/dl)	88.07 (4.35)	107.46 (2.77)	80.07 (7.66)	113.28 (4.41)
HDL cholesterol (mg/dl)	53.12 (3.52)	48.30 (1.65)	51.34 (5.73)	52.02 (2.34)
VLDL cholesterol (mg/dl)	18.66 (1.24)	23.45 (0.91)	22.36 (2.08)	25.28 (1.42)

Prevalence of dyslipidemia among the pre- and post-menopausal women is shown in Table 3. While hypercholesterolemia (pre-menopausal: 46.87%; post-menopausal: 69.56%) and hypertriglyceridemia (pre-menopausal: 11.46%; post--menopausal: 21.74%) both were found to be more prevalent among post-menopausal women, high LDL cholesterol (pre-menopausal: 50.00%; post-menopausal: 47.83%) and low HDL cholesterol (pre-menopausal: 24.17%; post-menopausal: 15.22%) occurred in more frequently among pre-menopausal women. However, when dyslipidemia was taken into consideration it was found that prevalence of high LDL cholesterol was highest among pre-menopausal and hypercholesterolemia was highest among post-menopausal women. Binomial test of equality of proportion was calculated between two groups to determine whether there were significant differences between proportions. It was found that difference between the proportions of hypercholesterolemia (0.22±0.09) and hyperglyceridemia (0.11±0.05) was significant, while difference between the proportions of high LDL cholesterol and low HDL cholesterol was not significant.

Table 3. Prevalence of dyslipidemia (in %)

Prevalence	Pre-menopausal women (n=96)	Post-menopausal women (n=46)
Hypercholesterolemia (mg/dl)	46.87	69.56
Hypertriglyceridemia (mg/dl)	11.46	21.74
High LDL cholesterol (mg/dl)	50.00	47.83
Low HDL cholesterol (mg/dl)	24.17	15.22

Venn diagrams were created to illustrate how various dyslipidemia-related factors overlapped in pre- and post-menopausal women (Figures 2a and 2b). Pre-menopausal 4.17% (n=4) and post-menopausal women 8.7% (n=4) showed the presence of three lipid abnormalities (hypercholesterolemia, hypertriglyceridemia and high LDL – cholesterol). About 19.79% (n=19) of pre-menopausal women and 17.39% (n=8) of post-menopausal women had normal levels of lipids. However, 4.17% (n=4) of pre-menopausal women and 6.52% (n=3) of post-menopausal women had all four lipid abnormalities (hypercholesterolemia, hypertriglyceridemia, high LDL-cholesterol, and low HDL-cholesterol).

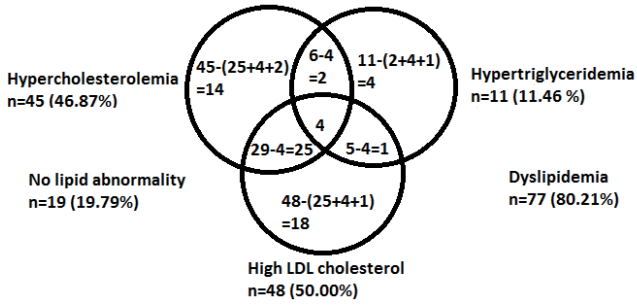

Fig. 2a. Overlap of individual components according to Venn diagram: pre-menopausal women

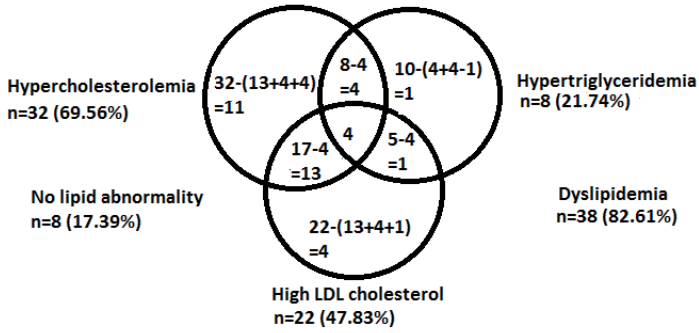

Fig. 2b. Overlap of individual components according to Venn diagram: post-menopausal women

Linear regressions were calculated to examine independent association of factors with lipid abnormalities for pre- and post-menopausal women (Tables 4a and 4b). Among pre-menopausal women BMI was significantly associated with serum cholesterol (SC) and HDL cholesterol. And diastolic blood pressure was significantly associated with LDL cholesterol (Table 4a). Among post-menopausal women waist-hip ratio was significantly associated with serum triglyceride (TG), diastolic blood pressure was significantly associated with LDL cholesterol, fasting plasma glucose was significantly associated with serum cholesterol and HDL cholesterol, and systolic blood pressure was significantly associated with LDL and HDL cholesterol (Table 4b).

Table 4a. Linear regression showing association of factors with dyslipidemia: pre-menopausal women

Factors	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)
Age (years)	R^2=0.26	R^2=0.27	R^2=0.009	R^2=0.16
	F=1.187	F=1.238	F=0.632	F=0.693
BMI	R^2=0.107	R^2=0.002	R^2=0.002	R^2=0.208
	F=5.297*	F=0.067	F=0.071	F=11.554*
Waist hip ratio	R^2=0.096	R^2=0.002	R^2=0.007	R^2=0.003
	F=2.098	F=0.087	F=0.303	F=0.112
Fasting plasma glucose (mg/dl)	R^2=0.051	R^2=0.057	R^2=0.005	R^2=0.008
	F=2.369	F=2.648	F=0.237	F=0.338
Systolic blood pressure (mm Hg)	R^2=0.032	R^2=0.015	R^2=0.057	R^2=0.015
	F=1.441	F=0.680	F=2.673	F=0.679
Diastolic blood pressure (mm Hg)	R^2=0.015	R^2=0.00	R^2=0.080	R^2=0.039
	F=0.674	F=0.011	F=3.842*	F=1.806

*Results significant at $p<0.05$ level

Table 4b. Linear regression showing association of factors with dyslipidemia: post-menopausal women

Factors	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)
Age (years)	R^2=0.008	R^2=0.002	R^2=0.001	R^2=0.006
	F=0.724	F=0.194	F=0.083	F=0.540
BMI	R^2=0.036	R^2=0.009	R^2=0.010	R^2=0.017
	F=3.529	F=0.852	F=0.903	F=1.659
Waist hip ratio	R^2=0.002	R^2=0.054	R^2=0.003	R^2=0.014
	F=0.210	F=5.337*	F=0.240	F=1.318
Fasting plasma glucose (mg/dl)	R^2=0.061	R^2=0.032	R^2=0.022	R^2=0.138
	F=6.147*	F=3.087	F=2.154	F=15.081*
Systolic blood pressure (mm Hg)	R^2=0.004	R^2=0.001	R^2=0.100	R^2=0.042
	F= 0.400	F=0.102	F=10.500*	F=4.101*
Diastolic blood pressure (mm Hg)	R^2=0.027	R^2=0.001	R^2=0.044	R^2=0.016
	F=2.578	F=0.088	F=4.375*	F=1.532

* Results significant at $p<0.05$ level

Discussion

An important modifiable risk factor for cardiovascular disorders is dyslipidaemia. On the other hand, menopause is the permanent cessation of menstruation at the end reproductive life due to loss of ovarian follicular activity. According to Kanwar et al. (2014), menopause-related hormonal changes have a significant impact on serum lipid levels, which are a key factor in most cases of cardiac diseases. A risk factors of coronary heart disease (CHD) include abnormalities of different cholesterol lipoprotein lipids, like high total cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides, and low (LDL) and HDL cholesterol. Raised LDL cholesterol has been found to be a significant predictor of the development of coronary atherosclerosis (Gupta et al. 2017).

Trends in total cholesterol levels were reported by the Global Burden of Metabolic Risk Factors Study between the years of 1980 and 2008. It was projected that throughout this time, total cholesterol levels rose in India as well as in other low- and lower-middle-income nations. Conversely, cholesterol levels decreased in the majority of high-income nations. These trends were discovered through mathematical modelling of sporadic epidemiological research conducted across several nations. The availability of high-quality data from high-income nations (such as the USA, the UK, Germany, Japan, etc.) was facilitated by regular national surveys. In contrast, good-quality epidemiological data are not accessible in low- and lower-middle-income countries, including India; therefore, trends there were determined using probabilistic estimations (cf. Gupta et al. 2017). The results of a study by Pandey et al. (2010) showed that post-menopausal women in Western India had a much greater prevalence of metabolic syndrome compared to pre-menopausal

women. Compared to pre-menopausal women, post-menopausal women exhibited significantly higher levels of serum TC, serum TGs, serum LDL, and serum VLDL lipids (Shenoy and Vernekar 2015). Between pre- and post-menopausal women in Kota, Rajasthan, Kanwar et al.'s (2014) study found no discernible change in blood lipid profiles.

The results of our study showed that dyslipidemia affected more than 82 percent of Paundra Kshatriya women, depending on whether it was present or absent. Both pre-menopausal and post-menopausal women may attest to this. In addition, it was found that post-menopausal women (82.7%) exhibited higher rates of dyslipidemia compared to pre-menopausal women (80.2%) although this difference was not statistically significant ($x^2 = 6.16$). The goal of this study was to determine the prevalence of dyslipidemia in pre- and post-menopausal women among the Paundra Kshatriya community of West Bengal's Sonarpur region, South 24-Parganas district.

Hypertriglyceridemia and hypercholesterolemia were found to be more common in post-menopausal women. In contrast, high LDL cholesterol and low HDL cholesterol were more common in pre-menopausal women compared to post-menopausal women. However, when dyslipidemia was included in the analysis, it was found that pre-menopausal women had the highest frequency of elevated LDL cholesterol while post-menopausal women had the highest prevalence of hypercholesterolemia. The prevalence of dyslipidemia in pre- and post-menopausal Paundra Kshatriya women appears to be on the rise. As a result, the findings of the current study do not support findings reported in other similar studies conducted in India (Pandey et al. 2010; Shenoy and Vernekar 2015; Kanwar et al. 2014).

In many emerging nations, changes in nutrition, demography, epidemiology, and socioeconomic conditions are taking place. Obesity and the metabolic syndrome are becoming more common in adults and children in emerging economy countries as the economic situation improves. According to Mishra and Khurana (2008), urbanization, nutrition change, and decreased physical activity are the key factors. It is interesting to observe that the prevalence of dyslipidemia in the peri-urban population was noticeably high despite adhering to a somewhat less stressful lifestyle exhibiting a high level of physical activity. Given the close proximity of Paundra Kshatriya to Kolkata's metropolitan core, it is reasonable to suppose that this group is experiencing a change in both eating habits and other aspects of lifestyle.

This study has some limitations. For example, this study was restricted to Paundra Kshatriya which is dominated by peri-urban area. In addition, the sample size was small and, thus, may not be representative of all Paundra Kshatriya population in West Bengal. Moreover, no comparison was made in terms of effects of lipid variation due to food consumption, physical activity, medication and other factors.

This study showed worrying prevalence of dyslipidemia among pre- and post-menopausal Paundra Kshatriya women. Presence of dyslipidemia was slightly higher among the post-menopausal women compared to the pre-menopausal women. Prime cause for dyslipidemia among the pre-menopausal women was high LDL Cholesterol and among the post-menopausal women was

hypercholesterolemia. The studied population follow a relatively less stressful and high physical activity lifestyle although it exhibited a very high level of lipid abnormalities. This should be noted that this population is undergoing changes in their lifestyle and dietary pattern due to nearness of urban centre, Kolkata.

Ethical clearance

Present work got ethical clearance from IHEC (Institutional Human Ethics Committee) of Anthropological Survey of India, Ministry of Culture, Government of India.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

VRR, DKA and NKB conceived the idea of study. NKB, SB and TKB analyzed the data. DKA and NB prepared the Tables and Figure. DKA, NB and TKB took part in drafting the manuscript. VRR, DKA, NKB, NB, SB and TKB all revised and finalized the manuscript.

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Anthropological Survey of India, Ministry of Culture, Government of India

Corresponding author

Dipak Kumar Adak, Anthropological Survey of India, 27 Jawaharlal Nehru Road, Kolkata, India, e-mail: adakdipak@gmail.com

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The diet of the human groups buried in a late- and post-Medieval rural parish cemetery in Libkovice (Czech Republic)

Aleksandra Karykowska[1], *Paweł Konczewski*[1], *Barbara Kwiatkowska*[1], *Joanna Witan*[2,3], *Aleksandra Lisowska-Gaczorek*[4], *Krzysztof Szostek*[4]

[1] Department of Anthropology, 5 Kożuchowska St., 51-631 Wrocław, Institute of Environmental Biology, Wrocław University of Environmental and Life Sciences
[2] Institute for Preservation of Archaeological Heritage of Northwest Bohemia, J. Žižky 835/9, 434 01 Most, Czech Republic
[3] Department of Archeology, University of West Bohemia in Pilsen, Univerzitní 2732, 301 00 Plzeň 3, Czech Republic
[4] Institute of Biological Sciences, Cardinal Stefan Wyszynski University in Warsaw, 1/3 Kazimierza Wóycickiego St., 01-938 Warsaw

Abstract: Libkovice is a village in the northwestern Czech Republic that was demolished at the end of the last century due to the expansion of a nearby mine. The former church cemetery has been a subject to bioarchaeological excavation and research, where some 850 burials from the 13th to the 19th Century have been discovered so far. With the application of stable isotope analysis, it has also been possible to uncover the dietary patterns of this exemplary rural Central European community, which was the aim of this study.

The materials analysed here consist of samples from long bones of 56 burials and 18 animal bones discovered in Libkovice during the 2019/21 excavations. It has been employed stable carbon ($\delta^{13}C$) isotope analysis to determine the average contributions of foods derived from the C3 plants.

Statistically significant differences were found between the analyzed fauna and human samples for nitrogen ($F=47.4$ $p<0.05$) and carbon ($F=19.18$ $p<0.05$). There were no statistically significant differences in the analyzed animal and human samples between the specify centuries. When considering the ages of various human individuals, the results indicated statistically significant differences in nitrogen isotopes ($F= 7.71$ $p<0.05$) between children from the infants I group and older children together with adults from the Middle Ages, as well as between children from the infants I group and adults ($F= 3.3$, $p<0.05$) from the modern times. The proportion of food from C3 plants that made up the diets of the studied population was on average 89%, and the potential proportion of freshwater fish in the diet could be higher than 80%.

The similarity between the chronologically diverse groups may indicate similar strategies for food acquisition. The results obtained for the population of Libkovice are very similar to the diets of the populations living in Central Europe broadly during the two periods.

Key words: human remains, isotopic analyses, rural communities, late- and post-Medieval period, Central Europe.

Original article

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Introduction

Libkovice (Ustecki Region, Most District) was a village in northwestern Czech Republic, which was occupied for approximately 800 years until the late 20th Century (Fig. 1).

At the end of the 20th Century, due to plans to expand a nearby open-pit lignite mine, the inhabitants of Libkovice were resettled, and the village was razed.

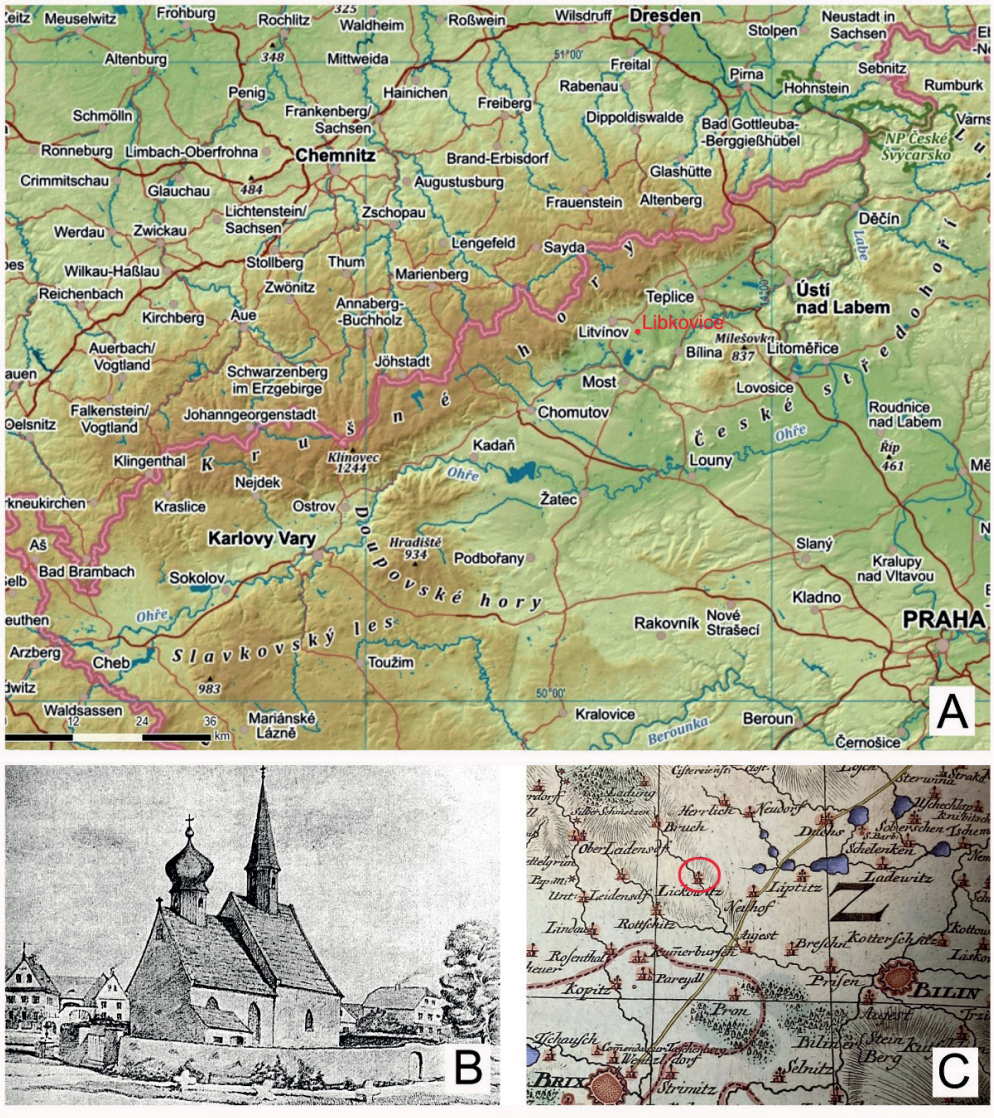

Fig. 1. Libkovice, Ustecky District: A – location of the village (red dot and caption) on the map of North-West Bohemia (source: mapy.cz), B – 19th century drawing of the St. Nicholas church (by Vařeka 2020), C – location of the village (red circle) on Petri's map from 1764 (by Kučera 2022)

The town's abandonment has created an opportunity for scholars to carry out a multi-faceted archaeological study of the entire area of the medieval and modern village, a region that until the 19th Century had a rural population (Biel 2021). Archaeological research carried out from 1995–1996 demonstrated that the central element of the layout of Libkovice was St. Nicholas Church since its foundation in the 13th Century (Vařeka 2020). Until around the mid-19th Century, there was a cemetery around the church, which was the burial place of successive generations of villagers. Comprehensive archaeological and anthropological research of this cemetery began in 2019, lasting until the end of 2022, which led to the discovery of over 850 burials in various states of preservation (Fig. 1). This research aimed to learn about funerary customs and changes in the management of the cemetery space throughout its use-life, as well as to learn about the biology, health, and demographics of the community buried there. Although the field research is ongoing, some preliminary results have already been published (e.g., pathology [Kwiatkowska et al. 2021]).

Isotopic studies are widely applied in biological anthropology and bioarchaeology and are applied broadly to various chronological periods. Isotopic analyses in anthropology are performed to study nutritional strategies of populations living in different historical periods, to reconstruct the process of weaning, or to describe migration phenomena.

Of particular use here, isotopic analysis can provide us with a direct insight into an individual's diet is possible (Schoeninger 2011), as the isotopic signature of the consumed food is reflected in predictable ways in the isotopic ratios in the body's tissues (Pate 1994; Schwarcz and Schoeninger 2012). Stable isotope analyses of human remains have been applied to a variety of cases with variable time depths. These include, but are not limited to, studies on the diet of Neanderthals (Richards and Trinkaus 2009), prehistoric hunter-gatherer populations (Pate 1998; Katzenberg et al. 2010), and Neolithic populations (Pearson et al. 2015), medieval populations (Reitsema et al. 2010; Tomczyk et al. 2021); and modern populations (Lamb et al. 2014; MacKinnon 2015; Tomczyk et al. 2020).

Based on previously published studies on the variability of the carbon isotopic ratios ($\delta^{13}C$), depending on which photosynthetic pathway is dominant in plants (Smith and Epstein 1971; van der Merwe and Vogel 1978), it is possible to determine the contributing proportions of C3 and C4 pathway plants in the diet based on the value of $\delta^{13}C$ bone collagen (e.g., Schwarcz and Schoeninger 1991).

The values of stable nitrogen isotopes ($\delta^{15}N$), on the other hand, demonstrate variation depending on the trophic level, which makes it possible to study the proportion of proteins of marine origin in the diet (Schoeninger et al. 1983; Choy and Richards 2009). The higher the trophic level, the greater the $\delta^{15}N$ differences among animal species. By comparing human nitrogen isotopic values with those of local herbivores (e.g., domesticated cattle), carnivores (e.g., dogs), or other omnivores (e.g., pigs), it is possible to determine the sources of protein in the human individuals' (Richards and Trinkaus 2009).

As such, this study aimed to reconstruct the diets and food preferences (e.g.,

% proportion of animal protein, C3 vs. C4 diet, and proportion of freshwater fish) of people buried in the Libkovice cemetery.

Material and Methods

Samples from long bones of 56 human skeletons (38 children and 18 female adults- selected randomly) discovered in the cemetery of the St. Nicholas Church in Libkovice (district Most, Czech Republic) were obtained for the study. The subjects were assigned to six age categories: 1) infans I (aged 0 to 7 years), 2) infans II (aged 7 to 12–14 years), 3) juvenis (aged 12–14 to 20–22 years), 4) adultus (aged 20–22 to 30–35 years), 5) matures (aged 30–35 to 50–55 years) and 6) senilis (over 55) (Malinowski and Strzałko 1989).

To build a local baseline, animal remains, including bone fragments from 18 domesticated animals (cattle, pig), from the same site were also analyzed. Both human and animal bones came from two historical periods, the Middle Ages (13th–15th Century) and modern times (16th–19th Century) (Tab. 1).

Isolation of collagen and measurement of stable isotopes

Bone collagen was isolated from cortical bone fragments using Bocherens' protocol (1997). Isotopic measurements were performed using a Costech ECS 4010 elemental analyzer coupled via a Thermo Scientific Conflo IV to a continuous flow Thermo Scientific Delta V Advantage mass spectrometer at the Scottish Universities Environmental Research Center (SUERC). The values of the isotopic composition of nitrogen and carbon were expressed in delta notation ($\delta^{15}N$, $\delta^{13}C$). The international laboratory standards used in the analyses were atmospheric nitrogen (AIR) and PeeDeeBelemnite (PDB).

Evaluation of diagenetic changes in collagen

To obtain reliable results from isotopic analyses, the C/N ratio was determined (DeNiro 1985; Ambrose and Norr 1993) and checked to ensure that it was within the acceptable range of 2.9 to 3.6 (van Klinken 1999). In addition, the percentage of carbon and nitrogen in collagen was determined for each sample. Acceptable values of these parameters were a minimum of 25% carbon and a minimum of 10% nitrogen in collagen (Ambrose 1990; Pate 1997; Van Klinken 1999; Pate et al. 2016).

Paleodiet reconstruction

A linear mixing model based on carbon isotopic values was used to determine the average proportion of foodstuffs from C3 plants (Dewar and Pfeiffer 2010; Pate et al. 2016). Experimental values from the populations from Miechow (southern Poland) were incorporated into the model (Mnich et al. 2020). As a result, no isotopic correction was necessary. Mixing models proposed by Hedges and Reynard (2007) and Fraser et al. (2013) were used to reconstruct the percentage of animal protein in the diet. Isotopic enrichment between the different trophic levels described by contemporary models should lie in the range of 3‰ – 5‰ (DeNiro and Epstein 1981; Sponheimer et al. 2003; Robbins et al. 2005). In this study, the differences between the individual trophic levels were assumed to be 4‰, which is a result consistent with the literature (DeNiro and Epstein 1981; Sponheimer et al. 2003; Robbins et al. 2005).

Statistical methods

Statistical analyses were performed using Statgraphics Centurion 18 software. These analyses included cluster analysis using Ward's method, linear models reconstructing the percentage of animal protein in the diet, and the proportion of C3 vs C4 plant food products, as well as the parametric ANOVA test. All tests were performed assuming a 95% confidence level.

Results

The values obtained for the samples' collagen C/N ratio were in the range of 3.1–3.4 (mean 3.25) and did not exceed the limit of the norm for diagenetically unchanged samples (2.9–3.6). The percentage of carbon in all samples was over 41% and nitrogen was over 14%. Therefore, all analyzed samples of human and animal bone collagen were not diagenetically altered and presented reliable data (Tab. 1, Fig. 2).

