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Asymmetry in Body Composition Variables of Youth Athletes

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ABSTRACT: Assessing bilateral differences in paired anthropometric features is an important methodological problem in sports anthropology. The present study included 128 adolescent female athletes (59 rhythmic gymnasts, 58 tennis players, and 11 swimmers). Body composition components were determined using multi-frequency bioelectrical impedance measurements (analyzer InBody 170). Asymmetry coefficients of muscle and fat mass accumulation in the upper (AA) and lower (AL) limbs were calculated using the Nacheva` equation (1986). The percentiles method was applied to distribute the bilaterally studied anthropometric features according to the mean values of the units of asymmetry (UA). Wilkoxon-test was used to assess the statistically significant differences in paired variables. Kruskal-Wallis test was applied to determine the differences in UA between three assessed athlete groups, depending on their age. The differences in body composition components between rhythmic gymnasts (RG), tennis players (TP), and swimmers (SW) were well expressed in all assessed age groups. The most considerable inter-group differences were observed in terms of the asymmetry coefficient in the lean body mass (LBM) with a right direction and body fat mass with a left direction for upper limb fat mass (%, kg), which have signed the highest values in the tennis players group, followed by the RG on the same age. Swimmers had significantly the lowest values of UA for all body segments. A close relation was found between asymmetry in body composition variables and the type of sports activity. Tennis was found as a sport with more pronounced inter-limbs asymmetry.

KEY WORDS: tennis players, rhythmic gymnasts, swimmers, asymmetry, body composition.

Original article

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Introduction

The assessment of bilateral differences in paired anthropometric features is an important methodological problem in anthropology. Bilateral asymmetry in human beings arose millions of years ago in the process of the evolution as an adaptive mechanism to the changing environmental factors (Zeidel et al. 2010). Anthropometric asymmetry provides information about changes occurring in the morphofunctional characteristics of the human body, according to the type and intensity of physical activity (labor, sports, etc). Assessing bilateral differences in paired anthropometric features is of great interest worldwide in biomedical sciences. Asymmetric changes, mainly in the limbs, have been observed in athletes competing in a variety of sports: volleyball, handball, javelin throwing, cricket, racket sports, rhythmic gymnastics, football, etc (Koley 2008, 2010; Stoykov 2012; Rynkiewicz 2013; Schluga-Filho et. al. 2016; etc.).

To achieve higher productivity in rhythmic gymnastics, gymnasts often perform repetitive motor actions, mainly with the dominant limb (Teixeira and Paroli 2000; Zaidi 2011). This leads to greater values of the thigh circumference and a greater angle of extension in the knee and hip joint compared to the contra-lateral limb (Georgopoulos et al. 1999; Douda et al. 2002; Frutuoso et al. 2016).

Tennis, similarly to rhythmic gymnastics, is a sport with an asymmetric etiology, especially existing in the upper limbs. The asymmetric accumulation of fat mass and lean mass between the right and left arms give rise to disproportions in the bones (Vergauwen 1998; Sánchez-Muñoz et al. 2007; Rogowski 2008, 2016; Abrahão et al. 2008; Berdejo-del-Fresno 2010). On the other hand, swimming is a sport that involves all muscles and provides a balanced body composition. Assessment of the effects of swimming training on body asymmetry in adolescents shows that continuous and intensive swimming during the growth periods does not cause body asymmetry in swimmers (Palomino-Martin et al. 2015).

Different studies associated the inter-limb asymmetries, of the lower-body greater than 10–15% with increased incidences of injury (Tyler et al. 2001; Bishop et al. 2018; Dos'Santos et al. 2019). The importance of the current research is to develop an accurate scale for assessing the directions and degree of the manifested morphological asymmetry, with aimed at preventing the health status of the athletes. In this reason:

The aim of the study is to evaluate the bilateral differences in body composition variables of adolescent athletes doing symmetric and asymmetric sports.

Material and Methods

The present study included 128 adolescent female athletes (59 rhythmic gymnasts, 58 tennis players and 11 swimmers). All athletes identified themselves as right-handed. Study participants were divided into three experimental groups according to the age classification in sports practice as follows: age group 1 includes all rhythmic gymnasts (RG) tennis players (TP) and swimmers (SW) from 8 to 10 years, the age group 2: 11–12 years and the age group 3: 12–14-year-old athletes. The criteria for inclusion were as follows: all athletes trained at least 5 times per week and competed regularly at the regional, national or international level championships. Their training experience in current sport was as follows: the mean training experience equaled 5.21 ± 1.2 years in TP, 4.92 ± 1.3 years in RG and 5.30 ± 1.3 years in SW. The girls and their parents completed informed consent and voluntarily participated in the study. The study protocol was reviewed and approved by the Ethical Committee of Institute of Experimental Morphology, Pathology and Anthropology with Museum-Bulgarian Academy of Sciences (Protocol № 22/07.02.2023) and was conducted in agreement with the principles stated in the Declaration of Helsinki for human studies (WHO 2008). Body mass index was calculated by a well-known formula using the mean height and weight values. Body composition components were determined by means of multi-frequency bioelectrical impedance measurements, which were taken with the use of In-Body (model: 170) analyzer, with eight electrodes. For accurate analysis, the following requirements have been met: the measurements of each athlete were made at least two hours after a meal and at least 12 hours before training. Statistical analysis was conducted using the SPSS software, version16.00, for Windows. Verification of the normal distribution of the anthropometric and body composition measurements and a non-normal distribution of one of the asymmetric coefficients prompted the use of the Kolmogorov-Smirnov and Shapiro-Wilk tests. Asymmetry coefficients of muscle mass and fat mass accumulationin the upper (UL) and lower (LL) limbs were calculated using the Nacheva` equation (1986):

$$\text{Units of asymmetry (UA)} = (\text{dominant limb}/\text{dominant limb} \cdot 100) - 100$$

The percentiles` method was applied to distribute the bilaterally studied anthropometric features according to the mean values of the units of asymmetry (UA). Wilkoxon-test was applied to assess the statistical significant differences in paired variables ($p \leq 0.05$). Kruskal-Wallis test ($p \leq 0.05$) was applied to assess the differences in UA between three assessed athlete` groups, depending to their age.

Results

All RG (mean age: 11.19±1.74 y; mean body height: 146.30±9.17 cm; mean body weight: 34.24±6.61 kg) had training in gymnastics six days per week, not less than 20 hours weekly. All TP (mean age: 11.24±1.72 y; mean body height: 153.60±9.43 cm; mean body weight: 45.13±1.02 kg) had training in tennis more than three times weekly and not less than 10 hours per week. The swimmers' training experience (mean age: 10.55±1.57 y; mean body height: 147.42±1.28 cm; mean body weight: 34.98±7.99 cm) was about five times per week and 90 minutes per training. Using results for height and weight, the mean values of BMI were observed. In TP it varied from 17.76 to 21.39 kg/cm^2 at the ages between 8 and 14. The results obtained for the other athletes' groups` were in the diapason from 15.31 to 16.90 kg/cm^2 and from 15.45 to 16.39 kg/cm^2 in RG and SW, respectively. TP exhibited the highest values of BMI compared to the RG and SW in all assessed age groups ($p<001$). During the assessed age period all athletes increased their BMI, most pronounced in the time between 11 and 14 years, due to the onset of puberty.

Bilateral differences in lean body mass and fat mass of the upper and lower limbs were presented in Fig. 1 (A, B, C). The largest differences between groups were observed for units of asymmetry, with a right direction for lean mass and a left direction for upper limb fat mass.

Units of asymmetry had significantly highest values in the tennis player group, followed by the RG` group (p<0.001). Swimming appears to be the sport with much lower levels of bilateral limb soft tissue differences.

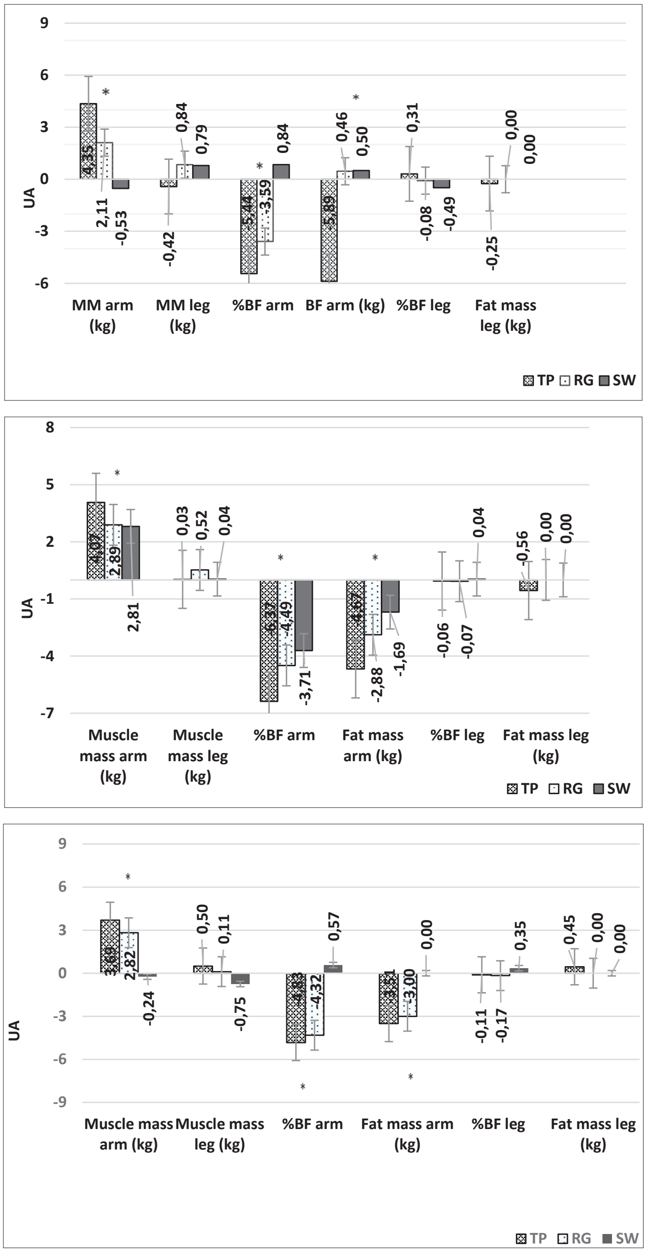

Fig. 1. Bilateral differences in lean body mass and fat mass in adolescent female athletes aged 8–10 years (1A), 11–12 years (1B), and 13–14 years (1C)

Based on the data on the percentile values of the UA, the profile of the anthropometric asymmetry of the athletes was established (Fig. 2A–Fig. 2C).

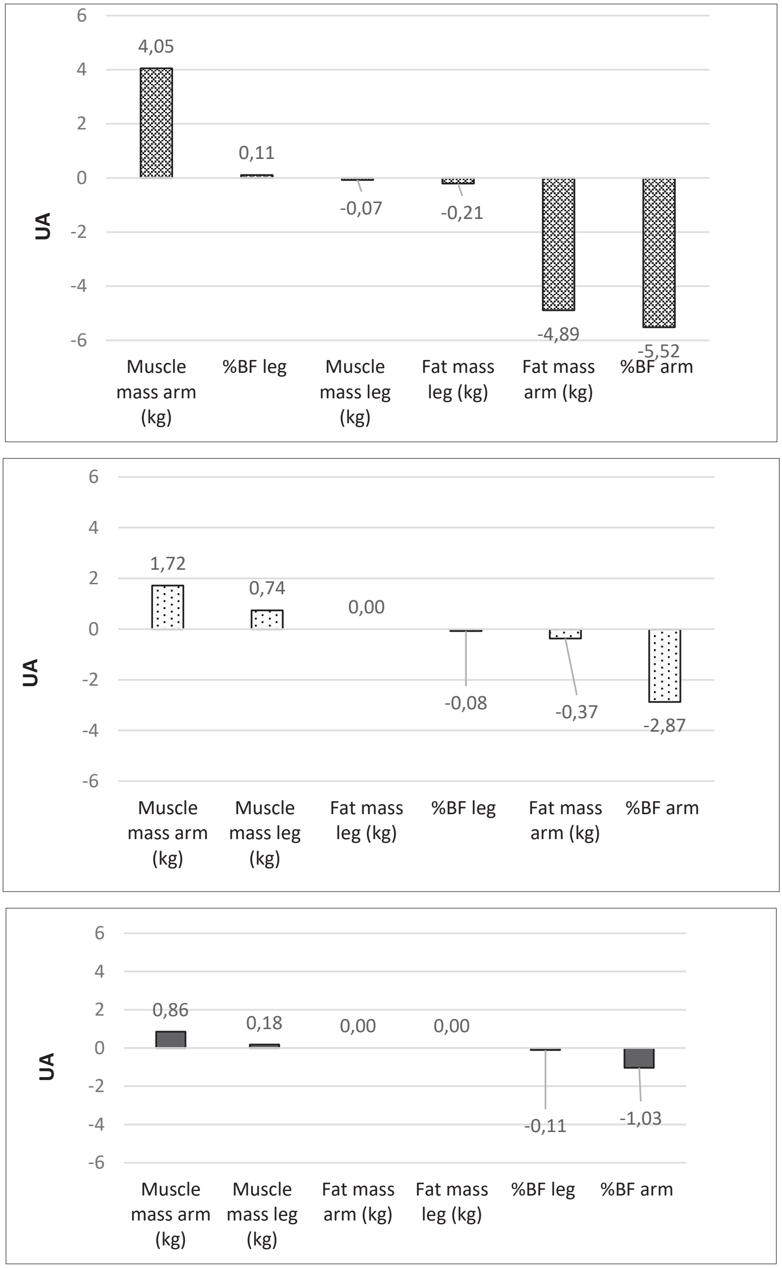

Fig. 2. Profile of the anthropometric asymmetry in: 8–14-years-old tennis players (2A), 8–14-years-old rhythmic gymnasts (2B), 8–14-years-old swimmers (2C)

The differences in body composition components between RG and TP were well expressed in the three age groups (p<0.001). Through all assessed age periods, TP had higher levels of asymmetry in muscle mass and fat mass of the upper limbs compared to the RG on the same age (p<0.001). Swimmers had significantly the lowest values of UA for all body segments (p<0.001).

Discussion

The study presented the bilateral differences in sport-specific morphological peculiarities in youth female competitors doing different sports. Some researchers declared that the asymmetry is more pronounced in the upper extremities in comparison to lower extremities with the right side prevalence (Tomkinson et al. 2003; Malina and Buschang 2004; Ulijaszek and Mascie-Taylor 2005). Schell et al. (1985) marked that morphological asymmetry is one of the main problems of anthropology and a suitable methodology is needed to analyze this phenomenon. Anthropometry is the most traditional method to assess the differences in paired parts of the body. The segmental bio-electrical impedance analysis (BIA) is a newer method to investigate regional diversification of the lean and fat mass of the body compartments. A comparative study of body composition (BC) in Slovak young women and men, with the use of BIA, showed significantly higher values of active body components in men. Opposite to that a significantly higher values of non-active body components were detected in woman. The authors reported a positive correlation between BC and indices (Falbová et al. 2022). A significant association of smoking with the body composition components was found, while it was responsible for higher adiposity in young adults. Higher values of all anthropometric parameters and indexes in smoking young adults compared to non-smokers were recorded, with statistically significant differences in waist circumference, BMI, waist to hip ratio, and fat mass indexes (Falbová et al. 2023). The segmental analysis of body composition, through the applied bioimpedance method, gave the opportunity to make a more detailed study about the manifestations of morphological asymmetry in the muscle and fat mass of the limbs in adolescent athletes.

The active physical load of certain muscle groups of the dominant upper limb in tennis players leads to a decrease in the %BF and better developed of muscle mass. Throughout the studied age period, upper limbs muscle mass and upper limbs fat mass (%) showed a strong to very strong level of asymmetry in tennis players and a moderate level in rhythmic gymnasts. The direction of the manifested asymmetry for muscle and fat mass is right-sided and left-sided, respectively. The lower limbs were characterized by relative symmetry in terms of the distribution of soft tissues in the studied sports activities. Kistrop et al. (Kistrop et al. 2000) found that measuring whole body and regional body composition by DEXA can improve the prediction of energy expenditure what could be helpful information about athletes who try to lose fat mass or gain lean muscle. Abrahao et al. (2008) made comparison of the young athletes playing tennis for up to two years (age 6–10 years) with the male instructors between 22 y and 37 years of age that have been engaged in playing tennis at least eight years. The authors reported the increase of the incidence of right somatic measures superior to the left, because the excessive time of training of asymmetric sport, which, according to the researchers, influenced postural deviation (Abrahao et al. 2008).

Conclusions

This study found a strong relationship between asymmetry in body composition variables and the type of sports activity. Tennis was found as a sport with more pronounced inter-limbs asymmetry. Established by us a body asymmetry profile allows summarizing and systematizing the information about the dimensions and direction of the manifested bilateral differences.

Conflict of interests

No potential conflicts of interests were reported by the author.

Ethical Approval

Ethical approval was given by the Ethics Committee of the Institute of Experimental Morphology, Pathology, and Anthropology with Museum–Bulgarian Academy of Sciences (Protocol № 22/07.02.2023) and was conducted in agreement with the principles stated in the Declaration of Helsinki for human studies (2008).

Authors' contribution

AD designed the study, collected the data, oversaw the statistical analysis/interpretation and agree to be accontable for all aspect of the work.

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

Age at pubarche and the risk of developing cardiometabolic complications among 50–52-year-old men from Krakow Longitudinal Study (Poland)

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ABSTRACT: Despite contradictory observations, it has been postulated that early age of adrenarche predisposes to an increased risk of cardiometabolic complications in further ontogeny due to greater body fatness. The aim of this study was to test the above postulates.

We present the results of research on 67 men aged 50–52 – participants of the Krakow Longitudinal Study conducted in the years 1976–2022 – from two birth cohorts 1970 and 1972. Boys were examined annually, aged 6–18, initially 940 people, at the age of eighteen – 358. They were examined again as adult men in 2004 (age 32–34) – 122 people and again in 2022 (age 50–52 years) 67 men. Based on the pubarcheal age, 50-year-olds were divided into 3 groups: early (11 people), average maturing (44 people) and (12 people), where the following were compared: resting systolic and diastolic blood pressure, basic parameters lipid profile – total cholesterol and its fractions, triglycerides, fasting glucose, body height and weight, waist and hip circumferences, indicators – Body Mass Index (BMI), Waist–hip Ratio (WHR), the thickness of 6 skinfolds and the prevalence of metabolic syndrome.

The results of the analyses showed that:

(1) there is a clear gradation, i.e., the earlier the age of pubarche, the worse the metabolic health of men;

(2) compared to the other groups, the total adiposity in men with early pubarche is slightly higher, with clearly marked abdominal obesity; BMI and WHR showed a contrasting picture.

At this stage of the analyses, it is difficult to clearly judge whether the cause of the increased cardiometabolic risk in the studied men with early pubarche is related to earlier age of adrenarche and the mechanisms and stimuli causing it, or to greater adiposity.

KEY WORDS: early adrenarche, metabolic syndrome, abdominal adiposity, blood pressure, triglyceride.

Original article

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Introduction

Two independent processes characterise the period of puberty. The first one is *adrenarche*, during which there is an increase in the activity of the adrenal glands and the secretion of steroid hormones; the second one – *gonadarche* – is characterised by an increase in the activity of the gonads. Witchel et al. (2020) state that the physiological mechanisms governing the onset of adrenarche remain unclear.

Adrenarche – chemical changes within the endocrine system, invisible at the macro level, observed in boys on average at the age of 7–8, which precede the visible changes in body morphology, i.e. increased growth rate, appearance of acne, increased activity of sweat glands, development of pubic hair – *pubarche* by approximately 1.5–2 years. Increasing production of adrenal androgens causes these physical manifestations of adrenarche. Research from recent years shows that the dominant bioactive androgen in children during normal and premature adrenarche is 11-ketotestosterone (Rege et al. 2018). After excluding diseases that increase the concentration of adrenal steroid hormones, e.g., androgen-secreting tumours, Cushing's syndrome and rare genetic disorders, premature adrenarche (PA) is currently considered as a variant of puberty (Potau et al. 1999; Utriainen et al. 2009) – before the age of 7. with. – and, consequently, premature pubarche (PP) – before the age of 9. According to Rosenfield (2021), the cause of premature adrenarche is still unknown. It is often associated with unfavourable metabolic features in children, such as hyperinsulinism, dyslipidemia, obesity (especially abdominal obesity) and later ovarian hyperandrogenism (Utriainen et al. 2015). Potau et al. (1999) observed that premature pubarche in boys, unlike girls, is not associated with a group of endocrine-metabolic abnormalities. Williams et al. (2015) found that metabolic syndrome was only observed in obese boys and girls, regardless of whether they had PA or not. Therefore, obesity appears to increase metabolic risk in the prepubertal population and not in PA. However, one study on a group of boys and girls conducted by Matzarapi et al. (2022) showed that PA-induced metabolic changes begin in childhood.

Do metabolic changes detected in children with PA persist into adulthood? Despite the existence of contradictory results (Bell et al. 2018), some authors postulated that early age of puberty in children predisposes to an increased risk of cardiometabolic complications in further ontogenesis, which is associated with higher adiposity (Widén et al. 2012; Prentice and Viner 2013). However, the authors of these studies used differing determinants of the pace of puberty, i.e., age at menarche (retrospective – recognising that it may be subject to memory errors), age at the peak of the pubertal spurt, skeletal age and others. In a review article, Rosenfield (2021) stated that the effects of premature adrenarche in adults have not been clearly defined.

Due to the existence of the mentioned above discrepancies in study findings, the aim of the presented study was to determine the following:

(1) whether the early age of adrenarche in boys, determined in our Krakow Longitudinal Study as the age of physical manifestation of this phenomenon in the form of pubarche, is associated with a greater risk of cardiometabolic complications in adulthood,

(2) whether it is accompanied by increased adiposity.

Due to the increasing prevalence of disorders in this area of health, the results of this study might be useful in early prevention, including mental health. Since the age of pubarche in boys worldwide has significantly decreased over the last few decades, these problems may affect an increasing number of boys and, later in life, men. For instance, over the period 40 years (1971–2010), in the Krakow population the age at the onset of pubic hair development decreased by more than one year: from 12.99 to 11.80 years (Kryst et al. 2012). Such a rapid acceleration of physical maturation is not accompanied by faster mental, emotional and cognitive development because this requires experience and, as stated by, among others, Dorn et al. (1999), Sontag-Padilla et al. (2012), Barendse et al. (2018), Marakaki et al. (2018), Ellis et al. (2019) and Michielsen et al. (2020), early puberty increases the risk of mental health disorders, such as anxiety, depression, oppositional defiant disorders, as well as antisocial and destructive behaviour. Similar association has been found regarding adrenal hormones, such as androgens and cortisol (Michielsen et al. 2020).

Material and methods

The research material consisted of data 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 (Krakow's Continuous Research [KBC 1976–2022]). The first series of annual surveys was performed in 1976–1988 (the age of the participants was 6–18 years), and the second series in the years 1980–1990 – the age of the respondents was 8–19 years. The present study combined data from men from two series examined in 2004 (32–34 years old) and re-examined in 2022 (50–52 years old). The number of examined boys and men are as follows:

- I series – from 480 at the age of 6 to 180 at 18,
- II series – from 460 at the age of 8 to 178 at 18,
- 122 men in 2004 and 67 men in 2022.

The numerical statement shows that the number of men in subsequent studies decreased, which is the expected trend in longitudinal studies. 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.

There was no morphological selection of men who participated in research conducted in 2022. The mean body height of 122 men studied in 2004 was 178.1 cm, and 177.9 cm for 67 study participants. The examined men were born in Krakow at the same time (1970 and 1972), grew up in a similar socio-economic reality and during childhood (developmental period) lived in the same district of Krakow (Nowa Huta) consisting of large blocks of flats, near a huge metallurgical and coking complex (the Vladimir Lenin Steelworks in Nowa Huta); they grew up and matured in the 70s and 80s of the 20th century. The first half of the 70s in Poland is often referred to as the "golden Gierek era", a period of temporary prosperity of the Polish society, during which a marked rise in the standard of life of the citizens was observed and a system of social care, such as free schooling system, free national healthcare system, hygiene-medical and dental care of children and youth in Polish schools, was developed. The modernization and industrialization of the country progressed. Since the mid-50s of the 20th century a number of industrial giants operated in Krakow and its immediate vicinity. During this period factory plants gradually increased their production, resulting in the discharge of many harmful substances into the environment, polluting the soil, water and air. An increase of morbidity rate among both adults and children became a problem which eventually forced the authorities to close down the department of electrolysis in the Aluminum Plant in Skawina (operated on the basis of obsolete Soviet technologies) in1981.

During annual examinations of children, anthropometric measurements were taken, such as body height (b-v distance), length of lower limbs (b-sy), body weight, waist and hip circumferences and thickness of skin-fat folds. In addition, in boys pubic hair development was assessed using the Tanner scale, which covers the period from pre-pubertal (stage I) to adult (stage V) – photos and drawings. The assessment was carried out by designated staff members. In addition to analogous anthropometric measurements, during the study of adult participants of this research (2022) blood pressure was measured (upper arm blood pressure monitor Omron M3 COMFORT). Body weight was measured using a Tanita Body Composition Analyser, type BC-418 MA.

Based on the age of pubarche, the participants were divided into three groups:

- early maturing, 10–11 years old – 11 people (no cases of premature pubarche were found in the sample),
- average maturing, 12–13 years old – 44 people,
- late maturing, 14–16 years old – 12 people.

In each of the three groups the following morphological characteristics were analysed: body height and weight, length of lower limbs (B-sy distance); waist and hip circumferences; thickness of 6 skinfolds, i.e., above the biceps and triceps, subscapular, abdominal, suprailiac and calf (measured with Harpenden skinfold calliper) and biomedical parameters: resting systolic (SBP) and diastolic (DBP) blood pressure, lipid profile: total cholesterol and its fractions (HDL, non-HDL, LDL), triglycerides and glucose concentration (blood taken on an empty stomach). The analyses were performed by the SYNEVO laboratory in Krakow.

To show the overall length proportions, the lower limb length index (relative legs length) was calculated: (B-sy/body height) x100. Body mass and the type of fat distribution around the waist and hips were estimated in accordance with WHO recommendations, based on body mass index (BMI) and the ratio of waist circumference to hip circumference (WHR).