Table 1. Human and animal skeletal material from Libkovice site subjected to carbon and nitrogen isotope analysis ($\delta^{15}N$ nitrogen isotope result, $\delta^{13}C$ – carbon isotope result, %N – percentage of nitrogen in the sample, %C – percentage of carbon in the sample, CNMolar – diagenesis index; C/N – ratio values of collagen in the sample

Sample ID	Sample Type	Age/Species	Gender	Dating	$\delta^{15}N$	$\delta^{13}C$	%N	%C	CN Molar
1013a	femur/ humerus	Infans I (18 months)		13-15th century	15,1	-19,8	14,9	43,4	3,4
1013b	femur/ humerus	3-4 years		13-15th century	12,9	-20,2	14,9	42,9	3,4
1049	femur	Infans I (5-6 months)		13-15th century	14,4	-19,8	14,6	41,4	3,3
1087	femur	5-6 years		13-15th century	12,9	-19,9	14,0	40,0	3,3
1089	tibia	4 years		13-15th century	13,3	-19,8	14,6	42,1	3,4
1093	femur	9-10 years		13-15th century	13,8	-19,8	14,7	41,8	3,3
1117	ulna	5-6 years		13-15th century	13,4	-19,6	14,6	41,6	3,3
1163	femur	Infans I (18-24 months)		13-15th century	14,5	-19,9	14,7	43,1	3,4
1191	femur/ humerus	Infans I ((24 months)		13-15th century	14,6	-20,1	14,9	42,8	3,4
1193	femur	10-12 years		13-15th century	13,8	-19,5	14,4	41,7	3,4
1259	femur	10-11 years		13-15th century	12,4	-20,2	15,0	42,5	3,3
1299	femur	(Infans I) 0-12 months		13-15th century	16,0	-19,5	14,5	41,0	3,3
1307	femur	6-7 years		13-15th century	13,1	-19,3	15,0	41,4	3,2
1309	femur	8-9 years	M	13-15th century	12,9	-19,9	14,5	40,6	3,3
1343	femur	Infans I (7-12 months)		13-15th century	15,6	-19,6	15,2	43,8	3,4

Sample ID	Sample Type	Age/Species	Gender	Dating	$\delta^{15}N$	$\delta^{13}C$	%N	%C	CN Molar
1349	femur	Infans I (0-1 months)		13-15th century	14,6	-19,1	15,3	43,2	3,3
1353	femur	Infans I (6 months)		13-15th century	12,0	-19,9	15,3	42,6	3,2
1369	tibia	13-14 years	M	13-15th century	11,1	-19,2	15,3	43,2	3,3
1371	femur	12-13 years		13-15th century	11,4	-19,8	15,1	42,7	3,3
1379	femur	7-8 years		13-15th century	13,3	-20,2	15,5	43,1	3,2
1179	clavicle	Maturus	F	13-15th century	11,4	-20,2	15,7	42,3	3,1
1401	tooth	Adultus	F	13-15th century	10,6	-19,5	15,7	42,2	3,1
1493	humerus	Maturus	F	13-15th century	13,1	-19,3	15,8	42,3	3,1
1553	fibula	Maturus	F	13-15th century	12,4	-20,0	15,6	43,1	3,2
1561	clavicle	Maturus	F	13-15th century	11,8	-20,0	15,7	42,2	3,1
1563	humerus	Adultus	F	13-15th century	12,0	-20,0	15,5	42,6	3,2
1579	ulna	Maturus	F	13-15th century	12,2	-20,0	16,0	42,2	3,1
1593	femur	Maturus	F	13-15th century	13,6	-19,7	15,4	42,9	3,3
1601	femur	Adultus	F	13-15th century	13,7	-19,3	15,3	42,1	3,2
1107	femur	Infans I/ Infans II		16-19 century	9,0	-20,6	14,0	40,8	3,4
1051	femur	0-3 months		16-19 century	12,6	-19,8	14,9	43,2	3,4
1025	femur	4-5 years		16-19 century	12,2	-20,0	14,4	40,9	3,3
1139	femur	Infans		16-19 century	15,5	-19,1	15,6	43,7	3,3
1145	tibia	9-10 years		16-19 century	11,3	-20,1	14,1	39,7	3,3
1189	femur	14-15 years		16-19 century	13,6	-19,6	14,0	40,7	3,4
1219	humerus	Juvenis		16-19 century	11,7	-19,8	14,0	40,7	3,4
1237a	humerus	8-9 years		16-19 century	13,4	-19,7	14,2	40,4	3,3
1237b	femur	7-8 years		16-19 century	13,3	-19,7	14,3	40,7	3,3
1247	femur	11-12 years		16-19 century	12,0	-19,4	14,1	40,4	3,3
1301	femur	2-3 years		16-19 century	14,1	-19,6	15,4	43,3	3,3
1315	femur	3,5-4,5 years		16-19 century	12,6	-20,1	14,6	42,3	3,4
1329	humerus	8-9 years		16-19 century	12,8	-20,3	15,1	42,4	3,3
1391	femur	13-14 years		16-19 century	12,8	-19,8	15,2	43,0	3,3
1393	humerus	6-8 months		16-19 century	15,7	-19,6	15,4	43,6	3,3
1405	femur	Infans II		16-19 century	13,9	-20,0	14,7	41,0	3,3
1411	femur	Infans II/Juvenis		16-19 century	12,0	-19,8	15,7	43,7	3,2
1443	femur	Juvenis		16-19 century	14,1	-19,8	15,2	42,4	3,3
1069	humerus	Adultus	F	16-19 century	12,7	-19,7	15,7	42,8	3,2
1101	ulna	Adultus	F	16-19 century	12,4	-19,7	15,3	43,7	3,3

Sample ID	Sample Type	Age/Species	Gender	Dating	$\delta^{15}N$	$\delta^{13}C$	%N	%C	CN Molar
1169	ulna	Maturus	F	16-19 century	13,4	-19,8	14,6	40,3	3,2
1197	ulna	Adultus	F	16-19 century	12,0	-20,1	13,6	38,5	3,3
1227	femur	Adultus	F	16-19 century	11,8	-19,7	15,2	42,6	3,3
1243	radius	Adultus	F	16-19 century	10,8	-20,2	15,6	41,7	3,1
1317	humerus	Adultus	F	16-19 century	12,1	-19,9	15,4	41,6	3,2
1383	ulna	Adultus	F	16-19 century	10,5	-20,0	15,2	41,9	3,2
1395	ulna	Adultus	F	16-19 century	13,1	-19,8	15,5	42,7	3,2
Q07	mandible/ tooth	Pig		16-19 century	9,5	-21,1	15,7	41,7	3,1
Q18	mandible/ tooth	Bovines		16-19 century	7,8	-20,8	13,9	38,6	3,2
Q27	tooth	Bovines		16-19 century	8,4	-21,4	15,0	40,1	3,1
Q29	mandible	Pig		16-19 century	6,1	-20,7	13,8	37,7	3,2
Q30	long bone	Bovines		13-15th century	7,1	-20,9	14,9	39,9	3,1
Q32	tooth	Bovines		13-15th century	9,2	-21,1	15,2	42,0	3,2
Q33	long bone	Bovines		13-15th century	6,3	-20,9	14,9	39,9	3,1
Q39	mandible	Pig		13-15th century	8,0	-20,0	14,5	39,8	3,2
Q40/41	tooth	Bovines		13-15th century	7,3	-20,9	15,5	42,3	3,2
Q47/56	long bone	Bovines		13-15th century	7,7	-20,2	14,8	41,2	3,2
Q48/57	tooth	Bovines		13-15th century	7,8	-20,5	15,5	41,8	3,1
Q66	tooth	Bovines		13-15th century	7,1	-20,1	15,2	40,7	3,1

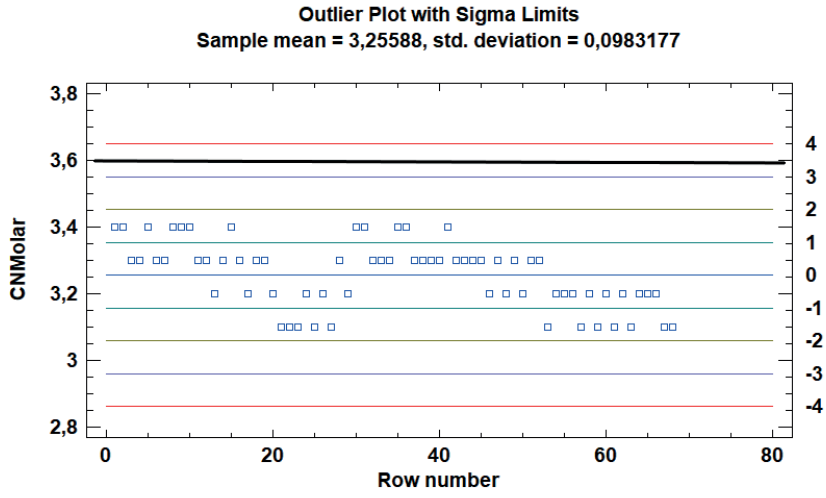

Fig. 2. Values of the diagenetic index C/N of all analysed bone samples. The black line shows the limit for post-mortem non-diagenetic samples (C/N=3.6) (The row number – the number of the next sample)

Reconstruction of diet

A comparison was made between carbon and nitrogen isotopic values obtained for human and animal samples from the two analyzed periods (the Middle Ages and modern times). The mean value of $\delta^{15}N$ for all human samples (without distinguishing the individuals by their ages) from the Middle Ages was 13.17‰ (SD=1.34), and in the case of modern samples, the mean was 12.53‰ (SD=1.52). The results obtained for animal samples in both periods were, respectively, 7.56‰ (SD=0.85) and 7.43 (SD= 1.19) (Fig. 3).

For carbon isotopes, the mean value of $\delta^{13}C$ for medieval human samples was -19.76‰ (SD=0.32), and for modern times samples -19.88‰ (SD=0.37). The results for animal samples were -20.57‰ (SD=0.43) and 20.97‰ (SD=0.38), respectively (Fig. 4).

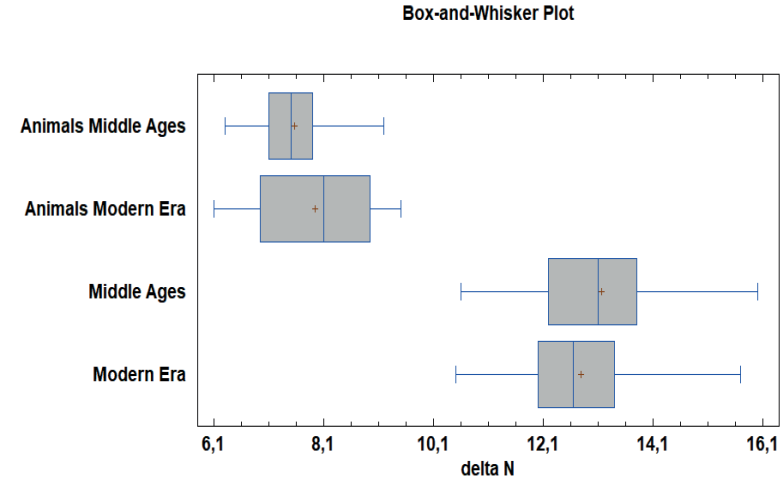

Fig. 3. Variation of nitrogen isotope levels in humans and animals in Libkovice (delta N – nitrogen isotope ratio)

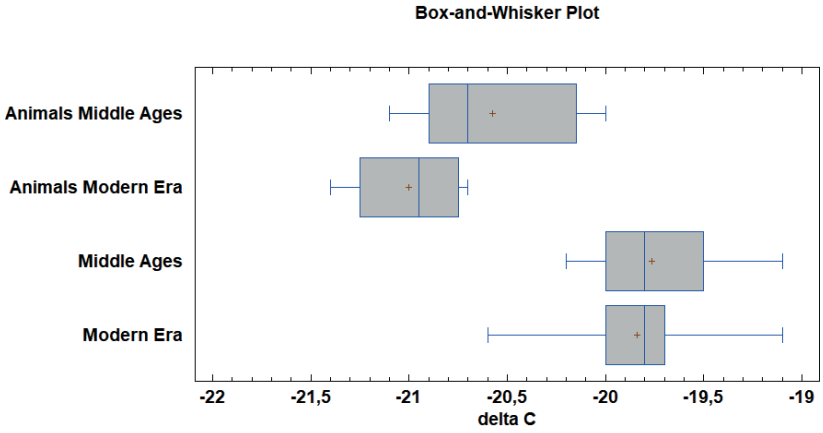

Fig. 4. Variation of carbon isotope levels in humans and animals in Libkovice (delta C – carbon isotope ratio)

Both nitrogen and carbon isotopes showed statistically significant differences between the analyzed fauna and human samples. For nitrogen it was $F=47.4$; $p<0.05$, and for carbon $F=19.18$; $p<0.05$. There were no statistically significant differences between the study periods, both for the analyzed animals and humans.

The diversity of trophic networks was analyzed via cluster analysis based on Ward's model (Fig. 5). The human samples formed a homogeneous cluster within which all individuals from medieval and modern times fit. The second cluster was formed by animal bones that differed in nitrogen and carbon levels. In the case of nitrogen isotopes, the differences were more than 4‰, indicating a trophic level shift.

At the border of the clusters was one individual (individual no. 4) from the infants I/II group who died in modern times. Such low values of both nitrogen and carbon isotopes suggest a completely different diet compared to the other individuals, that is, their diet included a lower proportion of proteins of animal origin.

The results of the isotopic analyses of samples from the Middle Ages, considering the age distribution, indicate statistically significant differences for nitrogen isotopes ($F= 7.71$ $p<0.05$) (Fig. 6).

They occur between the infants I group and older children together with adults. For the individuals from modern times, statistically significant differences were observed between infants I and adults ($F=3.31$, $p<0.05$).

The observed differences may indicate the effect of breastfeeding, which analyzes will be presented later in the other study. There were no statistically significant differences in carbon isotopes considering the age of individuals in the two historical periods analyzed.

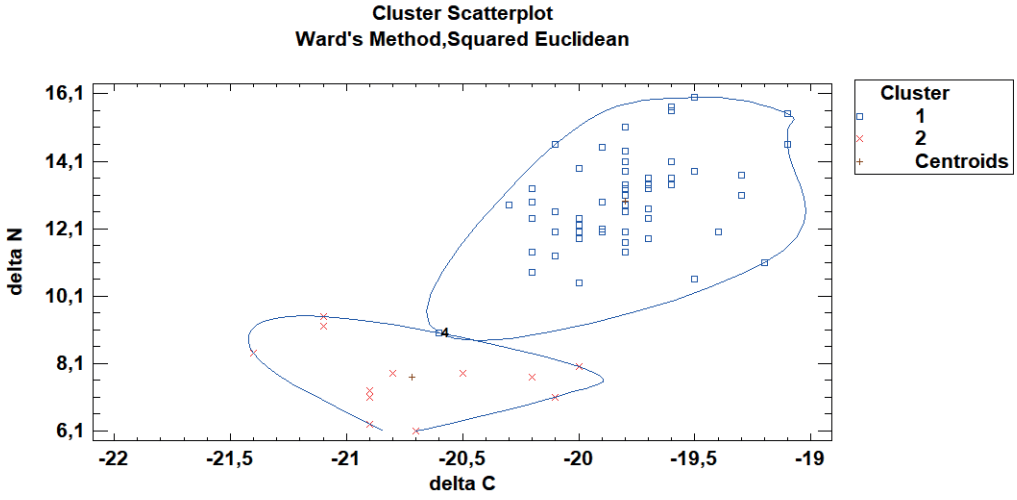

Fig. 5. Isotopic diversity of human and animal samples analysed. Cluster 1 – human specimens, cluster 2 – animal specimens

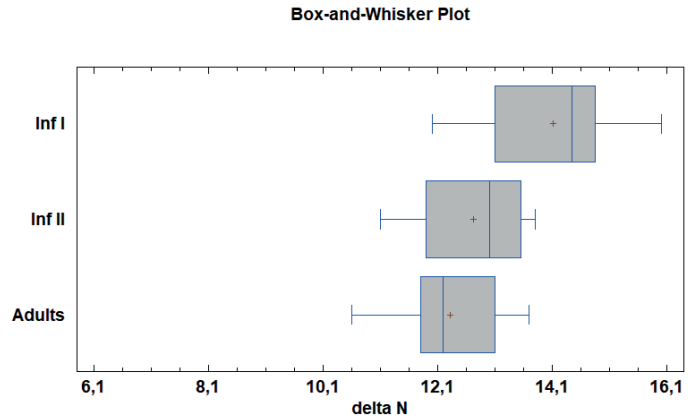

Fig. 6. Variation of nitrogen isotope levels in human samples from the Middle Ages by age in Libkovice. 1 – infans I; 2 – infans II; 3 – adults

The isotopic data obtained from the animal collagen samples do not differ from known isotope levels in medieval land animals from Poland (Reitsema et al. 2013; Krajewska 2015; Reitsema et al. 2017; Tomczyk et al. 2020). Isotopic values of carbon and nitrogen from the studied animal samples were typical for herbivores eating C3 plants (Polet and Katzenberg 2003).

C3 vs. C4 diets

To estimate the proportion of nutrients characteristic of the C3 photosynthetic pathway compared to products of C4 origin, a detailed analysis of isotopic variation was used based on the model proposed by Dewar and Pfeiffer (2010).

Considering the high probability of consumption of C4 products by individuals analyzed from the historical periods, a model was applied to the products of the C3 vs. C4 photosynthetic pathways (Pospieszny et al. 2020; Monk et al. 2020). Data from Mnich and colleagues were used to determine the carbon isotopic values within C4 plants (2020). The reconstruction was based only on adult bone samples due to the potential impact of breastfeeding on children and juveniles (Table 2).

Table 2. Estimated proportion of C3 food in the diet of studied adults (females) representing the medieval and modern periods from Libkovice based on stable carbon isotope data

		Isotopic data		Lower limit -21,4‰ (C3- based terrestrial diet, experimental data for cattle) Upper limit -7,4 ‰ (C4- based terrestrial diet experimental data lead out from millet. Mnich et al. 2020)	
Sample	n	mean $\delta^{13}C$ (‰)	range (‰)	mean C3 vs. C4-based plant and animal component (%)	Range C3 (%)
13-15th century	9	-19,8	-20,2; -19,3	88,4 C3 vs. 8,6 C4	91,4 – 85,0
16-19th century	9	-19,9	-20,2; -19,7	89,2 C3 vs. 10,8 C4	91,4 – 87,9

The analysis showed that within the study group, the proportion of C3 food was dominant, averaging 89%. In addition, the low inter-individual variability indicates that both the groups representing the Middle Ages and the modern times probably consumed food of similar quality with respect to the carbon isotopes studied. The results show that, with a high probability, the studied individuals were a socially homogeneous group which predominantly consumed food made from the C3 plants, regardless of the historical period in which they lived.

The proportion of animal protein in the diet in the light of the models

We propose two scenarios to verify the percentage of animal protein in the diets of the individuals studied, taking advantage of the fact that the $\delta^{15}N$ value increases by 4‰ on average with each successive trophic level (Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Sealy et al. 1987; Fraser et al. 2013). In the first model, a 100% plant-based diet was represented by the mean value obtained for herbivores. In this case, the starting point was the mean ratio of nitrogen isotopes obtained for all animals analyzed (medieval cattle = 7.5‰, modern times cattle = 8,1‰). Due to the fractionation between trophic levels (Δ plant-herbivorous fraction ≈ fractionation 4‰), the predicted nitrogen level of vegetation consumed by herbivores was reconstructed, equaling 3.5‰ for both periods studied.

An animal-specific enrichment of +4‰ was also added to the $\delta^{15}N$ value to determine the value of the endpoint $\delta^{15}N$ for the linear model. The endpoint of the model for the Middle Ages was $\delta^{15}N$ =11.5‰, while for modern times was $\delta^{15}N$ =12.1‰. The mean value of $\delta^{15}N$ for the medieval group was 12.31‰, while that of the modern population was 12.09‰.

The observed levels of $\delta^{15}N$ in the individuals studied here exceeded the assumed isotopic variability in herbivores (considering the 4‰ fractionation of isotopes) from Libkovice. The first scenario suggests that the fraction of animal protein in the diet of the medieval and modern groups probably came mostly from sources other than herbivore and omnivore meat. Similar results were obtained using the pig model. Therefore, a second scenario based on data from prehistoric freshwater fish was proposed, focusing on predatory freshwater fish such as Northern Pike (*Esox Lucius*) and Zander (*Sander lucioperca*) (Robson et al. 2016). Therefore, data from the work of Tomczyk and colleagues (2020b) were adopted as the endpoint. This point is the average of the literature data describing a potential diet consisting of 100% freshwater carnivorous fish protein. This made it possible to conclude that the hypothetical, predicted value of nitrogen isotopes for people consuming only freshwater fish was more likely. This model showed that the potential proportion of predatory freshwater fish in the diet could have been over 80%. Pike is a widely used supplementary fish in carp ponds. It allows higher production per hectare of pond and plays an important role in clearing the pond of insects, larvae and tadpoles, as well as undesirable species that come in with waters from natural reservoirs and compete with carp for food.

The observed similarity between the analyzed chronologically diverse groups is high, which may indicate similar food acquisition strategies despite the passage of multiple centuries.

Discussion

Population-based comparative analyzes were used to better understand the nutritional strategies of the analyzed community of Libkovice. It is interesting to compare the studied group against selected groups from the Middle Ages and modern times (Fig. 7).

The isotopic values of adults were compared with the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio data from several groups of modern animals from different ecosystems with different isotopic values (Fig. 7). A similar dispersion of the $\delta^{15}N$ values was observed for individuals from the Middle Ages and the modern times. The isotopic shifts recorded in the human samples from Libkovice (regardless of the period) against the results on animals from the same location prove that for both periods studied, cattle and pig meat were a component of the diet of the inhabitants of the area. The fact that the nitrogen isotopic ratios of the inhabitants of Libkovice are relatively high suggests that the predatory freshwater fish were also an important component of the local diet. This data calls into question the source and availability of freshwater fish for the inhabitants of Libkovice. Since its founding, the village was run by the Cistercian Order, located approx. 5 km north of the monastery in Osek (Vařeka 2020). Complexes of fishponds built in the late Middle Ages were part of the monastic property, the nearest of which was located 3–4 km east of Libkovice, on the border with the village of Liptice (Liptiz). The ponds can be seen on archival plans, e.g., on the map of Saxony published in 1764 by Issak Jacob von Petri (Kučera 2022).

Special attention should be paid to two ponds located directly in the village, included in the Ordonan Survey Map of 1842. The long tradition of small water reservoirs and a developed water management represented by mills in Libkovice is shown by a 1240 privilege issued by Slavko, abbot of the nearby Cistercian monastery in Osek and Prussian bishop ("villam Lubcowitz ... cum duobus molendinis ... Predictus vero Wazlaus curiam, ... piscinulam domi predicte ..." Friedrich and Kristen 1962).

The results of the isotopic analyses were plotted on a graph presenting data available for populations from Poland and other European countries from various periods (Fig. 7 and 8). The Medieval and modern populations of Libkovice were characterized by isotopic values most similar to the Polish population from Radom from the 16th–17th Centuries and the 18th–19th Centuries (Fig. 7 and 8). Similar values were also recorded in studies of a Medieval group of individuals from the village of Kaldus (Fig. 7), in the Kuyavian-Pomeranian Voivodeship. The results obtained from Solt-Tételhegy in Hungary on a medieval population (mean value of $\delta^{13}C$ for enamel – 11.1‰, for dentin – 17.4‰; mean value of bone apatite $\delta^{13}C$ – 10‰, mean value of bone collagen $\delta^{13}C$ – 17.1‰) also suggest that C3 plants were the predominant type consumed.

The $\delta^{15}N$ values of dentin and bone further indicated that animal protein constituted a moderate part of the diet of the study group. Despite signs of status differences indicated by burial location, the stable nitrogen isotope values suggest that individuals had relatively egalitarian access to animal protein (Gugora et al. 2018).

The similarity to the populations from Radom and Kaldus (Poland) can be attributed to the exploitation of protein resources from inland water reservoirs, such as rivers and lakes (Reitsema et al. 2017; Tomczyk et al. 2020). The monks arrived in nearby Osek (Osseg

in German) in 1197, and Libkovice was included in their estates (Vařeka 2020).

The inter-population comparison as well as the Cistercian fishponds in the vicinity of Libkovice, support the results of our isotopic analyses. The isotopic data obtained from the animal collagen samples do not differ from known isotopic levels in Medieval land animals from Poland (Reitsema et al. 2013; Krajewska 2015; Reitsema et al. 2017; Tomczyk et al. 2020). The carbon and nitrogen isotopic values of the animal samples tested were typical of C3 plant-eating animals (Polet and Katzenberg 2003). It should be emphasized that the state of preservation of the bone material was very good, and none of the analyzed samples exceeded critical diagenetic values.

The assumptions regarding a diet enriched with protein derived from fish are confirmed by the model approaches presented in the literature (Schulting 2018). According to Schulting (2018), the typical composition of a diet containing a variety of C3 and animal protein, derived mainly from land animals, is assumed for values of $\delta^{13}C$ below -18‰ and $\delta^{15}N$ below 12‰. In this study, the mean values obtained for nitrogen isotopes were higher, indicating the influence of protein from higher trophic levels.

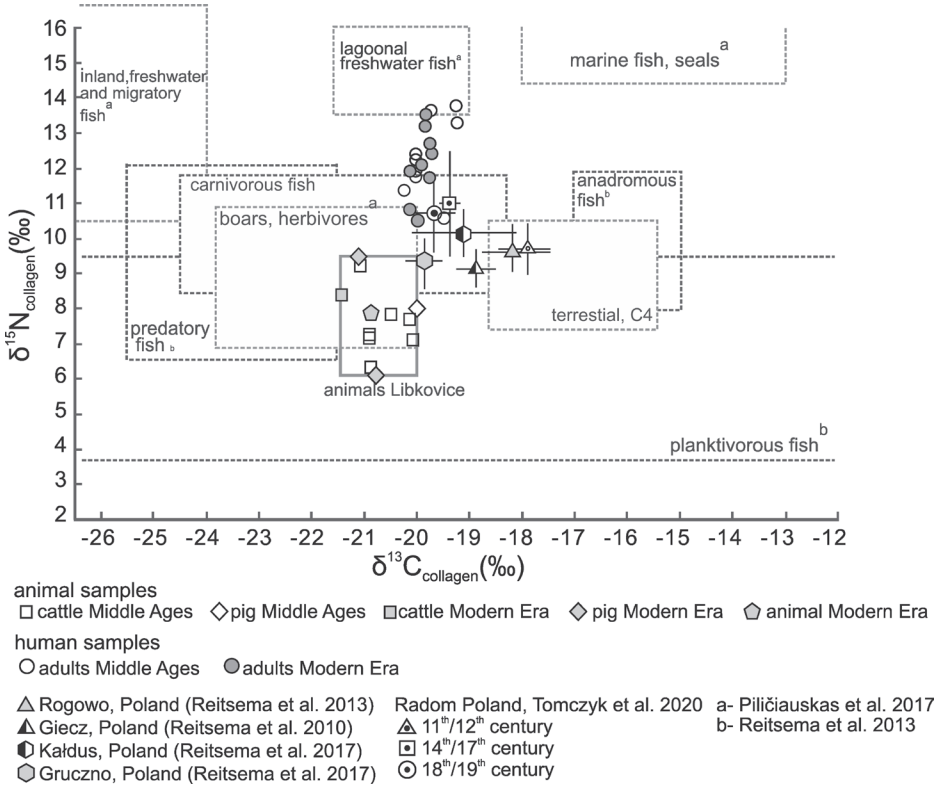

Fig. 7. C and N isotopic proportions of individuals buried at the Libkovice site compared with the environmental background of the site, isotopic ranges of different animal groups and chronologically similar populations

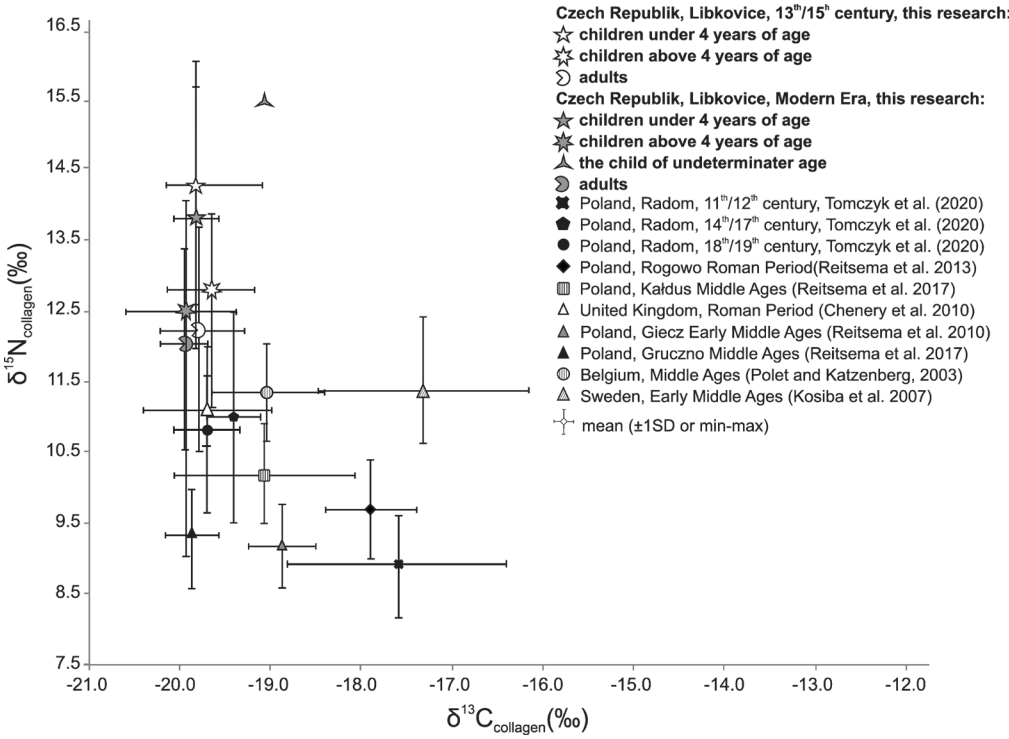

Fig. 8. Carbon and nitrogen isotopic values of adults and children from the Libkovice site in comparison with other selected European populations

Conclusions

The results of analyzes of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes obtained from skeletal remains excavated from the St. Nicholas Church cemetery in Libkovice indicate that the proportions of the studied isotopes differentiate well between the trophic networks characteristic of the analyzed historical fauna. The isotopic levels of the animals significantly differ from the human samples by more than 4‰.

The reconstruction of the diet indicates a general dominance of the C3 plants in the diet, reaching up to 90%, with practically imperceptible differences between the studied periods (Middle Ages vs. modern times). In addition, the low inter-individual variability indicates that individuals representing both historical periods consumed food of similar quality, with respect to the carbon isotopes studied.

Based on the results, it can be concluded with high confidence that the individuals studied were a socially homogenous group and predominantly consumed foods from C3 plants, with a small proportion of higher carbon isotope products (e.g., sugar from sugarcane, millet, etc.). It is unlikely that they consumed the meat of animals feeding on C4 plants, such as millet, for example, since the animals' $\delta^{13}C$ values are too low, thus excluding feeding on C4 plants. The variation in the percentage of the

C3 food components indicates that the populations' diets were similar over the periods under study.

The reconstruction of the percentage of animal protein in the diet showed that by using a model that included the consumption of freshwater fish, the proportion of the diet based on protein derived from aquatic organisms could have been high and reached over 80%. Certainly, however, the remaining protein was obtained from herbivores/omnivores feeding on C3 plants. Significant similarities were observed between the analyzed, chronologically diverse groups, which may indicate that these populations maintained their food acquisition strategies and culinary practices, despite the passage of centuries, as verified by comparative population analyses.

The dispersion of $\delta^{15}N$ values in individuals from the Middle Ages is similar to the values obtained for samples from the modern periods. In contrast, the isotopic shifts recorded in human samples from Libkovice, concerning the animal background data, prove that animal protein from cattle and pigs was one of the components, albeit a minor component, of the diet during the Middle Ages and modern times. Moreover, the nitrogen isotopic ratios of those buried in Libkovice were relatively high and demonstrated values about 3‰ above the ranges characteristic of predatory freshwater fish, which shows that predatory freshwater fish were an important component of the diet, in addition to terrestrial animal protein.

This research will be expanded in the future by broadening the source database by analyzing further samples from the cemetery at the St. Nicholas Church in Libkovice, as well as human remains from an early Medieval cemetery and a cemetery from the 19th–20th Century, both of which are located in the vicinity of Libkovice but are not yet excavated. Ultimately, this will allow us to verify the presented research results and paint a more complete picture of foodways in this region over time. Eventually, we aim to conduct archaeobotanical and zooarchaeological analyses to supplement the isotopic data, which will make it possible to obtain a full reconstruction of the diet and food production in the Central European rural community from Libkovice.

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Conflict of interests

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Authors' contribution

AK – Manager of funded project, originator; analysis of bone material, selection of research methods, preparation of introduction; PK – Supervision of the excavation work, preparation of the historical background of the work; BK – Substantive supervision of the work, preparation of the theoretical part of the work; JW – Excavation work, transportation of material from the Czech Republic, assistance in macroscopic analysis; AL-G – Preparation of material for isotopic analyses, isolation of bone collagen, preparation of discussion; KS – Preparation of material for isotopic analyses, isolation of bone collagen, preparation of discussion; substantive proofreading of the text.

Corresponding author

Aleksandra Lisowska-Gaczorek, Institute of Biological Sciences, Cardinal Stefan Wyszynski University in Warsaw, 1/3 Kazimierza Wóycickiego St., 01-938 Warsaw; e-mail: a.lisowska-gaczorek@uksw.edu.pl

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Relationship between body sway and body build in healthy adult men and women

Anna Lipowicz[1], *Monika N. Bugdol*[2], *Katarzyna Graja*[1], *Katarzyna Nowakowska-Lipiec*[3], *Katarzyna Jochymczyk-Woźniak*[3], *Dobrochna Fryc*[3], *Robert Michnik*[3], *Andrzej W. Mitas*[2]

[1] Department of Anthropology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
[2] Faculty of Biomedical Engineering, Department of Informatics and Medical Equipment, Silesian University of Technology, Gliwice, Poland
[3] Faculty of Biomedical Engineering, Department of Biomechatronics, Silesian University of Technology, Gliwice, Poland

Abstract: Studies investigating the relationship between balance ability and body size, build and proportions tend to concentrate on body mass and height rather than breadth parameters or size of individual body segments. The purpose of this study was to determine a relationship between the ability to keep balance and the size, build and proportions, based on breadth and length dimensions of the body in healthy adult men and women during a position of free standing. This study also aimed to investigate how the lack of visual control affects the analyzed relationship. The study group consisted of 102 adults of both sexes. The investigations encompassed anthropometric measurements of the body and the ability to keep balance. The analysis covered a of series anthropometric parameters, 9 indices of body proportions, mean velocity of the COP movement (MV) and ellipse area (EA). A statistical analysis of the results was carried out taking into consideration the division into groups due to sexes. The results of the Pearson correlation have revealed that there is a statistically significant correlation (weak or moderate degree) between anthropometric parameters of the body and stabilographic values. Results differ between sexes and depend on whether Romberg's test was performed with open or closed eyes. The obtained results showed that the surface area of ellipse significantly depends on the dimensions of these body elements which relate to the position of the centre of mass. The obtained results, which differ depending on sex, show that the values of the body sways in a position of free standing depend on breadth and length dimensions of the body, visual control and the analyzed parameter of balance.

Key words: anthropometry, body dimensions, stabilography, students, Romberg test.

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Introduction

Balance is defined as the ability to keep the centre of gravity of the body over the base of support, restricted by the outline of the feet. The sense of balance makes it possible to determine a position of the body and its individual parts in space, the movement of the body, a direction and velocity of changes. An efficient sense of balance enables keeping balance and stability in an automatic and continuous way in changing conditions (Hanes and McCollum 2006; Panankin 2018). The sense of balance is controlled by the organ of vision, the vestibular system of the ear as well as proprioceptors in muscles, joints and tendons (Peterka 2018). Ageing, disease or damage to any of the above-mentioned elements may result in balance disorders causing symptoms, such as difficulty keeping the right body posture, dizziness, disorders of vision and hearing, difficulty in concentration and memory (Loyd et al. 2021).

The primary sensory system is the organ of sight. It provides information on the surroundings and objects moving around the body, which gives a signal for the movement of the body (Peterka 2018). Central vision enables stabilization and control of spontaneous sways and rocking triggered by visual signals, on the other hand, peripheral vision makes it possible to control the body posture (Gaerlan et al. 2012).

Proprioceptive sensibility involves receptors, such as muscle and joint spindles, tactile and lamellated corpuscles, Ruffini corpuscles as well as Golgi tendon organs. These are specialized mechanoreceptors sensitive to stretching (extension) and changes in pressure within muscles, tendons and joints. A special role in keeping balance is played by signals from receptors located in the neck and ankles of lower limbs. The former informs about the direction in which the head is turning, the latter about the movement of the body, swaying, the surface area of standing including its features, such as hardness and adhesion (Peterka 2018).

The vestibular system of the ear consists of 3 semicircular canals, which transmit signals about the position of the head in three-dimensional space. The system also contains utricle and sacculus, which are responsible for vertical orientation and linear movement. Proper work of the receptors of both (right and left) vestibular systems consists in symmetrical and simultaneous transmission of signals to the brain (in the case of the head movements of different intensity on both sides) (Peterka 2018).

Information obtained through the visual channel provides details only about the surroundings. On the other hand, the vestibular system sends signals only about the head position. Balance can be kept thanks to simultaneous signals from these two sources supplemented with additional information from proprioceptors (Gaerlan et al. 2012). Nervous impulses are segregated in the brain, especially in the cerebellum, where they are integrated with previously learned pieces of information and habitual movements.

Because balance is a motor skill based on a very complex mechanism of nervous and muscular control, there are various factors which determine its diversity in the population. It can be intuitively divided into a group of internal factors, including measurable morphological parameters and possible pathologies occurring within the motor system and nervous system.

One of the best-studied factors affecting the postural stability is age. Healthy children reach an adult pattern of balance by the age of 10–12 years (Hum-

phriss et al. 2011). An optimum control of body balance is achieved at late adolescence and maintained until around 60 years of age (Gaerlan et al. 2012). A similarly strong differentiating demographic factor is sex. It is believed that women are characterized by better stability due to lower location of the centre of mass in their bodies (Greve et al. 2013; Puszczałowska-Lisis et al. 2018). Further factors influencing balance keeping involve parameters connected with the location of the centre of pressure (COP). The above-mentioned parameters include the body build and shape. It is assumed that mainly body sway is related to height according to inverted pendulum model (McGrath et al. 2015). The taller the person, the greater body sways he/she features (Alonso et al. 2015). Studies show that the sway values are also affected by the body mass; the greater the body mass, the higher the amplitude of sways (Hue et al. 2007). It is the most noticeable in obese people (Ku et al. 2012). Due to ambiguity of the BMI index (Body Mass Index), an attempt was made to differentiate the muscle content and fat tissue content from the total body mass. The conducted analyses only ascertained the influence of the above-mentioned parameters on the velocity of sways. Fat content percentage correlates negatively with sway velocity, whereas fat-free mass percentage shows positive correlation.

Among anthropometric parameters and posture-metric parameters, researchers analyzed postural features related, among other things, to spinal curvatures and pelvic asymmetry. The values which showed a negative impact on balance are: severe inclination of the sacral bone, backward deflection of the body and increased thoracic kyphosis (Walicka-Cupryś et al. 2013). In addition, patients with idiopathic scoliosis achieved significantly worse results in stabilometric tests (Catan et al. 2020).

Since balance is an ability which is strongly conditioned by the development of the nervous system, a considerable number of persons with different degrees of mental disabilities was tested. Those investigations revealed that patients with autism spectrum, borderline and other disorders of moderate degree show a significant loss of balance ability (Gouleme et al. 2017), and the value of such a loss correlates with the disability degree (Bibrowicz et al. 2019; Lipowicz et al. 2019a). In addition, patients that have experienced stroke suffer a reduction of postural stability. However, some studies reported that mentally disabled persons and those with damaged central nervous system tissue exhibit improved balance skills as a result of training (Kang 2015; Lee et al. 2016).

There have been few studies investigating the relationship between balance ability and body size, build and proportions. Instead, researchers have been concentrated on investigating other anthropological aspects, such as body mass and height, often neglecting breadth parameters or size of individual body segments. Moreover, research on the relationship between balance and the build and shape of the body in children and adolescents revealed that regardless of age, boys and girls who are characterized by smaller morphological parameters sway more than individuals with stronger body build (Lipowicz et al. 2019b). The present work aimed to determine the relationship between the ability to keep balance and the body size, build and proportions in healthy adult men and women in a position of free standing. In addition, this study aimed to examine the extent to which a lack of sight control affects the relationship analysed herein.

Material and methods

The investigations encompassed 102 adults of both sexes being the students of the Academy of Physical Education in Katowice (Department of Physiotherapy) and the Silesian University of Technology, at the age 20–24 years (body mass: 73±15 kg, body height: 172.65±8.59 cm). The study group consisted of 47 men (body mass: 82±13 kg, body height: 179.5±5.61 cm) and 55 women (body mass: 65±11 kg, body height: 166.79±5.94 cm). The tests involved anthropometric measurements of the body and the ability to keep balance. Students at both universities did not differ significantly in body build and balance.

All the test participants agreed to take part in the tests. The study design was approved by the Bioethical Committee at the The Jerzy Kukuczka Academy of Physical Education in Katowice before commencement of the study (decision no. 3/2019).

Balance measurements were conducted using the Zebris FDM-S measuring platform (Zebris Medical GmbH, Isny, Germany). Each participant's body was subjected to 26 measurements (Table 1). All measurements were carried out in accordance with the Martin technique. Mean values were adopted for the measurements done on both sides of the body. The measurements were conducted using an anthropometric equipment, such as anthropometer, callipers and centimetre tape measure. On the basis of the above-mentioned anthropometric measurements, 9 indexes of body proportions were calculated (Table 2).

Table 1. Characteristics of the examined material

	Men			Women			
	Mean	Min-max	SD	Mean	Min-max	SD	p
Age [years]	20.6	18.8-24.7	1.5	20.7	18.8-30.6	1.9	0.5929
Body measurements							
Body weight [kg]	81.9	55-117.5	13.9	65.4	48.0-106.0	10.7	<0.0001
Stature [cm]	179.5	168.5-194.4	5.6	166.7	154.3-179.0	6.0	<0.0001
Suprasternale height [cm]	145.7	136.5-159.1	5.1	135.7	127.0-146.0	5.1	<0.0001
Acromial height (standing) [cm]	146.6	135.5-161.0	5.2	136.0	127.8-147.5	5.5	<0.0001
Elbow height (standing) [cm]	113.2	100.5-127.9	5.0	105.4	98.5-114.8	4.2	<0.0001
Wrist height [cm]	87.6	76.0-98.8	4.4	82.1	71.4-89.6	3.9	<0.0001
Waist height (Natural) [cm]	113.6	102.0-123.8	4.4	106.6	98.6-115.1	4.7	<0.0001
Tibiale height [cm]	48.1	42.5-55.4	3.1	45.5	40.5-52.9	2.9	<0.0001
Mean Iliospinale height [cm]	101.1	91.8-11.8	4.3	94.0	56.9-103.2	4.2	<0.0001
Sitting height [cm]	94.5	89.8-102.3	3.1	89.2	81.4-96.7	3.2	<0.0001
Trunk length [cm]	54.5	48.3-65.9	4.1	51.1	45.3-57.5	2.9	<0.0001
Mean length of the upper body segment [cm]	90.3	81.5-99.7	4.6	82.1	74.1-90.9	3.8	<0.0001
Upper extremity length [cm]	77.7	61.5-85.7	4.3	70.7	47.3-85.6	6.5	<0.0001
Acromion-Radiale length [cm]	33.4	28.5-36.3	1.9	30.5	23.3-34.8	2.0	<0.0001

	Men			Women			
	Mean	Min-max	SD	Mean	Min-max	SD	p
Radiale-Dactylion III length [cm]	44.3	26.9-49.9	4.2	40.1	20.8-53.1	5.6	0.0001
Lower extremity length (Trochanterion) [cm]	89.2	80.4-100.8	4.4	84.5	73.4-93.9	4.3	<0.0001
Mean Thigh length [cm]	53.0	46.3-60.0	3.0	48.6	40.6-56.0	3.4	0.0023
Head and neck height [cm]	33.8	30.6-38.2	1.5	31.0	27.1-34.5	1.6	<0.0001
Biacromial breadth [cm]	40.2	33.2-44.1	2.3	36.4	33.0-39.7	1.6	<0.0001
Bideltoid breadth [cm]	47.3	40.4-55.2	3.3	41.8	35.5-50.7	2.8	<0.0001
Chest breadth [cm]	28.9	24.5-33.5	1.9	24.8	22.4-29.0	1.8	<0.0001
Chest depth[cm]	19.5	16.4-23.0	1.5	18.1	14.6-23.0	1.9	0.0003
Biiliocristale breadth [cm]	28.8	22.6-33.5	1.9/2	27.9	25.1-32.0	1.7	0.0239
Chest circumference (below bust) –rest [cm]	87.6	75.7-102.5	6.2	75.4	67.0-89.0	5.5	<0.0001
Chest circumference (below bust)-inhalation [cm]	93.1	83.0-109.0	6.0	79.8	71.5-92.0	5.2	<0.0001
Waist circumference [cm]	79.1	66.0-101.0	6.7	72.1	62.0-93.0	7.2	<0.0001
Buttock (hip) circumference [cm]	98.9	82.7-115.0	6.7	98.1	83.0-123.0	7.4	0.5558
Thigh circumference [cm]	57.6	47.8-68.5	4.8	56.8	45.0-77.0	5.5	0.4881
Indices							
BMI [kg/cm^2]	25.4	18.0-35.3	3.9	2.5	17.8-36.7	3.6	0.0154
Sitting Height Ratio (SHR)	52.4	41.4-55.1	2.0	53.3	40.8-56.1	2.1	0.0004
Skelic index	91.1	81.6-141.5	8.6	88.0	78.1-145.3	9.1	0.0003
Upper extremity length to stature index	43.3	32.7-47.2	2.5	42.4	29.4-51.5	3.4	0.1219
Arm length to height index	18.6	15.4-20.8	1.1	18.3	16.6-20.2	1.0	0.1373
Arm to forearm index	76.9	61.3-97.2	7.8	76.9	62.1-112.9	8.3	0.3126
Lower extremity length to stature index	56.3	54.0-59.8	1.3	56.4	53.7-59.3	1.4	0.5640
Width- breadth chest index	67.7	56.6-84.2	6.4	73.4	58.3-84.4	7.1	<0.0001
WHR waist to hip ratio	0.81	0.73-0.90	0.03	0.73	0.66-0.85	0.04	<0.0001
WTR waist to high ratio	138.6	123.4-157.8	6.5	127.0	110.5-157.4	9.2	<0.0001
Body balance parameters							
Sway path (SP) EO [mm]	326.3	183.5-592.9	81.8	345.4	179.7-563.4	70.0	0.2126
Sway path (SP) EC [mm]	380.0	217.9-716.2	101.9	395.913	272.3-606.0	83.8	0.3948
Elipse area (EA) EO [mm^2]	95.1	12.4-264.4	59.6	96.0	20.4-331.6	67.6	0.9451
Elipse area (EA) EC [mm^2]	127.1	21.6-458.8	101.0	113.0	26.4-285.2	68.1	0.4122
Mean velocity EO [mm/s]	5.4	3.1-9.9	1.4	5.8	3.0-9.4	1.2	0.2126
Mean velocity EC [mm/s]	6.3	3.6-11.9	1.7	6.6	4.5-10.1	1.4	0.3948

Table 2. Anthropometric indexes calculated on the basis of the conducted measurements

Indices	Calculation method
SHR sitting height ratio	([BS-v]/[B-v])*100 SHR, sitting height/Stature ×100
Skelic index	([B-v]-[BS-v]/[BS-v])*100 (limb length / body length with head) * 100 (length of the legs / length of the trunk with head) x 100
Upper extremity length to stature index	([a-daIII]/[B-v])*100 (Upper extremity and palm length / Stature) * 100
Arm length to height index	([a-r]/[B-v])*100 (Acromion-Radiale Length / Stature) *100
Arm to forearm index	([r-sty]/[a-r])*100 (forearm length / Acromion-Radiale Length) *100
Lower extremity length to stature index	([B-is]/[B-v])*100 (length of the lower limb / Stature) *100
Width-breadth chest index	([xi-ths]/[thl-thl])*100 (Chest depth / Chest breadth) * 100
WHR waist to hip ratio	Waist circumference / Buttock (hip) circumference
WTR waist to high ratio	Waist circumference / Thigh circumference
BMI body mass index	Body weight / (Stature in m)^2

The balance test (the analysis of the position of the resultant of ground reaction forces) was based on the Romberg test. During that test, a study participant was standing on their lower limbs, which were positioned as wide apart as the width of their pelvis, and their arms were hanging freely alongside their body. Romberg's test was conducted twice: with eyes open (EO) and eyes closed (EC). The time of each test equalled 60 seconds.

The analysis involved 2 parameters:

- mean velocity of the COP movement (MV) [mm/s] – total length of the path covered by the COP (the path covered by the centre of pressure of ground reaction force during the measurement) divided by the time of the test duration,
- ellipse area (EA) [mm^2] in which the COP was located during the test (the surface area of ellipse created by 95% of the COP positions during the test).

Analyzed values were obtained from a 30-second measurement (i.e., from 15. to 45. second).