To illustrate the level and distribution of subcutaneous fat, the following body fat indexes were constructed:

- total subcutaneous fatness (or total fatness), the sum of 6 skinfolds: biceps, triceps, calf, subscapular, abdominal and suprailiac;
- trunk fatness (the sum of 3 skinfolds: subscapular, abdominal and suprailiac);

- abdominal fatness (the sum of 2 skinfolds: abdominal and suprailiac);
- limbs fatness; the sum of 2 skinfolds: triceps and calf.

The distribution of subcutaneous fat, the relationship between peripheral fat (limbs) and central fat (trunk), was presented in the form of two indicators:

- (limbs fatness /trunk fatness) x 100,
- (limbs fatness /abdominal fatness) x 100.

The occurrence of metabolic syndrome (MS) was verified based on the presence of at least 3 of the following five factors, without indicating the dominant pathogenetic factor; the criteria were chosen following Kramkowska and Czyżewska (2014) and Dobrowolski et al. (2022):

- abdominal obesity (defined by waist circumference) in European men > 94 cm,
- increased triglyceride level, > 1.7 mmol/l,
- reduced blood concentrations of HDL cholesterol, < 1 mmol/l) for men,
- increased blood pressure – systolic ⩾ 130 mmHg and/or diastolic ⩾ 85 mmHg,
- abnormal fasting glucose, ⩾ 5.6 mmol/l.

Pearson's chi-square tests and ANOVA were used to analyze the differences between the singled-out groups of men. The calculations were conducted using the STATISTICA software, version 13 package. The MedCalc statistical software package for biomedical research (version 22.017) was used to analyze the odds ratio (OR) (MedCalc Software Ltd.).

To present a significant amount of information about various features of the body structure of the participants in one place, the results of the analysis of differences between groups were presented in the form of standardised profiles (Fig. 1 and Fig. 2). The mean values of individual characteristics of men with early and average age at pubarche (X) were standardised for the mean (μ) and standard deviation (SD) of the late pubarche age group ("Z-Score" standardization). Z-score was calculated according to the formula:

$$Z = X - \mu / SD,$$

where:
Z – is the Z-score (standard score), i.e., the number of standard deviations by which a given value of, for example, the body height of individuals in the early or average pubarche group differs from the average body height of individuals with late pubarche,
X – non-standardized variable – score, i.e., the body height values of males from early and late pubarche groups.

If the Z value is positive, it means that the score is above the average value for the reference group, while a negative Z value indicates that the score is below the average value.

Men entering pubarche late were characterised by the best ("healthiest") biomedical profile in terms of lipid profile components and blood pressure. Therefore, they were used as a reference group. Information about the level of education of the men was also provided. The descriptive characteristics of the analysed features were presented in summary tables.

Results

The average age of initiation of pubic hair development among 50–52-year-old men from Krakow's Continuous Research (KBC 1976–2022) was calculated using the probit analysis method; median Me = 12.51 years, SE = 0.10, which is almost identical to the result estimated for the entire study population during childhood N = 485, where Me = 12.49 years, SE = 0.05 (Bocheńska and Chrzanowska 1993). Table 1 shows the distribution of the rate of pubarche in subsequent ages in the studied adult men.

The level of education of the examined men is presented in Table 2. In all pubarche age groups, most people had higher education, while vocational education was the least prevalent. Differences in the level of education between age at pubarche groups were relatively small and statistically insignificant ($chi^2 = 1.082$, $p = 0.897$). The extreme maturation groups – early and late pubarche – were characterised by an almost identical distribution of individual education categories: over 60% with higher education (63.6% and 66.7%, respectively), 27.3% and 27.5.0% with secondary education while 9.1% and 8.3% with vocational education. Among people exhibiting an average pubarche age, there was about 10% fewer in the tertiary education category, but about 10% more had a secondary education. The number of individuals with vocational education was the most similar in each maturation category (Tab. 2).

Tab. 1. Frequency of pubarche in subsequent years of boyhood among 50–52-year-old men. (KBC 1976–2022)

Age* (years)	Total number	Post pubarche		
		n	cumulation	%
9.5	67	0	0	0.00
10.5	67	3	3	4.48
11.5	67	8	11	16.42
12.5	67	27	38	56.72
13.5	67	17	55	82.09
14.5	67	7	62	92.54
15.5	67	3	65	97.01
16.5	67	2	67	100.00

* middle of the age range

Tab. 2. Level of education of 50–52-year-old men in groups of different pubarche ages. (KBC 1976–2022)

Pubarcheal age		Level of education *			
		higher	secondary	vocational	Total
Early	n	7.0	3.0	1.0	11.0
	%	63.6	27.3	9.1	100.0
Average	n	24.0	17.0	3.0	44.0
	%	54.6	38.6	6.8	100.0
Late	n	8.0	3.0	1.0	12.0
	%	66.7	25.0	8.3	100.0

* The differences between groups not statistically significant, $chi^2 = 1.082$, P = 0.897

Figure 1 shows, in the form of normalised profiles, the differences between three groups of men exhibiting different pubarcheal ages in terms of lipid profile components, systolic and diastolic blood pressure, waist and hip circumferences.

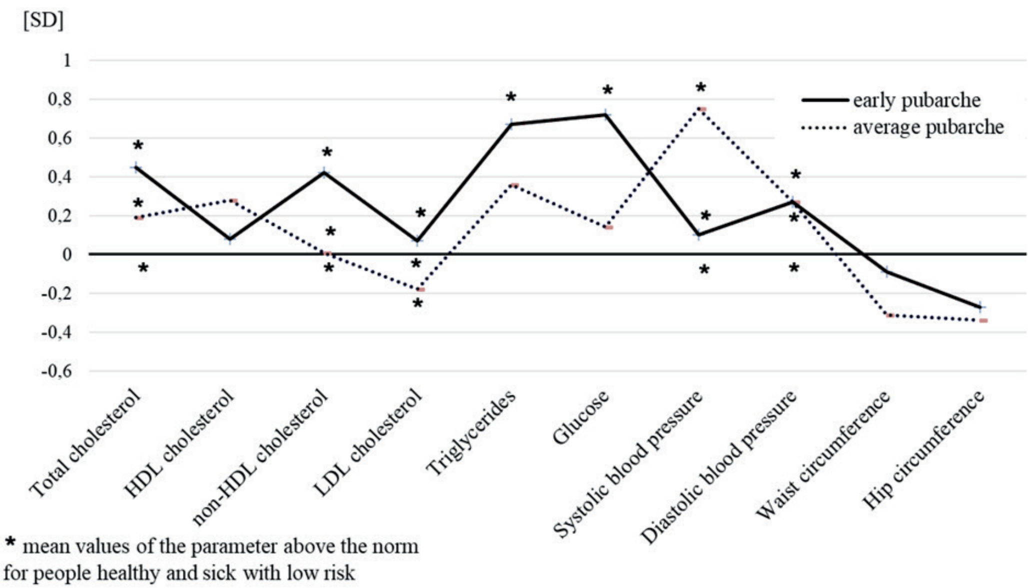

Fig. 1. The differences in terms of lipid profile components, systolic and diastolic blood pressure, and waist and hip circumferences between men aged 50–52 from the early and average pubarche groups in relations to the late pubarche category – normalised profiles. * Biomedical parameters, whose reference values (medical norms for healthy subjects and low cardiovascular risk group) are exceeded at the level of the average value by the groups with different pubarcheal age, are marked with an asterisk. (KBC 1976–2022)

Tab. 3. Mean, standard deviation (in parenthesis), median, Q1 and Q3 *quartiles* (in parenthesis) of lipidogram parameters, systolic and diastolic blood pressure and waist and hip circumferences among 50–52-year-old men at different ages at pubarche. (KBC 1976–2022)

Biomedical and physique parameters	Age at pubarche			Differences
	early	average	late	P #
Total cholesterol (mmol/l)	5.58 (1.24)	5.33 (1.18)	5.15 (0.96)	0.680
	5.40 (4.20; 6.30)	5.20 (4.70; 6.00)	5.00 (4.45; 5.95)	
HDL cholesterol (mmol/l)	1.35 (0.25)	1.42 (0.26)	1.32 (0.36)	0.503
	1.40 (1.22; 1.47)	1.40 (1.30; 1.66)	1.20 (1.01; 1.48)	

Biomedical and physique parameters	Age at pubarche			Differences
	early	average	late	P #
non-HDL cholesterol (mg/dl)	163.65 (50.87)	151.30 (45.96)	147.83 (37.89	0.679
	157.0 (126.0; 190.0)	147.0 (124.0; 176.0)	140.0 (117.0; 183.0)	
LDL cholesterol (mmol/l)	3.29 (0.95)	3.14 (0.92)	3.11 (0.99)	0.883
	3.40 (2.30; 4.00)	3.10 (2.40; 3.70)	2.80 (2.40; 4.10)	
Triglycerides (mmol/l)	1.81 (1.33)	1.59 (0.87)	1.34 (0.70)	0.505
	1.30 (1.10; 2.00)	1.40 (1.00; 1.90)	1.20 (0.80; 1.50)	
Glucose (mmol/l)	5.76 (1.05)	5.47 (0.57)	5.40 (0.50)	0.407
	5.50 (5.20; 9.00)	5.40 (5.10; 5.70)	5.40 (5.00; 5.80)	
Systolic blood pressure (mmHg)	139.18 (14.10)	147.70 (18.00)	137.83 (13.10)	0.477
	134.0 (129.0; 152.0)	148.5 (137.5; 158.0)	135.5 (127.5; 141.5)	
Diastolic blood pressure (mmHg)	92.00 (7.60)	92.00 (12.00)	89.70 (8.40)	0.615
	93.0 (88.0; 99.0)	91.0 (85.0; 97.0)	87.5 (83.0; 97.5)	
Waist circumference (cm)	97.77 (10.08)	94.76 (11.10)	98.96 (13.50)	0.114
	98.5 (91.5; 104.0)	94.2 (89.2; 101.0)	96.7 (90.0; 106.5)	
Hip circumference (cm)	101.50 (7.16)	100.95 (7.94)	103.50 (7.38)	0.803
	103.0 (94.0; 109.0)	100.7 (97.7; 104.2)	104.0 (97.5; 108.2)	

ANOVA test (analysis of variance)

In terms of lipid profile components at the level of average values (Fig. 1, Tab. 3), men from all maturation groups exceeded the norms for total cholesterol concentration, HDL and LDL. Triglyceride and glucose levels were additionally exceeded only by early maturing men. Thus, the group with early pubarche had the worst metabolic profile, deviating from the group with late pubarche (i.e., exhibiting the best metabolic condition) the most, within the range of +0.10 to +0.72 SD. The group with average pubarche age deviated slightly less, from -0.08 to +0.36 SD. Only the mean content of HDL was normal in all groups, and the differences between the categories ranged from +0.08 to +0.38 SD. Mean arterial blood pressure was elevated in both SBP (⩾130 mmHg) and DBP (⩾85 mmHg) in all three groups of men. The group with late pubarche had the lowest SBP and DBP, while slightly higher SBP and DBP values were exhibited by the group with early pubarche. The largest deviation (+0.8 SD) regarding SBP, averaging 147 mmHg, was exhibited by the group of average maturing age. Compared to late maturing men, men of early and average pubarche exhibited a worse metabolic condition and were characterised by a slightly smaller average waist and hip circumference as well as more clearly marked in the average age at pubarche category (Fig. 1, Tab. 3).

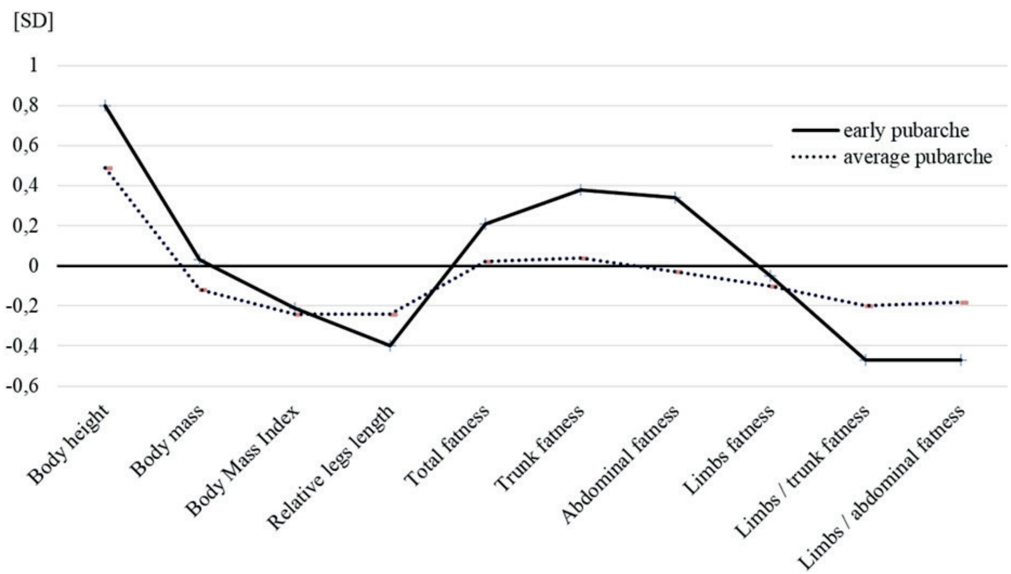

Fig. 2. The differences in selected features of body morphology between men aged 50–52 from the early and average pubarche groups in relations to the late pubarche category – normalised profile. (KBC 1976–2022)

Tab. 4. Mean, standard deviation (in parenthesis), median, Q1 and Q3 *quartiles* (in parenthesis) of body height and mass, BMI, relative legs length, and thickness and distribution of subcutaneous fat among 50–52-year-old men at different ages at pubarche. (KBC 1976–2022)

Physique parameters	Age at pubarche			Differ-ences
	early	average	late	P #
Height (cm)	179.25 (8,07)	178.02 (5.66)	176.02 (4.05)	0.803
	180.5 (175.4; 186.2)	177.9 (174.2; 181.7)	177.6 (173.0; 179.2)	
Weight (kg)	89.48 (15.68)	87.59 (15.24)	89.43 (15.63)	0.516
	91.9 (74.8; 102.0)	87.3 (78.6; 93.9)	86.2 (77.65; 102.2)	
BMI (kg/m²) *	27.76 (3.58)	27.59 (4.39)	28.96 (5.76)	0.473
	27.9 (24.1; 31.0)	28.0 (24.4; 30.15)	27.0 (25.45; 32.0)	
Relative legs length ** (%)	52.51 (1.60)	52.65 (1.18)	52.86 (0.88)	0.936
	52.4 (51.2; 53.9)	52.7 (51.9; 53.6)	52.9 (52.1; 53.7)	
Total fatness (cm) ***	10.09 (2.17)	9.44 (3.03)	9.38 (3.31)	0.270
	10.1 (7.7; 11.8)	9.0 (7.5; 11.5)	9.3 (6.2; 11.9)	
Trunk fatness (cm)	7.00 (1.42)	6.28 (1.94)	6.20 (2.10)	0.306
	6.9 (5.2; 8.3)	6.1 (5.5; 8.6)	6.1 (4.4; 7.9)	

Physique parameters	Age at pubarche			Differ-ences
	early	average	late	P #
Abdominal fatness (cm)	4.78 (1.03)	4.22 (1.33)	4.26 (1.52)	0.424
	4.9 (3.5; 5.6)	4.2 (3.3; 5.2)	4.4 (2.9; 5.6)	
Limbs fatness (cm)	2.35 (0.65)	2.30 (0.85)	2.40 (0.99)	0.899
	2.4 (1.7; 2.8)	2.1 (1.8; 2.8)	2.2 (1.5; 3.3)	
Limbs/trunk fatness (%)	33.31 (5.51)	36.74 (7.87)	39.32 (12.70)	0.667
	33.7 (30.1; 37.1)	35.0 (32.3; 41.0)	36.8 (29.3; 43.5)	
Limbs/abdominal fatness (%)	49.10 (8.53)	54.50 (12.63)	57.90 (18.9)	0.792
	50.0 (42.0; 53.9)	53.2 (46.2; 63.3)	57.1 (45.1; 61.6)	

* Body Mass Index; ** (B-sy/body height) x100; *** individual measures of body fatness are presented in the methods;

ANOVA test (analysis of variance)

Figure 2 illustrates the differences, in the form of normalised profiles, among selected features of body morphology between men from the early and average pubarche groups in relation to the late pubarche category. The highest average body height was exhibited by the early pubarche group, which was 3 cm higher compared to those with late maturation (+0.8 SD), with the same body weight as the reference group, which translates into a mathematical picture of a slimmer overall body figure in them (BMI was lower by 1.2 kg/m^2). However, the average BMI values of both groups were at the upper limit of the overweight category (Fig. 2, Tab. 4). The average lower limb index of -0.40 SD for the early pubarche age indicated that, given their greater average body height, study participants belonging to this category had relatively shorter lower limbs. This is somewhat less marked with regards to the average maturation group. The level and distribution of subcutaneous fat determined by the thickness of the skinfolds also differed. The average total, trunk and abdominal sum of skinfolds are the highest in early maturing men. The deviation of these parameters from the reference group averaged approximately +0.4 SD (difference from 0.5 to 0.8 cm, depending on the skinfold). However, the adiposity of the limbs was identical in both groups. The relationship between the adiposity of the limbs and of both the trunk and the abdomen suggested a greater tendency to accumulate fat in the central rather than peripheral deposits in the early maturing group, deviation +0.47 SD, with differences in the values of the indices of approximately 8%. This does not suggest that there was no such tendency in the other groups but, rather, indicates that this tendency was less pronounced. The average pubarche group was more similar to the early maturing group in terms of general body structure but more similar to the late maturing group regarding adiposity (Fig. 2, Tab. 4).

Tab. 5. The frequency of lipid and carbohydrate metabolism disorders and its manifestation in the form of changes in physique among 50–52-year-old men at different ages at pubarche. (KBC 1976–2022)

Biomedical parameters and physique indexes		Age at pubarche		
		early	average	late
Elevated total-cholesterol	n	8/11	28/43	6/12
> 5.0 mmol/l	%	72.7	65.1	50.0
Low HDL-cholesterol	n	2/11	2/43	2/12
< 1.0 mmol/l	%	18.2	4.6	16.7
Elevated non-HDL cholesterol	n	7/11	24/43	6/12
> 145 mg/dl	%	63.6	55.8	50.0
Elevated LDL-cholesterol	n	7/10	31/42	7/12
> 3.0 mmol/l	%	70.0	73.8	58.3
Elevated triglyceride	n	5/11	11/43	2/12
> 107 mmol/l	%	45.4	32.6	16.7
Elevated glucose level	n	5/11	19/43	4/12
⩾ 5.6 mmol/l	%	41.7	44.2	30.0
High systolic blood pressure	n	8/11	38/44	8/12
⩾ 130 mm Hg	%	72.7	86.4	66.7
High diastolic blood pressure	n	10/11	33/44	9/12
⩾ 85 mm Hg	%	90.9	75.0	75.0
BMI *	n	3/11	12/44	3/12
normal body weight 18.5 – 24.9	%	27.3	27.3	25.0
overweight + obesity	n	8/11	32/44	9/12
⩾ 25	%	72.7	72.7	75.0
WHR *	n	2/11	10/44	1/12
peripheral fat distribution < 1.0	%	18.2	22.7	8.3
central fat distribution	n	9/11	34/44	11/12
⩾ 1.0	%	81.8	77.3	91.7
Metabolic syndrome (MS) **	n	5/11	16/43	4/12
presence	%	45.4	37.2	33.3

Note: * standards according to Word Health Organization (WHO); BMI – Body Mass Index; WHR – Waist-to--hip ratio; ** verification criteria are described in the methods; presence of ⩾ 3 criteria within the group

Table 5 presents the prevalence of lipid and sugar metabolism abnormalities measured by exceeding the reference norms for a healthy population and low-risk patients, as well as the related unfavourable changes in body structure and arterial blood pressure in groups of men of different pubarcheal age. Compared to men with late pubarche, in the early maturing category, in terms of all biomedical parameters, the number of individuals exceeding the reference norms was higher, i.e., the severity of metabolic abnormalities was greater. Compared to men with late pubarcheal age, the chance of elevated triglyceride level is 2.86 times higher in men with early pubarcheal age (OR = 2.86; 95% CI: 0.35–22.7).

The prevalence of metabolic syndrome in this group was also slightly higher (45.4% vs. 33.3%), which is a logical consequence of the higher prevalence of abnormalities in individual biomedical parameters. Relative to men with late pubarcheal age, the chance of developing MS is 1.67 times higher in men with early pubarcheal age (OR = 1.67; CI: 0.31–9.01, P = 0.553), statistically insignificant. However, unfavourable health changes observed in body structure measured by the BMI showed an excessive body weight and determined by the proportion of waist to hip circumference (WHR $\geqslant$ 1.0) indicating the central (abdominal) fat deposit, occur with a slightly higher frequency in the late maturing group in relation to other groups. For most of the analysed parameters, men of average pubarcheal age exhibited an intermediate prevalence of the analysed abnormalities (Tab. 5).

Discussion

The aim of the present study was to determine (1) whether early age of adrenarche in boys, calculated as the age of physical manifestation of this phenomenon in the form of pubarche, is associated with a greater risk of cardiometabolic complications in adulthood, (2) whether early age of adrenarche is related to increased adiposity. There are few studies on this topic, especially regarding the male gender. This form of longitudinal research constituting systematic annual observations regarding the same group of study participants at the age from 6 (adrenarche time) to 18 years (the time of adolescence), followed by taking data on the same study participants at 30-year-olds and 50-year-olds, has not been found in the available literature. The discussion of the topic is limited.

Sixty-seven men, mostly with higher education (over 60%), participated in the last stage of the study, and groups with extreme maturation ages, early and late pubarche categories, were characterised by an almost identical distribution of individual education categories (Tab. 2). Suppose we assume that the level of education determines the intellectual level. In that case, it can be roughly said that the intellectual level of the respondents was similarly diverse, which may translate into comparable knowledge about the need for health-promoting behaviours. Such a high percentage of higher education exceeded the average for large cities and, to an even greater extent, the national average.

Regarding metabolic health, the early pubarche group had the greatest burden and the late pubarche group exhibited the best health parameters. At the level of average values, all parameters of the lipid profile, glucose concentration, SBP and DBP had higher values in the early pubarche group compared to the late pubarche category (reference group), deviating from +0.07 SD (LDL) to +0.7 SD for triglycerides and glucose and +0.4 SD for cholesterol and triglycerides (Fig. 1, Tab. 5). Similarly, compared to men with late pubarche, in the group with early maturation, the percentage of men exceeding the reference norms was higher regarding all biomedical parameters (Tab. 5). Thus, early pubarche was accompanied by a greater severity of metabolic abnormalities. The average maturation group was more similar to those in the late pubarche category. Therefore, there was a clear gradation: the earlier the age of pubarche, the worse the metabolic health of men and, consequently, the greater the risk of cardiovascular events (none of the studied men had premature pubarche).

Such a different metabolic picture presented by the separate groups of pubarche in terms of trends, even (or perhaps even more so) in the face of a small sample, allowed us to conclude that there is a relationship between the age of initiation of puberty (pubarche, and therefore the preceding adrenarche) and men's metabolic health.

The worse metabolic condition of the surveyed early maturing men is logically associated with the highest incidence of metabolic syndrome (MS). Therefore, MS was found to be the most common among them (45.4%), and among late maturing, the prevalence was the lowest (33.3%) (Tab. 5). The MS includes selected biomedical characteristics, i.e. increased triglyceride concentration, decreased HDL concentration, increased blood pressure – systolic and/or diastolic, abnormal fasting glycemia and increased waist circumference signalling abdominal obesity (Kramkowska and Czyżewska 2014). Their co-occurrence increases the risk of developing atherosclerotic cardiovascular diseases and type 2 diabetes (Kramkowska and Czyżewska 2014; Banach et al. 2021; Dobrowolski et al. 2022). Contrary to what could be expected, average absolute waist circumference was not the largest in men with the poorest metabolic health but in the healthiest ones – those with late pubarche. A similar situation concerned hip circumference (Fig. 1, Tab. 3). Therefore, WHR, commonly considered a determinant of fat distribution, showed a slightly higher prevalence of the central (abdominal) type of fat distribution, associated with a higher risk of metabolic disorders, in individuals with late rather than early pubarche (Fig. 2, Tab. 5).

Similarly, the average BMI was lower in men of early and average pubarcheal age compared to late maturing men, who were more corpulent according to this index (Fig. 2, Tab. 4). This results from a subtle mathematical play of body height and weight, where the squared value of body height significantly influences the quotient. In the study sample, early maturing men were, on average, 3 cm taller than those with late pubarche, with almost identical body weight (Tab. 4). According to some authors (Bulum et al. 2015), the BMI does not reflect the distribution of fat tissue, and the use of waist and hip circumference and WHR has its limitations in diagnosing central obesity. The value of waist circumference may be inadequate for exceptionally short or tall people with similar values of this measurement. In the light of our research, these statements seem to be correct. The results of the analysis of the level and distribution of subcutaneous fat based on the measurement of skinfold thickness seem to be in contrast to the assessment employing BMI and WHR (Fig. 2, Tab. 4). The average absolute value of the total sum of six skinfolds, the trunk and abdomen, were the highest in early maturing men (difference in skinfold thickness from 0.5–0.8 cm). However, the adiposity of the limbs was identical in both groups. The ratios of adiposity of the limbs to both the trunk and the abdomen showed a greater tendency to accumulate fat in the central rather than peripheral deposit in the group of early maturing men compared to the other categories (Fig. 2, Tab. 4).

In general, the results of our study show that: (1) adults that experienced early pubarcheal age have an increased risk of metabolic abnormalities and, consequently, a higher risk of cardiovascular events; (2) compared to individuals with a later pubarcheal age, subcutaneous fatness estimated from the skinfold thickness is slightly higher, with clearly marked abdominal obesity; (3) the results of body fat estimates in the form of BMI and WHR do not fully correspond to the results of skinfold-based measurements, which makes the interpretation of the findings more difficult.

The results of our study do not allow us to conclusively state whether the cause of the increased risk of cardiometabolic disorders in the examined men with early pubarcheal age is the mere fact of earlier adrenarcheal age and the mechanisms and stimuli causing it, or whether it is necessary for the early pubarche to coexist with the high fatness.

There are few reports on PA in boys. Data from the available literature regarding both sexes are contradictory. The international dialogue on the topic of the early age of adrenarche as a potential factor, the source of various disorders in the functioning of the child's body at different stages of ontogenesis, is briefly presented in the introduction. There have been many studies investigating the relationship between the advancement of sexual development and metabolic disorders and other dysfunctions, such as mental diseases, although they concerned other later stages of puberty based not on the pubarche's age but on the timing of the voice changes (mutation), later stages of pubic hair development, and the pace of puberty measured by the time of the onset of pubertal growth spurt. Various methods of assessing adiposity are used, most often based on BMI and WHR. The later phases of puberty are under the control of other areas of hormonal regulation – the HPG axis (hypothalamus-pituitary-gonadal), while pubarche is regulated by the HPA axis hypothalamus-pituitary-adrenal). Additionally, the results often depend on the research methods used in the study. According to some authors (Havelock et al. 2004; Belgorosky et al. 2008; Styne 2011) the time of the onset of adrenarche has no significant effect on the age of onset of gonadarche. Much evidence supports the existence of separate mechanisms controlling these two pubertal events.

Hormonal products of the adrenal cortex (HPA axis), that manage adrenarche participate not only in the child's sexual development but also in the regulation of metabolism, blood pressure, and, what is important, in managing the body under stress of chemical, physical and emotional nature (Carroll et al. 2011; Javorsky et al. 2011). The acceleration in the timing of increasing the body's androgen production, apart from the early appearance of pubic hair, often causes metabolic disorders similar to those that can be present in adults. This seems understandable due to the range of body functions under the adrenal glands' control, which may be disturbed by some individual or parallel stimulus/stimuli or exist in a cause-and-effect relationship.

Emotional stressors may be one type of such stimuli. Analysis of pubic hair development in a sample of 1146 school-age boys from Krakow (cross-sectional study from 2010) showed that, compared to their peers from complete families, boys growing up in single-parent families have a markedly accelerated development. Their pubarcheal age begins, on average, over a year earlier (10.70 years vs. 11.95 years) (Kowal et al. 2011a). Differences between them were also marked at the stage of intrauterine development. As newborns, they were characterised by a slightly smaller size compared to their peers from full families, with a slightly shorter gestation time (there were no cases with a weight below 2,500 g). However, their mothers and fathers were slightly taller and slimmer. The differences discussed were not large (-0.2 to -0.3 SD) and were statistically insignificant, except for weight gain during pregnancy – single mothers gained significantly more weight. The incidence of complications of pregnancy and delivery was similar. An in-depth analysis of pregnancy-related stress, assessed subjectively by mothers, showed a statistically significantly higher magnitude and frequency among women with single-parent family status, as well as a worse assessment of both family and material situation. In addition, single mothers were twice as likely to admit to smoking cigarettes during pregnancy. Stress and smoking during pregnancy have been shown to exert considerable adverse effects on offspring (Kowal et al. 2011b). It should be noted that not all married women are happy mothers. Despite existing stressful family conflicts, a significant number of mothers do not choose to separate or divorce.

Many studies have linked an unfavourable prenatal environment to the development of cardiometabolic disorders and neuroendocrine dysfunctions, as well as an increased risk of mental illness in later life (Van den Bergh et al. 2020; Eberle et al. 2021). Experiments in animal models have shown that prenatal exposure to stress or excess glucocorticoids can disturb the physiology of the offspring, resulting in reduced birth weight and consequently an increased likelihood of cardiovascular dysfunction, glucose homeostasis, HPA axis activity and anxiety-related behaviour in adulthood ('developmental programming' theory). Glucocorticoids are key mediators of stress responses mediating developmental programming (Cottrell and Seckl 2009).

Stress enhances the production of the CRH hormone (corticoliberin) in the hypothalamus, which stimulates the pituitary gland to produce adrenocorticotropic hormone (ACTH). ACTH subsequently stimulates the production and secretion of glucocorticosteroids, mineralocorticosteroids and androgens by the adrenal cortex in a related manner; there is no separate pituitary hormone inducing adrenal androgen production. Instead, inhibition of hormone secretion is mediated by negative feedback via the action of glucocorticosteroids on ACTH secretion (Javorsky et al. 2011). Thus, one could hypothetically assume that in boys entering school age and growing up in unfavourable family conditions exacerbating stressful situations, the HPA axis causes, in addition to an increase in cortisol, an increase in the production of androgens (adrenarche) that regulate pubic hair development (pubarche).

Children growing up amid strained family relationships and facing family breakdown are at risk of serious emotional and behavioural disorders, including depressive disorders, learning difficulties, and problems in relationships with peers as well as parents (Schor 2003). In a review article, Pietrzak (2020) showed that obesity in children co-occurs with their poorer emotional functioning. Several authors indicated that it is obesity which is the cause of this situation. Obese children are often discriminated against in their peer environment and experience a lot of psychological distress, which can consequently lead to serious emotional disorders requiring psychotherapeutic intervention. Negative attitudes (verbal and physical violence) towards overweight children start as early as pre-school age and increase among primary school children. Children experience negativity more acutely than adults, which increases their feelings of hopelessness and poor self-esteem (Pietrzak, 2020). In a review of articles, Godina-Flores and co-authors (2023), demonstrated that Mexican children and adolescents who are overweight or obese, are more likely to be depressed and report more depressive symptoms than normal-weight participants (Godina-Flores et al. 2023). Similar effects were observed in adults. A study by Rosmond et al (1998) on a group of 51-year-old men showed that abdominal obesity (and other markers of the metabolic syndrome) is associated with dysregulation of the HPA axis, which follows prolonged over-activation of the axis under factors such as environmental stress. Further analysis by Rosmond et al (1999) revealed that individuals with abdominal obesity show signs of HPA axis dysregulation following chronic, submissive stress, particularly evident in men with impulsive and anxious personality disorders. Men's perceived environmental stress depends on personality traits. Ortega-Montiel and co-authors (2015) found that self-reported chronic stress was an independent risk factor for obesity in Mexican men. Olszanecka-Glinianowicz (2008) concludes that despite shared pathophysiological pathways (abnormal HPA activation) and similar extrinsic factors influencing the development of obesity and depression, the question which of these diseases is the cause and which is the effect cannot be clearly answered. Still, their co-occurrence may lead to increased mortality, as both obesity and depression are independent risk factors for cardiovascular disease (Olszanecka-Glinianowicz 2008).

The results of our study may point to a similar kind of dilemma. On the one hand, psychological problems caused by obesity (lowered self-esteem, lack of acceptance of one's appearance and social isolation) can cause increased level of stress, lowered mood, and the occurrence of depression. On the other hand, chronic stress, lowered mood, and the occurrence of depression leading to changes in eating behavior (comforting with food and compulsive eating) may be the cause of the development of obesity (Olszanecka-Glinianowicz 2008). Obesity and depression are different, visible markers of the presence of a stress state in the body (disruption of homeostasis). Analogous conditions and neuroendocrine mechanisms could explain the observed phenomenon of accompanying early pubarche in men by the effect of increased tendency to abdominal obesity. The association between the two variables was found to be statistically insignificant, which may indicate that no such relationship exists. Psychosocial stress can result in accelerated adrenarche and subsequent early pubarche. Stress can also result in the appearance of increased abdominal obesity. Early pubarche and abdominal obesity are different, visible, external markers of the presence of a stress state in the human body. A direct, "statistically visible", cause-and-effect relationship between the two may not exist, but underlying both phenomena is stress, a disturbance in the activity of the HPA axis. The human body may react differently to prolonged stress with increased appetite or decreased cravings, or the two may alternate during life. As a result, some people will show a tendency to gain weight and others will lose weight.

We do not have more detailed information about the family, psychosocial environment during childhood – the peri-adrenarcheal period of the long-term group of 50-year-olds we studied. Considering the above-cited results of our own research on the Krakow population and world studies, the idea arises that in the early pubarche group more men may have grown up in a disadvantaged psychosocial situation than in the other pubertal groups.

Disturbances in the functioning of the HPA axis, and the subsequent growth and maturation of children, can also occur under the influence of factors of a chemical nature – unfavourable products of industrial activity. Editorial framework does not allow to present here another thread and a hypothetical, no less important disruptor. This will constitute the subject of the next paper, in the context of changes with age of selected body characteristics, including fatness, during the period of 8 to 17 years – the beginning and end of sexual maturation. Were the manifestations of abdominal obesity tendencies in boys already present during the developmental period?

Citing Kramkowska and Czyżewska (2014), metabolic syndrome is the main factor in the development of cardiovascular diseases, which are one of the leading causes of death in Western societies, so it is not surprising that this problem attracts the attention of the global medical community. Nevertheless, so far, the pathogenesis of this syndrome has not been fully understood.

Small sample size and, therefore, the low number of individuals in the extreme maturation groups limited the possibility of conducting statistical analyses and obtaining reliable estimates. The applied Pearson's chi-square tests and ANOVA showed the lack of statistical significance of differences between groups, even in the case of apparent differences in the mean values of analysed features, but this is the property of statistical tests due to the size of the groups; Therefore, the term "tendencies" was used. This is a limitation of this study.

It was investigated whether the group of 67 men was representative. The average height and weight, BMI, pubarche age and distributions of these characteristics in the group of adult males studied as 17-year-olds were compared to analogous characteristics in the entire group of long-term study boys at 17 years of age. No statistically significant differences were found between the groups.

Nevertheless, such research has great value, is highly time-consuming, takes decades and is labour-intensive and expensive. The studied group has the same, i.e. "closed" gene pool throughout the observation period. During the research, adaptive changes in the phenotype resulting from the interaction of genes with the environment, governed by epigenetic mechanisms that control gene activity, are observed (not the result of gene pool changes, as can occur in cross-sectional studies).

Another advantage is that the group conducting the research consisted mainly of the same people, which is essential, for example, in assessing the development of pubic hair, which is, to some extent, a subjective assessment.

Final conclusion

The revealed gradation, the earlier the age of pubarche, the worse the metabolic health of men, takes us back in time and shows us the source of the abnormalities – adrenarche – as a stage in ontogeny;

the earlier a child reaches this stage, the worse the health prognosis. However, early adrenarche is not the instrumental cause of the described difficulties; it is a manifestation, a result of the factors causing it; all we need do is discover them. It appears that the stage of adrenarche is a critical, open "developmental window" for cardiometabolic risk for the male sex. Further research is needed.

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Authors' contribution

BAS was the originator of this study, performer statistical analysis, interpreted the results, drafted the manuscript and wrote the papier; AW performer statistical analysis and interpreted the results; RŻ was the originator of research and manager, and proofreader; MK participated in collecting the bibliography. All authors participated in data collection, confirmed the correctness of the interpretation of the analysis results, revised the manuscript and approved the final version.

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The variability of anthropometric and body composition parameters in middle-aged women associated with menopause and smoking

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Abstract: Menopause and its related hormonal changes are associated with the variation of body composition, especially impacting adipose tissue metabolism and the reduction of lean mass. The purpose of the present study was to investigate the impact of smoking during menopause on the subsequent effects on body composition.

The sample comprised of 572 Slovak women aged between 39 and 65 years (49.67±6.2). Standard anthropometric techniques were used to collect anthropometric measurements, whereas bioelectrical parameters were measured utilizing a mono-frequency bioimpedance analyzer (BIA 101). Data on menopausal status, physical activity, and smoking habits were obtained via a specific questionnaire.

In postmenopausal women, our results showed a statistically significant difference between smokers and non-smokers in BMI, TBW%, ECW%, ICW%, MM%, FFM%, FM% ($p < 0.05$). No significant differences were observed in premenopausal women, although two-way analysis of covariance revealed a significant interaction between smoking and menopausal status on the FM% ($p < 0.001$), FFM% ($p < 0.001$), and MM% ($p = 0.002$), whilst controlling for age and physical activity.

In our sample group of middle-aged women, the combined impact of menopause and smoking appeared to influence anthropometric parameters and body composition.

Key words: Body composition, physical health, aging, physical activity, smoking.

Original article

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Introduction

Numerous factors, such as aging, health status, gender, genetic predisposition, and reproductive history influence body composition, which can be evaluated using anthropometric parameters such as body mass index (BMI), waist-to-hip ratio (WHR), as well as bioelectric impedance analysis (BIA) (Luptáková et al. 2013; Drozdová et al. 2016; Danková et al. 2017; Falbová et al. 2019; Vorobeľová et al. 2021, 2022). Compared to other techniques, BIA is a low-cost, readily accessible method which does not cause radiation exposure. The parameters collected and derived from BIA are the phase angle (PhA), reactance (Xc), resistance (R), fat mass (FM), fat-free mass (FFM), muscle mass (MM), and total body water (TBW) (Marini et al. 2020). Aging is one of the main determinants of body composition changes; part of this natural process in women is menopause, diagnosed after 12 months of amenorrhea with permanent termination of ovary functions (Greendale et al. 1999). Menopause is reportedly linked to an increase in BMI and body weight, specifically visceral fat mass (Donato et al. 2006; Dmitruk et al. 2018; Dehghan et al. 2021); the underlying cause is, however, still debated (Al-Safi and Polotsky 2015; Karvonen-Gutierrez and Kim 2016). These changes lead to the presentation of health conditions, such as metabolic syndrome and cardiovascular diseases (Matvienko et al. 2011; Opoku et al. 2023). Lovejoy et al. (2008) suggested that it is caused by lower energy expenditure and a decline in estrogen levels; others supported a similar hypothesis by documenting a rise of abdominal fat in response to hormonal changes (Svendsen et al. 1995; Kodoth et al. 2022) as reported by a 5-years prospective study where women who mitigated the decline of estrogen levels by using continuous hormone therapy did not experience weight gain (Guthrie et al. 1999). In a more recent study, Kwok et al. (2012) did not find an interaction between smoking and hormone activity (and smoking) on waist circumference and WHR while other studies have attributed an increase in BMI and obesity only to aging and not menopause (Trikudanathan et al. 2013; Greendale et al. 2019; Fenton 2021). Other changes in body composition associated with menopause include a reduction of lean mass (Sipilä et al. 2020), skeletal muscle mass, and total body water (Dmitruk et al. 2018). A factor closely related to obesity is a lack of physical activity, whereby its effects are amplified in postmenopausal women already impacted by aging and hormonal changes (Dubnov et al. 2003). A combination of abdominal adiposity, physical inactivity, and inflammatory markers have been related to sarcopenia (Maltais et al. 2009) while performing physical activity is crucial in preserving good health status in postmenopausal women. Physical activity not only has a profound effect on body composition by improving fat distribution (as reported by numerous studies conducted on women after menopause; Bendinelli et al. 2022; Juppi et al. 2022; Harraqui et al. 2023), but it also reduces mortality rates (Sherman et al. 1999). The positive effects of exercise in aging women are not limited to better fat distribution but also to improved FFM and skeletal MM, as reported by a study on a sample of Caucasian women conducted in Portugal who performed 12 months of a combination of step aerobics, muscle strength building and flexibility/postural control training (Aragão et al. 2014). Furthermore, according to a meta-analysis, high-intensity training, specifically cycling, benefits aging women's body composition (Dupuit et al. 2020). In contrast, it has been reported that smoking harms human health by increasing the risk of developing numerous maladies, such as cardiovascular diseases and cancer (Dai et al. 2022). Smoking also correlates with the prevalence of metabolic disorders and central obesity (Kwaśniewska et al. 2012), hence, smokers tend to have higher visceral fat mass and WHR than non-smokers (Falbová et al. 2023). Interestingly, smokers have also been observed to have a lower mean BMI compared to non-smokers due to an increased metabolic rate; this phenomenon has been reported in numerous cross-sectional and observational studies (Canoy et al. 2005; Clair et al. 2011; Efendi et al. 2018). However, after adjusting for confounding factors, other studies have reported no difference in fat mass between former smokers and persons who have never smoked (Akbartabartoori et al. 2005; Kim et al. 2012; Piirtola et al. 2018) providing further evidence regarding an increase in BMI due to quitting smoking. Specifically, in postmenopausal women who stopped smoking, the increase in fat mass and weight was associated with a higher muscle mass (Kleppinger et al. 2010). Moreover, among those trying to stop smoking women appeared to gain more weight compared to men, especially among older women compared to younger ones (McVay and Copeland 2011; Kasteridis and Yen 2012; Allen et al. 2014). In addition, an association between smoking and body composition has been reported by a cross-sectional study showing that after the cessation of smoking, BMI and FM increased (Stavropoulos-Kalinoglou et al. 2008). However, the literature on smoking in aging women is scarce and mainly focuses on the effect of developing early menopause (Hayatbakhsh et al. 2012). A proposed reason for smokers' early menopause is that smoking is detrimental to ovarian function and promotes follicular atresia (Mattison and Thorgeirsson 1978; Ginsberg 1991). Moreover, it has been shown that women who smoke experienced difficulties in conception and infertility; hence, these findings support the observed negative effect of smoking on the womens' reproductive systems (Olsen et al. 1983; Baird and Wilcox 1985). Although it is well known that menopause increases fat mass and decreases MM (North American Association for the Study of Obesity et al. 2000; World Health Organization 2011), smoking may have different effects on body composition parameters (Canoy et al. 2005), depending on gender, age category and menopausal status. Currently, studies regarding the effect of smoking on body composition in midlife women are rare and yield inconsistent result. For example, some of them concluded that smoking decreases FM (Ambikairajah et al. 2019), and another one suggested that smoking is associated with an increase in FM (Portugal et al. 2019). Unclear previous results are also characterized for MM (Kwaśniewska et al. 2012; Graff-Iversen et al. 2019).

Our hypothesis suggests that the significant interaction effect of these two factors – smoking and menopause, exists. Due to the inconsistent data in the literature on the relationship between smoking and body composition variables in middle-aged women, the present study aims to investigate this issue after adjusting for age and physical activity. In addition, to our knowledge, there has not been a single report on their combined effect on body composition, this issue should be investigated. Therefore, in this cross-sectional study, we analyzed the relationships between smoking and body composition in middle-aged women according to their menopausal status, and the interaction effect of smoking and menopause on body composition.

Materials and Methods

The investigated sample consisted of a homogenous sample of 542 middle-aged Slovak women between 39 to 65 years of age (mean age 49.67 ± 6.169 years). This study was based on data collected during two cross-sectional surveys in Slovakia. Participants were recruited from different localities in Slovakia's western, southern, and middle parts by invitation letter. The recruitment involved a non-random procedure based on volunteering and convenience. Collected data was anonymized and analyzed solely for scientific purposes. Data was collected between 2009 and 2015 by the Department of Anthropology at Comenius University in Bratislava and performed in cooperation with general practitioners. The sample included only women who provided written informed consent to participate in the study to adhere to the Declaration of Helsinki principles. Women who could not respond due to severe physical or mental illness and with whom anthropometry and body composition analysis could not be performed were excluded from the study. The women were interviewed using pre-tested, interviewer-administered questionnaires of their reproductive and menstrual history, socio-demographic background, lifestyle, and health status designed by Kaczmarek (2007) and validated in Polish studies (The Menopause-Specific Questionnaire, A. Mickiewicz University Poznań, Poland, Maria Kaczmarek). All socio-demographic and lifestyle variables were measured by self-reporting. Smoking status was categorized as current 'smokers' (smoking once a week to every day) and nonsmokers (never smoking). Physical activity was categorized into two groups, regular and never, including occasional. Women were divided according to their menopausal status into late pre-, peri, and postmenopausal groups. The late premenopausal group included women who had experienced regular menstruation during the last 12 months, which continued at the time of the study. The perimenopausal group included women who reported that their menstrual cycle length had become more irregular in the preceding 12 months or that they had stopped menstruating for between 3 and 12 months. Women were considered postmenopausal if they reported 12 consecutive months of amenorrhea before the examination (Vorobeľová et al. 2019). Due to the low number of perimenopausal women, this group was merged with the premenopausal group. This combined group was analyzed in an association study between smoking status and body composition parameters, in our efforts to compare women in the reproductive period (defined as the late premenopausal and perimenopausal) and in the postreproductive period (defined as postmenopausal).

Anthropometric and body composition analysis

The anthropometric measurements were taken after participants had removed shoes and heavy clothing. Data were taken by trained anthropologists using standard techniques; body height was measured within 0.5 cm accuracy by a Sieber and Hegner anthropometer at head level with the participant standing barefoot with feet together; body weight was measured on a personal balance scale within 0.1 kg accuracy; and BMI was calculated as body weight divided by height squared. The waist and hip circumferences were measured according to the NHLBI Obesity Education Initiative (Audrain-McGovern and Benowitz 2011) and WHO (World Health Organization 2011). WHR was calculated as the circumference of the waist divided by the circumference of the hips. Body composition measurements were carried out in the morning. The body composition was measured using the bioelectric impedance analyzer (BIA 101, Akern S.r.l.) at a signal frequency of 50 kHz, with a constant excitation current at 800 μA and a four-electrode arrangement. Bioimpedance is a complex quantity composed of resistance (R, Ohm) related to the quantity of fluids and reactance (Xc, Ohm) related to the capacitance of the cell membrane. Individual variables of body composition such as phase angle (PhA), fat mass (FM), fat-free mass (FFM), muscle mass (MM), body cell mass (BCM), total body water (TBW), extracellular water (ECW), intracellular water (ICW) were obtained using the software Bodygram program (Version 1.21, Akern S.r.l).

Statistical analysis

IBM SPSS for Windows (Statistical Package for the Social Science, version 20.0, Chicago, IL) was used for all statistical analyses, with statistical significance set at $p \leq 0.05$. Baseline descriptive statistics were performed on the entire sample; a division into two pre- and postmenopausal groups was subsequently performed. A one-sample Kolmogorov-Smirnov test assessed the normality assumption hypothesis for continuous variables. The parametric independent sample t-test, the non-parametric Mann-Whitney U test, and the unequal variances t-test tested the differences in smoking and non-smoking groups across the body composition variables. Furthermore, due to multiple statistical comparisons, to reduce the increased risk of a type I error, the Bonferroni correction (p-value $< 0.05/n$) was used by multiplying the p-value by the number of comparisons. Two-way ANCOVA analyses were conducted on the dependent variables FM%, FFM%, MM%, BMI, TBW%, ECW%, and ICW%, with age, education level, physical activity, hypertension, and chronic cardiovascular disease as covariates to evaluate the relationship between smoking status and body composition parameters. The variable age required logarithmic transformation because the values were not normally distributed.

Results

The sample encompassed 303 (52.97%) late pre- and perimenopausal women and 269 (47.03%) postmenopausal, out of which 11.71% declared to be regularly physically active, 88.29% not physically active or only occasionally, 30.07% smokers and 69.93% non-smokers. In addition, 78 (13.64%) declared to smoke occasionally and 95 (16.61%) daily, out of these 7 women reported to smoke between 1 to 5 cigarettes per day, 26 between 6 and 10 cigarettes per day, 34 between 11 and 20 cigarettes per day and only 2 smoke more than 20 cigarettes per day. In the pre- and perimenopausal group, 28.71% smoked, and 13.53% were regularly physically active; in the postmenopausal group, 31.60% were smokers, and 9.67% were regularly physically active. As expected, the mean age of the postmenopausal group was significantly higher (54.25 ±4.82) compared to the mean age in the pre- and perimenopausal group (45.62±4.04) (p <0.001). The BMI of the entire sample corresponded to the WHO category pre-obesity with a mean value of 27.07; moreover, post-menopausal women had a higher BMI (28.25) than pre- and perimenopausal women (26.02). This pattern was also observed in the WHR variables since it correlates to the BMI. Inversely, in the raw bioelectrical impedance data Xc, R, and PhA, higher values were reported in the postmenopausal women. Similar mean values across the two groups were found in the BCM%, with almost no difference, 47.13 in pre- and perimenopausal and 47.67 in postmenopausal. Moreover, postmenopausal women had a higher TBW% and ICW% but lower ECW% than pre- and perimenopausal women. MM% and FFM% were notably lower in postmenopausal women, whereas FM% was higher, aligning with the BMI, and WHR, values. Statistically significant differences among those described above were obtained by utilizing a Student's t-test on the variables: age (p <0.001), Xc (p = 0.048), R (p = 0.020), TBW% (p <0.001), ECW% (p = 0.001), ICW% (p = 0.001), MM% (p <0.001), FFM% (p <0.001) and FM (p <0.001); whereas, due to a non-normal data distribution, a Mann-Whitney U test was performed on the variables: BMI (p <0.001), WHR (p <0.001), and as well as PA, and BCM%, which were not statistically significant. After adjusting the p-value with Bonferroni correction, most of the differences remained significant, as shown in Table 1.

Tab. 1. Baseline characteristics of the participants

	Entire sample (N = 572)		Pre- and perimenopausal women (N = 303)		Postmenopausal (N = 269)		*p*	Adjusted p[a]
	Mean	SD	Mean	SD	Mean	SD		
Age	49.67	6.17	45.62	4.04	54.24	4.82	<0.001*	<0.001*
BMI	27.07	5.61	26.02	5.29	28.25	5.74	<0.001*	<0.001*
WHR	0.83	0.08	0.81	0.08	0.85	0.07	<0.001*	<0.001*
Xc	61.30	10.49	62.12	10.30	60.38	10.65	0.048*	0.714
R	543.53	68.45	549.78	68.00	536.49	68.40	0.020*	0.305
PhA	6.44	0.88	6.45	0.86	6.43	0.91	0.302	1.000
BCM%	47.39	2.88	47.13	1.95	47.67	3.63	0.998	1.000
TBW%	48.45	5.22	49.43	4.93	47.36	5.33	<0.001*	<0.001*
ECW%	44.77	2.92	44.41	2.80	45.19	3.01	0.001*	0.021*
ICW%	55.23	2.93	55.60	2.81	54.81	3.01	0.001*	0.019*
MM%	36.88	4.92	37.59	4.63	26.09	5.12	<0.001*	0.004*
FFM%	62.50	8.34	64.06	8.02	60.75	8.36	<0.001*	<0.001*
FM%	37.48	8.34	35.94	8.02	39.21	8.37	<0.001*	<0.001*

Notes: *marks a statistically significant difference a Bonferroni correction

Abbreviations: BMI: body mass index; WHR: waist-to-hip ratio; Xc: reactance; R: resistance; PhA: phase angle; BCM%: body cell mass percent; TBW%: total body water percent; ECW%: extracellular water percent; ICW%: intracellular water percent; MM%: muscle mass percent; FFM%: fat-free mass percent; FM%: fat mass percent.