Descriptive statistics, which are presented in Table 3, include mean, standard deviation and range values. Analyses were performed for each sex separately. Because of non-normal distribution of stabilographic parameters, MV and EA were logarithmically transformed. Furthermore, correlation between stabilographic and anthropometric parameters were studied, using Pearson's coefficients. Computations were made for the values obtained during tests with eyes open and closed. Next, for each obtained correlation coefficient a significance test was carried out. The test values at $*p<0.05$; $**p<0.01$; $***p<0.001$ were considered statistically significant.

Table 3. Pearson's correlation coefficient values between stabilographic parameters (MV and EA) in tests with eyes open (eo) and closed (ec)

	MV eo	EA eo	MV ec	EA ec
		Men		
MV eo	-	0.45**	0.76***	0.45**
EA eo	0.44**	-	0.44**	0.68***
MV ec	0.50***	0.28*	-	0.59***
EA ec	0.18	0.62***	0.40**	-
		Women		

Legend: MV – mean velocity of the COP movement [mm/s]; EA – ellipse area [mm^2]; eo – eyes open; ec – eyes closed; level of significance: ** $p<0.01$, *** $p<0.001$

Results

Table 3 presents the correlation values for the stabilographic parameters obtained in tests with open and closed eyes. In women, the correlation values were within the 0.18 to 0.62 range, whereas in men the values ranged between 0.44 and 0.76. The above-mentioned values indicated the lack of full dependence between the path length and ellipse area, which suggests a different impact of various factors on the balance parameters in both sexes in different conditions.

Table 4. Pearson's correlation coefficients between the body build and balance

	Men				Women			
	eyes open (eo)		eyes closed (ec)		eyes open (eo)		eyes closed (ec)	
	MV	EA	MV	EA	MV	EA	MV	EA
Body measurements								
Body weight [kg]	-0.33*	ns	ns	ns	-0.45**	ns	ns	ns
BMI	ns	ns	ns	ns	-0.51***	ns	ns	ns
Stature [cm]	ns	ns	ns	ns	ns	0.31*	0.30*	0.36**
Suprasternale height [cm]	ns	ns	ns	ns	ns	ns	ns	0.36**
Acromial height (standing) [cm]	ns	ns	ns	ns	ns	0.32*	ns	0.39**
Elbow height (standing) [cm]	ns	ns	ns	ns	ns	ns	ns	0.30*
Wrist height [cm]	ns	ns	ns	ns	ns	ns	ns	ns
Waist Height (Natural) [cm]	ns	ns	ns	ns	ns	ns	ns	0.33*
Tibiale height [cm]	ns	ns	ns	ns	ns	0.33*	ns	0.34*
Mean Iliospinale height [cm]	ns	ns	ns	ns	ns	ns	ns	0.29*
Sitting height [cm]	ns	ns	ns	ns	ns	ns	0.42**	0.33*
Trunk length [cm]	-0.29*	ns	ns	ns	ns	ns	0.35*	ns
Mean length of the upper body segment [cm]	-0.30*	ns	ns	ns	ns	ns	0.42**	ns

Tab 4 (cont.)

	Men				Women			
	eyes open (eo)		eyes closed (ec)		eyes open (eo)		eyes closed (ec)	
	MV	EA	MV	EA	MV	EA	MV	EA
Upper extremity length [cm]		ns	ns	ns	ns	0.31*	0.28*	0.39**
Acromion-Radiale length [cm]	-0.38**	ns	ns	ns	ns	0.36**		0.40**
Radiale-Dactylion III length [cm]		ns	ns	ns	ns		0.29*	0.32*
Lower extremity length (Trochanterion) [cm]		ns	ns	ns	ns	ns	ns	0.33*
Mean Thigh length [cm]	-0.31*	ns	ns	ns	ns	ns	ns	ns
Head and neck height [cm]		ns	ns	ns	.35*	0.28*	0.34*	ns
Biacromial breadth [cm]		ns	ns	ns	ns	ns	ns	ns
Bideltoid breadth [cm]	-0.29*	ns	ns	ns	ns	ns	ns	ns
Chest breadth [cm]		ns	ns	ns	ns	ns	ns	ns
Chest depth[cm]	-0.35*	-0.37*	ns	ns	-0.30*	ns	ns	ns
Biiliocristale breadth [cm]	-0.35*	ns	ns	ns		ns	ns	ns
Chest circumference (below bust) - rest [cm]	-0.34*	ns	ns	ns	-0.30*	ns	ns	ns
Chest circumference (below bust) - inhalation [cm]	-0.36*	ns	ns	ns	ns	ns	ns	ns
Waist circumference [cm]	-0.36*	ns	ns	ns	-0.44**	ns	ns	ns
Buttock (hip) circumference [cm]	ns	ns	ns	ns	-0.50***	ns	ns	ns
Thigh circumference[cm]	ns	ns	ns	ns	-0.51***	ns	ns	ns
Indices								
Sitting height ratio (SHR)	ns	ns	ns	ns	ns	ns	ns	ns
Skelic index	ns	ns	ns	ns	ns	ns	ns	ns
Upper extremity length to stature index	ns	ns	ns	ns	ns	ns	ns	0.29*
Arm length to height index	ns	ns	ns	ns	ns	ns	ns	ns
Arm to forearm index	ns	ns	ns	ns	ns	ns	ns	ns
Lower extremity length to stature index	ns	ns	ns	ns	-0.30*	ns	-0.37**	ns
Width-breadth chest index	ns	ns	ns	ns	ns	ns	ns	ns
WHR waist to hip ratio	ns	ns	ns	ns	ns	ns	ns	ns
WTR waist to thigh ratio	-0.31*	ns	ns	ns	ns	ns	ns	ns

Legend: MV – mean velocity of the COP movement [mm/s]; EA – ellipse area [mm^2]. Level of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns – not significant.

The tests of Pearson's correlation r (Table 4) revealed that correlation between body parameters and features connected with stability is statistically significant, in a small or moderate degree. Correlations of the highest level of statistical significance were observed only in women, in tests with open eyes. Such

correlations were related to the association between sway velocity (MV) and factors such as: body mass ($r = -0.45$, $p<0.001$), BMI ($r = -0.51$, $p<0.0001$), hip circumference ($r = -0.50$, $p<0.0001$), the largest circumference of the thigh ($r = -0.51$, $p<0.0001$) and waist circumference ($r = -0{,}44$; $p<0.001$). Negative values of the correlation coefficients revealed that higher velocity was shown by women having smaller body mass as well as smaller circumferences of waist, hips and thighs. Moreover, women who swayed more quickly had significantly smaller circumference of the chest and smaller depth of the chest as well as a longer head with the neck. In addition, the MV EO significantly depended on the proportions of the length of the lower limb in relation to the body height (women with relatively short legs were prone to swaying more quickly). After the closure of the eyes, the correlation values decreased and became statistically insignificant. None of the breadth dimensions and body circumferences showed any considerable influence on the velocity of sways. After the elimination of the sight control, the sway velocity path was affected by the length dimensions of female bodies. Taller women having longer spine and longer upper limbs were characterized by considerably higher velocity. Moreover, the MV EC significantly depended on the proportions of the length of the lower limb in relation to the body height, which means that women with shorter legs in relation to the body height were prone to swaying more quickly).

In women, ellipse area EA, contrary to MV, showed a significant correlation solely with length parameters, and not with breadth parameters of the body. A bigger surface area of ellipse in tests with open eyes was typical of taller women with a higher position of their shoulder, knee, and longer upper limbs. After the closure of the eyes, different measurements describing the height of the body and length of its individual segments gained on statistical significance in their relation to the ellipse area confirming greater sways in taller women with longer upper and lower limbs.

In tests with open eyes, greater velocity of sways was characteristic of men with smaller body mass ($r = -0.33$; $p<0.05$), shorter trunk, shorter upper limbs and shorter thighs. Moreover, those who swayed more quickly were characterized by smaller breadth dimensions of the body, such as: upper breadth of the body ($r = -0.29$, $p<0.05$), hip breadth ($r = -0.35$, $p<0.05$), chest depth ($r = -0.35$, $p<0.05$) and smaller circumferences of the body, such as: chest at rest ($r = -0.34$, $p<0.05$) and chest while breathing in ($r = -0.36$, $p<0.05$) as well as waist ($r = -0{,}36$, $p<0.05$). Quicker sways were characteristic of men with lower WTR values, i.e., a smaller circumference of the thigh in relation to the waist circumference. After the closure of eyes, none of the parameters of the body build and shape in men significantly influenced the velocity of sways. This fact suggests that in such a situation the men's body build lost its significance for the stability of the body and men with various types of body build swayed in a similar way with their eyes closed.

Among all analyzed dimensions of male bodies, such as length, circumference and breadth, none showed any significant relationship with the ellipse area in tests with open eyes. The only dimension that revealed some relationship was the depth of the chest. Men with more oval chests had a significantly larger ellipse area describing the sways. After the

closure of eyes, the men's body build and shape did not considerably affect the size of the ellipse area.

Moreover, it was observed that men revealed a significant relationship between sway velocity in tests with eyes opened (EO) and mainly the build of the upper part of their body, namely the length of trunk and upper segment of the body, the breadth of the upper part of the body, the circumference of thorax and waist as well as chest depth. On the other hand, in women, a significant relationship occurred both in the case of upper body dimensions (e.g., thorax and waist circumferences) and lower body dimensions (e.g., hip and thigh circumferences).

Discussion

Balance in terms of biomechanics is defined as ability to keep the centre of gravity of the body over the base of support. However, the borderline of stability does not coincide with the outline of the feet. Postural stability is one of the most important indexes of correct body posture and involves ability to regain balance. The size of sways is described by parameters connected with the stabilometric path, most often with the path length (or the velocity of sways – the value obtained from the division of the path length by the test time) and the size of the ellipse area describing maximum sways occurring in a position of standing (Jurkojć 2018). Velocity of sways and the ellipse area showed a moderate correlation (from 0.4 for women with eyes closed to 0.59 for men with eyes closed), which means that, for instance, study participants making quicker movements around the centre of mass (with a longer path of stabilogram) may achieve both large and small values of the ellipse area. The correlation values suggest that postural stability depends on various and not always the same factors. Literature mentions age and sex, efficiency of body functioning, proper posture, muscle strength as well as body build and shape (Wang et al. 2022).

The present study describes the ability to keep balance by means of the velocity of sways MV and the area of ellipse EA. The above-mentioned are indicated as the most informative parameter when body sway is assessed (Raymakers et al. 2005; Błaszczyk and Beck 2023). The obtained results showed a different influence of the body build on MV, and different on EA.

In general, the velocity of sways significantly depended on the dimensions describing the breadth of the body, for instance the breadth of the upper body (in men), hip breadth (in men), chest circumference (in both sexes), waist circumference (in both sexes), hip circumference and thigh circumference (in women). The smaller breadth dimensions in a tested person, the higher sway velocity (and the longer path of stabilogram) they showed. Similar results were obtained by Lipowicz et al. (2019a; 2019b) in the case of children and adolescence. Regardless of age, children and youth characterized by lower body circumferences (thorax, waist, hips, arms) swayed more, especially in medio-lateral plane. Also, Alonso et al. (2015) suggested that the fat mass concentration in the chest and abdomen (android shape) increases the load on the hips, explaining the larger stabilographic medio-lateral path. Smaller breadth dimensions may indicate weaker muscularity of the body, lower mass of muscles and more delicate skeleton structure (Xiao et al. 2005; Malakar et al. 2022). The dependence between the sway con-

trol and a relatively low muscle component was observed in the investigations of girl gymnasts, where ectomorphic subjects showed 72% of more body sway than endomorphic girls (Allard et al. 2001). It was also reported that there was a certain relationship between a degree of muscularity of lower extremity and sways (Muehlbauer et al. 2015). Weaker muscles of lower limbs are responsible for relatively greater sways, whereas strength training improves the postural stability of the body (Youssef et al. 2018). In addition, the results of Alonso et al. (2015) suggest that lower lean body mass can be a risk factor for the postural control. In addition, what cannot be excluded is greater tiredness of muscles in slimmer, less muscular subjects (Sterkowicz et al. 2016). This fact may cause greater difficulty in keeping motionless body posture and result in higher velocity of sways.

The ellipse area is a parameter describing the range of maximum sways which can be achieved by a person in a position of free standing. The obtained results show that the size of the ellipse area depends on the body elements connected with the location of the COP, namely the dimensions of the body height measured, for instance from the ground to the top of the head, shoulder, elbow, waist, knee, and correlated length of upper extremities. The higher the centre of mass is located, the greater ellipse area the body sways in free standing. Among the tested adults the ellipse area EA changed along with the length dimensions, such as the height of body, shoulder, knee, head with the neck as well as the length of upper extremity. In the test performed without eyesight control, the above-mentioned relationship only grew in importance. Generally speaking, the higher the measurement point was located in a tested person (e.g., the top of the head, jugular notch, iliac spine), the greater the ellipse area became in a standing position. These associations were statistically significant only in women. Similarly significant positive correlations of length dimensions (height and trunk-cephalic length), and not waist-hip ratio (WHR) with the COP area, were reported among adult men and women from Brazil in tests with open eyes (Alonso et al. 2015). From a biomechanical perspective, greater sways in tall and slim women result from a higher location of the centre of mass (COM) of the body. Such a postural sway can be explained by the inverted pendulum model, which is based on the relation between the motion of a pendulum and its length, mass, and stiffness. According to this model, in a position of free standing the body sways mostly around the ankle joint. It may be supposed that the fact that taller women are prone to greater sways results from behaviour. Shorter women far more often wear high-heeled shoes and thus most probably train the postural stability and cope with greater sways (Wan et al. 2019). However, whether foot shape and more flexible longitudinal arch observed in taller and heavier women leads to a greater postural sway (Aurichio et al. 2011; De Blasiis et al. 2023) is an area for further investigation.

Body mass and BMI are anthropometric variables which, next to body height, are the most often analyzed factors influencing the ability to keep balance. However, the results of investigations are not uniform. In the current work, the BMI turned out to be a vital factor affecting only the velocity of sways in women in the tests with open eyes ($r = -0.51$, $p < 0.0001$). The higher the BMI in women, the lower velocity of sways was

achieved by women. Among young men no significant relationship was revealed, either with MV or EA. Among Brazilian adults aged over 60, the BMI and fat mass did not seem to influence the balance during a one-leg stance task (Pereira et al. 2018). A different study ascertained that the body mass was an independent factor and accounted for as much as 52-54% of the variance of balance stability in group of men with a wide BMI spectrum (17.4–63.8 kg/m^2; Hue et al. 2007), in whom the decline of balance stability was strongly correlated with an increase in body weight. Moreover, Mainenti et al. (2011) showed that elderly women with more fat mass had larger balance sway. In addition, Neri et al. (2021) found that there is no differences between women with gynoid and android obesity. Winters and Snow (2000) reported that 31% of postural sway variability in premenopausal women was caused by the fat mass. Conversely, Farenc et al. (2003) analysing the influence of body characteristics of 20-60 years-old individuals on their upright stance, showed that thinner subjects have larger horizontal displacements of the centre of gravity (COG) than normal or corpulent subjects. Smaller sways in subjects with larger BMI, which were observed in the present work, may relate to a low variability of this feature in the studied population (young healthy persons, without overweight or obesity) and specificity of the BMI index measuring rather muscularity than fat content in young people.

Our study confirmed the conclusion drawn by Alonso et al. (2015) reporting that for the young adults, without major diseases or other abnormalities, the anthropometric variables had different relations to postural sway according to sex. For instance, men showed a statistically significant correlation between the velocity of sways and the dimensions of the upper parts of the body, whereas women revealed such correlation for both upper and lower parts of the body. The reasons for such dimorphic differences can be found in diverse distribution of fat tissue (android and gynoid type of the adipose tissue distribution) and muscle tissue as well as different proportions of the body in both sexes (broader shoulders in men, broader hips in women).

After closing their eyes, both men and women showed an increase in sway velocity and ellipse area. This fact confirms significance of the visual stimulus for the body stability. However, the elimination of vision had a different impact on the analysed relationship in both sexes. In men with closed eyes, the value of sways ceased to depend on their body build, while in the case of women with closed eyes, their body build began to play a greater role for their stability. This fact can be observed particularly in the ellipse surface area. Similarly, Chiari et al. (2002) showed that the postural sway parameters increase while in a position of standing with eyes closed, and further, that body size and body composition are strongly related to postural sway in conditions with eyes closed. However, Alonso et al. (2015), in their multi-factor analysis encompassing both men and women, stated the significance of trunk-cephalic length for sway velocity and the COP area in tests with eyes closed, whereas the tests with eyes open showed the importance of only body height. This phenomenon can be explained by the possibility of two diverse strategies (ankle and hip strategies) applied by both sexes to both testing conditions (which can be seen in the differences in the degree of muscularity and muscle training, body shape and

the point of the body mass weight, differences in the risk of falling at an elderly age between sexes). The literature reports some contrary observations showing that after the closure of eyes the stiffness decreases in the tarsal joint, which increases sways (Rothwell 2012), or vice versa, that the stiffness increases after the closures of eyes to reduce the risk of falling (Alonso et al. 2015). Regardless of the observations related to the change in body stiffness, it is clearly visible that when the visual information is omitted, signals from the somato-sensory and vestibular systems have a greater importance for the postural control, especially in women. An increased sensitivity to sensory information from proprioceptive and vestibular systems, activation of receptors placed in the muscles and joints, together with vestibular cues, provide the brain with information about where the body and its parts are located with respect to the gravitational environment (Tanaka et al. 2000). In addition, Alonso et al. (2015) suggested that ankle and hip strategies have opposite behaviours in relation to vision and the inverted pendulum.

Conclusions

From the perspective of postural correction therapy and the prevention of falls in persons with different types of disorders, investigating the relationship between body build and balance keeping is of considerable interest. Few studies investigating this issue have focused mainly on the relationships between sway values and body height, body mass and the BMI. Study participants of such studies tended to be characterized by specific features, for instance exhibiting obesity (Greve et al. 2007), disability (Lipowicz et al. 2019a), or focus on a specific age class, e.g., children (Lipowicz et al. 2019b; Plandowska et al. 2019) or the elderly (Jochymczyk-Woźniak et al. 2018).

This work, on the other hand, presents a relationship between balance parameters and a big number of measurements which precisely describe the body build of young adults, men and women, without balance disorders and with diverse body structure. The obtained results showed that the smaller breadth dimensions in a tested person, the higher velocity of sways (and the longer path of stabilogram) was observed. On the other hand, the ellipse area was substantially dependent on these body elements which is related to the location of the COP. The higher the position of the COP, the larger the ellipse area made by the body sway in a position of free standing. The pattern of dependence of sway values in adults was different in both sexes. It also depended on the visual control (eyes opened / closed) and the analysed balance parameter (sway velocity / ellipse area). These relations were often statistically significant although low; in general, they achieved higher values in women than in men.

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Ethics statement

The study design was approved by the Bioethical Committee at the The Jerzy Kukuczka Academy of Physical Education

in Katowice before commencement of the study (decision no. 3/2019). All the test participants agreed to take part in the tests.

Authors' contribution

AL – is the initiator of the work, participated in the collection of material; she is a co-author of the paper's draft and final versions; MNB – performed the statistical analyses; KG – took part in collecting material and writing the text; KN-L – participated in collecting material and writing the text; KJ-W – participated in collecting material and writing the text; DF – participated in collecting material and writing the text; RM – participated in the interpretation of the results; AWM – participated in the interpretation of the results.

Corresponding author

Anna Lipowicz, Department of Anthropology, Institute of Environmental Biology, Wrocław University of Environmental and Life Sciences; Kożuchowska 5; 51-631 Wrocław, Poland; phone: +48 71 320 5898; e-mail: Anna.Lipowicz@upwr.edu.pl

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Morphological and genetic aspects of Marfan Syndrome as demonstrated by a case of death during pregnancy with the discovery of two *de novo* missense mutations in the FBN1 gene

Isabella Aquila[1], *Matteo Antonio Sacco*[1], *Silvia Boca*[2], *Donatella Malanga*[3], *Giuseppe Viglietto*[3], *Ludovico Abenavoli*[4], *Martino Maesani*[1], *Elena Varotto*[5,6], *Francesco Maria Galassi*[7], *Pietrantonio Ricci*[1]

[1] Institute of Legal Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, Italy
[2] Department of Forensic Medicine, Asl Città di Torino, Turin, Italy
[3] Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, Italy
[4] Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy
[5] Archaeology, College of Humanities, Arts and Social Sciences, Flinders University, Adelaide, SA, Australia
[6] FAPAB Research Center, Avola (SR), Sicily, Italy
[7] University of Lodz, Faculty of Biology and Environmental Protection, Department of Anthropology, Poland

Abstract: Marfan Syndrome (MFS) is an autosomal dominant disease caused in most cases by mutations in the FNB1 gene, which encodes for fibrillin 1. MFS does not alway shows typical phenotypic signs. Indeed, the occurrence of sudden death of unknown cause is increasingly seen in young adults without *ante mortem* pre-existing pathology to explain the event. In many cases the diagnosis of Marfan Syndrome (MFS) is carried out *post mortem*, especially in cases where the disease's external phenotype is absent. Here is reported a case of a young woman who died during a twin pregnancy investigated with medico-legal and forensic anthropological procedures. The autopsy showed the absence of a typical marfanoid habitus and the presence of a dissecting aneurysm of the aorta with histopathological degeneration of the aortic elastic fibers. The genetic investigation revealed two previously undetected *de novo* mutations of the FBN1 gene: c.T6181C: p.C2061R and c.G1415A: p.C472Y. This new mutations, together with a comprehensive analysis, demonstrates the existence of a causal relationship between these mutations and the dissecting aneurysm of the aorta. This also stresses the importance of a combined multidisciplinary approach to this condition which includes morphological and genetic studies.

Key words: Marfan Syndrome, genes, mutation, dissecting aneurysm, legal medicine.

Original article

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Introduction

Marfan Syndrome (MFS) is an autosomal dominant disorder caused by genetic mutations. In 66-91% of cases, these mutations involve *FBN1,* a gene that provides instructions for making fibrillin-1 (15q21.1); less frequently, mutations involve *TGFβRl* or *TGFβR2* (also present in Loeys-Dietz syndrome) (Dean 2007). Fibrillin, an extracellular matrix glycoprotein which is a structural component of calcium-binding microfibrils, was discovered in the 1980s thanks to monoclonal antibody technology, whereas *FNB1* was directly linked to MFS in 1991 (Sakai et al. 1986). Fibrillins' domain organisation has been shown to have been conserved throughout evolution, with a structure constituted by 43 calcium-binding epidermal growth factor-like (cbEGF) domains interspersed with 7 transforming growth factor β-binding protein-like (TB) domains (Piha-Gossack et al. 2012; Jensen et al. 2021). Mutated *FBN1* alters the structure of fibrillin-1 or decreases its amount, thus compromising the tensile properties of the microfibrils (Frydman 2008). Since, as it will be explained subsequently, no palaeogenetic tests have been carried out so far on possible ancient MFS cases, no information is available regarding the exact first emergence of *FBN1* mutations in hominin evolution. Nevertheless, a study by Asgari and colleagues (2020) clearly demonstrated how a positively selected *FBN1* missense variant in a Peruvian sample contributes to reducing that population's stature, which could possibly speak for a major and long-standing role for altered forms of fibrillins in human evolution. Indeed, as Asgari and colleagues underlined (2020) "it is also possible that other FBN1-related traits like changes in cardiovascular system performance have offered an evolutionary advantage" in their studied population.

From a clinico-morphological perspective the disorder can involve several organs and systems: the skeleton (*pectus carinatum, pectus excavatum*, joint hypermobility, high palate with dental crowding, *protrusio acetabuli,* scoliosis, reduced elbow extension, *pes planus*), the eye (*ectopia lentis,* flat cornea, increased axial length of globe, hypoplastic iris or ciliary muscle), the heart (dilatation of the aortic root, mitral valve prolapse, dissection of the ascending aorta), the lungs (spontaneous pneumothorax, apical blebs), the skin (*striae atrophicae*) and the dura mater (lumbosacral dural ectasia). These alterations may coexist or not, with a very heterogeneous clinical presentation, which makes the correct diagnosis difficult (Dean 2007). Recent studies by the Milan anatomy research group (Dolci et al. 2018) have once more highlighted the relevance of a morphological assessment of MFS from the facial abnormalities by presenting a quantitative method of assessing the phenotype, whereas other clinical studies focus on the cardiovascular aspects on the disease (Bianucci et al. 2023).

In 2010, the diagnostic criteria for MFS were revised. The two cardinal features of MFS (aortic root aneurysm/dissection and *ectopia lentis*) assumed a greater role in diagnosing the syndrome and molecular genetic screening for *FBN1* and other relevant genes (e.g. *TGFβR1* and 2) became more important. Additionally, from the histological point of view, MFS is characterised by loss of elastic fibers, pseudocystic medionecrosis, with deposition of mucoid substances, as evidenced in our case and in other studies (Klintschar et al. 2009). However, these findings are not specific to MFS. Indeed,

they are present in other connective disorders (e.g. Ehler-Danlos) and, in the longer term, arterial hypertension, which speaks for the great heterogeneity of this condition, a characteristic which also clearly emerged from external morphological studies (Dolci et al. 2018). Early mortality from MFS results from aortic dilatation (Krause 2000).