Tab. 2. Pre- and perimenopausal smokers and nonsmokers

Body composition variables	Smokers (N= 87)		Nonsmokers (N= 216)		*p*	Adjusted *p*[a]
	Mean	SD	Mean	SD		
BMI	26.66	5.19	25.76	5.31	0.066	0.924
WHR	0.82	0.07	0.80	0.08	0.060	0.840
Xc	63.36	9.89	61.62	10.44	0.184	1.000
R	546.64	59.28	551.04	71.30	0.611	1.000
PhA	6.63	0.98	6.38	0.80	0.045*	0.630
BCM%	47.57	2.08	46.96	1.88	0.014*	0.196
TBW%	48.69	5.06	49.72	4.85	0.098	1.000
ECW%	44.13	2.72	44.52	2.83	0.266	1.000
ICW%	55.87	2.72	55.49	2.84	0.277	1.000
MM%	37.18	4.60	37.75	4.65	0.328	1.000
FFM%	62.87	8.16	64.54	7.93	0.101	1.000
FM%	37.13	8.16	35.46	7.93	0.101	1.000

Notes: *marks a statistically significant difference a Bonferroni correction

Abbreviations: BMI: body mass index; WHR: waist-to-hip ratio; Xc: reactance; R: resistance; PhA: phase angle; BCM%: body cell mass percent; TBW%: total body water percent; ECW%: extracellular water percent; ICW%: intracellular water percent; MM%: muscle mass percent; FFM%: fat-free mass percent; FM%: fat mass percent.

Menopausal status and smoking

The differences between the two groups (smokers and nonsmokers) across the variables under study in pre- and perimenopausal and postmenopausal women are summarized in Table 2. In pre- and perimenopausal women, the analysis of the variables BMI, WHR, and PhA, revealed a statistically significant difference only in PhA values of smokers and non-smokers, U = 8014.50, z = 2.005, p = 0.045, r = 0.15. Further tests were performed on the following variables: Xc, R, BCM%, TBW%, ECW%, ICW%, MM%, FFM%, and FM%. Statistical difference was obtained only for the variable BCM% (p = 0.014). The magnitude of the difference in the means (mean difference = -0.608, 95% CI: -1.093 to -0.124) was minimal (eta squared = 0.019). However, after adjusting the p-values with a Bonferroni correction, no statistical significance was reported in any of the variables. Similarly, the same analyses were performed in the postmenopausal group, as shown in Table 3. On the variables BMI, PhA, and BCM%, statistically significant differences were recorded in BMI (p = 0.001, r = 0.26) values of smokers and nonsmokers. On average, postmenopausal women who smoke had a significantly lower BMI 26.66 versus 28.9. Moreover, the following variables: Xc ($p = 0.001$), R ($p = 0.032$), ECW% ($p < 0.001$), ICW% ($p < 0.001$), MM% ($p < 0.001$), FFM% ($p < 0.001$), and FM% ($p < 0.001$), showed statistically significant differences between smokers and nonsmokers. The magnitude of the difference in the means of the variables was small: Xc (mean difference = -4.473, 95% CI: -7.175 to -1.772; eta squared = 0.038), R (mean difference = -19.153, 95% CI: -36.696 to -1.611; eta squared = 0.017), ECW% (mean difference = 1.389, 95% CI: 0.629 to 2.149; eta squared = 0.046), ICW% (mean difference = -1.389, 95% CI: -2.149 to -0.629; eta squared = 0.046), MM% (mean difference = -2.203, 95% CI: -3.500 to -0.906; eta squared = 0.040), FFM% (mean difference = -4.224, 95% CI: -6.326 to -2.123; eta squared = 0.055), and FM% (mean difference = 4.170, 95% CI: 2.064 to 6.276; eta squared = 0.053). In this case, smokers had higher values of Xc (63.44) compared to nonsmokers (58.96). In accordance with the results obtained in the variable BMI, it was observed that smokers had significantly more MM% and FFM% and lower FM% overall than nonsmokers. ICW% had a greater value in smokers at 55.76 compared to nonsmokers, whose value was 54.37; contrarily, ECW% was higher in non-smokers (45.63) compared to smokers (44.24). For WHR and TBW% a parametric test was used (due to the unequal variances), resulting in a statistically significant difference only in TBW% ($p = 0.001$), with higher scores in smokers at 49.13 than nonsmokers at 46.54. After calculating the adjusted p-value taking into account the following confounding factors: age, education level, physical activity, hypertension, and chronic cardiovascular disease, statistical significance was maintained across most of the variables BMI ($p = 0.009$), Xc ($p = 0.018$), TBW% ($p = 0.001$), ECW% ($p = 0.006$), ICW% ($p = 0.006$), MM% ($p = 0.002$), FFM% ($p = 0.001$) and FM% ($p = 0.001$). The association of smoking and menopausal status with body composition parameters was studied while controlling for age and physical activity (Tab. 4). A significant interaction effect was observed between menopausal status and smoking across all the dependent variables, except for ECW% and ICW%; FM%, $p < 0.001$ with a small effect size (partial eta squared = 0.025) indicating that the influence of menopausal status and smoking on FM% is not uniform across all individuals with an approximately 2.5% of variance; FFM%, $p < 0.001$ with the same effect size (partial eta squared = 0.025) confirming the impact of menopausal status and smoking on FFM%; MM%, $p = 0.002$ similarly with a small effect size (partial eta squared = 0.016) suggests similarly to FM% the presence of non-uniformity in the group and that only 1.6% of the variance in MM% is explained by the interaction effect between menopausal status and smoking; likewise results are found for the variable BMI, $p = 0.003$ (partial eta squared = 0.016) and TBW%, $p < 0.001$, (partial eta squared = 0.025) showing that TBW% is influenced as the previous variables by the interaction between menopausal status and smoking. To summarize, in postmenopausal women the interaction between menopause and smoking leads to significant changes in body compostion parametres such as FM%, FFM%, MM%, BMI and TBW%.

Tab. 3. Postmenopausal smokers and nonsmokers

Body composition variables	Smokers (N = 85)		Nonsmokers N = 184)		*p*	Adjusted *p*[a]	Adjusted p[b]
	Mean	SD	Mean	SD			
BMI	26.66	6.07	28.99	5.45	0.001*	0.014*	0.009*
WHR	0.83	0.08	0.85	0.07	0.065	0.910	0.290
Xc	63.44	11.56	58.96	9.92	0.001*	0.018*	0.018*
R	549.59	75.76	530.43	64.03	0.032*	0.448	0.094
PhA	6.60	1.08	6.35	0.81	0.104	1.000	0.209
BCM%	47.56	2.73	47.73	3.98	0.376	1.000	0.855
TBW%	49.13	6.28	46.54	4.63	0.001*	0.013*	0.001*
ECW%	44.24	3.19	45.63	2.82	<0.001*	0.005*	0.006*
ICW%	55.76	3.19	54.37	2.82	<0.001*	0.005*	0.006*
MM%	37.60	5.78	35.39	4.64	<0.001*	0.013*	0.002*
FFM%	63.64	10.01	59.41	7.11	<0.001*	0.001*	0.001*
FM%	36.36	10.01	40.53	7.14	<0.001*	0.002*	0.001*

Notes: *marks a statistically significant difference a Bonferroni correction; b adjusted for age, education level, physical activity, hypertension, and chronic cardiovascular disease by analysis of covariance

Abbreviations: BMI: body mass index; WHR: waist-to-hip ratio; Xc: reactance; R: resistance; PhA: phase angle; BCM%: body cell mass percent; TBW%: total body water percent; ECW%: extracellular water percent; ICW%: intracellular water percent; MM%: muscle mass percent; FFM%: fat-free mass percent; FM%: fat mass percent.

Tab. 4. An association of smoking and menopausal status on body composition parameters

Dependent variables	Predictors	Observed power	Partial η2	F	*p*
FM%	Smoking	0.371	0.103	2.669	0.103
	Menopausal status	0.087	0.001	0.320	0.572
	Physical activity	0.974	0.026	15.309	<0.001*
	logAge	0.645	0.010	5.454	0.020*
	Menopausal status x smoking	0.966	0.025	14.372	<0.001*
FFM%	Smoking	0.386	0.005	2.798	0.095
	Menopausal status	0.091	0.001	0.351	0.554
	Physical activity	0.975	0.027	15.477	<0.001*
	logAge	0.647	0.010	5.483	0.020*
	Menopausal status x smoking	0.969	0.025	14.691	<0.001*

Dependent variables	Predictors	Observed power	Partial η2	F	*p*
MM%	Smoking	0.461	0.006	3.475	0.063
	Menopausal status	0.110	0.001	0.507	0.477
	Physical activity	0.944	0.022	12.614	<0.001*
	logAge	0.248	0.003	1.637	0.201
	Menopausal status x smoking	0.861	0.016	9.307	0.002*
BMI	Smoking	0.271	0.003	1.823	0.178
	Menopausal status	0.105	0.001	0.472	0.493
	Physical activity	0.955	0.023	13.375	<0.001*
	logAge	0.718	0.011	6.465	0.011*
	Menopausal status x smoking	0.857	0.016	9.189	0.003*
TBW%	Smoking	0.374	0.005	2.692	0.101
	Menopausal status	0.113	0.001	0.533	0.466
	Physical activity	0.973	0.026	15.214	<0.001*
	logAge	0.570	0.008	4.582	0.033
	Menopausal status x smoking	0.964	0.025	14.224	<0.001*
ECW%	Smoking	0.884	0.017	9.989	0.002*
	Menopausal status	0.182	0.002	1.104	0.294
	Physical activity	0.754	0.012	7.034	0.008*
	logAge	0.987	0.030	17.644	<0.001*
	Menopausal status x smoking	0.340	0.004	2.401	0.122
ICW%	Smoking	0.880	0.017	9.868	0.002*
	Menopausal status	0.178	0.002	1.071	0.301
	Physical activity	0.750	0.012	6.957	0.009*
	logAge	0.987	0.030	17.580	<0.001*
	Menopausal status x smoking	0.346	0.004	2.448	0.118

Notes: *marks a statistically significant difference FM%: fat mass percent; FFM%: fat-free mass percent; MM%: muscle mass percent; BMI: body max index; TBW%: total body water percent; ECW%: extracellular water percent; ICW%: intracellular water percent.

Discussion

Menopause is a physiological aging process that occurs in women. Nonetheless, numerous aspects are still unclear and insufficiently studied. The first finding of our research was that postmenopausal women had, as expected, a higher BMI, WHR, and FM% compared to premenopausal women. This finding corresponds to data reported by Juppi et al. (2022), who reported in a short and long-term follow-up research an increased in BMI of between 1% and 3% ($p < 0.001$) as well as a significant increase of total, regional, and subcutaneous fat tissue. The analysis was conducted on a sample of 316 postmenopausal women from two longitudinal cohort studies and a short-term follow-up study on 230 perimenopausal women who were researched leading up to menopause. Furthermore, Greendale et al. (2019) studied a sample of 1,246 pre and postmenopausal women of different ethnicities and observed that during menopause, the average woman's FM rise rate nearly doubled from 1%–1.7% per year, resulting in a 6% overall gain in FM. The cause of this change in body composition is still debated although it tends to be attributed to aging and not menopause (Luptáková et al. 2012, 2013; Danková et al. 2014) but the increase in central adiposity is associated with a possible change in FM distribution after menopause. These changes are most likely to be the result of hormonal shifts during mid-life when women have a higher testosterone-to-estradiol ratio after menopause, which has been linked to increased central adiposity deposition, as reported by Ambikairajah et al. (2019) in a systematic review that analyzed 201 cross-sectional studies, collaborating a sample size of 1,049,919 individuals. In our study, no statistical significance was found across the body composition variables mentioned above in the groups of smokers and nonsmokers in premenopausal women. Similar results were obtained by Portugal et al. (2019), with no significant differences in TBW, ECW-to-ICW ratio, FM, and FFM in both women and men, between the categories never smoker, former smoker, and current smoker. In contrast, postmenopausal women who smoke were found to have a significantly lower BMI value ($p = 0.014$), and lower FM% ($p = 0.002$), whereas MM% ($p = 0.013$) and FFM% ($p = 0.001$) had higher values compared to nonsmokers. In addition, after adjusting for age, education level, physical activity, hypertension, and chronic cardiovascular disease (CVD) in the covariance analysis, the menopause and smoking-associated differences in anthropometric and body composition parameters were consistent. The finding that cigarette smokers are generally leaner compared to non-smokers is consistent with many previous studies (Akbartabartoori et al. 2005; Danková et al. 2014). Lower BMI in smokers was also reported by Kwaśniewska et al. (2012) in a sample of 7,792 Polish women and by Graff-Iversen et al. (2019) in a cross-sectional study conducted in Norway on 22,294 women. More in-depth cross-sectional analyses (Piirtola et al. 2018) on twins compared three groups: current-never, former-current, and former-never smokers, finding that even though current smokers tend to have a lower BMI. Some studies observed a lower amount of FM, especially in visceral areas. Onat et al.´s study (Jandíková et al. 2014) established that smoking Turkish adult women had a lower visceral adipose tissue area than those who never smoked. Therefore, the authors concluded that body FM and visceral fat accumulation are inhibited by cigarette smoking in women. However, the results related to visceral fat among smokers and nonsmokers are often contradictory, as reported in a cross-sectional study of women aged 18–80 years from different ethnicities (Brand et al. 2011). Therein, smoking caused an increase in visceral fat mass conversely and thus represented a metabolic and cardiovascular risk factor. A study by Graff-Iversen et al. (2019) supported this suggestion by showing that waist circumference and WHR was larger for current smokers than in those who never smoked. On the other hand, the same study also observed a negative association between smoking and hip circumference. In the perspective of research concluding that a higher percentage of gluteofemoral fat is linked to lower cardiovascular disease risk, the authors further suggested that smoking could be a modifying cardiovascular disease risk factor through mechanisms that reduce fat storage capacity in the lower body region. Although a large number of studies have reported an association between smoking and lower adiposity, most of these studies did not take into account the effect of menopausal status (Van Geel et al. 2009). Considering our results, we hypothesize that this association concerns postmenopausal rather than late pre- and perimenopausal women. According to the review by Audrain-McGovern et al. (2011), most of the effects of smoking on body weight and fat deposits are likely to be mediated through nicotine. An old animal study on rodents demonstrated that nicotine increases sympathetic nervous system activity and thermogenesis in adipose tissues, thus increasing whole-body metabolism, which might subsequently affect the decrease in adiposity (Yuki et al. 2015). Moreover, nicotine intake is known to result in an increase in fat oxidation and a decrease in fat accumulation (Stachenfeld et al. 1998; Lee and Choi 2019). Androgens hormones might mediate the link between smoking and the greater amount of MM and FFM observed in our study. It has been reported that current smokers have higher circulating levels of testosterone and free testosterone (Stachenfeld 2008). Similarly, Jandikova et al. (2014) found higher levels of androgens in smoking postmenopausal women. Furthermore, limited data indicated there may be a positive association between testosterone with MM and lean body mass in older women (Serra-Prat et al. 2019; Hioka et al. 2021). In addition, it has also been observed that a low-free testosterone level appears to be a significant predictor of loss of appendicular muscle in Japanese women (Park et al. 2021). Therefore, we hypothesize that androgens could also play an important role in smoking – higher MM association in our study sample. However, despite the described above studies and our results, smoking was conversely reported by Lee et al. (2019) to accelerate MM loss in currently smoking middle-aged women, compared to past and never smokers. Our study also concluded that postmenopausal smokers had higher TBW% ($p = 0.013$) and ICW% ($p = 0.005$) but lower ECW% ($p = 0.005$) than nonsmokers. The relationship between hydration and smoking in postmenopausal women, to the best of our knowledge, has not been studied. However, it is known that body fluid distribution is associated with sex hormones, where estrogen increases and progesterone tends to decrease its levels (Stachenfeld et al. 1998, 1999; Stachenfeld 2008). The population-based study of postmenopausal women found that current smokers had higher circulating levels of estradiol compared with non-smokers (Olsen et al. 1983). Endogenous female sex hormones may thus provide one plausible mechanism through which cigarette smoking influences TBW% in postmenopausal women. Furthermore, in a more recent study, Serra-Prat et al. (2019) observed a decline in TBW and ICW in the elderly (both men and women) which was associated with decreased muscle strength and mass. In addition, Hioka et al. (2021) observed in correlation analyses on a sample of < 65-year-old women a significant negative correlation between the total body ECW/ICW ratio and handgrip strength associated with deterioration of muscle quality. Moreover, individuals with sarcopenia had a higher prevalence of abnormal ECW/TBW ratio (Kleppinger et al. 2010).

Limitations and recommendations for future research

The results of our study enrich the literature on the topic. However, this study examined data from a cross-sectional study that can only examine correlations in data; it does not confirm a causal relationship between smoking, menopause, and body composition. In addition, the same individuals were not surveyed over time, and the lifestyle and personal data on smoking, physical activity, and menopause were gathered through self-reporting, which means that there may have been inaccuracies and/or other factors might have influenced the trends observed in our data. Moreover, the research parameters were non-specific; data on the frequency and intensity of physical activity was not gathered, as well as the number of cigarettes smoked per day and whether the nonsmokers have ever smoked in the past. Long-term follow-ups and more detailed data collection can overcome these limitations. Furthermore, due to the missing data on hormone levels in our study sample, our hypothesis about the relationship between smoking, sex hormones, and body composition parameters should be examined in future research. Considering the findings from the literature that the association of smoking with body fat can differ depending on the location of the FM, further studies regarding this relationship in postmenopausal women would be relevant. Another potential limitation of our study is the lack of data on dietary habits, calorie intake and water consumption, which are important predictors of body composition and could be important covariates to include in the study of the relationship between menopause, smoking and anthropometric parameters. Moreover, the methodology used, BIA, since it tends to underestimate FM, is recommended to perform further testing with other methodologies such as DEXA or specificBIVA (Marini et al. 2013) a valid technique for evaluation of FM% and ICW/ECW. We also suggest that cut-off values for FM and MM should be reconsidered in postmenopausal smokers, lower for FM and higher for MM, in future clinical research.

Conclusions

The results obtained in this study can aid healthcare professionals in tailoring recommendations aimed for postmenopausal women regarding lifestyle changes, preventive measures as well as counseling and support for smoke cessation. Such recommendation could be especially important to postmenopausal midlife women who regularly smoke, as these individuals may show lower FM and higher MM that are indicators usually associated with better health outcomes and may mistakenly point to a better health status in these women. Moreover, the findings can provide a basis for evidence-based public health policies and interventions that can raise awareness of the adverse effects on body composition of smoking during menopause. However, due to the limitations of our study, future research is needed to examine the reported in our study associations.

Authors' contribution

Simona Sulis was responsible for the statistical analysis, writing of the manuscript, analysis, and interpretation of data. Petra Švábová participated in data collection, analysis, and interpretation of data. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

Morphological structure and ethnic background of the Sudanese *Shagia* tribe (East Africa)

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ABSTRACT: North Sudan, especially the 4th Nile Cataract region, is home to one of the world's most isolated human populations. This study aimed to clarify the ethnic background of the *Shagia* based on anthropological analyses. This study provides a morphological and ethnographic characterisation of the previously unstudied *Shagia* tribe. Head and body measurements were conducted among 64 adults from three villages. There were observable but relatively small admixture proportions of non-African population genes (light skin colour, narrow noses and masculine proportions) in their morphological build. The *Shagia's* uniqueness may have been the result of severe genetic drift episodes resulting from founding events, such as long-term isolation and traditionally small population size. It is useful to trace the ethnic history of Africans and, specifically, for the tribal members of *Shagia* so that they may better understand and learn about their history. This study shows that the *Shagia* tribe was displaced from their territory due to the construction of the Merowe Dam. Thus, the results of this research fulfil the assumptions of urgent anthropology, as it contributes to the protection of the heritage of the 4th Nile Cataract region: an area of historical value to the study of the evolution of contemporary civilisation.

KEY WORDS: 4th Nile Cataract region, Shagia tribe, morphological structure, anthropometry, Sudan.

Original article

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Introduction

Africa is believed to be the cradle of humankind. One-eighth of the world's population lives in Africa, primarily along the north and west coasts and along fertile river valleys. Sudan is one of the largest countries and is ethnically very diverse. Sudan was divided in 2011, which resulted in the creation of South Sudan as an independent country. This division was preceded by years-long armed conflicts between the north and south of the country. Before the division, the north was inhabited by Sudanese Arabs (49% of the total population) and the Kushites (the Beja and Nubians). In contrast, the south was inhabited by the Nilotes (Nuer, Dinka, Shilluk, etc.) and Sudanese tribes (the Azande and the Fur). Today, post-division, North Sudan is predominantly Muslim, while South Sudan is primarily Christian (Fadlalla 2004; Herman 2015; Cockett 2016).

Prior to the division of Sudan, for centuries the northern region had been inhabited by a Semitic tribe, the *Shagia*. The 4th Nile Cataract region, due to its geographical location at the junction of two deserts (N 18º 56.413′ and E 032º 07.547′) (Fig. 1), left the *Shagia* tribe genetically and culturally isolated. The *Shagia* are known for both their nomadic and sedentary lifestyles. In the 4th Nile Cataract region, the *Shagia* are farmers, and their estates exist along the Nile, where families have lived together for generations. Each village consists of several farms and 40–60 people.

In 2005, as a consequence of the Merowe High Dam construction (Failer et al. 2006; Hildyard 2008), the possessions of numerous *Shagia* families were confiscated, and the people were displaced from the 4th Nile Cataract region. The government settled the *Shagia* in three locations in the Nubian Desert: Al-Multaqah, Al-Makabrab and Al-Muqadam and provided them with access to schools, mosques, hospitals, electricity and a primitive water supply system. However, the unrecorded history and culture of this tribe have been allowed to disappear. Therefore, the primary aim of this interdisciplinary research was to characterise the morphology of the *Shagia* people and provide an ethnographic description of their geographically, genetically, and culturally isolated population. Importantly, this research cannot be replicated as the *Shagia* villages disappeared once they were moved to multi-cultural settlements.

Material and methods

Subjects

During a scientific expedition organised in February 2005 by the Museum of Archaeology in Gdańsk, Poland (before the displacement of *Shagia* from the 4th Nile Cataract region and the division of Sudan), anthropometric measurements were taken from individuals that occupied three villages (Abu Haras, Shibabit and El Higiena) (Fig. 1). A total of 64 adults underwent anthropological analysis. The study participants (n=29 men and 35 women, mean age = 23.9 ± 17.3 years, median age = 20 years) identified themselves as members of the *Shagia* tribe.

This research was conducted in accordance with the principles of the Declaration of Helsinki and local law. Informed consent was obtained from all participants. If study participants were unable to read, oral consent was obtained in the presence of a local government representative. The study received approval from the Bioethical Commission of the Pomeranian Medical University in Szczecin, Poland (KB–0012/129/10).

Fig. 1. Geographical location of Shagia tribe. (Photos, ©2023 TerraMetrics, Map data ©2023 Google)

Ethnographic characteristics

The *Shagia* are farmers, and their homesteads are located along the Nile River, from Kurtia to the 3rd Cataract. Their spoken and written language is Arabic but there are few literate individuals in the sparsely populated villages. The houses are constructed of bricks made from mud or animal dung, and the roofs are thatched. The thatch provides insulation and helps to regulate the temperature inside the house by reducing heat transfer. The villages consist of farmsteads located near fields irrigated by the waters of the Nile. Each village is inhabited by 40–60 people. Families have multiple generations living together. The leaders are men in their prime (over 35 years of age) and are often not the family's most senior members. Most work, including fieldwork, is performed by women. Newborns are given strings of colourful beads, which serve as amulets. Girls also have their ears pierced but, instead of earrings, they wear pieces of string due to impoverishment. Sometimes children undergo skin scarification in the cheek area, but this tradition is disappearing.

According to custom, girls are circumcised at the age of a few years (up to 5 years of age). The procedure includes clitoridectomies, as well as removing and sewing up the labia minora, and is an occasion celebrated by the entire village.

Our observations in the villages of Abu Haraz, Shibabit and El Higiena showed that the *Shagia* typically have a vegetarian diet consisting primarily of leguminous plants (beans, broad beans and crown vetch). While the country is rich in citrus fruits (grapefruits, oranges and lemons), they are rarely eaten because, due to their price, most *Shagia* cannot afford them. Vegetables cultivated in the fields are mainly Dutch tomatoes, onions and chives, and are the main sources of vitamins. On special occasions, such as weddings, christenings and the Ram Holiday, mutton or goat meat is eaten. The staple food is a flour-based bread called 'gurassa' and an overcooked broad bean called 'ful'. After every meal, black tea with milk and sugar is drunk. An equally popular drink is an infusion of mint or chokeberry.

The faces of older adults exhibit scarification (Fig. 2), which takes the form of three horizontal cuts on the cheeks. Their bodies are decorated with henna, which is also used to underline the eyes of small children. Women may wear gold, which is a sign of prosperity.

There is a sharp divergence between having access to medical services and using them in practice. Doctors employed in medical centres (located in larger villages) are mainly those who are obliged to work in the provinces for several years after finishing their studies. They are responsible for administering vaccinations and providing prenatal and primary health care services. However, local people typically do not use the available for them services. Disease is narrowly defined among the *Shagia* people, with every non-traumatic pathology called malaria. The *Shagia* often use medicines received from citizens of Western countries, such as members of scientific expeditions. Local people use these medicines (without regard for contraindications and expiry dates) and treat every pill as a remedy. However, even doctors in health care centres may use antimalarial drugs for other diseases.

While Islam allows for polygamy, most men from the villages have only one wife due to a lack of wealth. On average, there are from one (young couples) to eleven children in a family (Fig. 3). The ratio of boys to girls is similar.

Our survey found a high degree of familial connections and consanguineal relationships between Abu Haraz villagers. However, despite the sparse population of Abu Haraz, our survey did not uncover marriages between close relatives.

Fig. 2. Face scarification in Shagia tribal members

Fig. 3. Shagia family

Anthropometric measurements

Body and head measurements were taken using anthropometric equipment in compliance with the principles of anthropometry (Malinowski et al. 2000; Tovee 2012). All measurements were performed using standard anthropological methodology based on standard anthropometric points using a medical scale, anthropometer, and digital or spring callipers. The measurement principles, recording method, and the instruments used were first developed by Martin (1928) and, with some modifications, are still widely used today (Malinowski et al. 2000; Tovee 2012). Measurements were labelled with the abbreviations of the anthropometric points that characterise them. Each participant assumed an anthropometric position (i.e., at attention, barefoot, with the upper limbs hanging freely along the trunk, hands close to the thighs and the head in the Frankfurt plane) for examination. Assuming this position allows for standardised measurements and observations during examinations and facilitates accurate comparisons and analyses across individuals.