These diagnostic and interpretative difficulties must be considered relevant not only to diagnosticians in their attempt at a more refined diagnosis which could help avoid sudden death in MFS patients (i.e. the role of an early diagnosis), but also to (forensic) anthropologists, morphologists and geneticists studying the disease both in modern and ancient populations in an attempt to determine its antiquity and evolutionary course. Hence a multidisciplinary investigation approach ought to be considered optimal for this rare disease which, due to the relative lack of raw date on the quality of life (QOL) of patients, has been the subject of a recent comprehensive study in Poland (Trawicka et al. 2022). This showed how MFS patients have a reduced QOL with subsequent need for medical and psychosocial assistance.

Moreover, Wozniak-Mielczarek et al. (2022) in a morphological study of a Polish population indicated how the diagnosis of MFS still remains "sophisticated" and the differentiation between MFS patients and those who simply present with a marfanoid *habitus* (i.e. they externally appear to have MFS but do not) cannot be established merely on the basis of an analysis of external body features. It thus follows that the definitive diagnostic tool is genetic analysis, which confirms of the disorder in order to make an early diagnosis and recommend genetic counselling to the patient and their relatives. Genetic counselling allows physicians to give information on the nature, inheritance pattern and implications of the genetic disorder so as to help them make informed medical and personal decisions (Dietz 2001).

For this reason, identifying new mutations linked with MFS and better describing its genotypic background represents a new and fruitful research avenue. In this article we present a recent medical case study and offer both anthropometric, histologic and genetic data, which we believe could be to benefit both of established pathologists and MFS diagnosticians as well as biological anthropologists attempting to apply present-day methodologies to the reconstruction of this antiquity and evolution of MFS.

Case presentation and Methods

A 35-year-old woman with a diagnosis of MFS and a positive family history was hospitalised at 23 weeks pregnant with twins because of vomiting and epigastric and spinal pain. She died during an investigation by an angio-CT scan, 4 days after being admitted to hospital. Physicians suspected an aortic dissection to be her cause of death. The patient had undergone cataract surgery years before her death and physicians over time had suspected MFS to be presenting in her. A medicolegal autopsy and a forensic anthropological assessment were carried out. Research of genetic mutations associated with cardiovascular defects was performed. The panel analyses the coding regions of 404 genes related to cardiovascular alterations, as well as 94 noncoding loci implicated in the pathogenesis of cardiovascular diseases and 2 expansion regions of *DMPK* and *CACNA1A* genes. The analysis of the sequences focused on coding regions of 16 genes associated with Marfan's Syndrome, Loeys-Dietz Syndrome, aneurysms and dissections of

thoracic aorta: *ACTA2*, *CBS*, *COL3A1*, *COL5A1*, *FBN1*, *FBN2*, *FLNA*, *MED12*, *MYH11*, *MYLK*, *NOTCH1*, *PRKG1*, *SKI*, *SLC2A10*, *SMAD3*, *SMAD4*, *TGFB2*, *TGFBR1*, *TGFBR2*. The employed technique was Next Generation Sequencing (NGS) and validation of the pathogenetic variants through automated sequencing according to the Sanger method (Tipu et al. 2015). Research of mutations of the genes included in the panel was carried out through automated sequencing according to the Sanger method. This research was made by means of a set of primers (Ion AmpliSeqTM Cardiovascular Research Panel) producing 1043 amplicons. Analysis was performed according to the protocol of manufacturer using the Ion-Torrent PGM platform. This technique is based on sequencing by synthesis and detection trough semiconductor chip method. Coverage >20; Quality Control (QC) >20 are the criteria used for calling the variants. The presence of surely pathogenetic ones was confirmed by means of PCR amplification and traditional sequencing in accord to the Sanger method, using an automatic fluorescent technology analyser.

The NGS technology can lead to errors at different stages of the process, and it has got a sensitivity equal to 95% (Qin 2019).

Results

1. Visual examination of the cadaver

The corpse belonged to a female individual assessed to be 35 years old at the time of her death. The total length of the corpse measured on the autoptic table was 185 cm. The colour of the iris was brown and a subluxation of the crystalline lens emerged from the analysis of the medical record. Inspection of the oral cavity showed no abnormalities worthy of note and the absence of dental prostheses. The examined body appeared to be characterised by regular nutrition and showed muscular trophism. The *panniculus adiposus* was regularly present. *Striae gravidarum* (stretch marks) were noted. Normally formed external genitalia were recorded.

2. Anthropometric data

Upper limbs = 97 cm (measuring the distance between the humeral head and the distal phalanx of the middle finger of the hand).

Lower limbs = 97 cm (measuring the distance between the anterior superior iliac spine and the sole of the foot, medially, at the level of the tuberosity of the scaphoid bone).

Right wrist circumference =17 cm.
Left wrist circumference = 17 cm.
Left ankle circumference = 23 cm.
Right ankle circumference = 23 cm.

3. Autoptic and histological findings

During the dissection of the cadaver, only the presence of a dissecting aneurysm of the aorta, from the intrapericardial ascending trait to the aorto-iliac bifurcation of this artery was revealed. According to the Stanford classification, this dissection was catalogued as type A, with generation of a false lumen. At sections of the aortic lumen, both real and false one, the medium adventitial dissection was 45 cm long. The section of the intrapericardial anterior wall of the aortic root disclosed the presence of an ulcerated atheromatous plaque with a contextual intramural blood collection and a haemorrhagic suffusion in the periaortic adipose tissue and subepicardial area.

At histological examination, hematoxylin-eosin staining of the media of the aorta showed pseudocystic degeneration with deposition of mucoid substances (Figs. 1–2), and dissection of the aorta

(Figs. 3, 5). Moreover, Weigert van Gieson stain evidenced disorientation and fragmentation of the elastic fibers (Fig. 4).

All these findings together allow us to postulate the presence of the Marfan's Syndrome, as the main condition affecting the deceased patient. Specifically, regarding her cause of death, based on the evidence emerged from her autopsy, acute cardio-respiratory failure appears to be the most likely explanation. This was caused by a dissecting aneurysm of the aorta with an identified entrance in an ulcerated atheromatous plaque in the intrapericardial ascending aorta due to MFS.

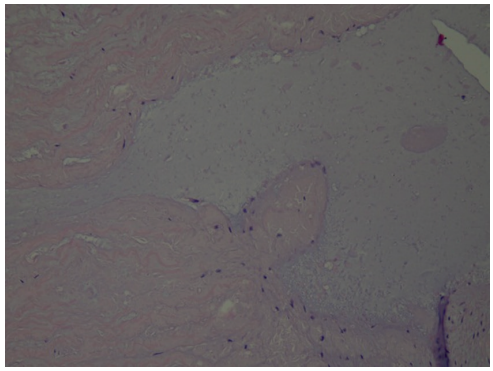

Fig. 1. Histological image of intimomedial mucoid degeneration of the aorta. Hematoxylin-eosin (H/E) stain.

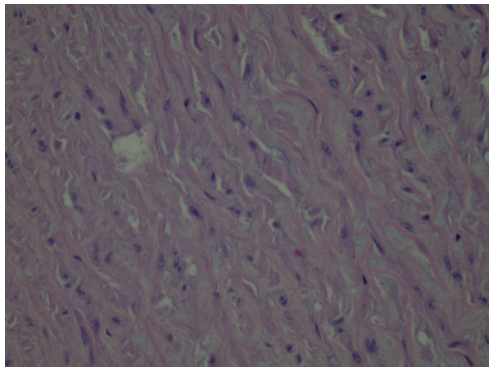

Fig. 2. Histological image of deposition of mucoid substances. H/E stain.

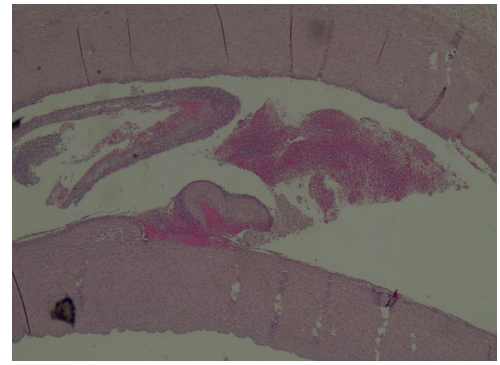

Fig. 3. H/E: Histological image of dissection of the aorta. H/E stain.

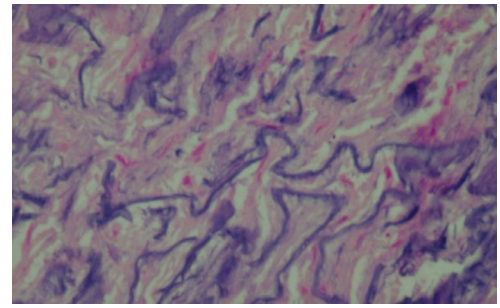

Fig. 4. W: Histological image of fragmentation of elastic fibers. H/E stain.

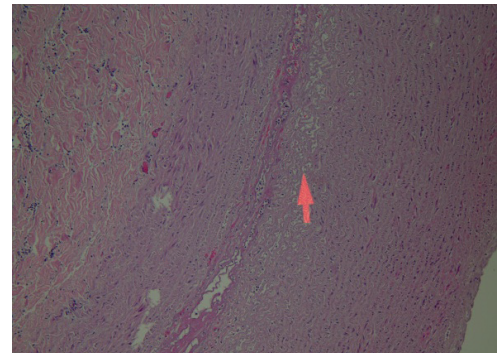

Fig. 5. H/E: Histological image of the point of least resistance in the aorta. H/E stain.

4. Genetic analysis

The FBN1 gene showed the presence of two de novo missense mutations: *C.T6181C:P.C2061R E C.G1415A:P.C472Y.*

Discussion and Conclusion

The antiquity of MFS is currently being disputed with some maintaining that the disease can be confidently retrospectively diagnosed in ancient human remains and those who consider the provided evidence simply too scant. This applies to two cases, one a skeleton, the other a mummy respectively from the Middle Ages and the 19th century AD. In both cases, the importance of confirmatory palaeogenetic analyses has been underlined as a valuable solution to the diagnostic difficulty (Bianucci et al. 2023). Other proposals for MFS in the past have been made through the so-called "indirect" palaeopathological sources (artworks, archival and historical information) on such historical characters as Akhenaten, Paganini and Lincoln, but no conclusive evidence has so far been put forward in the available published literature (Bianucci et al. 2023).

Notwithstanding, despite its still unclarified antiquity, MFS can determine serious consequences as ventricular arrhythmias and sudden cardiac death (Hoffmann et al. 2013), for these reasons it is important a correct and multidisciplinary management of the disease. The multidisciplinary approach, as it has been illustrated above, should always take into account a potential patient's external anatomy as well as his/her anthropometric features, although these features alone may not be sufficient to perform a definitive diagnosis and may not always allow physicians and anthropologists alike to distinguish an MFS phenotype from a marfanoid one.

In this case, analysis of the *FBN1* gene showed the presence of two *de novo* missense mutation: c.T6181C:p.C2061R e c.G1415A:p.C472Y. These pathogenetic variants destroy a cysteine residue and so they are within the allocation criteria for *de novo* pathogenetic variants. To the best of our knowledge, there are no studies detailing the biological and clinical significance of both these variants. In this case, the two mutations did not cause an exuberant, externally observable marfanoid phenotype (with the exception of subluxation of the crystalline) but may reasonably have contributed to the overall pathologic habitus. Indeed, precisely thanks to the *post-mortem* autoptic examination, it can be asserted that the presence of fragmentation of the elastic fibers of aortic wall and myofibrils of the cardiac wall where aortic dissection is present are directly associated with the two aforementioned *de novo* mutations. In particular, despite from a genetic point of view they may fall into the category of the so-called Variants of Unknown Significance (VUS: variants which do not have sufficient information for attributing to them a clinical significance), it can be argued that the destruction of the cysteine residue is directly related to a significant functional mutation in *FBN1*.

The identified variants of *FBN1*, without an externally visible marfanoid phenotype, cause a fibrillin pathological dysfunction related to a predisposition to the development of aneurysms and/or aortic dissections, which may be fatal if it not promptly identified. For this reason, these two newly discovered mutations should be added to the body of knowledge on MFS.

In this preventive perspective, it is important to underline how several studies have shown favourable outcomes in pregnant patients with aortic dissection affected by MFS (Thakur et al. 2012; Allyn 2013; Chuan-Yaw et al. 2013). Surgical treatment has shown some success in type A dissection (Urbanski et al. 2013).

In conclusion, this case also shows that in subjects without symptomatic or externally evident MFS prevention is indeed crucial in order to:

- Reduce fatal events
- Improve outcomes in the short term and long term
- Set early lifestyle and the appropriate medical and surgical therapy
- Expand the search of the disease to family members.

Therefore, the discovery of these two *de novo* new mutations, provided by an autoptic and forensic anthropological study, could also improve the effectiveness of genetic screening, especially in patients who might not be straightforwardly classified as MFS sufferers based on gross clinical examinations (Aquila et al. 2020).

This case also shows how a confirmatory diagnosis of MFS requires the interplay of medicine (clinical and forensic), the morpho-anthropological sciences and genetics in order to attain a more precise understanding of the phenotype and genotype of this still mysterious condition, a challenge both to physicians and anthropologists.

Conflict of interest statement

The authors have no conflict of interest to report.

Authors' contribution

I.A. – conceptualisation, writing of the first draft, diagnostic workflow; data acquisition and analysis; I.A., M.A.S., S.B., D.M., G.V., L.A., M.M., E.V., F.M.G., P.R. – critical revision of the manuscript, editing, revision of the first draft and response to the reviewers' comments, data analysis, data acquisition; P.R. – senior supervision.

Corresponding author

Isabella Aquila, Institute of Legal Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; e-mail: isabella.aquila@unicz.it

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Reliability of retrospective assessment of the age of first menstruation

Ryszard Żarów, Agnieszka Woronkowicz, Barbara Spring, Małgorzata Kowal, Janusz Brudecki

Department of Anthropology, Institute of Biomedical Sciences,
University School of Physical Education, 31-571 Krakow, Av. Jana Pawła II 78

Abstract: The age of the first menstruation is one of the indicators for assessing the course of puberty. It is also a sensitive indicator of the economic situation of individual professional groups or societies, and the low average age of menarche is widely recognized as a marker of society's well-being. The aim of the study was to analyse the reliability of the retrospective method of assessing the age at menarche by comparing the results to the age obtained from continuous research. Data regarding the age at menarche came from longitudinal somatic development and physical fitness studies conducted between 1976 and 2022. In 2022, 47 women were examined. In continuous studies, the prospective method was used in the assessment of the age of first menstruation while in the 2004 and 2022 studies a retrospective method was applied. Only in 4 out of 47 women the age of the first menstruation declared in 2004 and 2022 (the women were 32–34 and 50–52 years old, respectively) was consistent with the one found in continuous studies. In other cases, there was a discrepancy between the age found in continuous studies and self-reported in 2004 or 2022 or between the age stated in 2004 and 2022. Of those women who were present for the 2022 study, 36 had information about the age of first menstruation from continuous studies and the age of menarche in 2004 was given. For this sample the arithmetic mean and the standard deviation of the age at menarche were calculated. It was found that the retrospective method often used in the assessment of the age of the first menstruation is not fully reliable, as the average discrepancy in the assessment ranged from nearly 1 month (0.05 years) to over 2 months (0.19 years) compared to the prospective method. Women surveyed in 2004 determined the age of the first menstruation more accurately compared to statements obtained 18 years later from the same women. This study suggests that long-term memory (LTM) of a significant life event of every woman is unreliable, as indicated by the difference in the declared age of the first menstruation of women examined in 2004 and 2022, which, in individual cases, was up to 3, 4 or 5 years.

Key words: menarche, prospective method, retrospective method, continuous research.

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Introduction

The age of the first menstruation is one of the indicators for assessing the course of puberty. It is also a sensitive indicator of the economic situation of individual professional groups or societies, and the low average age of menarche is widely recognized as a marker of the well-being of society. It is sometimes included in the analysis of secular trends, acceleration or deceleration of development (Gomuła and Kozieł 2018; Brix et al. 2019; Liu et al. 2021; Pop et al. 2022; Wu et al. 2022 et other). However, regardless of the purpose of the research, the reliability of the results depends on the methods used to assess the age at menarche.

There are three main methods of assessing the age of menstruation: the status quo method (responders answer a question about the occurrence of the first menstruation of girls, most often used at the age of 9–16 years), the retrospective method (responders answer a question of when the first menstruation occurred in girls and women, most often used in participants after 16 years of age and the prospective method (based on a question when the first menstruation occurred, asked during longitudinal studies including girls from about nine years of age. The retrospective method is the most commonly used methods used to estimate the age at menarche. Girls/women are asked to provide the age of the onset of menstruation to the nearest year and month, e.g., I was 12 years and 4 months (12.33 years). It is very rare for a woman to remember the age of her first menstruation to the nearest day. To calculate the average value in the sample of adult women, potentially all study participants are considered for analyses because all of them are post-menarche. The question arises: what size of menarche age memory error can be expected in women asked about it at different stages of life? Could this error be influenced by the woman's age at the time of the examination, i.e., the temporal distance from the date of menarche? For instance, the longer period of time has passed since the time of menarche, the more the recalled (retrospective) age differs from the actual age at menarche, i.e., prospective one. This problem can only be recognized in longitudinal studies (as in this study), which there are relatively few (Livson and McNeil 1962; Damon et al. 1969; Damon and Bajema 1974; Casey et al. 1991; Must et al. 2002; Żarów and Cichocka 2008). In the available studies, the differences between prospective and retrospective age at menarche ranged from -0.50 to +0.17 years. The size of the differences is significant, which is a subject for further analysis. In continuous studies of a selected group of girls/women from Krakow, which have been ongoing since 1976, the age at menarche was obtained at 3 ontogeny points, including 2 checking long-term memory (retrospective method) in relation to the well-established actual age of menarche (prospective method).

The aim of the present study was to determine whether the date of menarche recreated in women's memory differs from the actual date determined by prospectively, in adolescence, and what are the magnitude and direction of the memory errors. This study adds to the debate regarding how reliable the retrospective age at menarche is as a measure of changes in the rate of puberty in girls when used to track successive birth cohorts in a sample. This study also aims to determine whether in the study of the inheritance of the rate of puberty between mothers and daughters, the obtained results may be distorted by the age of the individuals at the time of examination.

Material and methods

Research material consisted of data derived from a longitudinal study focused on somatic development and physical fitness of girls and boys, conducted in 1976–2022 by the Department of Anthropology of the Institute of Biomedical Sciences of the University School of Physical Education in Krakow (KBC 1976–2022). The first series of annual surveys was performed in the years 1976–1988 (age of the participants 6–18 years), and the second series in the years 1980–1990; the age of the respondents was 8–19 years. The study analysed data combined from two series examined in 2004 (age 32–34 years) and re-examined in 2022 (age 50–52 years). Sample size and age of the examined girls and women:

- I series – data collected from 455 girls at the age of 6 to 142 aged 18,
- II series – data collected from 360 girls at the age of 8 to 108 women aged 18,
- 103 women in 2004 and 47 women in 2022.

Descriptive statistics, such as mean and measures of variability were calculated. Statistical significance of the differences between the analyzed assessment methods was calculated using the Student's t-test for dependent samples. The analyses were conducted using the Statistica 13.0 software. The numerical statement shows that the number of women in subsequent studies decreased, which is the expected trend in longitudinal studies. In the first and second series of the study, information about the age of the first menstruation was collected using the prospective method. In the studies conducted in 2004 and 2022, a retrospective method was used. Of the 47 women surveyed in 2022, 25% reported secondary education, and the remaining 75% (35 people) stated higher education. All studies were conducted with the consent of the girls' parents and test subjects. The consent of the Bioethics Committee at the Regional Medical Chamber in Krakow was obtained for the examination in 2022 (consent no. 65/KBL/OIL of April 11, 2022). All procedures contributing to the study complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Results

Table 1 shows individual data on age and menstruation according to the prospective and retrospective methods of the surveyed girls and women.

Of 47 women examined in 2022, only four cases in the age of menarche found in the longitudinal study was consisted with the one self-reported by the participants in 2004 and 2022. Ten women declared the same age of menarche while examined in 2004 and 2022 aged 32–34 and 50–52 respectively. In the remaining 33 cases, there was a discrepancy between the age found in the continuous surveys and the age declared in 2004, between the age found in the continuous tests and in 2022, or between the age stated in 2004 and in 2022. Of the 47 women who participated in the 2022 study, 36 also had information about their age of the first menstruation available in the data from continuous studies regarding the reported age of menarche in 2004. For this sample, arithmetic mean, standard deviation and range of the age of menarche were given at three points in women's ontogeny – as a teenager, at 32–34 and at 50–52 years old (Table 2).

Table 1. The age of the first menstruation data according to the prospective method and the retrospective method – age in years (KBC 1976–2022)

Serial number	Test No	Continuous tests 1976-1988 and 1978-1990	Research in 2004 Women aged 32-34	Research in 2022 Women aged 50-52	Difference between continuous testing and 2004	Difference between continuous testing and 2022	Difference between 2004 and 2022
Age of first menstruation consistent in all studies							
1	768	12	12	12	0	0	0
2	324	13	13	13	0	0	0
3	181	15	15	15	0	0	0
4	410	16	16	16	0	0	0
Age of first menstruation consistent in 2004 and 2022 surveys, and divergent in relation to continuous studies							
5	394	11.08	12	12	-0.92	-0.92	0
6	431	11.42	11	11	0.42	0.42	0
7	684	11.75	13	13	-1.25	-1.25	0
8	998	11.92	11	11	0.92	0.92	0
9	757	12.33	12	12	0.33	0.33	0
10	650	12.5	12	12	0.5	0.5	0
11	605	13.42	14	14	-0.58	-0.58	0
12	399	13.75	14	14	-0.25	-0.25	0
13	746	13.92	13.58	13.58	0.33	0.33	0
14	437	14.67	13	13	1.67	1.67	0
Age of first menstruation divergent across all studies							
15	888	10.5	10	10.08	0.5	0.42	-0.08
16	918	11.58	11.5	11.58	0.08	0	-0.08
17	60	11.58	.	10.25		1.33	
18	237	11.67	12.5	12	-0.83	-0.33	0.5
19	442	12.17	.	15		-2.83	
20	871	12.25	12.42	13	-0.17	-0.75	-0.58
21	457	12.33	.	10.25		2.08	
22	314	12.5	.	12.25		0.25	
23	313	12.58	12.5	11.83	0.08	0.75	0.67
24	785	12.58	11	10.5	1.58	2.08	0.5
25	752	12.83	12.33	14.5	0.5	-1.67	-2.17
26	659	12.83	14	12.92	-1.17	-0.08	1.08
27	214	12.92	16	11	-3.08	1.92	5
28	777	13	14	13.5	-1	-0.5	0.5

Serial number	Test No	Continuous tests 1976-1988 and 1978-1990	Research in 2004 Women aged 32-34	Research in 2022 Women aged 50-52	Difference between continuous testing and 2004	Difference between continuous testing and 2022	Difference between 2004 and 2022
29	415	13.08	14	13.92	-0.92	-0.83	-0.08
30	349	13.17	.	13.5		-0.33	
31	633	13.33	14	14.75	-0.67	-1.42	-0.75
32	671	13.5	13	17	0.5	-3.5	-4
33	173	13.5	13	14	0.5	-0.5	-1
34	523	13.58	12.67	12.08	-0.92	1.5	0.58
35	629	13.67	13.50	14.17	0.17	0.5	-0.67
36	890	13.75	13.58	12.75	0.16	1	-0.75
37	623	13.75	14	14.92	-0.25	-1.17	-0.92
38	74	13.75	.	12.83		0.92	
39	343	13.83	14.08	13.92	-0.25	-0.08	0.17
40	847	13.92	.	14.5		-0.58	
41	387	13.92	13	16	0.92	-2.08	-3
42	524	14.08	14	14.67	0.08	-0.58	-0.67
43	790	14.08	.	16.42		-2.33	
44	601	14.42	.	14.25		0.18	
45	646	14.42	15	16	-0.58	-1.58	-1
46	965	14.58	.	16		-1.42	
47	67	14.92	.	16.67		-1.75	

. did not participate in the study

Table 2. Descriptive statistics of women's age of the first menstruation; age is stated in years (KBC 1976–2022, n=36)

Parameter	Continuous testing Prospective method	2004 Women aged 32-34 Retrospective method	2022 Women aged 32-34 Retrospective method
Arithmetic mean	13.05	13.10	13.24
SD	1.15	1.36	1.68
Range	10.50 – 16.00	10.00 – 16.00	10.08 – 17.00

Table 2 shows that the average age of menarche that was self-reported in 2004 was higher by almost one month (0.05 years), and in 2022 – by more than two months (0.19 years) from the corresponding age calculated from the longitudinal data. Thus, older by 18 years women reported a later age of menarche. For the current study group, the value of the Student's T-test for dependent samples

was t=0.9875, p=0.33, the number of degrees of freedom df=35, i.e., a statistically insignificant difference between the data given in found in continuous studies and found in 2022. The differences between the arithmetic mean of the continuous surveys and the mean of the 2004 surveys (t=0.3346, p=0.74, df=35), as well as between the means in 2004 and 2022, were also not statistically significant (t=0.6269, p=0.53, df=35).

Discussion

The age of the first menstruation is influenced by both genetic factors and the external environment. The relationship of the age at menarche with body structure, diet quality, physical activity, personality type, geographical environment, socio-economic status of parents, as well as the impact of psychosocial stimuli has been reported in many studies (e.g., Rees 1995; Gonzales et al. 1996; Tahirović 1998; Chowdhury et al. 2000; Cichocka and Żarów 2002; Barkai et al. 2007; Nieczuja-Dwojacka et al. 2018; Durda-Masny et al. 2019; Karim et al. 2021; Glass et al. 2022).

As can be seen from the data presented in table 2, the results obtained using the two methods varied although differences between the data self-reported in 2004 and 2022 were statistically insignificant. These differences would potentially reach a significance level if the number of examined individuals (i.e., sample size) exceeded 140 people (i.e., with the number of n=144, the value of the t statistic=1.996, with p=0.05 [exact value - 0.048]). However, collecting so much data in such long-term continuous studies is extremely difficult. Still, the difference in arithmetic means in 2004 and 2022, amounting to 13.10 and 13.24, respectively, would be statistically significant with the sample exceeding 350 respondents (i.e., with n=360, t-statistic value =2.008 with p=0.05). Such, and larger, samples are regularly reported in comparative cross-sectional studies involving analyzes of secular trends in the age of menarche. The mean age at menarche obtained using the retrospective method was reported as later than the one determined using the prospective method (Table 2), and this difference was smaller if the examination date was closer in time, than the first menstruation. A similar tendency was observed by Koo and Rohan (1997), but the interval after the repeated questions regarding the age of menarche was much shorter – nearly 1 year and almost 2 years. With an average time interval of 323 days, 66.1% of study participants were able to recall the age at menarche correctly, while with an average interval of 649 days, only 44.8% of the subjects were able to do that. In our study, only 11% of women aged 32–34 reported the exact age of menstruation (4 out of 36 women) determined in continuous studies, and at the age of 50–52 years only 10.6% did so (5 out of 47 women) although the average error in the 2022 study was greater.

A greater overestimation of the average age of menarche may be due to a greater memory error of older women. The complicated relationship between the age of the first menstruation and the time of collecting information about this age was the subject of analyses by Mirzaei et al. (2019), who found that the more distant the studies were from the occurrence of menarche, the greater the memory error. At this stage of knowledge about the mechanisms of memory and their changes with age, it is difficult to explain why, over time, on average women con-

tinuously perceive their age at menarche as older. This tendency may be reflected in various studies, including analyses of secular changes in the age of menarche. When we examine the retrospective age of menarche (and this is the only age we have at our disposal in studies of adult women) in subsequent birth cohorts of women in the sample, we find the phenomenon of acceleration of puberty, i.e., the younger the years of birth, the earlier the age at menarche. It cannot be ruled out that this pattern of secular changes is caused by the tendency of women's memory to delay the age of menarche, i.e., the older the age of the respondents, the greater the delay. Estimating the pace of the changes per decade in such a way is subject to error. In our analysis, the average assessment error (overestimation) was 0.19 years, i.e., over 2 months. Kraków is one of the best-researched populations in Poland in terms of determining intergenerational changes in the pace of children's maturation. Between 1971 and 2010, 4 large series of cross-sectional studies have been conducted monitoring age at menarche using the status quo method (no recall bias). The decrease in the age of puberty in this period was 0.44 years, i.e., an average of 0.11 years per decade, or 1.5 months (Cichocka et al. 2012). The changes were therefore subtle and reliably determined, 1–2 months per decade. The magnitude of the trend per decade was similar to the magnitude of the age at menarche memory error in women at 50–52 years old. A question can be asked – did women in the prospective study tend to underestimate or overestimate their age at menarche compared to the age assessed prospectively? The analysis presented in Table 1 shows that in 2004 (women aged 32–34 years) 15 women underestimated the age of the first menstruation and 17 overestimated it, while in 2022 (women aged 50–52 years) women more often overstated the age of menarche; 19 women underestimated and 12 overestimated it while 1 woman reported the same age. The sample has been reduced by 4 women who reported the same age in all study series. There was also no clear difference in the assessment of the age at menarche between early and late maturing women. Out of these 36, early maturing women were conventionally defined as those whose first menstruation occurred before the age of 12.50 and those whose first menses happened after the age of 13.50 were classified as late maturing. Among the 10 early maturing women, 1 provided the exact age at menarche, 4 overestimated and 5 underestimated the age of menarche. In the group of late maturing women there were 2, 5 and 6 women in the same recall categories, respectively. Longitudinal studies in general, including ours, have weaknesses mainly because they are difficult to implement, time-consuming and expensive, and over time, an increasing rate of loss of participants (dropouts, loss to follow-up) is a common problem. This means that after several decades of collecting longitudinal data, only several dozen participants usually remain from the original sample. For these reasons there are not many such studies. Our research seems to be unique because we have 3 points in ontogeny, temporally distant, examining the age of menarche, including 2 testing long-term memory. An additional advantage of our study is that the research was largely conducted by the same people, which inspired trust among the surveyed women, helping them answer the questions, often involving personal matters, with more confidence and honestly.

Conclusions

1. The retrospective method that is often used in assessing the age of first menstruation is not fully reliable, as the average discrepancy in assessment ranges from nearly 1 month (0.05 years) to more than 2 months (0.19 years) compared to the prospective method.
2. Women aged 32–34 years more accurately stated the age of the first menstruation compared to statements obtained from the same women18 years later.
3. Long-term memory (LTM), even regarding such a significant event in the life of every woman, is often unreliable, as indicated by the difference in the declared age of the first menstruation of the same women surveyed in 2004 and 2022, in some cases amounting to 3, 4 or 5 years.

Overall, in the analysis of secular trends of the age of menarche, it is worth remembering to determine the size of the trend using the same method of assessing the age of menarche. The use of various methods can lead to unreliable results, as we have already previously shown (Żarów and Cichocka 2008; Cichocka et al. 2012), and the magnitudes of the trend can be subtle. As a result, this may cause an erroneous assessment of the conditions of intergenerational changes of the age at menarche.

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Conflict of interest

The authors declare no conflict of interest.

Author's contributions

RŻ: conceptualization, methodology, investigation, software, formal analysis, validation, interpretation of the data and results, writing of the original draft, project administration, data curation; AW: investigation, software, writing of the original draft; BS: investigation, formal analysis, validation, interpretation of the data and results, data curation; interpretation of the data; MK: investigation, software, interpretation of the data; JB: investigation, software, interpretation of the data; all authors reviewed and edited the manuscript.

Corresponding author

Ryszard Żarów, Department of Anthropology, University School of Physical Education of Krakow, Jana Pawła II 78, 31-571 Kraków, Poland, phone: 693741775; e-mail: wazarow@cyf-kr.edu.pl

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Cardiometabolic risk assessment in Eastern Slovak young adults using anthropometric indicators

Michaela Zigová, Eva Petrejčíková, Marta Mydlárová Blaščáková, Jana Gaľová, Hedviga Vašková, Soňa Kalafutová, Miriama Šlebodová

Department of Biology, Faculty of Humanities and Natural Sciences, University of Prešov, Prešov, Slovakia

Abstract: *Introduction:* Selected anthropometric indicators, such as anthropometric measurements, indices, or ratios could be reliable predictors of future cardiometabolic risk in primary prevention, especially in young adults.

Aim: This study aimed to establish cardiometabolic risk status in young Eastern Slovak adults according to anthropometric indicators.

Material and methods: Indicators used in this study, such as heart rate, blood pressure, five anthropometric measurements, as well as a total of 23 anthropometric indices and ratios were selected based on the available literature. These indicators were analyzed in 162 young adult participants of both sexes with a mean age of 20.78±2.22 years. The analyzed indices and ratios were calculated by routine anthropometry and were correlated with blood pressure and heart rate in the whole research group as well as among subgroups divided according to sex, obesity and hypertension status.

Results: Our results showed frequently higher values of input characteristics in males (71.88%), and statistically significant differences between sexes in 81.25% of the characteristics. The values of systolic blood pressure were above the norm in all males, and they also dominated in the obesity group. Correlation analyses conducted on all participants and in subgroups indicated a positive statistical significance in several indicators. The vast majority of the anthropometric indicators were significantly correlated with physiological indicators in almost all subgroups. Only A body shape *index* (ABSI) correlation coefficients did not show a significant correlation with physiological indicators in all analyzed subgroups. The correlations tended to be stronger among subgroup exhibiting potential to obesity. All analyzed indices and ratios were significantly correlated ($p \leq 0.05$), predominantly with blood pressure components rather than heart rate, especially in participants with the potential for disease complications than in participants without them.

Conclusion: The analyzed indicators are noninvasive and useful although they may be at different levels of association and clinical significance for various conditions. Thus some of the indicators may be standardly used in the early diagnostic process for monitoring cardiovascular health and risk stratification of patients.

Key words: Anthropometry, Cardiometabolic complications, Asymptomatic individual, Primary prevention, Young adulthood.

Original article

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Introduction

The consequences of the COVID-19 pandemic have rapidly translated into the health of the global population, including cardiometabolic health (Pina and Castelletti 2021). The current pandemic situation in the world and Slovakia has forced many to think about what important changes need to be made in the field of civilizational disease prevention. A deterioration in the availability of health care during the pandemic period showed the need for reliable monitoring and assessment of cardiometabolic status, especially in asymptomatic young adults. Young age is a period that allows early detection of future cardiometabolic complications, their prevention, and successful treatment if they are recognized in time (Tanrikulu et al. 2017; Barden et al. 2022). In this context, we can propose alternative approaches for the primary prevention of cardiovascular risk by analyzing anthropometric indicators, such as linear and curvilinear measurements, indices, and ratios. This noninvasive approach may provide valuable information about body size, shape, composition, development, and health, including cardiometabolic and nutritional status, even before any complications appear (Roriz et al. 2016; Piqueras et al. 2021; Minetto et al. 2022). In this context, the aim of our study was to analyze the importance of selected anthropometric indicators to predict cardiometabolic risk status in Eastern Slovak young adults.

Material and Methods

The first step of our research was the selection of indicators that are methodologically undemanding and could be commonly implemented in the first step of primary prevention of cardiometabolic disease conditions. All relevant information was searched in research databases such as NCBI, PubMed, and ScienceDirect® by entering the keywords anthropometry, anthropometry index, indices of adiposity, cardiometabolic risk, and their combinations. Our search strategy allowed us to select anthropometric indicators (i.e., five anthropometric measurements, and 23 indices and ratios) relevant to our study which were then calculated and correlated with physiological indicators (blood pressure and heart rate).

The study was performed among a group of 162 individuals of both sexes in the age range of 18–26 years who were interested in participating in our research activities. The implementation of the research and all procedures performed in the study were in accordance with ethical standards established by the institutional ethics committee (ECUP-022023PO). Participation in the research was anonymous, voluntary, and conditional on the signing of an informed consent form. The condition for participation in the study was the provision of information on sex, age, blood pressure, heart rate, body weight, height, and circumference measurements (waist, hip, and neck circumferences) and stating no acute or chronic disease at the time of obtaining data. To ensure the reliability and consistency of the data and minimize measurement error, we calculated the average value of three measurements of each variable. For statistical analysis, all participants were divided into six different subgroups according to sex, BMI ($\geq$ 25 kg/m^2), and blood pressure values (sBP/dBP $\geq$120/80 mmHg): males and females; obesity$^+$ and obesity$^-$, hypertension$^+$ and hypertension$^-$.

Standard procedures and tools (digital personal scale Omron BF-511 T, Seritex anthropometer GPM MODEL 100, Cescorf flexible steel tape, SencorS-BP 690 digital blood pressure monitor) were used to obtain information about physiological variables such as heart rate (HR; bpm), systolic and diastolic blood pressure (sBP and dBP; mmHg), measurements of body height (Ht; cm), body weight (Wt; kg), waist circumference (WC; cm), hip circumference (HC; cm), and neck circumference (NC; cm). Anthropometric data were collected following the recommendations of the *International Standards for Anthropometric Assessment* from 2011 (Stewart et al. 2011). These data were obtained from all participants and were used to calculate 23 anthropometric indices and ratios as indicators of cardiometabolic risk based on:

1. Body height and weight:
   - *Body mass index* (**BMI**) and optimized alternatives *new BMI* (**nBMI** $= 1.3 \times (Wt_{kg}/Ht^2_{m})$ and *Waist-corrected BMI* (**wBMI** $= WC_{m} \times (Wt_{kg}/Ht^2\ m)$ and *BMI multiplied by the square root of WC* (**BMI$_{\sqrt{WC}}$** $= (Wt_{kg}/Ht^2\ m) \times \sqrt{WC_{m}}$),
   - *Triponderal mass index* (**TMI** $= Wt_{kg}/Ht^3\ m$),
   - *Weight-adjusted waist index* (**WWI** $= WC_{cm}/\sqrt{Wt_{kg}}$)
2. Waist or hip circumferences:
   - *Abdominal volume index* (**AVI** $= [2\times WC^2_{cm} + 0.7 \times (WC_{cm} - HC_{cm})^2]/1000$),
   - *Body adiposity index* (**BAI** $= [HC_{cm}/Ht^{1.5}_{m}] - 18$),
   - *Body roundness index* (**BRI** $= 365.2 - 365.5 \times \sqrt{\{1 - [(WC_{m}/2\varpi)^2]/(0.5 \times Ht_{m})^2]\}}$),
   - *Conicity index* (**CI** $= WC_{m} / [0.109 \times \sqrt{(Wt_{kg}/Ht_{m})}]$,
   - *Hip index* (**HI$_{female}$** $= HC_{cm} \times Wt^{-0.482}_{kg} \times Ht^{0.310}_{cm}$; **HI$_{male}$** $= HC_{cm} \times Wt^{-2/5}_{kg} \times Ht^{1/5}_{cm}$),
   - *Fat mass* (**FM$_{female}$** $= 11.817 - 0.041 \times Age_{year} - 0.199 \times Ht_{cm} + 0.610 \times Wt_{kg} + 0.044 \times WC_{cm}$; **FM$_{male}$** $= -18.592 - 0.009 \times Age_{year} - 0.080 \times Ht_{cm} + 0.226 \times Wt_{kg} + 0.387 \times WC_{cm}$),
   - *Skeletal muscle mass* (**SM$_{female}$** $= 2.89 + (0.255 \times Wt_{kg}) + (-0.175 \times HC_{cm}) + (-0.0384 \times Age_{years}) + (0.118 \times Ht_{cm})$; **SM$_{male}$** $= 39.5 + (0.665 \times Wt_{kg}) + (-0.185 \times WC_{cm}) + (-0.418 \times HC_{cm} + (-0.0805 \times Age_{years})$,
   - *Relative fat mass* (**RFM$_{female}$** $= 64 - [20 \times (Ht_{cm}/WC_{cm})] + 12$; **RFM$_{male}$** $= 64 - [20 \times (Ht_{cm}/WC_{cm})]$),
   - *Waist to hip ratio* (**WHR**),
   - *Waist to hip to height ratio* (**WHHR**),
   - *Waist to height ratio* (**WHtR**) and its optimized alternatives *new waist to height ratio* (**WHT.5R** $= WC_{cm}/Ht^{0.5}_{cm}$) and *Waist to the square of the height ratio* (**WHt²R**)
3. BMI index:
   - *A body shape index* (**ABSI** $= WC_{cm}/(BMI^{0.66}\ kg/m^2 \times Ht_{m})^{0.5}$),
   - *Body fat percentage* (**BFP** $= (1.20 \times BMI\ kg/m^2) + (0.23 \times Age_{years}) - (10.8 \times Sex) - 5.4$, $Sex_{male} = 1$ and $Sex_{female} = 0$),
   - *Body surface area* (Mosteller, **BSA** $= (Ht_{cm} \times Wt_{kg}/3600)^{1/2}$),
   - *The Clinica Universidad de Navarra-body adiposity estimator* (**CUN-BAE** $= -44.988 + (0.503 \times Age_{years}) + (10.689 \times Sex) + (3.172 \times BMI\ kg/m^2) - (0.026 \times BMI^2\ kg/m^2) + (0.181 \times BMI\ kg/m^2 \times sex) - (0.02 \times BMI\ kg/m^2 \times Age_{years}) - (0.005 \times BMI^2\ kg/m^2 \times Sex) + (0.00021 \times BMI^2\ kg/m^2 \times Age_{years})$ $Sex_{male} = 0$ and $Sex_{female} = 1$).

Indices were calculated according to mathematical algorithms recommended in relevant studies (Bergman et al. 2011; Falhammar et al. 2011; Gómez-Ambrosi et al. 2012; Fu et al. 2014; Jelena et al. 2016; Peterson et al. 2017; Antonini-Canterin et al. 2018; Tran et al. 2018; Abolnezhadian et al. 2020; Van Haute et al. 2020; Kang 2021; Wu et al. 2021; Christakoudy et al. 2022; Minetto et al. 2022). Cardiometabolic complications were assessed based on values of standardly analyzed indicators (BMI, WHR, WHtR, WC, HR, and BP) according to generally accepted cut-off values mentioned below in the Table 3 (WHO 2000; WHO 2008; Ashwell et al. 2012; Egan and Stevens-Fabry 2015; Brugada et al. 2020). Data were checked for normality using the Kolmogorov-Smirnov test of normality and statistically evaluated using an online calculator (*https://www.socscistatistics.com*) while MS Office and Excel v.1808 were used to calculate descriptive statistics, t-test for data comparison between sexes, Pearson's correlation for association computation. The interpretation of the correlation coefficient sizes was based on Cohen's criteria (Cohen 1988). An informative value of anthropometric indices and ratios were interpreted according to the strength of correlation with physiological indicators, direction of correlations and statistical significance. All results with a p-value of ≤ 0.05 were considered statistically significant and to have higher informative value.

Results

Descriptive characteristics of research group participants

Our research aimed to analyze cardiometabolic risk status in young adults of both sexes, aged from 18 to 26 years, without confirmed acute or chronic disease, according to selected indices and ratios calculated on routine anthropometry. A group of 162 individuals of both sexes with a mean age of 20.78 ± 2.22 years participated in the study. The mean values of variables characterizing our research group are shown in Table 1 and Table 2. Our results showed that the mean values of 71.88% of the input characteristics, including age, were higher in males compared to females, which was also confirmed by the statistical analyses. The mean values of the indices and ratios ABSI and WHHR were equal in subgroups according to sex (Table 2). Statistically significant differences in mean values of the characteristics between sexes were confirmed in 81.25% of cases, except for dBP and the indices and ratios ABSI, BAI, WHHR, WHt^2R, and FM. Statistically significant intersexual comparisons with a p-value of < 0.001 were confirmed in the 4 out of 6 indices and ratios based on body height and weight, the 9 out of 13 indices and ratios based on waist or hip circumferences, and in all indices and ratios based on BMI calculation except for ABSI. All participants were divided into obesity$^+$ and obesity$^-$ subgroup according to BMI risk values of 25 kg/m^2 and above (41 and 121 individuals, respectively), and according to blood pressure values that indicated hypertension (sBP/dBP ≥120/80 mmHg), into hypertension$^+$ and hypertension$^-$ (97 and 65 individuals, respectively). Males dominated the obesity$^+$ group (73.17% of participants) and, on the other hand, females dominated the hypertension$^+$ group (55.67% of participants).

Table 1. Main characteristics of Eastern Slovakia study participants

	All participants (N = 162)				Male (N = 63)				Female (N = 99)				Statistic		
	$\bar{x}$	SD	MAX	MIN	$\bar{x}$	SD	MAX	MIN	$\bar{x}$	SD	MAX	MIN	T test	95% CI	P value
Age [years]	20.78	2.22	26	18	21.41	2.34	25	18	20.38	2.04	26.00	18.00	-2.957	-1.7179 to -0.3421	**
Ht [cm]	171.72	8.85	201	151	179.58	6.84	201	165	166.72	5.83	183.00	151.00	-12.786	-14.8463 to -10.8737	***
Wt [kg]	69.29	17.11	136	45	82.69	15.59	136	51.1	60.76	11.71	128.90	45.00	-10.194	-26.1785 to -17.6815	***
WC [cm]	78.22	11.99	127	57	87.1	10.11	121	70	72.57	9.39	127.00	57.00	-9.318	-17.6095 to -11.4505	***
HC [cm]	98.85	10.36	145	70	104.84	9.40	135	90	95.03	9.05	145.00	70.00	-6.625	-12.7341 to -6.8859	***
NC [cm]	34.41	3.87	45	28	38.25	2.83	45	33	31.97	2.01	40.00	28.00	-16.499	-7.0317 to -5.5283	***
HR [bpm]	78.15	13.01	120	46	75.57	12.06	120	52	79.80	13.33	109.00	46.00	2.042	0.1392 to 8.3208	*
sBP[mmHg]	121.72	11.65	166	95	126.40	10.04	155	100	118.75	11.62	166.00	95.00	-4.302	-11.1621 to -4.1379	***
dBP [mmHg]	76.09	9.85	109	47	75.70	9.76	109	60	76.34	9.89	104.00	47.00	0.404	-2.4919 to 3.7719	ns

Abbreviations: dBP – diastolic blood pressure; HC – hip circumference; HR – heart rate; Ht – body height; MAX – maximum; MIN – minimum; N – number; NC – neck circumference; ns – not significant; sBP – systolic blood pressure; SD – standard deviation; WC – waist circumference; Wt – body weight; $\bar{x}$ – mean; 95% CI – 95% confidence interval; * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$

Table 2. Calculated indices and ratios of study participants

	All participants (N = 162)				Male (N = 63)				Female (N = 99)				Statistic		
	$\bar{x}$	SD	MAX	MIN	$\bar{x}$	SD	MAX	MIN	$\bar{x}$	SD	MAX	MIN	T test	95% CI	P value
BMI	23.29	4.48	43.27	16.30	25.60	4.44	41.58	18.27	21.83	3.84	43.27	16.30	-5.729	-5.0695 to -2.4705	***
nBMI	23.10	4.30	42.81	16.35	24.85	4.35	41.09	17.70	21.98	3.87	42.81	16.35	-4.383	-4.1631 to -1.5769	***
wBMI	18.71	6.99	54.95	10.01	22.70	6.91	50.32	13.22	16.17	5.75	54.95	10.01	-6.509	-8.5114 to -4.5486	***
BMI√WC	20.81	5.78	48.76	13.14	24.06	5.67	45.74	15.58	18.74	4.82	48.76	13.14	-6.390	-6.9643 to -3.6757	***
TMI	13.56	2.48	25.07	9.70	14.26	2.55	24.02	10.15	13.11	2.33	25.07	9.70	-2.951	-1.9195 to -0.3805	**
WWI	9.44	0.61	11.33	7.85	9.62	0.59	11.33	8.59	9.33	0.59	11.19	7.85	-3.050	-0.4778 to -0.1022	**
AVI	12.85	4.06	32.48	6.62	15.63	3.75	29.30	10.24	11.08	3.16	32.48	6.62	-8.302	-5.6324 to -3.4676	***
BAI	25.96	4.09	45.95	14.44	25.62	3.99	37.33	17.98	26.18	4.14	45.95	14.44	0.851	-0.7394 to 1.8594	ns
BRI	2.63	1.19	8.88	0.87	3.16	1.16	7.87	1.42	2.30	1.07	8.88	0.87	-4.826	-1.2119 to -0.5081	***
CI	1.13	0.08	1.37	0.93	1.18	0.07	1.37	1.07	1.10	0.07	1.35	0.93	-7.091	-0.1023 to -0.0577	***
HI	51.11	2.39	59.03	41.07	50.81	2.20	58.25	43.89	49.05	3.01	60.28	41.41	-4.008	-2.6273 to -0.8927	***
FM	19.54	7.12	62.67	7.45	19.24	7.01	42.37	7.45	19.73	7.18	62.67	10.04	0.427	-1.7745 to 2.7545	ns
SM	25.38	7.14	50.46	16.72	32.83	5.78	50.46	20.59	20.64	2.11	29.83	16.72	-19.11	-13.450 to -10.930	***
RFM	26.66	5.95	48.82	11.66	22.28	4.42	35.39	11.66	29.45	5.05	48.82	17.40	9.238	5.6372 to 8.7028	***
WHR	0.79	0.06	0.99	0.62	0.83	0.06	0.99	0.74	0.76	0.05	0.89	0.62	-8.029	-0.0872 to -0.0528	***
WHHR	0.46	0.04	0.56	0.36	0.46	0.04	0.56	0.38	0.46	0.04	0.55	0.36	0.000	-0.0127 to 0.0127	ns
WHtR	0.46	0.06	0.74	0.34	0.49	0.06	0.70	0.38	0.44	0.06	0.74	0.34	-5.171	-0.0691 to -0.0309	***
WHT.5R	0.60	0.09	0.97	0.44	0.65	0.08	0.92	0.52	0.56	0.07	0.97	0.44	-7.543	-0.1136 to -0.0664	***
WHt^2R	27.10^{-4}	4.10^{-4}	43.10^{-4}	20.10^{-4}	27.10-4	4.10-4	40.10-4	21.10-4	26.10^{-4}	4.10^{-4}	43.10^{-4}	20.10^{-4}	-1.551	-0.0002 to 0.0000	ns
ABSI	0.07	0.004	0.09	0.06	0.07	0.004	0.09	0.07	0.07	0.004	0.08	0.06	0.000	-0.0013 to 0.0013	ns
BFP	23.51	5.73	52.04	11.46	20.40	5.78	39.10	11.46	25.48	4.73	52.04	18.30	6.106	3.4369 to 6.7231	***
BSA	1.81	0.25	2.64	1.39	2.02	0.21	2.64	1.53	1.67	0.16	2.49	1.39	-11.997	-0.4076 to -0.2924	***
CUN-BAE	24.92	6.84	53.48	8.82	21.50	6.80	42.65	8.82	27.10	5.92	53.48	16.30	5.537	3.6026 to 7.5974	***

*Abbreviations: ABSI – A body shape index; AVI – Abdominal volume index; BAI – Body adiposity index; BFP – Body fat percentage; BMI – Body mass index; BMI√WC – BMI multiplied by the square root of waist circumference; BRI – Body roundness index; BSA – Body surface area (Mosteller); CI – Conicity index; CUN-BAE – The Clinica Universidad de Navarra-body adiposity estimator; FM – Fat mass; HI – Hip index; MAX – maximum; MIN – minimum; nBMI – new BMI; ns – not significant; RFM – Relative fat mass; SD – standard deviation; SM – Skeletal muscle mass; TMI –Triponderal mass index; wBMI – waist-corrected BMI; WHHR – Waist to hip to height ratio; WHR – Waist to hip ratio; WHT.5R – New waist to height ratio; WHt^2R – Waist to the square of the height ratio; WHtR – Waist to height ratio; WWI – Weight-adjusted waist index; $\bar{x}$ – mean; 95% CI – 95% confidence interval; * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$*

The frequency of cardiometabolic complications

The percentage of participants who were evaluated according to recommended classification criteria (WHO 2000; WHO 2008; Ashwell et al. 2012; Egan and Stevens-Fabry 2015; Brugada et al. 2020) of traditional indicators of cardiometabolic risk like BMI, WC, WHR, WHtR, BP, and HR as participants at potentially increased or high risk is presented in Table 3. According to BMI, preobesity and obesity status were predicted in 19.14% and 5.55% of all participants, respectively. Values of BMI predicted more cases of males with the potential for preobesity and obesity. Waist circumference and WHR were relatively high in the group of females (both in 8.08% females). The risk of central obesity, according to the WHtR index, was predicted predominantly in males (28.57% males). The most frequently confirmed complication in our research group was increased blood pressure (57.41% for sBP, 23.46% for dBP, and 24.07% for both sBP and dBP). The sBP values of all males were above the norm (≥ 120 mmHg). An increase in both blood pressure components (sBP and dBP, respectively), was found in 19.75% of all individuals, with a predominance in females (24.24% of females). Hypertension-risk values of both blood pressure components were confirmed in only 3.03% of females and 6.35% of male participants. Information about heart rate predicted supraventricular tachycardia and increased future cardiovascular risk in 6.79% of individuals (2.02% of females and 14.29% of males). The values of all the mentioned indicators of cardiometabolic complications (BMI + WC + WHR + WHtR + sBP + dBP) were increased above the recommended norms only in 1.23% of participants (1 female and 1 male). From a comprehensive point of view, in males, there were confirmed risk values for the analyzed indicators more often than in females.