The following cephalometric measurements (Farkas et al. 2005) were taken: head width (*eu-eu*), head length (*g-op*), minimum frontal breadth (*ft-ft*), face width (*zy-zy*), the morphological height of the face (*n-gn*), soft nose width (*al-al*) and nose height (*n-ns*).

Body height (*B-v*) was measured with an anthropometer with an accuracy of 0.1 cm. The following body measurements were taken: trunk length (*sst-sy*), biacromial (shoulder) width (*a-a*), biiliocristal width of the pelvis (*ic-ic*), diameter of the chest (chest depth) (*xi-ths*), chest width (*thl-thl*), arm length ($a\text{-}da_{III}$) and leg length (*B-tro*).

Examined indicators

Somatic indices were calculated from the anthropometric measurements. The results of the individual ratios were assigned according to corresponding scales (Tab. 4, 5 and 6).

The cephalic index is an objective parameter for determining skull shape (Tab. 1). The minimum frontal-breadth / face-breadth index illustrates the ratio between the smallest breadth of the forehead and the width of the face. The facial index is a ratio of facial height to breadth and is used to determine various facial types. The nasal index differentiates sexual and ethnic changes and is an important measurement used in forensic science.

Rohrer's index is an indication of a person's weight relative to their height and is used as a proxy measure of adiposity. The trunk-length index shows the relative length of the trunk in relation to the total height. The acromial-height index indicates the relative width of the shoulders in relation to height, and the pelvic-height index shows the relative pelvic width to height. The arm-to-body ratio is defined as total arm length to total height, and the leg-to-body ratio is defined as the ratio of total leg length to total height (Versluys et al. 2018) (Tab. 2).

Tab. 1. The craniofacial anthropometric indexes – ranges

Index	Classification	Range	
		M	F
Cephalic index (by Martin-Saller)	dolichocephalic	x – 75.9	x – 76.9
	mesocephalic	76.0 – 80.9	77.0 – 81.9
	brachycephalic	81.0 – 85.4	82.0 – 86.4
	hyperbrachycephalic	85.5 – x	86.5 – x
Minimum frontal-breadth / face-breadth index (by Lundborg-Linders and Saller)	very narrow	x – 69.9	x – 71.9
	narrow	70.0 – 74.9	72.0 – 76.9
	medium	75.0 – 79.9	77.0 – 81.9
	wide	80.0 – 84.9	82.0 – 86.9
	very wide	85.0 – x	87.0 – x
Facial index (by Garson)	hypereuroprosopic very wide	x – 78.9	x – 76.9
	europrosopic wide	79.0 – 83.9	77.0 – 80.9
	mesoprosopic medium	84.0 – 87.9	81.0 – 84.9
	leptoprosopic narrow	88.0 – 92.9	85.0 – 89.9
	hyperleptoprosopic very narrow	93.0 – x	90.0 – x
Nasal index (by Martin)	very narrow	x – 54.9	
	narrow	55.0 – 69.9	
	medium	70.0 – 84,9	
	wide	85.0 – 99.9	
	very wide	100.0 – x	

Tab. 2. Anthropometric indexes – ranges

Index	Classification	Range	
		M	F
Rohrer Index (by Wanke (M) and Kolasa (F))	slender type	x – 1.37	x – 1.24
	medium type	1.38 – 1.58	1.25 – 1.36
	stout type	1.59 – x	1.37 – x
Trunk-length index (by Wanke (M) and Kolasa (F))	short trunk	x – 29.5	x – 29.4
	medium trunk	29.6 – 31.2	29.5 – 30.7
	long trunk	31.3 – x	30.8 – x
Acromial-height index (by Brugsch)	narrow shoulders	x – 21.9	x – 21.5
	medium shoulders	22.0 – 23.0	21.6 – 22.5
	wide shoulders	23.1 – x	22.6 – x
Pelvic-height index (by Vallois)	narrow pelvis	x – 15.9	x – 16.5
	medium pelvis	16.0 – 17.9	16.6 – 17.5
	wide pelvis	18.0 – x	17.6 – x

Index	Classification	Range	
		M	F
Arm-to-body ratio (by Vallois)	short arms	x – 44.9	
	medium arms	45.0 – 46.9	
	long arms	47.0 – x	
Leg-to-body ratio (by Malinowski)	short legs	x – 54.9	
	medium legs	55.0 – 56.9	
	long legs	57.0 – x	

Indexes of sexual dimorphism were also calculated (Tab. 3) The pelvic-shoulder index is the relative width of the pelvis compared to the width of the shoulders, and the chest-flattening index shows the proportion of chest width to depth.

Tab. 3. Indexes of sexual dimorphism – ranges

Index	Classification	Range	
		M	F
Pelvic-shoulder index (by Wanke (M) and Kolasa (F))	masculine proportions	x – 71.5	x – 79.3
	intermediate proportions	71.6 – 76.1	79.4 – 84.5
	feminine proportions	76.2 – x	84.6 – x
Chest-flattening index	very flat chest	x – 64.9	x – 65.9
	flat chest	65.0 – 68.4	66.0 – 68.9
	medium flat chest	68.5 – 71.4	69.0 – 70.9
	barrel chest	71.5 – 74.9	71.0 – 74.4
	protruding chest	75.0 – x	74.5 – x

The somatic structure of each individual was determined using the Adam Wanke's model. This is based on a comparison of the values of five indicators calculated for an individual to standard values characteristic of 'pure' types. The classification considers the body's shape and the proportions of its various parts. Wanke characterised four body types labelled with the letters I, A, V and H. In this study, these values were presented according to the Wanke system for men and the Kolasa system for women (Malinowski and Bożiłow 1997).

Ethnographic method

The ethnographic method is only used in studies conducted in the natural environment (Angrosino 1997). This study used ethnographic research and participant observation. The ethnographic method involves collecting information about the values, beliefs, social relations, material goods of the community under study and provides a thorough description of the culture of the tribe. The current research applied ethnographic observations, which involved the regular recording of facts or events as a result of participating in tribal life over a period of time.

Participant observation was in open form and supported by a photographic archive. All respondents gave their consent to be photographed.

Statistical analysis

Statistical analyses were performed using the STATISTICA software, version 9.0 (Statsoft, Inc., Hamburg, Germany). All data were categorised by gender, expressed as arithmetic means and presented as percentages.

Results

Morphological structure

Head and facial soft tissue measurements were conducted to determine reference values in the population. The results of the craniofacial anthropometric ratios for all 64 subjects are presented by gender in Table 4. The cephalic index indicated that the dolichocephalic (long head) type was most common among both men and women. The facial index classified *Shagia* faces as wide and very wide, with females having wider faces. The frontal breadth was classified as medium in the *Shigia* (in both men and women). Interestingly, given the climate they inhabited, they were characterised by narrow noses.

The mean height of the men was 170.08 cm; for women, it was 157.39 cm. The tallest man in Shibabit village was 188.4 cm, and the shortest (158 cm) came from Abu Haraz. The tallest woman was 171.4 cm, and the shortest was 146.1 cm. The *Shagia* men weighed, on average, 64.06 ± 10.14 kg, whilst women weighed 56.69 ± 11.21 kg. These measurements were also used to calculate body mass index (BMI). The BMI values showed that 17.24% of men and 28.57% of women were underweight, while 24.14% of men and 14.29% of women were overweight. Obesity was observed in 6.90% of men and 11.43% of women. Most of the population was well-nourished.

The most notable differences between the sexes were observed in Rohrer's index. According to the averages for this index, men had a slender body build while women had a stout one. Residents of the villages were characterised by a short trunk, narrow shoulders and pelvis, with short lower and long upper limbs (Tab. 5).

Tab. 4. The craniofacial anthropometric data of the Shagia tribe

Index	Male N (29)		Female N (35)	
	N	%	N	%
Cephalic index (by Martin-Saller)	**18**	**62.07**	**18**	**51.43**
	10	34.48	13	37.15
	1	3.45	2	5.71
	-	-	2	5.71
Minimum frontal-breadth / face-breadth index (by Lundborg-Linders and Saller)	1	3.45	-	-
	3	10.34	4	11.43
	12	**41.39**	**18**	**51.43**
	10	34.48	11	31.43
	3	10.34	2	5.71

Index	Male N (29)		Female N (35)	
	N	%	N	%
Facial index (by Garson)	11	37.93	**16**	**45.72**
	14	**48.27**	11	31.43
	1	3.45	3	8.57
	2	6.90	3	8.57
	1	3.45	2	5.71
Nasal index (by Martin)	6	20.69	7	20.00
	19	**65.52**	**23**	**65.71**
	4	13.79	5	14.29
	-	-	-	-
	-	-	-	-

bold indicates the most common type

Tab. 5. Anthropometric data of the studied Shagia tribe

Index	Male N (29)		Female N (35)	
	N	%	N	%
Rohrer Index (by Wanke (M) and Kolasa (F))	**18**	**62.1**	6	17.1
	8	27.6	9	25.7
	3	10.3	**20**	**57.2**
Trunk-length index (by Wanke (M) and Kolasa (F))	**27**	**93.1**	**31**	**88.6**
	2	6.9	3	8.6
	-	-	1	2.9
Acromial-height index (by Brugsch)	**20**	**69.0**	**25**	**71.4**
	6	20.7	7	20.0
	3	10.3	3	8.6
Pelvic-height index (by Vallois)	20	69.0	20	57.2
	7	24.1	4	11.4
	2	6.9	11	31.4
Arm-to-body ratio (by Vallois)	8	27.6	13	37.2
	9	31.0	9	25.7
	12	**41.4**	**13**	**37.2**
Leg-to-body ratio (by Malinowski)	**11**	**37.9**	**14**	**40.0**
	9	31.0	9	25.7
	9	31.0	12	34.3

bold indicates the most common type

Genetic sexual dimorphism (Tab. 6) influenced the bi-directional development of morphological and physiological traits. Sexual variation in body shape is highly dependent on physical workloads, and the *Shagia* had protruding chests and masculine proportions.

Body types (according to the Wanke method) are presented in Figure 4. Among men, the type I body build was the most common. Type I is characterised by an i.a. narrow shoulders. Whereas among women, the most common was type H, which is characterised by an i.a. short trunk and protruding chest. It is rare to find a pure type. Typically, only if the percentage is above 68% can the individual be classified as pure. Otherwise, individuals are considered mixed. Among men, a mixed I-V type body build was the most common; among women, it was type H-V.

Tab. 6. Indexes of sexual dimorphism of the Shagia tribe

Index	Male N (29)		Female N (35)	
	N	%	N	M
Pelvic-shoulder index (by Wanke (M) and Kolasa (F))	**12**	**41.4**	**24**	**68.6**
	6	20.7	5	14.3
	11	37.9	6	17.1
Chest-flattening index	2	6.9	1	2.9
	1	3.5	7	20.0
	3	10.3	2	5.7
	4	13.8	5	14.2
	19	**65.5**	**20**	**57.2**

bold indicates the most common type

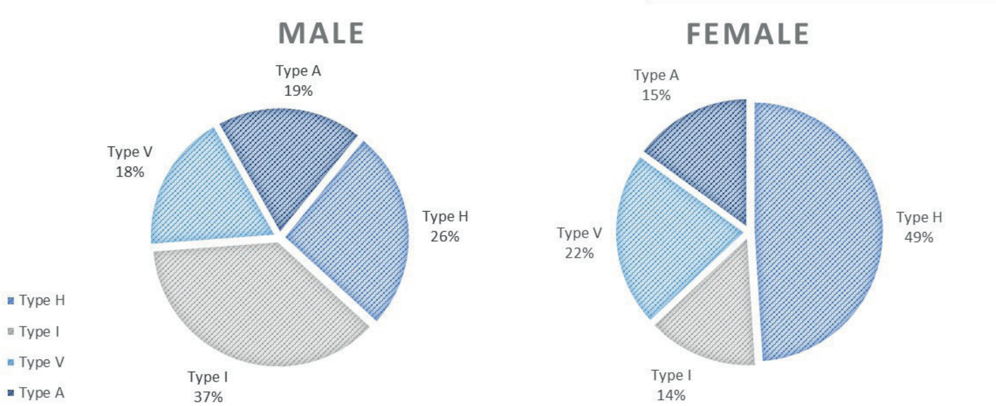

Fig. 4. Body types according to the Wanke method

Discussion

The difficult conditions of the natural environment influenced the *Shagia's* morphological build, while deeply rooted tribal affiliation helped maintain characteristic features (with relatively small admixture proportions for non-African genes) for this indigenous ethnic group.

Members of the *Shagia* tribe have long heads, either wide or very wide faces, mid-sized or wide foreheads, and narrow noses. Interestingly, narrow noses naturally occur in dry and cold climates due to natural selection (Coop et al. 2009), and this nasal shape is most common in individuals from East Asia (Dhulqarnain et al. 2020). Specific conditions of their bio-geographical environment have likely caused the characteristic somatic build of the *Shagia*. Residents of the villages were of medium height. The mean value of the pelvic-shoulder index in the *Shagia* was the same as men of Egyptian origin, who have a lighter skin complexion that is associated with Caucasian or European ancestry. Narrow hips and relatively long lower limbs, accompanied by short height, are likely related to bio-geographical origin and are typical of individuals whose ancestry is primarily from the African continent and possess dark skin pigmentation, regardless of their gender and geographical location.

Among the *Shagia* from the 4th Cataract region, some anthropometric characteristics indicated a bio-geographical origin from East Asia and the Mediterranean region. Anthropometric features characteristic of East Asians among the examined individuals of the *Shagia* can be explained historically. In pre-Islamic times, trade routes from Aksum and the Red Sea crossed at Meroë, and ships sailing from Arabia to Lake Chad to Kanem anchored in the Red Sea. The first migration of southern Arabian tribes from Arabia to East Africa occurred in 1000 BC, as recorded in the Sabanese inscriptions of South Arabia and Aksum. At the beginning of the new era, there was heavy migration to Nubia, Kurdufan and Darfur via Axum and Atbara along the Blue Nile (Fadlalla 2004).

It is unclear what role body proportions play in the bio-geographical diversity of modern humans. However, for the isolated *Shagia*, who have not been previously described anthropologically, anthropometry can provide clues to explain the history of the group. Moreover, recent advancements in genetic research have provided additional insights into human variation that complement anthropometric studies (Rindos 1992). In our previous research (Kempińska-Podhorodecka et al. 2008), we found a single delta32 allele of the human chemokine receptor 5 (CCR5) gene in the *Shagia* population, which is consistent with other studies investigating the rarity of this allele in African populations (Galvani and Novembre 2005). This allele is a 32-base-pair deletion that results in natural resistance to HIV infection among delta32 homozygotes (Glass et al. 2006). In the *Shagia*, the mutation may be related to their nomadic lifestyle and possible European admixture.

Similarly, the genetic polymorphisms of the Duffy antigen receptor for chemokine genes, which successfully protect against blood-stage infection by *Plasmodium vivax* (Donahue et al. 1968), has been identified in *Shagia* tribes and may also be a consequence of admixture with non-Africans (Kempinska-Podhorodecka et al. 2012a). This is consistent with the anthropological data and is likely due to the free flow of genetic material in the population. The hypothesis that

Shagia individuals were separated from other tribes in the region of the 4th Nile Cataract is also consistent with studies showing genetic variance in glucose dehydrogenase 6-phosphate (G6PD) that, in humans, determines the response to malaria exposure (Kempinska-Podhorodecka et al. 2013). The identification of a single G6PD mutation in the *Shagia* suggests that there was long-term assimilation with other tribes. It should be emphasised that the culture of today's Sudanese tribes is not homogeneous because even before the arrival of Arabs, indigenous populations demonstrated a vast array of customs and languages. Thus, the impact they made on newcomers would have been varied. However, it is surprising that the *Shagia* people of today do not identify with other tribes (Hildyard 2008) in Sudan. Hence, the focus on small and isolated populations can provide important insights into the factors affecting the distribution of heritable traits in Africa.

The results of our previous genetic studies (Kempińska-Podhorodecka et al. 2008; 2011; 2012a; 2012b; 2013; 2014) showed that *Shagia* individuals were separated from other tribes in the region of the 4th Cataract. These genetic results are consistent with the anthropological data and suggest an admixture of non-African populations over specific periods (Campbell and Tishkoff 2008; Lambert and Tishkoff 2009). However, the lack of genetic variation observed in this population may be due not only to the ethnic roots of the *Shagia* people but also to the geographical isolation of the villages. This can be partly explained by the familial relationships in the Abu Haras village, which had a high degree of consanguinity among the inhabitants (marriage only within their tribes). Cultural conditioning favoured intra-village marriages that were virtually free of extraneous genetic material. However, it is essential to approach the study of human variation with caution, as it should not be used to perpetuate stereotypes or discriminatory practices. It is vital to consider individual variation and the complex interplay of genetic, environmental and cultural factors that contribute to human differences.

It is difficult to clearly establish the ethnic affiliation of the *Shagia* tribe due to their nomadic lifestyle and historic assimilation with other populations. On the one hand, this obstructed access to the population. On the other hand, these features were a prerequisite to the population's uniqueness. Furthermore, our analysis of the *Shagia* people from three isolated villages has made it possible to preserve their cultural heritage. Following this study, the *Shagia* tribe was displaced from their territory due to the construction of the Merowe Dam. As a result, their homeland was flooded with a newly formed lake. Thus, the *Shagia* in these villages migrated to various areas occupied by other tribes, preventing the possibility of further research on the *Shagia* people.

The available literature provides little information about the *Shagia*, and the existing reports are usually historical. Unfortunately, this information gap is difficult to fill given that, following resettlement, the *Shagia* have been living with populations of thousands of individuals from various tribes and have established new social orders, traditions and cultures.

Overall, this study characterises a previously unexplored ethnic group in Sudan and provides important insights into the anthropological diversity of the local population. Human diversity is of immense importance to scientists for the effective diagnosis and treatment of diseases and for classifying people based on ethnicity. It should be also noted that the villages of the *Shagia* have never been mapped. Thus, from a scientific perspective, the *Shagia* are interesting because they were a homogeneous population resulting from their geographical, genetic, and cultural isolation. The *Shagia* constitute an example of how genetic attributes may mirror morphology and socio-cultural features in human populations. Furthermore, our findings are valuable for tribal members to understand and learn about their history.

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Authors' contribution

Conceptualisation, A.K.-P.; methodology, A.S. and A.L.; validation, A.K.-P.; formal analysis, A.S., A.L. and A.K.-P.; investigation, A.S. and A.K.-P.; resources, A.K.-P.; data curation, A.S.; writing original draft preparation, A.S. and A.K.-P.; writing review and editing, all authors; visualisation, A.S., A.L. and A.K.-P.; supervision A.K.-P.; project administration, A.K.-P.; funding acquisition, A.K.-P. All authors have read and agreed to the published version of the manuscript.

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Declarations of interest

The authors declare no conflict of interest.

Ethical statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Bioethical Commission of the Pomeranian Medical University in Szczecin, Poland (KB-0012/129/10).

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

Possible future evolutionary consequences to *Homo* as a result of the implementation of biotechnology

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Abstract: Biotechnology has become one of the most powerful forces on the planet, since it is capable of altering life processes at a molecular level. Since human bodies are dynamic biological systems, medicine requires to understand the evolutionary antecedents of *Homo*, especially in relation to neurohormonal regulation. Furthermore, increasing human dependence on biotechnology has led to relaxed natural selection in *Homo*, with subsequent increase of genetic load. In this paper, we speculate on the possible consequences of the application of parsimoniously derived biotechnologies onto the biological system of humans, with special attention to three areas: 1. human brain augmentation; 2. biotechnology and public health; 3. relaxed natural selection and genetic load. Human ability to manipulate and alter the structure and function of the body may not only make natural selection redundant but will be guided by a teleology whose purpose will seek to improve upon nature's design.

Key words: brain augmentation, brain-machine interfaces, cosmetic neurology, relaxed natural selection, genetic load, bioethics.

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Introduction

Human bodies are dynamic biological systems. According to systems theory (von Bertalanffy 1957) a change to any function or any element of a system elicits changes in other elements of that system. Human technologies are constructs resulting from discoveries of basic properties of objects reached by the process of abstraction – isolating essential properties of objects and relations of those objects to others from all possible properties and relations. This approach in science is known as the rule of parsimony, 'Ockham's Razor', the simpler, the better (Tornay 1938; Burgess 1998). Construction and use of technologies to control the world was crucial for humans becoming the dominant vertebrates (Laland and Seed 2021). However, when it comes to the use of technologies directly interfering with biological processes of ecosystems and individual organisms, problems occur because biological entities are systems, hence not abstract isolated entities (Bateson 1973; Charlton 2008; Saniotis 2011). Therefore, what was successfully abstracted from to produce technological objects and processes, comes back to interact with these processes and results of those interactions are largely unpredictable because they were abstracted from, not included, into the design of technologies. Vertebrate brains, including human ones, are processing information along multiple intersecting pathways stimulated by external sensory inputs and modulated by physiology of organisms (Saniotis and Henneberg 2011a). Therefore, they are complex systems.

In this paper, we speculate on the possible consequences of the application of parsimoniously derived biotechnologies onto the biological system of humans, with special attention paid to three areas: 1. human brain augmentation; 2. biotechnology and public health; 3. relaxed natural selection and genetic load.

Unknown evolution: brain augmentation

Since the early Neolithic period humans have been able to genetically alter dozens of plant and animal species for their benefit. Although genetic manipulation of plants and animals was pivotal for human population increase during the 1st epidemiological transition, which later gave rise to civilisations, proposed genetic engineering of humans may have future evolutionary and public health consequences. While biotechnologies have received much theoretical attention, there have been relatively few studies assessing biotechnologies from an evolutionary viewpoint. Numerous biotechnological companies now develop, facilitate and spread various kinds of biotechnologies on a global scale. Their impact is both profound and increasing (Tab. 1).

Proponents of biotechnologies such as brain-machine interfaces (BMIs), cosmetic neurology and genetic engineering aim at augmenting human brain's abilities. This includes the idea of downloading the brain into robots (Bostrom 2003) or replacing it with a nanotech brain (Kurzweil 2000). Currently, novel BMIs are being developed to provide movement for quadriplegics or amputees (Shin et al. 2012; Bouton et al. 2016; Sharma et al. 2016; Wojtalik et al. 2016; Bundy et al. 2017). Within the next fifty years various brain chips will be developed to prevent or reduce the onset of neurodegenerative diseases (i.e. Alzheimer's disease, Parkinson's disease, multiple sclerosis) and brain trauma (i.e. stroke). These may include nano/neuroprosthetic devices (Saniotis et al. 2018, 2020) to enhance cognitive and neuromotor functions.

Tab. 1. List of top 10 biotechnologies being used in the developing world. Modified from Acharya et al. (2004)

Top 10 biotechnologies to improve health in developing countries
1. Molecular diagnostics
2. Recombinant vaccines
3. Vaccine and drug delivery
4. Bioremediation
5. Sequencing pathogen genomes
6. Female-controlled protection against sexually transmitted infections
7. Bioinformatics
8. Enriched genetically modified crops
9. Recombinant therapeutic proteins
10. Combinatorial chemistry

High brain bandwidth optimisation may be achievable leading to improved inter-neuron transmission (Saniotis et al. 2018; Kurzweil 2000). Improvements in neuropathological markers, albeit beneficial in improving our current knowledge of cortical processes, have yet to recognize their evolutionary precursors (Saniotis et al. 2014). Furthermore, neuroscience has yet to produce a theory for assessing neurohormonal regulation of higher cortical processes (Saniotis et al. 2018).

Pharmacological approaches to brain augmentation have been ongoing over the last few decades. Giurgea (1972), who first coined the term "nootropic" to mean a substance which can initiate enhancement of cognitive abilities, has yet to spread. While substances such as methylphenidate (Ritalin) Modafinil and dextroamphetamine (Adderall) have been widely researched, they have failed to enhance human intelligence.

Chatterjee (2016) offers a poignant vignette regarding the prescribing of pharmacological substances to a businessman in order to improve his study of Arabic language. Notwithstanding this, several authors have argued that there are short term and long-term effects of psychostimulants such as anxiety, cerebrovascular disease, nausea, depression, higher stroke risk, psychosis, seizures and Parkinsonian symptoms (Lappin and Darke 2017; Lappin and Sara 2019).

Short-term adverse effects of psychostimulants include cerebrovascular disease, anxiety, initiation of mental illness, insomnia, diarrhoea and nausea, their long-term consequences include higher stroke risk, cognitive impairment, and psychosis, stroke, Parkinson's disease and seizures (Ballon and Feifel 2006; Sahakian and Morein-Zamir 2007).

The effectiveness of current synthetic psychostimulants lies in their ability to amplify neurochemical alterations (Smith et al. 2017; Lappin and Sara 2019). It has been argued that the modern brain is unable to efficiently metabolise the toxic substrates of synthetic psychostimulants – a poignant example of evolutionary mismatch (Saniotis et al. 2014). If this is the case, the current prevalence of psychostimulants may have the potential to dysregulate neurohormonal mechanisms in *Homo*. Despite evidence of methamphetamine induced epigenetic alterations (Smith et al. 2017; Jayanthi et al. 2018; Limanaqi et al. 2018; Krasnova et al. 2020), more research needs to be conducted in relation to cause-effect dynamics between genetic and epigenetic aspects and possible long-term changes.

However, the desire for brain augmentation must also be critiqued due to recent brain evolution where it has been reported that during the Holocene period (last 10,000 years) the human brain has actually shrunk by approximately 10% (100–150 ml) (Brown 1992; Henneberg 1988; Ruff et al. 1997; Saniotis et al. 2020). This reduction in brain size has come at a time when civilisations and their concomitant technologies and science have arisen (Henneberg and Steyn 1993) (Fig. 1).

Second, there has been a decline in genotypic intelligence in various countries, resulting in lowered average values of intelligence quotients (IQ). For example, from 1975 to 2003, 11–12 years old children from the United Kingdom had an IQ decline by 12 points (Shayer et al. 2007), while Danish conscripts in 2004 had had a decline in IQ by 1.6 points (Teasdale and Owen 2005). More recent research (Bratsberg and Rogeberg 2018) has noted a decline of the "Flynn effect" in the Norwegian population. The Flynn effect refers to the increase of IQ during the 20th century (Flynn 2009; Pietschnig and Voracek 2015). This time period witnessed a rapid rise of IQ in several western nations by approximately 3 points per decade (Shayer et al. 2007). However, the last two decades have seen a reversal

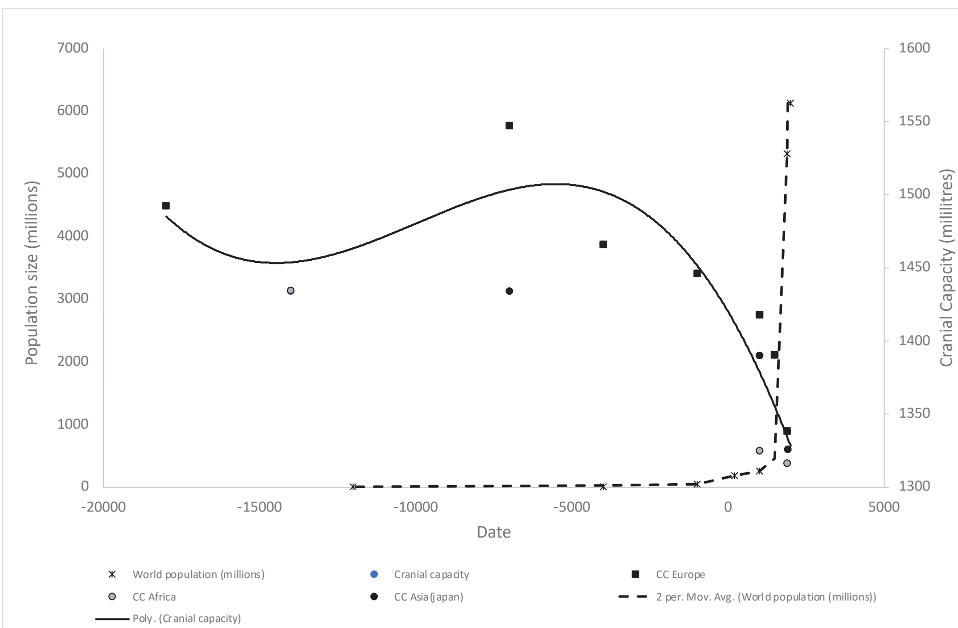

Fig. 1. Changes in the brain size (cranial capacity) on three continents and the world population during the last 22, 000 years. Data for cranial capacities from: Henneberg and Steyn (1993, 1995) and Data for World population from UN sources and Gapminder (www.gapminder.org)

of the Flynn effect (Dutton et al. 2016). A decline in IQ has deleterious consequences across the spectrum of human scientific, economic and other areas of life. This is a concern since decline in IQ has come at a time when humanity is facing serious global challenges such as biodiversity loss, climate change, ecological degradation, growing wealth inequity between developed and undeveloped nations, food insecurity, pandemics and the erosion of democratic governance.

Third, a major problem with brain augmentation technologies (which have yet to become a reality) is that science has yet to know the evolutionary antecedents of the human brain. This makes 'tweaking' the brain environment problematic. Saniotis et al. (2014) offer a poignant caveat on this theme when they note that while nootropic agents may offer possibilities in maximising human cognitive performance, this should not go beyond the brain's evolutionary capacities. On account of the brain's complexity, it is yet unclear how the brain may respond to such substances. Consequently, we should err on the side of caution since nootropic agents may lead to unbalancing the delicate neurohormonal environment (Saniotis et al. 2014). We should remember that there are several psychiatric disorders which exemplify "neurochemical aetiologies" (i.e. bi-polar disorder, schizophrenia, major depressive disorder, obsessive compulsive disorder) (Knable and Weinberg 1997; López-Figueroa et al. 2004; Berk et al. 2007; Saniotis et al. 2014). For instance, alterations in multiple neurotransmitters (i.e. dopamine, acetylcholine, GABA, serotonin, glutamate) are involved in schizophrenia (Brisch et al. 2014); dopaminergic and serotonergic pathway changes are associated with bi-polar disorder and manic depressive disorder (Benedetti et al. 2020); glutamate is implicated in obsessive compulsive disorder (Shugart et al. 2009; Wang 2010) while depletion of serotonin, dopamine and norepinephrine are linked to major depressive disorder (Hasler 2010).

Furthermore, tampering with the brain's neurohormonal regulation poses an ethical quagmire. Do extant humans have the right to change the brain in a way which may affect future evolution of the human species? Additionally, there is the possibility that in the future enhanced humans will have an unfair advantage over non-enhanced humans in all sectors of society. The question remains whether brain augmentation will become mandatory for future humans? Will brain chips be necessary in order to deal with the sheer volume of information necessary to live in a high-tech world? If so, will human reliance on augmentation technologies further affect genotypic intelligence? One lesson that history has taught us is that technology is often a twin edge sword and that our reliance on it may expose us to unforeseen consequences – the atom bomb being science's biggest caveat par excellence.

Misuse of Biotechnology and public health

Illegal and unethical aspects of biotechnologies are no longer an intellectual concern of bioethicists but a reality. Misuse of genetics was evident in the early twentieth century by the Soviet scientist Ilya Ivanov who attempted to create a human-chimpanzee embryo – a "humanzee" (Rossiianov and Kirill 2003). The depraved medical experiments of Nazi physicians (Mellanby 1947; Roelcke 2004) and the decades long Tuskegee experiment in the United States further exemplify contravention of ethical practice (Tobin 2022). Although stringent international rules and regulations forbid the use of non-therapeutic gene technology, increasing commercialisation of genetic material is testing ethical boundaries (Borry et al. 2018).

Recently, the Chinese scientist Jina-kui He claimed to have produced the first children using germ line gene editing. According to He, twin girls were designed to have a natural immunity against the HIV virus. However, He was able to circumvent Chinese and international regulations banning clinically based gene-editing methods on human embryos (Zhejiang 2019).

For many bioethicists He's cavalier behaviour is the 'stuff of nightmares'. The question beckons, if a scientist can create designer babies in a country which explicitly proscribes gene editing on human embryos, what can we expect from countries where there are less distinct guidelines on this kind of biotechnology? The apparent ethical failings in this case demand not only better scientific governance by all interested stakeholders, but also a change in our thinking regarding genetic engineering and its socio-political and evolutionary consequences.

Biotechnology, relaxed natural selection and genetic load

Humans in the 21st century CE are facing multiple global challenges such as climate change, ecological collapse, loss of arable land, diminishing water resources, over population and environmental pollution. In the last twenty years approximately thirty novel pathogens have arisen such as MERS-CoV, SARS, Ebola and the recent coronavirus (SARS-CoV-2). Many global problems are seemingly insurmountable and will affect our current way of life. It has been conjectured that these multiple challenges could be solved via synthetic biology. Various authors (Ehrlich 2000; Savulescu 2003; Saniotis 2007a,b) have stated that the use of transgenic technologies in humans could be a way for adapting to long term climate change as well as leading to greater ecological awareness. Additionally, our evolved human perceptual systems are inefficient in detecting our current social and environmental challenges (Ehrlich 2000).

Could genetically augmented humans be better equipped in responding to them? The answer to this question remains open to debate. What can be answered is that humans have since the advent of the Holocene period been actively engaged in genetically modifying plants and animals in order to improve food procurement and animal domestication. Second, gene therapies and genetic screening are two current ways in which our genetic gaze is being focused to the human realm. Although, many genetic therapies are still in an emerging state of development their use is imminent.

Increasing human dependence on biotechnology has led to relaxed natural selection in *Homo*. To illustrate this point only approximately 50% of neonates survived past 15 years of age prior to the Industrial Revolution (mid 19th century). Later the survivorship increased tenfold mainly due to improvements in public health, diet and medical intervention. Consequently, child mortality has decreased between 1890 and 2017 by >50% (from 12.6 million to 5.4 million) (Budnik et al. 2004; Saniotis and Henneberg 2011b; Roser et al. 2013). (Fig. 2).

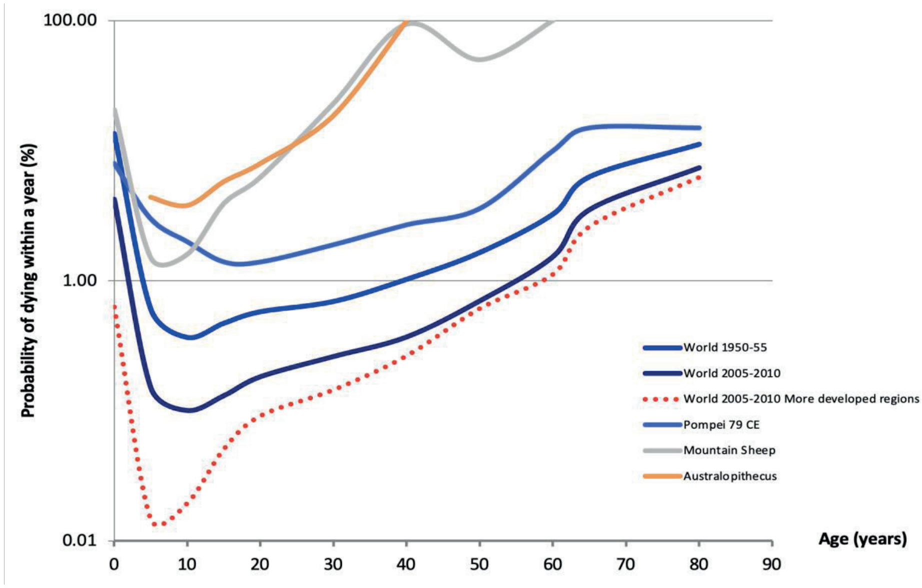

Fig. 2. Probabilities of dying within one year by age in various human populations over time compared to that of a mammal (mountain sheep). Data from the *World Health Organisation* (labelled "World" and Saniotis and Henneberg 2011b)

Although modern biotechnology has allowed more humans to reach reproductive age this has come at a cost of enabling less adaptive alleles to be circulated into the human gene pool. This accumulation of fitness reducing alleles in *Homo* has resulted in genetic load with subsequent reduction in human fitness according to earlier standards of what is fit (Agrawal and Whitlock 2012; Saniotis and Henneberg 2020). For instance, recent studies by You and Henneberg (You and Henneberg 2016, 2017) of 190 countries have identified an association between accumulated deleterious mutations due to relaxed selection and incidence for several kinds of cancer and type-1 diabetes.

Moreover, since the last quarter of the twentieth century there has been an increasing shift from curative medicine towards medical technology modifying the structure and function of the human body (Ehrlich 2000). While this shift is currently being facilitated by improvements in cosmetic surgical techniques, gene therapies are being proposed to eventually dominate body augmentation. There is no little doubt that western societies' fixation with youthfulness and concomitant disparaging of aging will continue to inform biomedicine's refocus on improving upon countering the body's physical and cognitive limitations.

Conclusion

Relaxation of natural selection combined with increasing genetic load means that humans will become more dependent on biotechnologies in many areas of life. Biomedicine's movement from therapeutic to prosthetic techniques is changing discourses on the human body, since this movement embodies the idea of transformation (Saniotis and Henneberg 2017).

The novelty of brain augmentation technologies will require more medical effort in understanding the evolutionary antecedents of *Homo*, especially since our knowledge of neurohormonal regulation of the brain is still unclear. Although, there have been some developments in neuroprosthetics in the last generation, these devices have mainly focussed on increasing a recipient's motor control. It may be years before machine-brain-interfaces can be developed to enhance higher order abilities, if at all. A similar challenge is facing cosmetic neurology – the use of pharmacological substances in order to "enhance" human cognitive abilities. Despite research into various touted nootropic substances, there is currently no substance that is a verifiable brain enhancer. One reason is that our knowledge of neurotransmitters and their interaction with neural circuits is relatively poor. Consider that medical science has yet to find a pharmacological cure for devastating mental disorders which have been earlier discussed. Perhaps, advancements in artificial intelligence in combination with gene technology, nanotechnology and virtual reality technology could lead to a substantial modification in human cognitive abilities. However, this is speculative as we have little knowledge on how the human brain has been shaped during evolution. Lastly, there is a possibility that any attempt in tweaking the brain at genetic and molecular levels may result in the incidence of new mental disorders or provoking already prevalent mental disorders.

Second, novel biotechnologies must be conducted within existing ethical guidelines. The introduction of non-therapeutic biotechnologies will, by their nature, be antithetical to evolution. Human ability to manipulate and alter the structure and function of the body will further relax natural selection making extant humans more dependent on medical interventions.

Conflict of Interest

None

Acknowledgements

None

Authors' contribution

A.S.: Conceptualisation, data analysis, writing of the first draft, critical revision; F.M.G.: data analysis, critical revision of the manuscript and literature, contribution to the writing of the second draft; M.H.: conceptualisation; data analysis, critical revision of the manuscript and literature, contribution to the writing of the second draft, supervision.

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

AI-generated faces show lower morphological diversity than real faces do

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Abstract: Some recent studies suggest that artificial intelligence can create realistic human faces subjectively unrecognizable from faces of real people. We have compared static facial photographs of 197 real men with a sample of 200 male faces generated by artificial intelligence to test whether they converge in basic morphological characteristic such as shape variation and bilateral asymmetry. Both datasets depicted standardized faces of European men with a neutral expression. Then we used geometric morphometrics to investigate their facial morphology and calculate the measures of shape variation and asymmetry. We found that the natural faces of real individuals were more variable in their facial shape than the artificially generated faces were. Moreover, the artificially synthesized faces showed lower levels of facial asymmetry than the control group. Despite the rapid development of generative adversarial networks, natural faces are thus still statistically distinguishable from the artificial ones by objective measurements. We recommend the researchers in face perception, that aim to use artificially generated faces as ecologically valid stimuli, to check whether their stimuli morphological variance is comparable with that of natural faces in a target population.

Key words: geometric morphometrics, GAN, artificial intelligence, human face, morphology, symmetry.

Original article

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Introduction

In recent years, internet users have become increasingly familiar with online content synthesized by artificial intelligence (AI). AI engines write songs, create art pieces, poems, or love letters (Cetinic and She 2022). Many online services that use content-generating AIs have become widely popular. People are getting used to the idea that independent creativity, once believed to be an exclusive domain of humans, can be 'extracted' from the human brain and replicated by well-trained, hierarchically organized pieces of code.

This is unlikely to be the final stop on the highway of human-to-AI devolution. Numerous industries keenly took up the new opportunities. AI-generated content is becoming popular in media and entertainment, in marketing, advertising, cinema production, retail, and the list could go on. The potential of a special type of AI engines, so-called generative adversarial networks (GAN), is in high demand in 3D modelling, and it can generate images of living entities nearly indistinguishable from real ones (Anantrasirichai and Bull 2022).

Recent generations of GAN have shown remarkable progress in replicating human morphometric features and generating realistic images of faces and bodies – both in 2D and 3D – of people who never existed but look amazingly life-like (Anantrasirichai and Bull 2022). This phenomenon raises a number of ethical questions, mostly related to the use of deep fakes (Mustak et al. 2023), which also leads to increased investment in cybersecurity industry and related fields whose aim is to protect individuals against nonconsensual use of their private biometric data and the public against disinformation (Pasquini et al. 2023; Wong 2022).

In February 2022, the media widely reported about a University of Texas study (Nightingale and Farid 2022) which suggested that synthesis engines have now left the "uncanny valley," meaning they are past the point where robots that look almost but not completely human-like evoke in people a negative emotional response (Geller 2008). The Nightingale and Farid study suggested that AI engines are now capable of creating fictional faces which are not only indistinguishable from, but even more trustworthy than the faces of real people. People are thus not only prone to perceive the artificial faces as real ones, but they also considered artificial faces more trustworthy-looking, i.e., having facial features that inspire confidence. This research was based on a survey where people evaluated faces and marked them as either real or artificially created. We have noticed some peculiarities in the approach to compiling the dataset of natural and AI-generated faces in this survey: the AI-created faces looked more smiley and generally more friendly than the natural ones did, which created a potential for bias in the judgements. Still, several other studies provided further support to the claims made by Nightingale and Farid (Sergi D Bray, Johnson, and Kleinberg 2023; Lago et al. 2022; Rossi et al. 2022; Tucciarelli et al. 2022).

More recent study also claims that AI-generated faces are now indistinguishable from human faces, however it points the possible limitation (Miller et al. 2023). As far as modern AI engines are trained disproportionally on photos of individuals with a white skin, generated white AI faces may look especially realistic. The authors (Miller et al. 2023) proposed a term AI Hyperrealism to describe the discovered effect when white

AI faces are judged as human more often than real human faces.

On the other hand, there are several papers indicating that people still do not perceive AI-generated faces as trustworthy (Liefooghe et al. 2023). One of the studies (Moshel et al. 2022) investigated how human brain processed artificially generated images based on brain imaging data. It shows that AI generated faces may be reliably recognized using people's neural activity, and peoples' subjective judgement do not always correlate with these data, as far as they perform near chance classifying real faces and realistic fakes. These findings may illustrate the fact that we still cannot state AI generated faces to be indistinguishable from real ones. The studies by Nightingale and Farid 2022, Miller et al. 2003, and some others focused mainly on an evaluation of the photorealistic qualities of artificially synthesized faces as well as respondents' subjective perceptions and judgements. In contrast, our objective here is to assess the degree to which real human faces and those generated by AI techniques are comparable in terms of objectively quantifiable parameters of facial morphology. To do so, we explore an alternative way of assessing differences between artificial and real faces, a method based on geometric morphometrics.

For the purposes of our study, we generated research objects using two prominent AI engines: Open AI's DALL·E 2 (Marcus, Davis, and Aaronson 2022) (henceforth DAL) and StyleGAN2 (henceforth NVID) developed by the NVIDIA team (Karras, Laine, and Aila 2019). DAL is a comprehensive network that is becoming extremely popular in the artistic community for its ability to create realistic images and arresting art pieces based on a description in natural language. The former GAN literally allows the artist to be the master of AI by providing it with detailed instructions how to create, edit, or adjust images. The latter is a project of NVID, a company whose main product are graphic processors and systems-on-chip. It allows users to generate extremely realistic yet totally fake portraits of people, down to details as minute as a realistic simulation of skin texture.

Despite the existence of various facial databases (e.g., 16–19), the acquisition of facial portraits from various understudied local populations may still be organizationally and timely demanding. The obvious advantage of artificial stimuli is also that they are just avatars and do not represent real human beings which may save time to researchers asking for approval to institutional review boards to work with human subject as well as taking off burden from ethical committees. AI faces could thus be well used for psychological research as a substitute for real human faces. One can thus easily imagine their implementation in research dealing with the relationship between facial appearance and associated personality factors. However, if one decides to use artificial stimuli, the crucial question is their ecological validity for perceivers from a target population.

In this study, we wanted to investigate whether the mean morphometric features – including symmetry and shape variance, here measured as morphological disparity – of AI-generated faces are the same as those of natural faces. Unlike several previous studies (Bray, Johnson, and Kleinberg 2023; Lago et al. 2022; Nightingale and Farid 2022; Rossi et al. 2022; Tucciarelli et al. 2022), we used standardized synthetized faces with a neutral expression and compared them to natural faces selected from our database of standardized facial portraits. Recent studies have shown that humans are no longer able to distinguish artificially generated facial stimuli from portrait photographs of real human beings. We therefore hypothesize that comparison of morphometric features measured directly from the faces should corroborate these recent findings.

Material and Methods

Facial datasets

Control group of faces (CTLR)

As a control group of natural faces, we used 197 male facial portraits (mean age ± SD = 26.62 ± 8.81) of European (Czech) origin, which we had used in our previous studies (Kleisner 2021; Kleisner, Pokorný, and Saribay 2019; Linke, Saribay, and Kleisner 2016). With the sample of natural faces, we wanted to cover a representative age range (from 19 to 59) that would approximately correspond to the range covered by the faces generated by GANs. Of course, the age of artificial faces cannot be objectively determined: it can only be approximately assessed based on the presented facial image, which is why the notion of age range in AI-generated faces must be taken with a grain of salt.

Artificial faces

DAL has an intuitive user interface for artists hosted on playgroundai.com, where it comes in a drop list along with another AI model. We used the version current as of December 2022. The query for the present study was "ID photo of a European man, enface." This led to the generation of dozens of faces comparable with the face settings in our database of standardized natural facial portraits with a neutral expression. In total, the DAL generated for us around two hundred facial portraits, some examples are provided in Figure 1.

Fig. 1. Examples of AI-generated faces

During data preparation, several images from the 200 generated by the DAL were excluded due to excessively cropped facial parts, such as the chin or the forehead. Then we chose 100 images for the purpose of the present study.

The NVID AI-engine in a public version for December 2022 also functions in conjunction with a basic user interface hosted on thispersondoesnotexist.com. This AI engine has no filtering options for age, biological gender, skin color, or any other criteria, but it generates faces that look astonishingly natural, including detailed skin features. For the morphometric purposes of the current study one hundred synthesized portraits with neutral emotional expression and appropriate face settings were selected.

We excluded AI-generated faces (of both DAL and NVID origin) whose apparent age and emotional expression fell outside the range of the control dataset: our aim was to make sure that the AI-generated and natural faces datasets do not excessively differ in their apparent age range and emotional display. This was done by a panel of six persons, and only AI-generated faces that fell within the range defined by the set of real faces were kept for further analyses.

Ethics

This study was performed in line with the principles of the Declaration of Helsinki. All procedures mentioned and followed were approved by the Institutional Review Board of the Faculty of Science of Charles University (protocol ref. number 2023/06). All photographed individuals gave informed written consent to use their portraits in scientific research. This particular study does not include information or images that could lead to the identification of any real person, because we do not use original photographs of real human beings but only shape coordinates defined on the faces that were already published within our previous works, e.g. (Kleisner et al. 2021, 2023).

Geometric morphometrics

Procrustes Analysis

Facial morphology was characterized by 72 landmarks, including 36 semilandmarks that denote curved features and outlines. Shape coordinates of all 397 facial configurations were entered into a Generalized Procrustes Analysis (GPA) using the gpagen() function in the Geomorph package in R (Adams and Otárola-Castillo 2013; Schlager 2017). Procrustes-aligned configurations were projected into a tangent space. Semilandmark positions were optimized based on minimizing the bending energy between corresponding points. See supplementary figure S1 for the visualization of the principle component analysis (PCA) on Procrustes residuals, i.e., aligned shape coordinated after Procrustes fit.

Morphological disparity (MD)

Based on symmetrized Procrustes residuals of facial configurations, morphological disparity was calculated separately for the group of artificial and natural faces using the morphol.disparity () function within the geomorph R package.

Asymmetry

Procrustes residuals were first laterally reflected along the midline axis. The corresponding paired landmarks on the left and right sides of faces were then relabeled, and the numeric labels of landmarks on the left side swapped for the landmark labels from the right side and vice versa. To measure asymmetry, we calculated Procrustes distances between the original and the mirrored (reflected and relabeled) configuration, whereby larger values indicated greater facial asymmetry.

Statistical analysis

To test the difference between the AI-generated and control group means, we conducted a Procrustes ANOVA with Procrustes residuals as a response variable and group factor as an independent variable. The analysis was done by procD.lm () function in the geomorph R package (Adams et al. 2023; Baken et al. 2021). The effect size was assessed as adjusted coefficient of determination (R^2). To test the difference in the levels of facial asymmetry between the AI and CTRL groups, we used a nonparametric Kruskal-Wallis test test based on Procrustes distances between the left and right side of the face. P-values for testing the group differences in morphological disparity were estimated by permutation test (based on 10,000 iterations) using morphol.disparity () function within the geomorph R package.

Results

The artificial faces were statistically different from the natural faces in terms of facial morphology ($F_{1,395}$ = 44.588, $p < 0.001$, R^2 = 0.101); see also Figure 2 for a two-group PCA plot and supplementary figure S1 for a PCA plot with separate visualization of DAL and NVID-generated faces.

The morphological disparity of the control group ($MD_{CTRL=}$ 0.00396) was significantly higher ($p < 0.001$) than the morphological disparity of AI-generated faces (MD_{AI} = 0.00289), indicating that the facial shape of real individuals is more variable than the shape of AI-generated faces. When images generated by the DAL (MDDALL = 0.00143) and the NVID (MDNVID = 0.00173) were compared with natural faces separately, it turned out that both groups of artificial faces significantly differ ($p < 0.001$) in the level of morphological disparity from the control group. On the other hand, the DAL and NVID faces did not mutually significantly differ in the level of morphological disparity ($p = 0.24$).

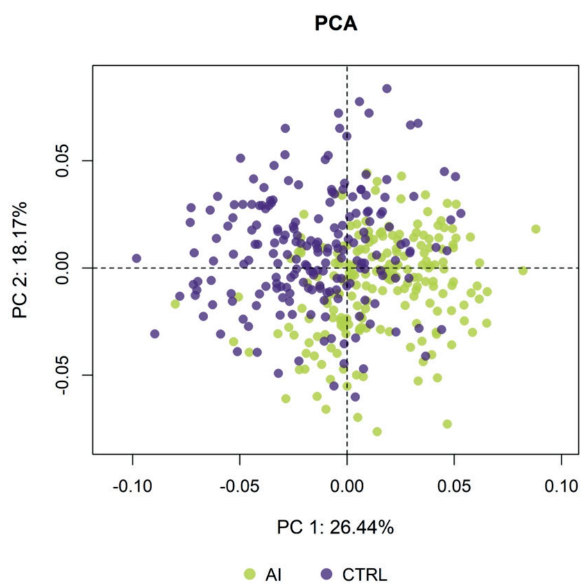

Fig. 2. A visualization of the principal component analysis (PCA) on Procrustes residuals showing the first (PC1) and second (PC2) principal component, which together explained 44.6% of overall shape variation. Dots depict individual faces shown in different colors based on whether they were AI- generated or natural, i.e., from the control group (CTRL)

The AI-generated faces also showed lower levels of facial asymmetry than the control group (ΔmeanCTRL–AI = 0.007, $W = 12446$, $p < 0.001$); see Figure 3. This held also when all three groups were compared separately (Kruskal-Wallis chi-squared = 49.512, df = 2, $p < 0.001$), see also supplementary Figure S2).

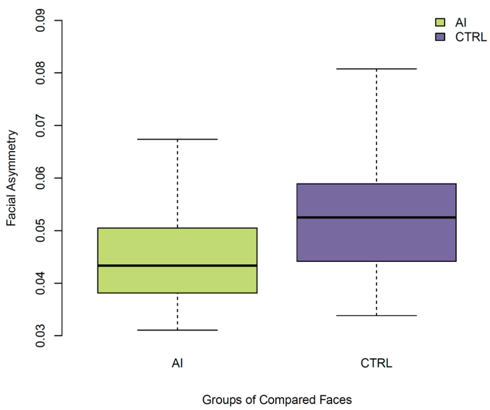

Fig. 3. Boxplots comparing the distribution of the asymmetry scores of faces generated by AI and control group of natural faces (CTRL). Thick solid lines between within the boxes indicate group medians. Whiskers denote the upper and lower quartiles

Discussion

Using geometric morphometrics based on faces with a neutral expression, we showed that AI-generated faces statistically differ from natural faces in terms of their facial shape. Moreover, AI-synthetized faces are less variable in facial shape, i.e., show lower morphological disparity, and have lower levels of facial asymmetry that natural faces do. From the perspective of objectively quantifiable morphometric measurements, artificial and natural faces are still distinguishable, although people cannot see these differences (Bray et al. 2023; Lago et al. 2022; Nightingale and Farid 2022; Rossi et al. 2022; Tucciarelli et al. 2022).