Table 3. Cardiometabolic complications of study participants

Indicator	Classification	Interval	All (N = 162)	Male (N = 63)	Female (N = 99)
BMI	Preobesity (increased risk)	25.0 – 29.9 kg/m^2	19.14%	34.92%	9.09%
	Obesity class I. (moderate risk)	30.0 – 34.9 kg/m^2	3.09%	7.93%	0.00%
	Obesity class II. (severe risk)	35.0 – 39.9 kg/m^2	1.23%	1.59%	1.01%
	Obesity class III. (very severe risk)	≥40 kg/m^2	1.23%	1.59%	1.01%
WC	High risk	♀ ≥80 cm ♂ ≥ 94 cm	6.79%	11.11%	4.04%
	Very high risk	♀ ≥88 cm ♂ ≥102 cm	7.41%	6.35%	8.08%
WHR	Moderate risk	♀ 0.81 – 0.85 ♂ 0.96 – 1.0	7.41%	4.76%	9.09%
	High risk	♀ > 0.85 ♂ > 1	4.94%	0.00%	8.08%
WHtR	Central obesity (increased risk)	≥0.5	16.67%	28.57%	9.09%

Indicator	Classification	Interval	All (N = 162)	Male (N = 63)	Female (N = 99)
sBP	Prehypertension (increased risk)	120 – 139 mmHg	52.47%	92.06%	27.27%
	Hypertension (high risk)	≥140 mmHg	4.94%	7.94%	3.03%
dBP	Prehypertension (increased risk)	80 – 89 mmHg	16.05%	7.94%	21.21%
	Hypertension (high risk)	≥90 mmHg	7.41%	7.94%	7.07%
sBP+dBP	Prehypertension (increased risk) sBP/dBP	120 – 139/80 – 89 mmHg	19.75%	12.70%	24.24%
	Hypertension (high risk)	≥140/≥90 mmHg	4.32%	6.35%	3.03%
HR	SVT (increased risk)	≥100 bpm	6.79%	14.29%	2.02%
BMI+WC+ WHR+WHtR+ sBP+dBP	Increased risk	All values above the norm	1.23%	1.59%	1.01%

Abbreviations: BMI – Body mass index; dBP – diastolic blood pressure; N – number; WHR – Waist to hip ratio; WHtR – Waist to height ratio; HR – heart rate; sBP – systolic blood pressure; SVT – supraventricular tachycardia, WC – waist circumference; ♀ – female; ♂ – male

Heart rate and blood pressure correlations with analyzed indicators

The relationship of anthropometric measures, indices, and ratios versus HR and BP was confirmed by correlation analyses in all participants and in six different subgroups (males and females according to sex; obesity$^+$ and obesity$^-$ according to BMI; hypertension$^+$ and hypertension$^-$ according to blood pressure). The correlation analysis confirmed statistical significance in several indices and ratios, especially with BP (Table 4 and Table 5). From the total number of 567 calculated correlation coefficients, 38.80% cases were found to be significant at $p \leq 0.05$. Our results highlight the positive correlation across the vast majority of indicators. A significant inverse correlation was predicted only in the cases of NC and HR in the group of all participants; in the cases of NC, CUN-BAE, BFP, and HR in the obesity$^-$ group of participants; and in the cases of Wt, WC, HC, NC, BSA, SM, and HR in the group of participants from the subgroup hypertension$^+$. According to our data, there was a predominantly weak and moderate correlation. Only 0.53% of coefficients indicated a strong positive correlation relationship ($r \geq 0.5$), namely in the index CUN-BAE and HR and also in the cases of FM, CUN-BAE, and dBP, but only in the obesity$^+$ subgroup. The strongest correlation from our results was observed in the obesity$^+$ subgroup in the cases of FM and dBP ($r = 0.5372$; $p \leq 0.001$), CUN-BAE and HR ($r = 0.5109$; $p \leq 0.001$) and also CUN-BAE and dBP ($r = 0.5065$; $p \leq 0.001$).

The vast majority of the indicators that we analyzed were significantly correlated with dBP in almost all subgroups. Only in the participants without obesity and in the participants with potential for hypertension (hypertension$^+$ subgroup) were the vast majority of indicators significantly correlated with sBP. The heart rate was the least significantly correlated parameter, with statistical significance observed only in 3.88% of all 567 correlation coefficients. A nonsignificant relationship between all the analyzed indicators and HR was observed in both sex-based subgroups and in the hypertension$^-$ subgroup.

Table 4. Correlation analyses in the whole research group and in both sexes

	All (N = 162)			Male (N = 63)			Female (N = 99)		
	HR	sBP	dBP	HR	sBP	dBP	HR	sBP	dBP
Wt	ns	0.3851***	0.2298**	ns	0.2634*	0.2482*	ns	0.2503*	0.3865***
WC	ns	0.3603***	0.2606***	ns	ns	0.3233**	ns	0.2176*	0.3629***
HC	ns	0.3028***	0.1964*	ns	ns	ns	ns	ns	0.2926**
NC	-0.1697*	0.3036***	ns	ns	ns	ns	ns	ns	ns
BMI	ns	0.3406***	0.3102***	ns	0.2638*	0.3054*	ns	0.2325*	0.3925***
nBMI	ns	0.3131***	0.325***	ns	0.2559*	0.3124*	ns	0.2224*	0.3858***
wBMI	ns	0.3487***	0.3037***	ns	0.2934*	0.3505**	ns	0.2109*	0.3659***
BMI√WC	ns	0.3497***	0.3069***	ns	0.2811*	0.3337**	ns	0.2234*	0.3811***
TMI	ns	0.2783***	0.3341***	ns	ns	0.3161*	ns	0.2103*	0.3756***
WWI	ns	ns	0.2036**	ns	ns	0.2883*	ns	ns	ns
AVI	ns	0.3496***	0.2647***	ns	0.2629*	0.3344**	ns	ns	0.3408***
BAI	ns	ns	0.2479**	ns	ns	ns	ns	ns	0.2596**
BRI	ns	0.2934***	0.3112***	ns	ns	0.3656**	ns	ns	0.3322***
CI	ns	0.2435**	0.1787*	ns	ns	0.2674*	ns	ns	0.2005*
HI	ns	ns	ns	ns	ns	ns	ns	ns	0.2063*
FM	ns	0.2264**	0.3637***	ns	0.2635*	0.3151*	ns	0.2441*	0.3924***
SM	ns	0.3837***	ns	ns	0.2622*	ns	ns	0.2561*	0.3555***
RFM	0.1802*	ns	0.2946***	ns	ns	0.3168*	ns	0.2072*	0.3549***
WHR	ns	0.2681***	0.2199**	ns	ns	0.3221*	ns	ns	0.2408*
WHHR	ns	ns	0.2397**	ns	ns	0.3309**	ns	ns	ns
WHtR	ns	0.3068***	0.3115***	ns	ns	0.3539**	ns	ns	0.3444***
WHT.5R	ns	0.3413***	0.2880***	ns	ns	0.3431**	ns	0.2068*	0.3566***
WHt^2R	ns	0.1939*	0.3184***	ns	ns	0.3534**	ns	ns	0.3066**
ABSI	ns	ns	ns	ns	ns	ns	ns	ns	ns
BFP	0.155*	ns	0.3127***	ns	ns	0.2944*	ns	0.19992*	0.3634***
BSA	ns	0.3806***	0.1821*	ns	ns	ns	ns	0.2493*	0.3689***
CUN-BAE	ns	ns	0.3175***	ns	ns	0.2606*	ns	0.2260*	0.3860***

*Abbreviations: ABSI – A body shape index; AVI – Abdominal volume index; BAI – Body adiposity index; BFP – Body fat percentage; BMI – Body mass index; BMI√WC – BMI multiplied by the square root of waist circumference; BRI – Body roundness index; BSA – Body surface area (Mosteller); CI – Conicity index; CUN-BAE – The Clinica Universidad de Navarra-body adiposity estimator; dBP – diastolic blood pressure; FM – Fat mass; HC – hip circumference; HI – Hip index; HR –heart rate; MAX – maximum; MIN – minimum; nBMI – new BMI; NC – neck circumference; ns – not significant; RFM – Relative fat mass; sBP – systolic blood pressure; SD – standard deviation; SM – Skeletal muscle mass; TMI –Triponderal mass index; wBMI – waist-corrected BMI; WC – waist circumference; WHHR – Waist to hip to height ratio; WHR – Waist to hip ratio; WHT.5R – New waist to height ratio; WHt^2R – Waist to the square of the height ratio; WHtR – Waist to height ratio; Wt – body weight; WWI – Weight-adjusted waist index; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$*

Table 5. Correlation analyses in the participants according to obesity and hypertension status

	Obesity^{+} (N = 41)			Obesity^{-} (N = 121)			Hypertension $^{+}$ (N = 97)			Hypertension^{-} (N = 65)		
	HR	sBP	dBP	HR	sBP	dBP	HR	sBP	dBP	HR	sBP	dBP
Wt	ns	0.3542*	0.3379*	ns	0.2200*	ns	-0.2482*	0.3213***	ns	ns	0.4028***	0.3995***
WC	ns	ns	0.3612*	ns	0.2309*	ns	-0.2139*	0.3477***	ns	ns	0.3214**	0.3287**
HC	0.3439*	ns	0.3277*	ns	ns	ns	-0.2341*	0.2396*	ns	ns	0.2869*	0.2822*
NC	ns	ns	ns	-0.2008*	ns	ns	-0.3269**	0.3573***	ns	ns	0.2572*	ns
BMI	0.4232**	ns	0.4798**	ns	ns	ns	ns	0.3395***	ns	ns	0.3098*	0.3679**
nBMI	0.4374**	ns	0.4909**	ns	ns	ns	ns	0.3334***	0.2531*	ns	0.2630*	0.3413**
wBMI	0.3522*	ns	0.4389**	ns	0.1969*	ns	ns	0.3339***	ns	ns	0.3447**	0.3796**
BMI√WC	0.3776*	ns	0.4569**	ns	ns	ns	ns	0.3397***	ns	ns	0.3336**	0.3768**
TMI	0.4418**	ns	0.4914**	ns	ns	ns	ns	0.3215**	0.3096**	ns	ns	0.3048*
WWI	ns	ns	ns	ns	ns	ns	ns	0.2189*	0.2715**	ns	ns	ns
AVI	ns	ns	0.3677*	ns	0.2153*	ns	ns	0.3316***	ns	ns	0.3327**	0.3400**
BAI	0.4495**	ns	0.4184**	ns	ns	ns	ns	ns	0.3380***	ns	ns	ns
BRI	ns	ns	0.4015**	ns	ns	ns	ns	0.3194**	0.2732**	ns	ns	0.2904*
CI	ns	ns	ns	ns	0.1882*	ns	ns	0.2692**	ns	ns	ns	ns
HI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
FM	0.4948**	ns	0.5372***	ns	ns	ns	ns	ns	0.3245**	ns	ns	0.3655**
SM	ns	ns	ns	ns	0.2939**	ns	-0.3080**	0.3301***	ns	ns	0.4243***	0.3504**
RFM	0.3874*	ns	0.4249**	ns	ns	ns	0.1998*	ns	0.4782***	ns	ns	ns
WHR	ns	ns	ns	ns	0.2380**	ns	ns	0.3419***	ns	ns	ns	ns
WHHR	ns	ns	ns	ns	ns	0.1994*	ns	0.2408*	0.3624***	ns	ns	ns
WHtR	ns	ns	0.4016**	ns	ns	ns	ns	0.3341***	0.2567*	ns	ns	0.2842*
WHT.5R	ns	ns	0.3895*	ns	0.2069*	ns	ns	0.3457***	ns	ns	0.2831*	0.3144*
WHt^{2}R	ns	ns	0.3920*	ns	ns	ns	ns	0.2840**	0.3837***	ns	ns	ns
ABSI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
BFP	0.4953***	ns	0.4943**	ns	-0.2412**	ns	ns	ns	0.4221***	ns	ns	ns
BSA	ns	0.3461*	ns	ns	0.2222*	ns	-0.3048**	0.3009**	ns	ns	0.4013***	0.3768**
CUN-BAE	0.5109***	ns	0.5065***	ns	-0.2051*	ns	ns	ns	0.4094***	ns	ns	ns

Abbreviations: ABSI – A body shape index; AVI – Abdominal volume index; BAI – Body adiposity index; BFP – Body fat percentage; BMI – Body mass index; BMI√WC – BMI multiplied by the square root of waist circumference; BRI – Body roundness index; BSA – Body surface area (Mosteller); CI – Conicity index; CUN-BAE – The Clinica Universidad de Navarra-body adiposity estimator; dBP – diastolic blood pressure; FM – Fat mass; HC – hip circumference; HI – Hip index; HR –heart rate; MAX – maximum; MIN – minimum; nBMI – new BMI; N – number; NC – neck circumference; ns – not significant; RFM – Relative fat mass; sBP – systolic blood pressure; SD – standard deviation; SM – Skeletal muscle mass; TMI –Triponderal mass index; wBMI – waist-corrected BMI; WC – waist circumference; WHHR – Waist to hip to height ratio; WHR – Waist to hip ratio; WHT.5R – New waist to height ratio; WHt^{2}R – Waist to the square of the height ratio; WHtR – Waist to height ratio; Wt – body weight; WWI – Weight-adjusted waist index; * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$

Key results from correlation analysis after categorization of anthropometric indicators into three groups (indices and ratios based on body height and weight, indices and ratios based on waist or hip circumferences, and indices and ratios based on BMI index calculation) are presented in Table 6. All three groups of indices and ratios that were calculated in our study were predominantly significantly correlated with BP rather than HR, and a greater number of significant correlation coefficients were calculated in the whole research group (55.56%) than in the male and female subgroups (34.57% and 45.68%). The analyzed indicators were significantly correlated with obesity and hypertension status more frequently than in subgroups without these complications.

Table 6. Key results of correlation analysis after division of the analyzed indices and ratios

Status	Indices and ratios based on body height and weight	Indices and ratios based on waist or hip circumferences	Indices and ratios based on BMI calculation
Sex	• a higher correlation with sBP in whole research group than in sex-based subgroups; • a higher correlation with dBP in females compared to males and to the whole research group;	• not significantly correlated with HR in the sex-based subgroups; • less significantly correlated with sBP in the sex-based subgroups;	• more significantly and closely correlated with BP components in females than in males; • not significantly correlated with HR in the sex-based subgroups;
Obesity	• significantly correlated with HR only in the obesity$^+$ subgroup; • the highest correlation coefficients with the HR and dBP in the obesity$^+$ subgroup; • almost completely not significantly correlated in the obesity$^-$ group;	• the highest correlation coefficients in the obesity$^+$ subgroup; • no significant correlation with sBP;	• the highest correlation coefficients with the HR and dBP in the obesity$^+$ subgroup • not significantly correlated with the HR and dBP in the obesity$^-$ group;
Hypertension	• more significantly correlated with sBP in the hypertension$^+$ subgroup;	• more correlated with both components of BP in the hypertension$^+$ subgroup;	• relatively low number of significant correlations; • the highest correlation coefficient in relation to HR;
Informative value	• the WWI index had the lowest informative value; • the highest correlation in the case of TMI and dBP;	• the HI index had the lowest informative value; • the highest correlation in the case of FM and dBP;	• the ABSI index without any calculated significant correlation in any subgroup; • The CUN-BAE the highest correlation with HR and dBP in the hypertension$^+$ subgroup;

Abbreviations: ABSI – A body shape index; BMI – Body mass index; BP – blood pressure; CUN-BAE – The Clinica Universidad de Navarra-body adiposity estimator; dBP – diastolic blood pressure; FM -Fat mass; HI – Hip index; HR – heart rate; sBP – systolic blood pressure; TMI – Triponderal mass index; WWI – Weight-adjusted waist index

Discussion

The purpose of the current study was to analyze anthropometric indicators in the context of cardiometabolic health based on an examination of whether physiologic characteristics, such as heart rate and blood pressure, five anthropometric measurements, and 23 indices and ratios could be useful in the noninvasive prediction of cardiometabolic risk status in the group of 162 Eastern Slovakia participants of both sexes with a mean age of 20.78±2.22 years. Several studies have reported that many anthropometric indicators based on measurements of body weight and height, waist and hip circumferences reflect cardiometabolic status in different age, sex, and ethnic subgroups and may be associated with each other or with other health indicators (Fu et al. 2014; Tran et al. 2018; Padilla et al. 2021; Wu et al. 2021; Casadei and Kiel 2022; Minetto et al. 2022). On the other hand, our results are in line with other studies showing that the frequency of cardiometabolic complications is heterogeneous in various research groups (Mladenova 2019; Nişancı Kılınç et al. 2019; Mangalavalli et al. 2021; Lahole et al. 2022).

From the point of view of all the analyzed indicators of cardiometabolic complications in our study, male participants were evaluated as a potentially higher-risk subgroup, and increased values of sBP were recorded in all males. The results of this study showed that especially values of BMI, WC, WHR, WHtR, sBP, and dBP, of some participants may be at potentially increased or high cardiometabolic risk and should be monitored in the future. Preobesity and obesity status, according to BMI values, were predicted for 19.14% and 5.55% of all participants, respectively. Waist circumference and WHR were increased in 14.20% and 12.35% of all participants, respectively, and an increased risk of central obesity according to the WHtR index was predicted in 16.67% of participants. Prehypertension, according to blood pressure values, was observed in 19.75% of individuals, with a predominance in females, and hypertension was observed in 4.32% of individuals, especially in males. The risk of supraventricular tachycardia was evaluated at 6.79%.

Lahole et al. (2022), in their cross-sectional study of 1,000 students with a mean age of 21.3±2.0 years, calculated increased risk mean values of BMI, WC, and WHR indices. The mean values of sBP and dBP were more favorable than the values in our research group (115.7±12.6 and 73.6±8.9 mmHg vs. 121.72±11.65 and 76.09 ±9.85 mmHg in our research group). A comparison of the mean values of the analyzed indices in both sexes did not result in significant results. The highest percentage of students with obesity status was predicted by WHR (57.30% of students), and the lowest percentage was predicted by NC (8.4% of students). The prevalence of hypertension and obesity was higher in the Lahole et al. (2022) research group compared to the results of our study and varies according to anthropometric indices.

Similarly to the results of our study, the mean values of BMI, neck circumference, and WHtR were higher in males in the Nişancı Kılınç et al. (2019) study of 4873 university students with a mean age of 20.58±1.86 years. Their results indicated that more male students were at increased or high risk of obesity.

In the Mladenova (2019) study the prevalence of anthropometric and cardi-

ovascular risk factors in a group of 386 Bulgarian students with a mean age of 21.20±2.4 years was analyzed. This study showed that mean values of the analyzed characteristics were higher in males, and these differences were statistically significant. Overweight and obesity, according to BMI, were predicted in 26.94% of participants and more frequently in males. Risk values of WHtR were predicted at 20.1% and prehypertension and hypertension were predicted according to blood pressure in 33.2% and 5.6% of cases, respectively (Mladenova 2019).

A study by Mangalavalli et al. (2021) analyzed 150 young students for blood pressure and routine anthropometric measurements, including the calculation of BMI in the context of obesity and prehypertension estimation. According to values of blood pressure, prehypertension was observed in 33.33% of students, predominantly females. Except for traditional indicators of cardiometabolic risk (BMI and waist circumference) determining the level and distribution of obesity, the neck circumference was a promising indicator, predicting obesity in more than half of the research group. Pearson's correlation analysis showed a significant, strong positive correlation between NC and systolic and diastolic blood pressure.

In our study, NC was correlated with heart rate and blood pressure, but not in all analyzed subgroups. According to our results, NC was better correlated with sBP in the subgroup of participants with the potential for hypertension (hypertension^{+}) than in the subgroup with obesity (obesity^{-}).

Anthropometric markers of obesity such, such as weight, height, WC, HC, BMI, WHR, and NC were also analyzed in the Hingorjo et al. (2012) study of 150 participating students aged 18 to 20 years. The mean values of the analyzed indicators were higher in males, except for hip circumference. In this study statistically significant differences between male and female mean values of NC and WHR were calculated at the $p \leq 0.001$ level. In contrast, the authors of the the Hingorjo et al. (2012) study did not report significant results after comparing BMI, WC, and HC mean values. A similar percentage of participants as in our research were categorized as overweight or obese according to BMI values.

The potential of using anthropometric indicators (BMI, WC, WHtR, WHR, new BMI, BAI, CUN-BAE, ABSI) as predictors of cardiometabolic risk was analyzed in a research group consisting of 550 British young individuals aged between 18 and 25 years (Amirabdollahian and Haghighatdoost 2018). The results showed that indicators based on body weight were in stronger association with measurements of body fat than indices related to body shape. According to their results, the authors presented the WHtR index as the best indicator of cardiometabolic risk, which together with WC had a better diagnostic capability for identifying cardiometabolic risk in young adults (Amirabdollahian and Haghighatdoost 2018).

Another study focused on the anthropometric indices HI, ABSI, and WHtR in 3844 Spanish Caucasian individuals reported that ABSI and WHtR but not HI were associated with high cardiovascular risk (Corbatón-Anchuelo et al. 2021).