Our finding of a greater shape variation of natural faces compared to artificial ones seems to support the findings of a perception study by Nightingale and Farid (Nightingale and Farid 2022). The lower shape variation of AI-generated faces points to their higher levels of averageness and since objects closer to the average are more common, people are likely to perceive them as more typical and therefore more natural (and more 'real'). In other words, natural faces are more variable, which also implies that in a sample of natural faces one finds more distinctive faces than in an AI-generated sample – and because distinctive faces are encountered less frequently, they are perceived as less natural, and therefore also less trustworthy (Sofer et al. 2015).

This leads us back to the "uncanny valley" phenomenon (Geller 2008). During the evolution of robotics, this term was used to describe a hypothesized relationship between a machine's appearance and the emotional response it evokes. It has been speculated that a robot's appearance and movements, which are somewhere in-between "somewhat human" and "fully human," make people feel apprehensive and insecure. Once, however, a robot or another synthetic object becomes sufficiently human-like, the "uncanny valley" ends.

Our results show that AI-generated human faces have by now emerged from the uncanny valley not just because GANs make them perfectly realistic, but in part also due to quite the opposite. Artificial faces are perceived as more trustworthy also because they show a lower variation and thus higher levels of averageness (lower distinctiveness) than natural faces do. Human-like generated images are in increasing demand in marketing communication, mass media, social media, and entertainment industry. Therefore, these results could motivate the developers of advanced AI engines for algorithms improvement and teach their machines create more natural looking content.

At the same time, our study has several limitations. First, we based our analysis solely on male faces. This was driven chiefly by the fact that female faces generated by GANs – especially those produced by the NVID platform – almost always smile. Therefore, we could not easily compare them with our sample of natural faces with a neutral expression. Another limitation is that we used only faces of European origin: future morphometric studies may improve on this by including different ethnicities and genders, as was in fact done in Nightingale and Farid's perception study (Nightingale and Farid 2022). Nevertheless, using Czech faces instead of faces of various European origins for comparison with artificially generated faces provides a more rigorous test of the observed phenomenon regarding the smaller shape variation of artificial faces. The Czech population is relatively homogeneous and potentially less variable compared to the broader European population. If we were to use a more diverse European sample, the difference in morphological disparity between the control group and artificial faces would be even more pronounced. Similarly, this logic applies to the seemingly wider age range of artificial faces. A greater age range would typically result in higher shape variation. However, the shape variation of artificial faces was lower compared to natural faces.

Experimental studies of facial perception that would in future use artificially generated facial stimuli should first calibrate them to levels of symmetry and overall morphological variation similar to those found in local populations of natural faces in order to ensure an appropriate level of ecological validity. For that purposes researchers may use already existing facial databases with clear geographical information about the origin of photos (Courset et al. 2018; Lakshmi et al. 2021; Ma et al. 2015; Saribay et al. 2018). The final question is whether researchers should use artificially generated faces as fully ecologically valid stimuli in perceptual task-oriented research. Probably yes, but only with great caution. However, one can expect that at least in the case of European faces, the synthetic and natural morphologies will be soon indistinguishable.

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Data availability

All data and R code are available at https://osf.io/5p2qf/

Ethical Approval

This study was performed in line with the principles of the Declaration of Helsinki. All procedures mentioned and followed were approved by the Institutional Review Board of the Faculty of Science of Charles University (protocol ref. number 2023/06). This study does not include information or images that could lead to the identification of any real person.

Authors' contribution

OB generated artificial faces, and manually applied landmarks on facial configurations; KK provided natural faces, performed geometric morphometrics and statistical analyses. Both authors interpreted the results and wrote the article.

Conflict of interest statement

We have no known conflict of interest to disclose.

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The presence of *Homo* in Sicily: evidence, hypotheses and uncorroborated ideas. An archaeo-anthropological perspective

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Abstract: This article summarises the main findings and data on the ancient peopling of the Mediterranean island of Sicily through an archaeo-anthropological perspective. The hypothesis surrounding the presence of the Lower Palaeolithic in Sicily with more ancestral species of *Homo* is also extensively reviewed and it is explained why there are not sufficient elements to maintain it. Finally, future multidisciplinary proposals are made to fill the gap on Sicilian cave archaeology.

Key words: archaeology, caves, chronology, *Homo*, palaethnology, peopling, prehistory, Sicily.

Original article

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Introduction

Sicily has long been the subject of anthropological research due to its long history and frequent population movements occuring throughout its territory both in prehistoric and historic times (Baker 2000). Besides classical ethnological and anthropological studies, bioarchaeological research and palaeopathological studies have been growing in recent years (Varotto et al. 2021; Fiorentino et al. 2022; Melintenda et al. 2023). The present study, focusing on the early arrival of humankind to the island, aims to highlight the phases and methods of the peopling of Sicily in prehistoric times by offering an examination of the most updated scientific literature and comparing it to the relevant theories proposed in the 20th century. The long-standing controversy regarding the possible existence of *Homo* species predating *Homo sapiens* in Sicily, which was rejected in the past due to questionable archaeological criteria, will also be discussed. We also evaluate the interpretations regarding the possible presence of more ancestral human species in Sicily. The history of the first settlements in Sicily is still a topic of considerable interest in the field of archaeological and anthropological research. This is mainly because there is an undeniable lack of systematic studies of the territory, which could reliably confirm or deny the presence of other species of the genus *Homo* that predated *sapiens* on the island. Yet, in the previous two centuries, there was no lack of interest on the part of scholars in studying many caves of the territory, since they were considered the first humankind's "dwelling place". It should be mentioned, nonetheless, that cave exploration is not necessarily in itself a "privileged" form of enquiry because nomadic prehistoric populations performed most of their activities outside caves; indeed, caves were no longer used for their original purpose already in proto-historic times. Only if rediscovered many centuries later without signs of being looted in antiquity (or more modern times) and depending on the state of preservation of the remains found therein, caves can indeed be considered places where reading the archaeological record is often possible.

What is more, in the first half of the 19th century, the history of European archaeo-anthropology (at the time still often referred to as palaethnology) began with caves, and the same path of research was followed in Sicily. Between the 19th and 20th centuries, there was a particular interest in examining the caves of western Sicily, which were investigated in order to study and learn more about Quaternary faunas (Privitera 2007). The archaeo-anthropological heritage preserved in the island's caves also attracted foreign scholars such as Hugh Falconer (1808–1865), after whom the *Elephas falconeri* would be named, and Ferdinand von Andrian (1875–1951), who joined Italian scholars in the effort, often transferring the recovered materials to museums of natural history and/or archaeology according to an unclear bureaucracy and communicating what was found in an incomplete manner, which caused a loss of precious contextual data (Battaglia 2014).

The fervour of studies that interested early Sicilian archaeo-anthropology led to the excavation of caves that proved fundamental to the understanding of certain aspects of Sicilian prehistory (Privitera 2007). Specifically, the cave of San Teodoro (Messina) was investigated in the first decades of the last century and between 1937 and 1942 the remains of seven individuals buried within it were brought to light (Garilli et al. 2020 and references therein); the Genovese cave (Trapani) was discovered in 1949 and its findings made an important contribution to the history of rock art and the study of Quaternary faunas. The excavation of the Chiusazza cave (Siracusa) made it possible to understand and clarify the succession of Sicilian *facies* from the Neolithic to the Bronze Age (Privitera 2007); the investigation of the Uzzo cave (Trapani), begun in the second half of the 1970s, contributed to understanding the transition from the Mesolithic to the Neolithic in Sicily and in general the island's cultural transformations between continuity and external contributions (Tagliacozzo 1994; Mannino et al. 2015).

The role of caves in the Sicilian settlement dynamics

In the Upper Palaeolithic, the first hunter-gatherer communities used caves as sedentary sites and settled mainly in coastal areas; they almost certainly explored the inland areas of the island and its relief, as already noted by Vaufrey in the 1930s (Martini et al. 2007). Final Upper Palaeolithic groups had to settle at higher altitudes, probably not only because of the presence of wildlife, but also for the procurement of raw materials, such as the flint sources near Monte Cervi, in the Madonie area (Belvedere and Forgia 2010). Groups settled near watercourses and hunting activities had to take place in the surroundings. The cave was a privileged environment, although there was no lack of living facilities in more or less deep rock shelters, of which few traces remain in the archaeological record, such as hearths. Even in a period as ancient as the Upper Palaeolithic, humankind exploited the large space offered by caves to perform burial rituals, evidence of which remains in the San Teodoro cave and the Grotta d'Oriente (Martini et al. 2007).

When the first human groups arrived in Sicily during the Final Epigravettian (ca. 16,000 years ago), the island must have been characterised by strong climatic instability with alternating warm interstadial phases and cooler steps (Romano et al. 2021). These arrangements led to the formation of steppe-like environments, especially at the Younger Dryas (dated to a time interval of 12,900 to 11,700 years ago) that closed the Late Glacial Period. This first phase of peopling was characterised by an uneven distribution of sites across the territory, mostly on the northern and eastern coasts. On a geological level, there was a progressive uplift of the coastline and the disappearance of continental shelves in front of the current coastline; a comparison with marine variations in the lower Tyrrhenian slope led to the hypothesis of a sea level lowering of around 40 m during the Late Glacial Period. Absolute dating (AMS^{14}C) on human bones to date indicates the oldest site in Addaura Caprara (Monte Pellegrino, Palermo), dated to 16,060–15,007 (calibrated–BP), while the San Teodoro site (Acquedolci, Messina) has been dated to around 14,500 years (Sineo et al. 2014); ST1 has been dated to 15,232–14,126 (calibrated–BP) (Garilli et al. 2020; Catalano et al. 2022). At the current state of research, the best investigated Epigravettian and Mesolithic sites are located in the western part of the island, in the Trapani area (Uccerie cave; Cala dei Genovesi cave; Oriente cave; Uzzo cave; Racchio-Isolidda cave; Cala Mancina cave), in the Palermo area (Termini Imerese Castle shelter; Addaura cave; Niscemi cave) and in the Agrigento area (Acqua Fitusa cave). Few sites are found in eastern Sicily and have been identified in the Messina area (San Teodoro cave; Sperlinga di San Basilio), in the Catania area (Perriere Sottano), in the Siracusa area (Giovanna cave; Canicattini Bagni; San Corrado cave), and in the Ragusa area (Riparo Fontana Nuova) (Lo Vetro and Martini 2022).

During the Palaeolithic period, therefore, caves were mainly used as settlement sites. Their use changed from the Upper Palaeolithic to the Bronze Age, but often persisted over time (e.g., cave churches and cave dwellings from the Middle Ages) (Patti 2013).

In later periods, and especially from the Copper Age onwards, caves played a complementary role to other settlements, being used as temporary shelters, perhaps during transhumance, as in the Palermo area (Battaglia 2014), or becoming "appendages" of villages (warehouses and/or workplaces) (Privitera 2007); some continued to be used as burial sites (from the Palaeolithic to the Bronze Age, in conjunction with rock necropoles), others as places where funerary ritual practices took place (e.g., the Petralia cave, in the Catania area (Palio 2014), the Fontanazza cave on the slopes of Monte Grande in the province of Caltanissetta (Panvini 2014), the Chiusazza cave in the Siracusa area (Tanasi 2008) and the Calaforno hypogeum (Ragusa) (Varotto et al. 2022).

The use of caves at high altitudes (around 1000 m), such as the Grotta del Santo in Adrano (Catania), the Grotta del Lauro on Monte Crasto (Messina), and the Grotta del Vecchiuzzo near Petralia Sottana (Palermo), has been documented since the Late Copper Age. In all probability, these were seasonal shelters linked to the movement of flocks (Cazzella and Maniscalco 2012). In general, for all the caves on the island, it can be said that their use varied on the basis of needs and beliefs, not only over the long term, but also within the same prehistoric *facies* or chronologically close phases (Privitera 2007).

The first peopling of Sicily: solid evidence

Most prehistory scholars accept the theory that no hominins arrived in Sicily before *Homo sapiens*. The event would have occurred during the final Epigravettian, ca. 16,000 years ago. Since that time there would have been a constant presence of human groups on the island, which would have gradually changed various ecosystems on the island, such as soils and vegetation. For instance, from 7,000 years ago throughout around the middle of the 5th millennium BC (Middle Neolithic) there would have been particularly intense agropastoral exploitation (Pasta and Speciale 2022). The expansion of *Homo sapiens* on the island occurred during the climatic alternations of the Bølling-Allerød (warm fluctuation) and Younger Dryas (cold fluctuation) (Sineo et al. 2014). The first hunter-gatherers inhabiting Sicily were characterised by low genetic diversity, which suggests a small effective population size of about 70 individuals (Posth et al. 2023). The main communication bridge must have been represented by what is now known as the Strait of Messina, separating Sicily from Calabria: most of the animal species present in Calabria in the Middle and Upper Pleistocene passed through Sicily, and it seems that the channel linking the Ionian and Tyrrhenian seas was crossed more easily and more often than previously thought, particularly in the "saddle" area, characterised by relatively shallow waters and a short distance between the southernmost tip of Calabria and the north-eastern cusp of Sicily (Marra 2009). Just as animals could cross this briedge, hominins could follow the same route. According to a study by D'Amore et al. (2009), there is a morphological continuum, based on skull morphologies of examined prehistoric individuals, between the Palaeolithic hunter-gatherers of the island and the Magdalenian people of continental Europe, suggesting a penetration and dispersion in Italy and Sicily of homogenous peoples or at least not entirely isolated from one another. These data were obtained by comparing the skulls from the San Teodoro cave with others found in western European contexts (D'Amore et al. 2009). Therefore, it is the Late Pleistocene phase that saw the entry of Upper Palaeolithic human groups into Sicily, a phase characterised based on the lithic industry by a unified cultural physiognomy (Petruso et al. 2011). Other land bridges have been suggested to have played an important role in the ancient peopling of Sicily is the vast area of the Sicilian Channel, which, along with the Strait of Gibraltar, Babel-Mandeb and the Sinai Peninsula, links the African continent to the European one and allows for the transition of bands of Palaeolithic explorers.

Nonetheless, there is no evidence of migrations from Africa to Sicily (and more generally Europe) from those routes (Sineo et al. 2014).

Pre-Epigravettian Sicily – uncertain evidence generating speculative theories

The final Epigravettian is widely accepted as the time of the entry of human groups into Sicily. Thus, most members of the scientific community believe that the discovery of out-of-context human lithic artefacts (Fig. 1) typologically attributed to the Lower Palaeolithic, therefore to the Middle Pleistocene, is not sufficiently strong evidence to affirm that pre-Epigravettian peoples ever existed on the island.

Such artefacts should be found in association with the *Elephas falconeri* Faunal Complex, dating to the early Middle Pleistocene, based on an absolute dating applied to the tooth enamel of the dwarf elephant *Palaeoloxodon falconeri* (455,000 ± 90,000 BP) (Sineo et al. 2014). Despite the lability of the available data, some scholars in the past supported the idea of older Pleistocene phases for Sicily, which will be examined here. One of the first was the famous archaeologist Paolo Orsi (1859–1935), who in 1899 published a report on the discovery of a lithic axe in the Alcamo area (Trapani), attributing it to the Lower Palaeolithic (Orsi 1899). This attribution, confirmed by the palaethnologist Ugo A. Rellini (Rellini 1924), was refuted by Raymond Vaufrey, a French palaethnologist, who judged the axe to be instead a Neolithic artefact (Vaufrey 1928; Vaufrey 1929).

In the 1960s, the debate on the first peopling of the island was reopened by Gerlando Bianchini, a Sicilian palaethnological enthusiast from Agrigento. In the area of San Giovanni Gemini (Contrada Rocca del Vruaro, Agrigento), in a layer of red sand also containing the remains of large mammals in a poor state of preservation, he found a bifacial, carved from a quartzite pebble (common among the alluvial material of the Platani river), currently on display at the Regional Museum of Agrigento, while a copy was on display at the Musée de l'Homme in Paris.

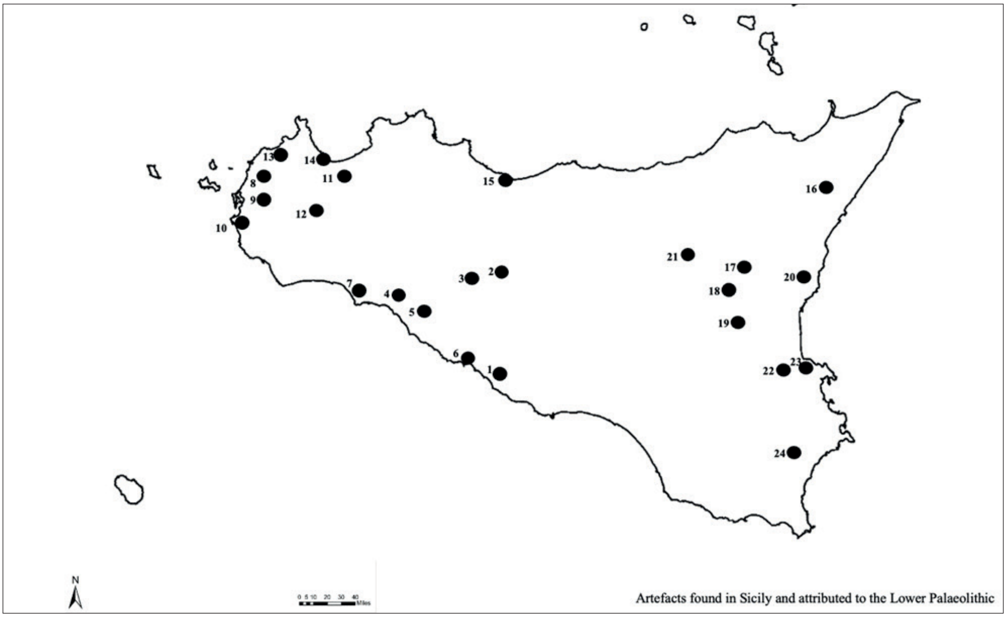

Fig. 1. Artefacts found in Sicily and attributed to the Lower Palaeolithic. Agrigento: 1. Maddaluso (Agrigento), 2. Rocca del Vruaro (San Giovanni Gemini), 3. Diga del Leone (Santo Stefano Quisquina), 4. Ribera, 5. Eraclea Minoa and Capo Bianco (Cattolica Eraclea), 6. Contrada Pergole and Capo Rossello (Realmonte), 7. Bertolino di Mare and Contrada Cavarretto (Menfi); Trapani: 8. Contrade Malummeri (Paceco), 9. Guarrato (Misiliscemi), 10. Contrade Chinisia and Granatello (Marsala), 11. Contrada Mulinello (Alcamo), 12. Contrade Bovara and Carnemolla (Salemi), 13. Grotta Emiliana (Mt. Erice), 14. Scopello (Castellammare del Golfo); Palermo: 15. Giancaniglia (Termini Imerese); Catania-Messina: 16.Valle dell'Alcantara; Catania: 17. Valle del Simeto (Poggio Monaco, Castellaccio, Fontanazza e Piccone), 18. Fiume Dittaino (Muglia Bassa and Muglia Nord), 19. Montagna di Ramacca, 20. Valverde; Enna: 21. Agira; Siracusa: 22. Lentini-Piana di Catania (Piano Meta, San Giorgio, San Basilio), 23. Contrada Piano Torre (Augusta), 24. Noto antica.

A further instrument, described as a small amygdala from the Diga del Leone, near Santo Stefano di Quisquina, was also collected by Bianchini in the 1970s. It was dated to the Lower Palaeolithic (Sineo et al. 2014). Bianchini also found some hachereaux in the ancient Agrigentine neibourhood of Maddaluso (Baldini et al. 1976). In 1961, Meli published reports on some bifacials from the basin of the Platani river, in the territory of Ribera (Agrigento): two were found in the riverbed and four near the mouth (Sineo et al. 2014).

Again, in the Agrigentine area, tools described as belonging to the Acheulean lithic industry were found at Eraclea Minoa (found in the mortar used by the Romans to build dwellings), Realmonte and Pergole. In the 1960s De Miro found artefacts in the Capo Bianco locality, which appear to be datable to the lower Palaeolithic (Baldini et al. 1976). In 1972, Biddittu and Piperno found limestone and quartzite tools at Bertolino di Mare (between Sciacca and Menfi) and quartzite tools at Contrada Cavarretto. No association with fossil fauna could be described. Pebble culture tools were found by the aforementioned Bianchini near Capo Rossello (Di Maida 2020). Other reports of lithic industries related to the Lower Palaeolithic come from the Trapanese area (contrade Malummeri, Guarrato, Chinisia and Granatello), where pebble and flake artefacts were collected; from Contrada Mulinello, along the course of the Fiume Freddo river, quartzite flake lithic industry was collected, attributed to the Clactonian tradition. On the river terrace of the Fiume Grande, in Salemi, pebble, discoidal and flake tools were found in the vicinity of Bovara, Fiume Grande and Carnemolla, outside the stratigraphic context (Tusa 1999). A quartzite instrument was found on the beach at Scopello (Castellammare del Golfo), albeit showing signs of extensive exposure to water (Baldini et al. 1976).

In 2005, during excavations in Grotta Emiliana, along the northern slopes of Mount Erice, in a layer containing remains of Pleistocene fauna characterised by the presence of hippopotami, three beaked pebbles, one lithic nucleus and one flake fragment were found, which the excavators attributed, albeit with great uncertainty, to the Lower or Middle Palaeolithic periods. The uncertainty was linked to the smallness of the sample (only five pieces), the poor conservation of the materials and the lack of clear comparisons that could be proposed for the Sicilian territory (Filippi 2014).

In the Palermo area, in 1961, Meli discovered what appeared to be choppers and bifacials in the locality of Giancaniglia at Termini Imerese. These were also attributed to the Lower Palaeolithic by Blanc and Cardini, while other authors were perplexed at the attribution of such finds (Baldini et al. 1976). In the Alcantara Valley, between the provinces of Catania and Messina, Tomarchio reported on the presence of tools attributable to the Lower Palaeolithic and the Pebble culture (Baldini et al. 1976).

Moreover, in the Catania area, in the 1970s, tools of the lythic industry attributable to the Lower Paleolithic were found. Once again these were surface basalt and quartzarenite finds, unsupported by stratigraphic data or certain osteological data. The artefacts found (181 in total) were exhibited during the scientific meeting of the Italian Institute of Prehistory and Protohistory in March 1975 in Florence and were examined by Graziosi, Guerri, Radmilli and Tinè resulting in a positive judgment by the said scholars (Revedin and Mella 1984). Here follow the localities where these tools were found: Poggio Monaco, Castellaccio, Fontanazza and Piccone (along the Simeto river); Muglia Bassa and Muglia Nord (in the proximity of the Dittaino river); Piano Meta, San Giorgio and San Basilio (in the vicinity of the ancient marshes of Lentini and the Catania plain); Montagna di Ramacca and contrada Piano Torre (respectively at the Gornalunga and Porcaria rivers).

These are somewhat large flake-shaped lithic industries, mostly scrapers and denticulate tools and there is no shortage of choppers and chopping tools. In addition, an artifact was also found by Lino in the Valverde locality (Catania) (Baldini et al. 1976), while Revedin and Mella reported the presence of Lower Palaeolitic quartzite and flint artefacts at Agira (Enna) and Noto Antica (Siracusa) (Revedin and Mella 1984).

According to Tusa, bifacials arrived in Sicily in the Lower Palaeolithic at the time of the Mindell (600,000–400,000) and Riss (320,000–140,000) ice ages; however, the number of finds is very small (Tusa 1999). In their study, Lo Vetro and Martini (2012), largely accepting the thesis already put forward by Palma di Cesnola in the 1990s (Palma di Cesnola 1994), identified three migratory phases that occurred during the Lower Palaeolithic, during which human groups from the European continent supposedly colonised the island. The first phase is the so-called Pebble Culture, which has been dated between 850,000 and 500,000 years ago on the Italian peninsula; the lithic industry consists of choppers and chopping tools, i.e., pebbles chipped on one or both sides in such a way as to obtain a sharp edge. The second phase is defined by an industry of flake tools without bifacials, which refers to the Clactonian industry and would date back to between 400,000 and 180,000 years ago; finally, the third phase would be that of the Acheulean, of which only faint traces remain (Lo Vetro and Martini 2012). Following Bianchini's reports, other scholars published studies on the discovery of Lower Palaeolithic industries between the 1970s and the 1990s (Bagnone 1981; Bidittu and Piperno 1972; Broglio et al. 1992; Recami and Baldini 1977) describing the discovery of choppers, chopping tools and bifaces, identified as Lower Palaeolithic industries.

Moreover, the re-examination of some of these lithic tools allowed to attribute these artefacts to the Campignien period, clearly a later *facies*, chronologically subsequent to the Mesolithic (Nicoletti 1997). The scepticism shown towards the theory of the island's peopling dating back to the Middle Palaeolithic was also poured over theories of a peopling of the island by species predating *Homo sapiens*. Indeed, many of the previously listed artefacts were found out of context and not associated with any Faunal Complex; furthermore, some scholars have ruled out the possibility that they could be authentically attributable to the Lower Palaeolithic (Nicoletti 1997; Piperno 1997; Tusa 1999; Leighton 1999). Many scholars, therefore, refured the claim that there were Lower and Middle Palaeolithic phases in Sicily. In recent years, the Aurignacian phase has also been refuted, after the Fontana Nuova lithic complex was attributed to the Epigravettian. Radiocarbon dating tests carried out on individuals and zoological remains have provided dates between 9,910–9,700 cal. BP and 8,600–8,480 cal. BP, confirming that Fontana Nuova was occupied by Mesolithic and not by Aurignacian hunter-gatherers (Di Maida et al. 2019).

It can be affirmed that studies published on lithic finds are merely typological and the provided descriptions lack stratigraphic references and the support of other associated data, such as fundamental faunal complexes (Di Maida 2020). Moreover, lack of any stratigraphic association and the total absence of butchering marks, probably testifying their actual use, make it difficult to consider the tools found in Sicily as certain data and support the hypothesis that ancestral industrial modes can actually have been produced later, for punctual exigencies.