Our study showed that of the three categories of indices and ratios, the ones that were based on body height and weight were more strongly correlated with blood pressure compared to indices and ratios based on waist and hip circumferences

or based on the calculation of BMI. The vast majority of the analyzed indicators were significantly more correlated with blood pressure compared to heart rate in almost all subgroups. The indicators were significantly correlated with obesity and hypertension status more frequently compared to status without these complications. The strongest correlation regarding HR and dBP was observed in the subgroup of participants with obesity. A stronger correlation was observed in the obesity^{+} subgroup regarding FM in relation to dBP and CUN-BAE in relation to both HR and dBP. The ABSI index had the lowest informative value as the correlation values were nonsignificant in all of the analyses. For comparison, amongst all of the indices analyzed in 550 British young individuals, CUN-BAE could be a new indicator of adiposity, and ABSI had the weakest correlation with adiposity (Amirabdollahian and Haghighatdoost 2018). In addition, Dominguez et al. (2021) demonstrated that increased adiposity estimated according to CUN-BAE has a predictive value for incident hypertension. The researchers of this study reported that a 2-unit increase in the CUN-BAE index values increased hypertension risk by 27% and 29%, respectively, according to sex (Dominguez et al. 2021). Another study showed a significant association between WC and sBP in females and WC and dBP in males, but other anthropometric indicators such as BMI and WHtR were nonsignificant in relation to blood pressure (Mladenova 2019). In a study by Chaudhary et al. (2019) BMI, WC, and WHR values increased in a linear relationship with blood pressure. According to the study by Gutema et al. (2020) the indicators BMI, WC, WHR, and WHtR were useful predictors of high blood pressure.

Conclusion

In recent years a lot of indicators reported in research studies have proven to be more useful in the association with cardiometabolic complications. Our study, based on the analysis of indicators, including 23 anthropometric indices and ratios, confirmed that from a total number of 567 calculated correlation coefficients, 38.80% of cases were with $p \leq 0.05$. All analyzed indices and ratios were significantly correlated, predominantly with blood pressure components rather than heart rate, especially among participants with the potential for disease complications. To conclude, the quantitative measurements of the body, calculated indices and ratios are non-invasive and useful indicators, although they may be at different levels of association and clinical significance for various conditions. Thus, some of the indicators may be standardly used in the early diagnostic process for monitoring cardiovascular health and risk stratification of patients.

Conflict of interest statement

Authors declared no conflict of interests.

Authors' contribution

All authors contributed to the planning of the research, discussed the problem, and contributed to the final manuscript. MZ supervised the study and was a major contributor to writing the manuscript, and MZ was also the corresponding author. JG, HV, and MŠ were responsible for data obtaining and anthropological indices and ratio calculations. EP, SK, and MMB were responsible for statistical analyses, language corrections, and data interpretation.

Corresponding author

RNDr. Michaela Zigová, Ph.D., Department of Biology, Faculty of Humanities and Natural Sciences, University of Prešov, 17. november 1, 08001 Prešov, Slovakia; e-mail: michaela.zigova@unipo.sk

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The age difference in 2D:4D among the Polish population: An exploratory study

Paulina Pruszkowska-Przybylska[1], *Magdalena Kobus*[1], *Elżbieta Żądzińska*[1,2], *Iwona Rosset*[1], *Milena Pruszkowska*[3], *Wojciech Kuczyński*[3], *Aneta Sitek*[1]

[1] University of Lodz, Faculty of Biology and Environmental Protection, Department of Anthropology, 90-237, Poland
[2] Biological Anthropology and Comparative Anatomy Research Unit, School of Medicine, University of Adelaide, South Australia 5005, Australia
[3] Medical University of Lodz, Department of Sleep Medicine and Metabolic Disorders, Lodz, Poland

Abstract: In this study a widely debated association between 2D:4D digit ratio and age was investigated. The study material included 960 individuals (530 females and 430 males) from Central Poland aged between 6–79 years. The information about age was obtained via survey filled in by study participants or, if underaged, their parents. The direct measurements of the second and fourth finger were performed to assess the 2D:4D digit ratio. The 2D:4D digit ratios for the left hand were significantly correlated with age both among females and males. In women the 2D:4D digit ratios for the right hand were significantly correlated with age. There were also significant differences in digit ratio between age groups. The results of our study suggest that there might be an association between digit ratio and age, and the direction of the correlation might be related to the phase of the ontogenesis.

Key words: digit ratio, prenatal testosterone, prenatal sex hormones proportion.

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Introduction

The second to fourth finger ratio (2D:4D) is a proportion known since the second half of the nineteenth century. It has been shown that the prenatal exposure to sex steroids affects the 2D:4D ratio. For instance, higher exposure to androgens affects longer fourth finger and in opposite more estrogens affect shorter fourth finger resulting in typically man (2D:4D<1) and woman (2D:4D≥1) proportion (Lutchmaya et al. 2004). However, it is not certain to what extent the 2D:4D ratio precisely indicates the current sex steroids proportion (Manning et al. 2014). Some studies suggest that prenatal sex steroid exposure is associated with neither the level of circulating androgens nor estrogens (Muller et al. 2011; Hönekopp et al. 2007; Kowal et al. 2020).

Numerous studies have reported that 2D:4D digit ratio does not change during human ontogenesis (Manning et al. 2014; de Sanctis V et al. 2017). Moreover, there are studies indicating that estrogen and androgens levels during postnatal period are not related to 2D:4D, and thus do not affect 2D:4D digit ratio during this period (Muller et al. 2011; Hönekopp et al. 2007; Kowal et al. 2020; Richards et al. 2017). However, some studies reported the opposite pattern showing that 2D:4D ratio may change with age (Kyriakidis 2021).

Hand morphogenesis occurs between 6 and 14 weeks of gestation and it consists of three main stages: shape forming (from 6 to 10 weeks), the appearances of creases (from 10 to 13 weeks), and development of ridges (from weeks 13 onward). This process is regulated by many biochemical factors, such as protein Sonic hedgehog (Shh) and wingless-type mouse mammary tumor virus integration site family member 7a (Wnt-7a), which induces transcription of the factor LIM homeobox transcription factor that takes part in dorsalization of the limb bud (Lacroix et al. 1984).

Rodent studies investigating hand morphogenesis have shown that length of the fourth finger is determined by the balance of testosterone to estrogen during a small window in fetal development probable around 14th week of gestation. Androgen and estrogen receptor activity is greater in the development of digit 4 compared to digit 2. Sex steroids regulate a network of genes that are involved in chondrocyte proliferation which leads to the growth of digit 4 independently of sex. Higher levels of androgens simulate chondrocytes proliferation of the fourth finger and leads to an increased 2D:4D ratio and oppositely in the case of higher level of estrogen (Brown et al. 2002; Zheng and Cohn 2011). Beside the time of establishment of 2D:4D ratio, it is not clear whether 2D:4D proportion is changing from generation to generation.

The aim of this study was to evaluate the differences in digit ratio among Poles in three age groups: children, young adults and adults.

Material and methods

The data comes from three cohorts investigated in years 2015–2021 in Lodz (city in Central Poland, population 680,000). The information about individuals age was obtained via survey filled in by study participants or, if underaged, their parents.

Cohorts' characteristics

Our cross-sectional study included 960 ethnically homogeneous healthy Caucasians (530 females and 430 males) aged

between 6–79 years divided into three groups: children (6–13 years), young adults (18–29 years) and adults (30–79 years).

The youngest cohort was instigated in years 2015–2017 in randomly selected primary schools in Lodz and included 611 children (319 girls and 292 boys). All measurements were carried out by the employees of the Department of Anthropology of the University of Lodz.

Individuals in the 18–30 years age range were measured in Lodz between September 2020 and March 2021 by medical students (Medical University of Lodz) and by the employees of the Department of Anthropology of the University of Lodz. There were investigated 167 individuals (104 females and 63 males).

The oldest cohort, aged over 29 years, included 175 adults (101 females and 74 males) investigated between July 2020 and September 2020 in Lodz was carried out by the employees of the Department of Anthropology of the University of Lodz.

Measurements

The direct measurements of the second and fourth finger were performed by qualified staff using a sliding calliper (Vernier calliper) with an accuracy of 0.001 m. Based on values of the fingers length, the 2D:4D index was calculated as a quotient of the length of the second digit and the fourth digit (mm).

The Consent of the Bioethics Committee at the Medical University of Lodz (RNN/374/19/KE and RNN/394/19/KE) and by the Ethical Commission at the University of Lodz (19/KBBN-UŁ/II/2016) were obtained. Written informed consent was obtained from all study participants or their parents in the case of children.

Statistical analysis

Due to lack of normal distribution of 2D:4D for right and left hand and age, the non-parametric tests were used.

The Kendall Tau correlation was applied to evaluate correlation between age and 2D:4D finger ratio for both hands.

Due to statistically significant differences between 2D:4D digit ratio for right and left hand ($t=-2.106$; $p=0.035$), further analyses were conducted separately for the right and left hand. However, among both sexes 2D:4D of left and right hand were positively correlated (females: $r=0.610$; $p<0.001$; males: $r=0.596$; $p<0.001$).

The Mann Withney test was used to determine whether there were dimorphic differences in 2D:4D ratio of right and left hand with regards to age.

The Kruskal Wallis test (H) with Tukey's post hoc tests were used to calculate differences between the three age groups.

The Cohen's d values were calculated to calculate the effect size for each comparison.

All statistical analyses were performed using the Statistica ver. 13.0 software.

Results

Although there were no significant age differences between males and females, there were dimorphic differences regarding 2D:4D (R) and 2D:4D (L) ratios. For example, females were characterized by higher 2D:4D (R) and 2D:4D (L) compared to males (Table 1).

Table 1. Statistical characteristics of the age and finger index (2D: 4D) of the right and left hand in the studied groups

sex	variables	N	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std. Dev.	Females vs Males		Cohen's d
										Z	*p*-value	
Females	Age	530	20.365	11.641	5.936	79.000	8.494	22.000	17.729	1.390	0.164	0.110
Males		430	18.479	11.374	5.919	79.000	8.404	21.000	16.584			
Children F	Age	319	9.334	9.012	5.936	13.303	7.726	11.036	1.941	-1.278	0.201	0.102
Children M		292	9.534	9.163	5.919	13.148	7.866	11.442	1.994			
Young adults F	Age	104	21.356	21.000	19.000	28.000	20.000	22.000	1.751	0.469	0.639	0.074
Young adults M		63	21.508	21.000	19.000	29.000	20.000	23.000	2.341			
Adults F	Age	107	52.299	51.000	30.000	79.000	43.000	62.000	12.564	0.921	0.357	0.116
Adults M		75	50.760	47.00	30.000	79.000	40.000	64.000	13.946			
Females	2D:4D (R)	530	0.986	0.986	0.881	1.095	0.966	1.000	0.033	4.985	<0.001	0.333
Males		430	0.975	0.975	0.867	1.058	0.952	1.000	0.033			
Females	2D:4D (L)	530	0.987	0.986	0.838	1.097	0.970	1.000	0.031	4.457	<0.001	0.290
Males		430	0.978	0.978	0.883	1.092	0.961	1.000	0.031			

The 2D:4D digit ratios for the left hand were statistically significantly correlated with age both among females (Table 2) and males (Table 3). In the case of females, the 2D:4D digit ratios for the right hand were also statistically significantly correlated with age (Table 2). Correlation between digit ratio and age within each of the age groups was non-significant (Table 2 and 3).

Table 2. Co-variability with age of 2D:4D finger ratio for the right and left hand in females

Groups	N	2D:4D R & age			2D:4D L & age		
		Kendall Tau (τ)	Z	*p*	Kendall Tau (τ)	Z	*p*
Females (total)	530	0.082	2.834	0.005	0.137	4.716	<0.001
children	319	0.032	0.857	0.392	0.066	1.757	0.079
young adults	104	0.048	0.720	0.472	0.050	0.735	0.451
adults	107	0.027	0.413	0.679	0.049	0.748	0.454

Table 3. Co-variability with age of 2D:4D finger ratio for the right and left hand in males

Groups	N	2D:4D R & age			2D:4D L & age		
		Kendall Tau (τ)	Z	*p*	Kendall Tau (τ)	Z	*p*
Males (total)	430	0.039	1.221	0.222	0.083	2.579	0.010
children	292	0.027	0.710	0.480	0.043	1.092	0.275
young adults	63	-0.028	-0.329	0.742	-0.095	-1.103	0.270
adults	75	-0.145	-1.842	0.065	0.022	0.282	0.778

Kruskal Wallis test showed that young adult females had higher 2D:4D (R) compared to children and adults (Table 4, Figure 1). In the male group the 2D:4D (R) was higher in young adults than in children (Table 4, Figure 2). In the case of 2D:4D (L) there were the following differences according to age groups: female children had lower digit ratio (L) than young adults and adults (Table 4, Figure 3) and young adults males had higher digit ratio (L) compared to children (Table 4, Figure 4).

Table 4. Differentiation of 2D: 4D finger ratio of the right and left hand between the groups distinguished by age.

Age group	Variables	N	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std. Dev.	St. Error	H	*p*
Females												
children	2D:4D (R)	319	0.982	0.983	0.881	1.063	0.964	1.000	0.030	0.002	36.383	<0.001[1]
young adults		104	1.004	1.000	0.929	1.095	0.985	1.028	0.033	0.003		
adults		107	0.982	0.986	0.892	1.060	0.958	1.000	0.035	0.003		
Males												
children	2D:4D (R)	292	0.973	0.976	0.867	1.058	0.950	1.000	0.033	0.002	6.053	0.049[2]
young adults		63	0.986	0.986	0.922	1.054	0.961	1.013	0.032	0.004		
adults		75	0.974	0.974	0.893	1.042	0.949	1.000	0.035	0.004		
Females												
children	2D:4D (L)	319	0.981	0.983	0.838	1.058	0.967	1.000	0.029	0.002	21.338	p<0.001[3]
young adults		104	0.998	1.000	0.920	1.097	0.972	1.016	0.033	0.003		
adults		107	0.994	0.986	0.910	1.057	0.971	1.015	0.033	0.003		
Males												
children	2D:4D (L)	292	0.975	0.977	0.883	1.053	0.956	1.000	0.030	0.002	9.097	0.011[4]
young adults		63	0.989	0.986	0.938	1.056	0.971	1.014	0.029	0.004		
adults		75	0.982	0.977	0.902	1.092	0.960	1.000	0.034	0.004		

Statistically significant post hoc tests:

[1] 2D:4D (R) Females
young adults and children p<0.001
young adults and adults p<0.001

[2] 2D:4D (R) Males
young adults and children p=0.046

[3] 2D:4D (L) Females
Adults and children p=0.005
young adults and children p<0.001

[4] 2D:4D (L) Males
young adults and children p=0.011

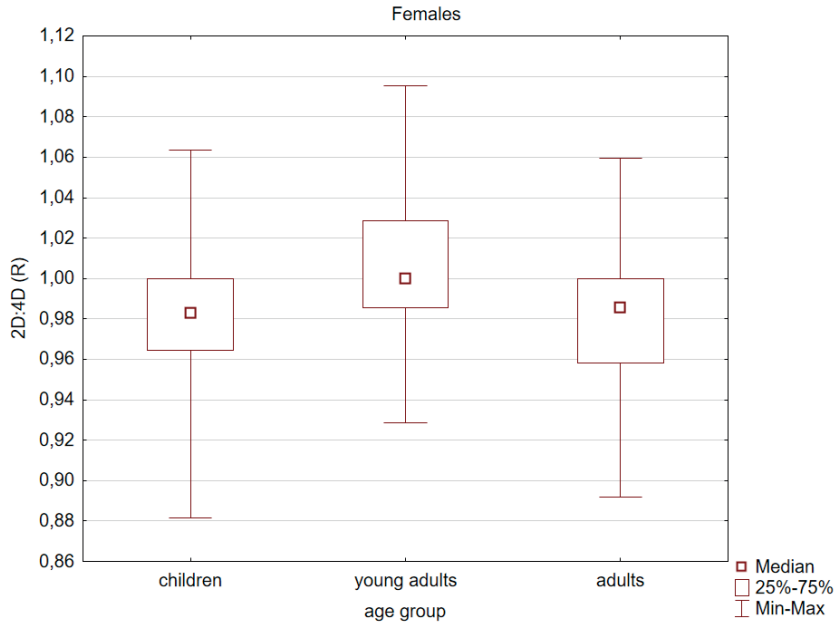

Fig. 1. Differentiation of 2D: 4D finger ratio of the right hand between the three age groups. Statistically significant effects: young adults and children ($p<0.001$) and young adults and adults ($p<0.001$)

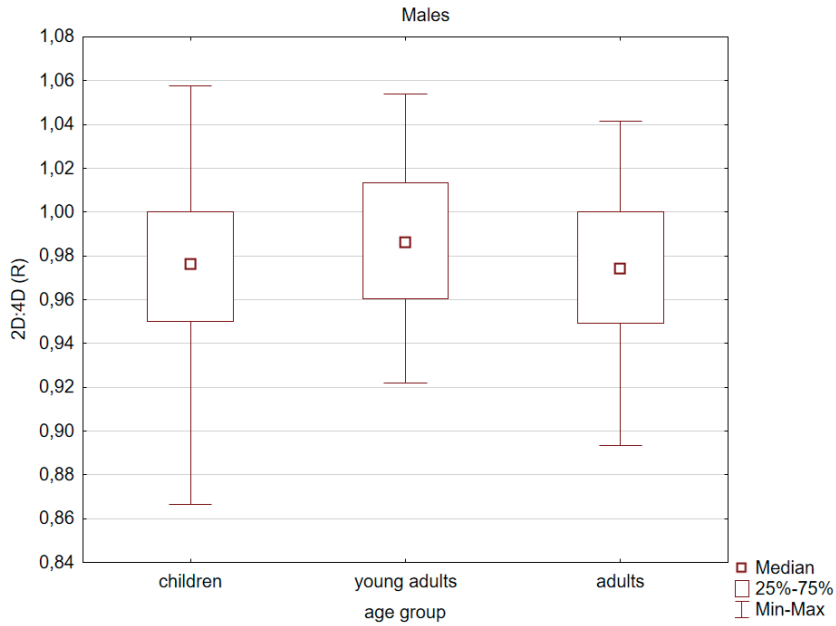

Fig. 2. Differentiation of 2D: 4D finger ratio of the left hand between the three age groups among males. Statistically significant effects: young adults and children ($p=0.046$)

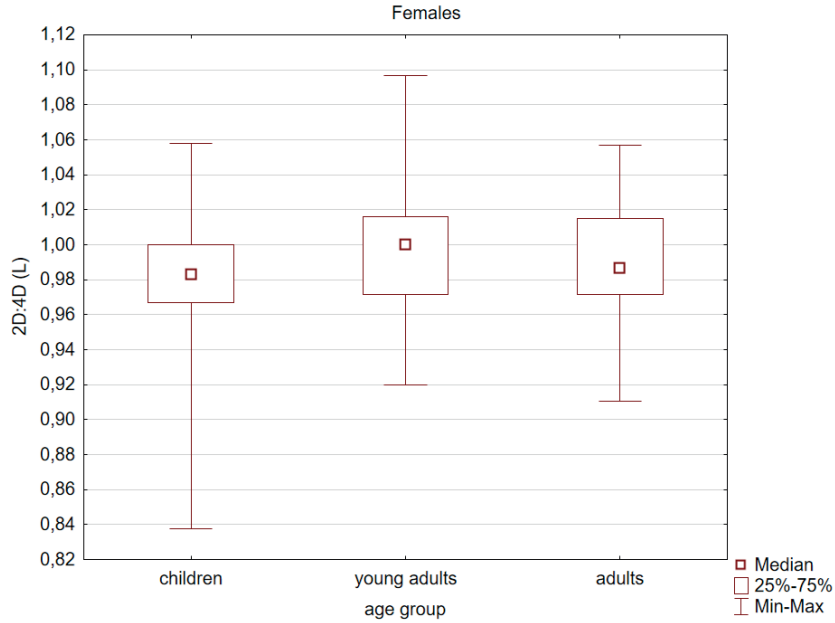

Fig. 3. Differentiation of 2D: 4D finger ratio of the left hand between the three age groups among females. Statistically significant effects: adults and children ($p=0.005$) and young adults and children ($p<0.001$)

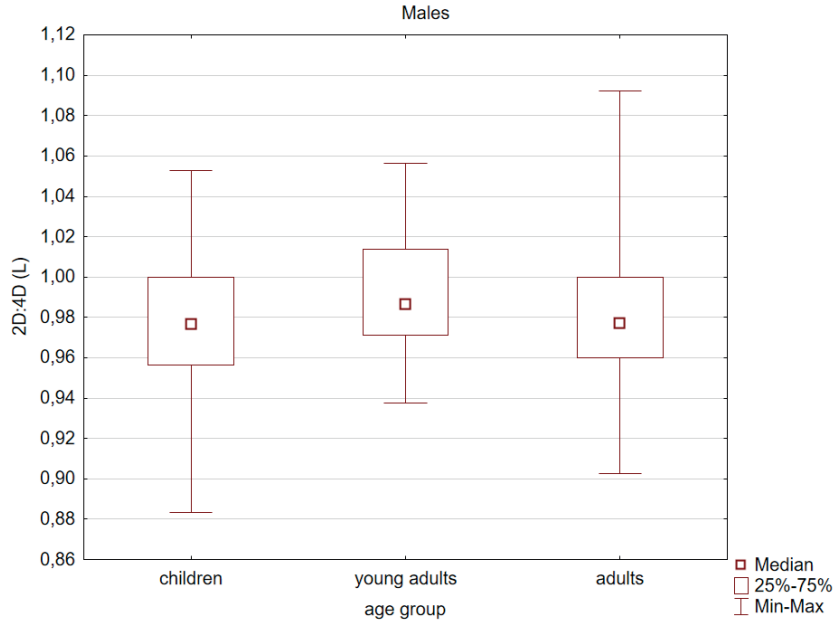

Fig. 4. Differentiation of 2D: 4D finger ratio of the left hand between the three age groups among males. Statistically significant effects: young adults and children ($p=0.011$)

Discussion

Although the relationship between the 2D:4D digit ratio and biology, behaviour and health has been widely investigated (Pruszkowska-Przybylska et al. 2008; Pruszkowska-Przybylska et al. 2021; Kasielska-Trojan et al. 2020; Sitek et al. 2018; Kobus et al. 2021) the association between 2D:4D and age is poorly understood mainly because studies have reported inconsistent results.

Regardless of the ethnic group, some changes in the 2D:4D digit ratio may occur during the prenatal period, the first two years after birth, and in later life (Butovskaya et al. 2021; Knickmeyer et al. 2011). The results of our study show that there are generation differences in the 2D:4D digit ratio for both hands between children and young adults. Older individuals seem to have higher digit ratios independently of sex possibly because there is an estrogen impact on digit development observed during late adolescence. In addition, Kyriakidis et al. (2021) reported that Greeks aged 38–63 years old had significantly higher 2D:4D ratio compared to a group aged <38 and ≥64 years old. In contrary, a study conducted by Kobus et al. (2021) showed no statistically significant correlation between 2D:4D observed across generations in a group of Poles aged 18–76 years. Similarly, Manning and Fink (2018) reported insignificant influence of age and no interaction effect of age and sex on 2D:4D in children or adults. The relationship between 2D:4D digit ratio and age has been also investigated in several longitudinal studies suggesting that 2D:4D digit ratio is not unstable in children and adolescents (Trivers et al. 2020; McIntyre et al. 2006; Trivers et al. 2006; Králík et al. 2017; Körner et al. 2020; Guo et al. 2021). One study by Richards et al. (2017) showed that 2D:4D among adult males was negatively correlated with age. The results of this study also show a negative, although non-significant, trend among adult males regarding the association of 2D:4D (right hand) with age. It is possible that chondrocytes are regulated during adulthood by testosterone, which, compared to children, is at a higher and more stable level in adults.

The time of the hand morphogenesis corresponds to the prenatal sex hormone exposure although there is no evidence that proportion of second and fourth finger remains equal during the entire ontogenesis. Moreover, longitudinal cohort study by ethnicity is needed to determine possible fluctuating changes of 2D:4D ratio.

We underline that confirmation of the correlation between 2D:4D digit ratio and age does not exclude the 2D:4D value as a determinant of the influence of sex hormones in prenatal development. However, each analysis should always be started by checking the relationship of the finger index in a given studied group with age and, if there is such a relationship, control for the influence of age on the value of this indicator while examining other effects.

Developing research in this area is necessary to create universal correction tool for each investigated group for 2D:4D research.

Limitations

The limitation of the study might be not equal number of individuals from each stage of the ontogenesis.

Due to cross sectional nature of the study the effect that was observed could be a secular effect within age sections of a population.

Another limitation of the study might be a wide range of age that could also have biological implication. For instance, the pattern of relationship with age might have higher order differences between younger and older age groups. Thus, longitudinal studies that include the same individuals are needed to provide information to supplement our findings.

Conclusions

There might be a possible association between digit ratio and age, and direction of the correlation might be related to the phase of the ontogenesis. The results of our study suggest that controlling the influence of age on the 2D:4D digit ratio value while examining other effects is important.

Conflict of interests

The Authors declare no conflict of interests.

Authors' contribution

P.P.-P. designed the study, collected the material, analyzed the data, and prepared the draft and final version of the manuscript. M.K. collected the material and participated in preparing the draft of the manuscript. E.Ż. participated in preparing the manuscript and provided critical comments on the manuscript. I.R. and M.P. collected the material. W.K. participating in the study designing A.S. designed the study, collected the material, participated in preparing the manuscript and provided critical comments on the manuscript.

Corresponding author

Paulina Pruszkowska-Przybylska, Department of Anthropology, Faculty of Biology and Environmental Protection, University of Lodz, 90-237, Poland; e-mail: paulina.pruszkowska@biol.uni.lodz.pl

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