Pre-Epigravettian Sicily: theories on the population of species predating *H. sapiens*

No osteological evidence of species belonging to the *Homo* genus other than *sapiens* has ever been produced for Sicily. The discovery of a few uncertain lithic tools from the Lower Palaeolithic cannot be considered sufficient proof for the contrary, as these are mainly based on unreliable data. Most scholars believe that there were sporadic human populations due to the variable accessibility of the Strait of Messina, and therefore came from the European continent (Burgio 1997; Sineo et al. 2014; Lo Vetro and Martini 2012), while a now fading minority insisted that there was a displacement of human groups from the African continent (Alimen 1975).

The discovery from Grotta Emiliana (Trapani) of fossil hippopotamus bones (pertinent to the *Elephas mnaidriensis* Faunal Complex), however lacking traces of butchering, in association with three beaked pebbles, one probable lithic nucleus and one pseudo-Levallois flake fragment, could provide further evidence for a Pleistocene settlement of the area (Chilardi et al. 2012), yet no more convincing data have been published so far on the matter.

According to Tusa, the absence of contextual relationships with the Middle and Upper Pleistocene fauna could indicate the presence of humans on the island between the *Elephas falconeri* Faunal Complex and the *Elephas mnaidriensis* Faunal Complex, thus in a phase dating back to around 300,000 years ago, as if humans had found a way to survive at the same time as the extinction of the macrofauna. Such a chronology would be compatible with the evidence of remains belonging to non-*sapiens* individuals in the Mediterranean basin. *Homo ergaster* appeared on the shores of the Mediterranean presumably not long after its dispersal from East Africa. In Dmanisi, Georgia, remains of *Homo erectus* dating from 1.8–1.78 million years ago were found (Vekua et al. 2002). From Atapuerca, Spain, come bone and stone remains of two different species belonging to the genus *Homo*: *H. antecessor* and *H. heidelbergensis* (Rodriguez et al. 2010). *Homo neanderthalensis* reached as far as Apulia: the famous Altamura Man lived between 180,000 and 130,000 years ago, when there was still a land link between Calabria and Sicily; Neanderthals dwelled the Calabria coasts (Bonfiglio et al. 1986) and presumably observed and evaluated the possibility to reach Sicily. No evidence of the Levallois technique has ever been described in the island.

However, this does not offer a valid explanation for the disappearance of human groups in the middle to upper Pleistocene and their subsequent reappearance in the Epigravettian period (Tusa 1999).

Sicily and its connections

According to some scholars, Sicily could be reached either from the European continent, through the Strait of Messina, or from Africa, through the Strait of Sicily, although virtually no scholar agrees today on the existence of the latter bridge. In the Sicilian Channel, the lowering of the sea level that occurred during the Ice Ages may greatly reduced the distance between Africa and Sicily in the Lower Pleistocene. During the peak of the last glaciation (late Wechselian), the sea level would have dropped by about 120 m, to the point where the distance between Cape Bon and south-western Sicily was reduced to about 60 km. In the Sicilian Channel, the arm of the sea was involved in active Plio-Pleistocene tectonic deformations with consequent bathymetric variations. This sea arm, at the beginning of its development, was less wide, shallower and sometimes characterised by discontinuous land bridges. Favourable conditions also occurred during the glacial periods of the Middle Pleistocene, when the lowering of the sea level was considerably significant (Abbate and Sagri 2012). Based on the above, Iovino and Marziano hypothesised an Africa-Sicily-Malta connection during the marine regressions that occurred in MIS 22, MIS 18, and MIS 16 (Lower Palaeolithic), phases in which there must have been small archipelagos between the Tunisian shelf and Malta that acted as bridges between Africa, the island of Malta and Sicily (Iovino and Marziano 2008).

Nonetheless, these hypotheses were not widely embraced by other scholars and currently are not considered valid solutions to the problem of the peopling of Sicily due to their speculative nature (Sineo et al. 2014).

The faunal turnover from the Lower Pleistocene (Monte Pellegrino) to the Late Upper Pleistocene (Castello) shows that the isolation of Sicily was not as marked as previously thought. In particular, some species derived from North African taxa are included in the Monte Pellegrino Faunal Complex, implying a connection with Africa (Marra 2009). Furthermore, the spread of humans and fauna must have followed the same routes and times, especially during periods of marine regressions (Iovino and Marziano 2008). A land bridge could have been formed in the area of the Straits of Messina (Antonioli et al. 2014). It was during the Late Palaeoglacial that an important geological event occurred: in this period, Sicily went from a phase of connection with southern Calabria, via a probable emerged bridge, to one of isolation (Petruso et al. 2011); it is probable, therefore, that the connection that still existed between Sicily and Calabria in the earlier phase had favoured the arrival of new cultural and technological stimuli (Tusa 1999).

Conclusions

Flakes, pebbles and bifacial lithic complexes were identified in Sicily over the last 70 years. However, caution should be taken while identifying these first communities that populated the island which geography differed from today due to land bridges that were connected to the mainland. Today, the majority consensus of the scientific community dealing with such problems has it that that no hominins came to Sicily before *Homo sapiens*, and that the latter reached the island coming from Europe (Burgio 1997; Sineo et al. 2014), not Africa (Alimen 1975; Iovino and Marziano 2008; Abbate and Sagri 2012). Although not all the found items can be clearly attributed to the Lower Pleistocene and some from the Agrigento area were found where land had not yet fully emerged at the time to which they are ascribed (Sineo et al. 2014), yet the presence of lithic industry from the Lower Pleistocene leaves a non-negligible question mark on Sicilian prehistory. Can it indicate that they were left on the island at a time preceding the final Epigravettian? Was this the result of Sicily being a land of passage? These questions appear legitimate. Although the undeniable presence of species of the genus *Homo* along the shores of the Mediterranean Basin would logically suggest an affirmative or, at least, possibilistic answer, the lack of any conclusive evidence shall leave the question open until further (potential) discoveries be made. As is always the case in archaeological and anthropological research, one should proceed cautiously when addressing such problematic possibility: there is still too little data available to prove that human groups predating *Homo sapiens* indeed settled on the island. While the absence of evidence does not necessarily equal the evidence of absence, the available findings, nowadays supported by more recent techniques, such as ancient DNA and radiocarbon dating, point towards a non-pre-Epigavettian peopling of Sicily by groups belonging to *Homo sapiens* and originating from Europe. Overall, the absence of precise answers to the aforementioned problem of probable Lower Palaeolithic tools should stimulate further research into the matter. Moreover, a greater interdisciplinarity in such research should be encouraged so that the search for skeletal remains or lithic artefacts is not neglected; censuses of caves in the territory of archaeological interest; systematic excavations of a greater number of Sicilian caves and the recovery of those (few) data concerning the areas where Lower Palaeolithic lithic tools were found.

Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contribution

CP conceptualisation, writing of the first draft; EV conceptualisation, editing and critical review of the first draft, supervision, data analysis, LS editing and critical review of the first draft, supervision, data analysis, FMG editing and critical review of the first draft, data analysis, supervision

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Tuberculous spondylitis: Macromorphological and radiological studies on a skeleton from the Late Iron Age monument of Nor Armavir, Armenia

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Abstract: The skeleton in question derives from the Late Iron Age monument of Nor Armavir and was unearthed from burial No. 19. The deceased was buried in an unusual position. In this article, we characterize the pathological bony changes indicative of tuberculous spondylitis. The skeleton was subject to a detailed macroscopic investigation. Besides age at death estimation and sex determination, a careful palaeopathological evaluation was performed on the bone remains. In addition, volumetric (3D) computed tomography was carried out on four lumbar vertebrae (L2–5) to complement the macromorphology-based diagnosis.

Key words: Armenia, Late Iron Age, Bioarcheology, Paleopathology, Tuberculous Spondylitis.

Original article

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Introduction

Tuberculous spondylitis is the most common form of bone and joints tuberculosis (An and Seldomridge 2006; Karadimas et al. 2008). The disease was already mentioned by Hippocrates and Galen (Turunc et al. 2007; Gouliouris et al. 2010) but the first detailed description of its main symptoms (hump and associated paralysis) was given in 1779 by the English surgeon Percivell Pott, after whom the disease was named (Pott's disease).

Tuberculous of the spine, or tuberculous spondylitis, is an infectious disease caused by *Micobacterium tuberculosis* and *Micobacterium bovis*, which cause clinically indistinguishable from each other diseases (Torres-Gonzalez et al. 2016). Molecular analysis of DNA has been carried out on archaeological samples and no evidence of *Micobacterium bovis* has been found (Zink et al. 2007). Tuberculous of the spine is characterized by the formation of specific granulomas and the progressive destruction of bone, leading to pronounced organic and functional disorders of the affected part of the skeleton (Pereira and Lynch 2005). The thoracic spine (60%) is the most common site of lesions, followed by the lumbar spine (30%). The cervical spine (5%) and the sacral spine (5%) are affected to a lesser extent (Mushkin et al. 2012; Ratobylsky et al. 2012). The number of vertebral bodies affected varies widely. Vertebral bodies of 2–3 vertebral bodies are most commonly found in first-time cases (65% of cases), and destruction of a single vertebral body is found in 1–3% of cases (Kotze and Erasmus 2006; Huang et al. 2009; Barinov and Malchenko 2013). The incidence of tuberculous spondylitis is higher in men than in women (Burrill et al. 2007; Cottle and Riordan 2008).

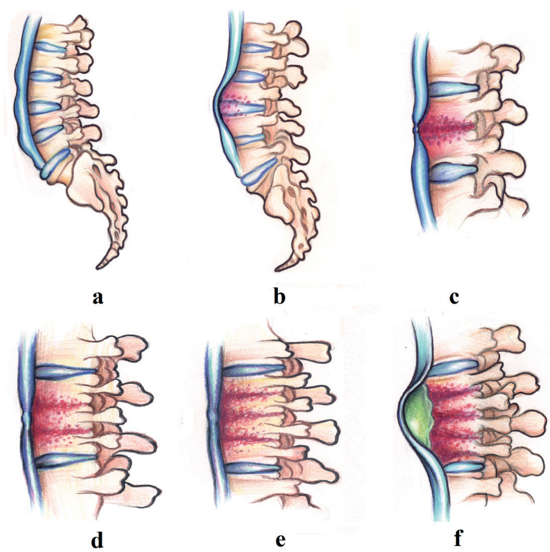

Fig. 1. Schematic representation of the development of tuberculous spondylitis: a) unaltered spine, b) lesion of two adjacent vertebral bodies, c) reduced height of the affected vertebral bodies and involvement of the intervertebral disc, d) lesion of the third adjacent vertebral body, e) lesion of the fourth adjacent vertebral body, f) formation of a paravertebral abscess under the anterior longitudinal ligament and irreversible wedge-shaped deformation of the affected vertebrae at several levels

Tuberculous spondylitis occurs as a result of reactivation and transfer of the source of infection from the primary complex, which may be located in the lungs or in another organ (Diachenko 1958). Tuberculous inflammation develops in the vertebral bodies. Initial foci occur in the marginal regions of the vertebral bodies close to the intervertebral discs. Mycobacteria may 'nest' in the bone marrow even though the bone tissue is not anatomically or clinically altered (Dyachenko 1958; Nather et al. 2005; Lee et al. 2015). In the bone system (including the spine), the onset of the local pathological process is expressed by a reactive inflammatory process around the pathogen and the development of an infectious granuloma. As the inflammation progresses, areas of necrosis appear.

The tuberculous nidus may develop in the central parts of the vertebral body-central type of vertebral lesion with a small involvement of intervertebral discs, but more often, the process is localized in the adjacent parts of the vertebral bodies and in the intervertebral disc-intervertebral type of tuberculous spondylitis. The intradiscal route is characterized by the primary involvement of the cartilaginous intervertebral disc with the hyaline boundary lamina. The boundary lamina acts as a buffer, but in adults it is weaker and less homogeneous than in children, which favours the growth of a tuberculous granuloma from the primary focus, which may spread vertically. This subchondral spread of the process disrupts the bone-cartilage junction, affecting the nutrition of the lamina and reducing its stability. As a result, the cartilaginous lamina degenerates, elements of tubercle tissue sprout through it, and the process moves to the nucleus pulposus and through it to the second lamina and the adjacent vertebra. The observed decrease in the height of the intervertebral cartilage is one of the early signs of spondylitis and depends primarily on a decrease in the elasticity of all its elements, especially the nucleus pulposus, which, when it germinates or perforates the cartilaginous lamina, eventually loses its dense covering, spreads and softens. The changes in the intervertebral disc are predominantly necrotic and curdled (Kornev 1953).

In the extradiscal route from the primary focus, granulomas are observed to sprout through the cortical layer of the vertebral body in its anterior, lateral or posterior regions. Most often, the primary focus is located closer to the anterior surface, which, on the one hand, leads to the weakening of the anterior stability of the vertebral body and its wedge-shaped deformity and, on the other hand, to the spread of the process to the anterior or lateral surface of the body under or through the periosteum, with the subsequent formation of "superfusion". If the granuloma occurs to the lateral surface of the vertebra not covered by the anterior longitudinal ligament, the abscess forms close to the vertebra–paravertebral, first under the periosteum and then beyond it. In such cases, the abscess (unilateral or bilateral) may remain in situ and take on a globular "swallow's nest" shape. In those anatomical areas where muscles attach to vertebral bodies, abscesses may spread and migrate through the intermuscular spaces (Kornev 1953).

The changes described above lead to marked destruction of the vertebrae. The anterior vertebral body is compressed and becomes wedge-shaped, forming a typical angular hump (Fig. 1).

In this work, we have applied a modern method of radial diagnostics, which allows us to obtain a volumetric (3D) image of the pathology of the spine of a Late Iron Age individual from the Nor Armavir monument. Archaeological excavations headed by S.A. Hmayakyan (with the participation of archaeologists N.G. Tiratsyan, M.S. Hmayakyan) were carried out in the territory of the Nor Armavir monument (Armavir Province) in 2022. Armavir Province (western part of Armenia) is located in the Ararat Plain, dominated by Mount Ararat to the south and Mount Aragats to the north; the province's capital is the city of Armavir (Fig. 2). The province shares a 72 km border with Turkey to the south and west. The territory of Armavir has been inhabited since the 5th millennium BC. The ancient Urartian settlement of Argishtikhinili (Nor Armavir) was founded in 776 BC by King Argishti I. One of the oldest written records of the region was found in the inscriptions of the Urartian king Rusa II (685–645 BC). The area was one of the most important regions of ancient Armenia since the Urartu period. Nor Armavir is a village in the Armavir province of Armenia. The Nor Armavir necropolis includes primary burials and cremations; the latter are represented by burnt bones while flesh, sometimes of multiple individuals (Khudaverdyan et al. 2022).

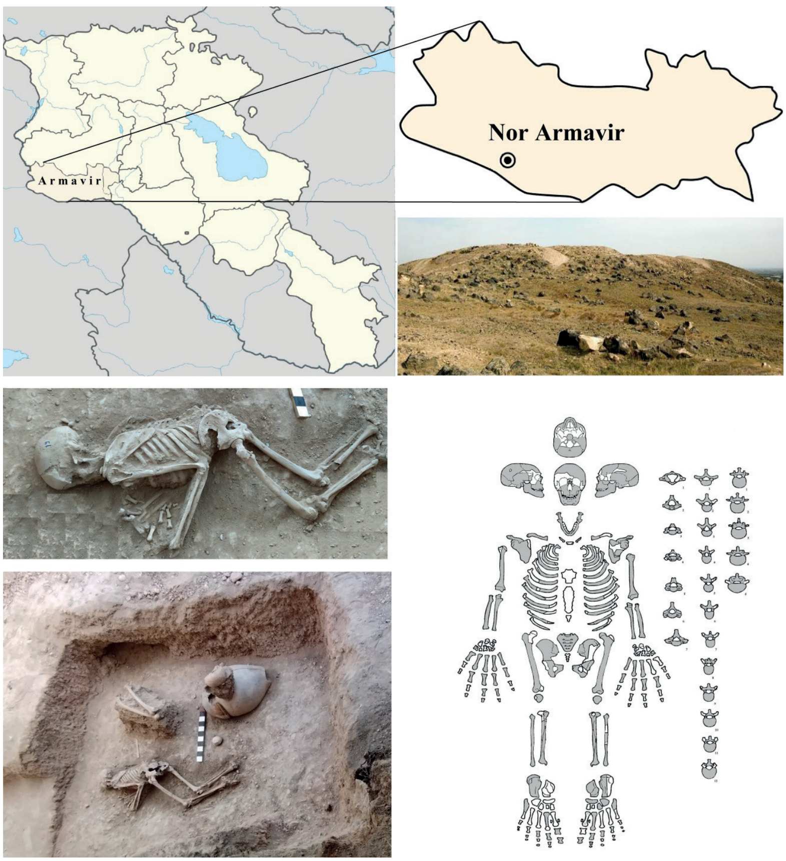

Fig. 2. Map showing the location of Armavir province (Armenia) where of Nor Armavir monument is located. Burial 19 (skeleton location). Diagrammatic illustration of the skeleton of No. 19. Missing parts are intentionally left blank; preserved ones were marked in gray

Evidence from the skeletal remains of these inhabitants of Armenia can add to our understanding of their lives. Here, we describe the remains of an adult skeleton with spinal anomalies. The study of human biological material from archaeological contexts is in a key position to facilitate cross-disciplinary research, to understand the past and present populations through ancient remains, and to make substantial theoretical contributions to the broader social sciences (Larsen 1997; Buikstra and Beck 2006).

Material and methods

Burial 19 from Nor Armavir monument contained a partially complete but well-preserved (grade 1 according to McKinley et al. 2004) skeleton of an individual (Fig. 2). The remains in burial no. 19 were lying in an unnaturally bent position with the arms and head clearly pulled down and the head turned to the west. The lumbar spine and hips were elevated. A small bronze necklace was found around his neck.

Sex and age at death were estimated using standard anthropological methods. Sex was determined based on cranial and pelvic morphology (Phenice 1969; Buikstra and Ubelaker 1994). Age at death was determined based on the degree of obliteration of the cranial sutures (Meindl and Lovejoy 1985; Buikstra and Ubelaker 1994) and dental wear (Cox and Mays 2000; AlQahtani et al. 2010). The stature was reconstructed on the basis of the long bones after Trotter and Gleser (1958).

3D computed microtomography (MicroCT) was used at the Institute of Applied Problems of Physics of NAS RA to assess the condition of intervertebral discs. Computed tomography allowed high-resolution (55 micrometers) observation and analysis of the condition of the intervertebral discs, which was simultaneously used to generate virtual 3D reconstructions. This method allows for conducting a detailed topographic analysis of the texture of the vertebral different slices, edge morphology, fracture angles and bone deformation.

Results

The remains from the burial belonged to a man aged 40–49 years (Fig. 2). The metric variables and the values measured on the hip bones are shown in Table 1. Pathological lesions are located exclusively in the spine (Fig. 3), particularly in the lumbar region. He was found to have a severe pathology – fusion of several lumbar vertebrae (L2–L5). The vertebrae of this segment had been deformed and fused together during the individual's lifetime. The bodies show both osteoblastic and osteoclastic activity (Fig. 3).

Tab. 1. Postcranial measurements of skeleton

		Right	Left
	Humerus		
1	Maximal length	326	331
2	Total length	322	329
3	Upper epiphysis breadth	–	53.2
4	Maximal midshaft breadth	63.2	64.2
5	Largest diameter Ø of the middle diaphysis	23.8	24.6
6	Smallest Ø of the middle diaphysis	20	19.9

		Right	Left
	Humerus		
7	Minimal midshaft breadth	65	65.5
7a	Midshaft circumference	70	72
7:1	Robusticity index	19.94	19.8
6:5	Cross-section index	84.1	80.9
	Radius		
1	Maximal length	253	252
2	Physiological length	242	242
4	Cross-section diameter	12.2	12.8
5	Sagittal shaft diameter	16	14
3	Minimal shaft circumference	40	39.5
3:2	Robusticity index	16.6	16.4
5:4	Cross-section index	131.2	109.4
	Ulna		
1	Maximal length	277	–
2	Physiological length	243	238
11	Sagittal diameter	14.7	13.8
12	Transverse diameter	16.5	18
13	Upper transverse diameter	21.6	21.7
14	Upper sagittal diameter	24	26.8
3	Minimal shaft circumference	36.5	36
3:2	Robusticity index	15.1	15.2
11:12	Cross-section index	89.1	76.7
13:14	Platyleny index	90	80.98
	Femur		
1	Maximal length	–	461
2	Natural length	–	452
21	Condylar breadth	–	85
6	Sagittal diameter of midshaft	31.7	32
7	Transverse midshaft diameter	27.8	28
9	Upper transverse shaft diameter	33	35.7
10	Upper sagittal shaft diameter	26.5	27.5
8	Midshaft circumference	93	95
8:2	Robusticity index	–	21.1
6:7	Pilastry index	114.1	114.3
10:9	Platymery index	80.4	77.1
	Tibia		
1	Full length	–	370
2	Condylo-talar length	–	350
1a	Maximal length	–	371.5
5	Upper epiphysis breadth	–	83

		Right	Left
	Tibia		
6	Lower epiphysis breadth	51.5	49.4
8	Sagittal diameter at midshaft level	33	34
8a	Sagittal diameter at the nutrient foramen level	39	41.3
9	Transverse diameter at midshaft level	20	21
9a	Transverse diameter at the nutrient foramen level	23	25.5
10	Midshaft circumference	85	90
10b	Minimal shaft circumference	77	77.5
9:8	Cross-section index	60.7	61.8
10b:1	Robusticity index	–	20.95
9a:8a	Cross-section index	58.98	61.8
10:1	Robusticity index	–	24.4
	Fibula		
1	Maximum length	–	357
	Skeletal proportions		
R1:H1	Brachial index	77.7	76.2
T1:F2	Tibio-femoral index	–	81.9
H1+R1/F1+T1	Intermembral index	–	70.2
H1+R1/ F2+T1	Intermembral index	–	70.93
H1:F2	Humero-femoralindex	–	73.3
R1:T1	Radio-tibial index	–	68.2
	Body length	166.3	

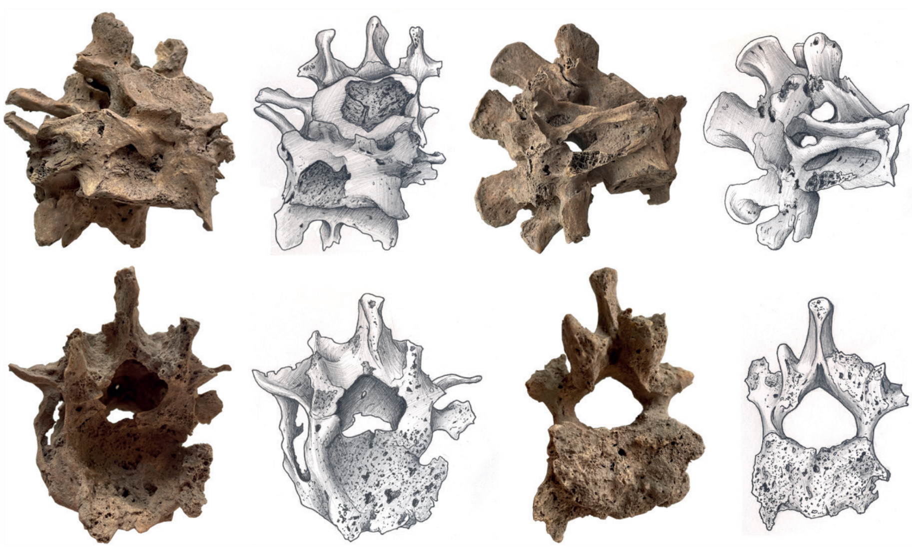

Fig. 3. Pathological lesions located in the spine. The destruction of the L2, L3, L4, and L5 bodies resulted in the sharp angular kyphosis in the upper lumbar region typical of the Pott's disease

The intervertebral discs of the lumbar vertebrae (L2–L5) have lost their basic properties (elasticity and firmness) and have undergone decay. On direct projection images, the height of the intervertebral disc is reduced unevenly; in lateral projection, the discs are compressed anteriorly more than posteriorly, as the anterior parts of the cartilage are loaded and destroyed more than the posterior ones. There was involvement of adjacent vertebrae in the inflammatory process and formation of destruction of adjacent closure plates. The images show foci of destruction in the vertebral bodies (Fig. 3). During the destruction, the vertebral bodies were, to a large extent, destroyed and currently represent a large number of chaotically arranged dense bone fragments. The peripheral parts of the vertebral body are "eaten away" with sequestrum formation in caries-type destruction. This occurred as a result of the spread of the tuberculous granuloma under the ligamentous (under the anterior or posterior longitudinal ligament) from the primary destroyed vertebra to the neighboring vertebrae or through the disc in the area of the pulposus nucleus with subsequent destruction of the entire intervertebral disc.

The sequestrum has a rounded shape and looks like a "melting sugar cube" (Fig. 3). On the image, the sequestrum has a heterogeneous structure. The affected vertebral bodies wedge into each other, with the formation of an angular bend of the spinal axis with the apex pointing backwards (hump), which leads to irreversible deformation of the spine. The size of the hump depends on the number of vertebrae destroyed, and its presence is the most characteristic sign of tuberculous spondylitis. Defects in vertebral bodies are partially filled with newly formed bone trabeculae. Characteristic is irreversible deformation of the spine, pronounced degenerative-dystrophic changes in bone tissue.

Due to the application of technical features, the multi-slice X-ray tomography (3d) enables detecting all changes more clearly compared to standard radiography of the spine (Fig. 4). Figure 4 shows a cross-section of the spine with some adjacent vertebrae attached. The use of a MicroCT scan of the relevant area of the spine allowed us to look at the ankylosis. Upon external examination, the bodies of these vertebrae were not clearly visualized as part of the deformity, which is probably due to osteomalacia (destruction, fusion) of their larger part and further formation of secondary bone callus. MicroCT allowed us to detect the mass resulting from osteomalacia of the bodies of the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} lumbar vertebrae, the nature of the fusion of the bodies and what was left of them. The pathology resulted in the formation of a bone mass approximately comparable in volume to the body of a single vertebra. The deformity was fused to the bone callus anteriorly. Bone layering, fine porosity and porosity are visible on the surface of the formation. There was probably a previous haemorrhage. Increased vascularisation of the bone promoted bone callus growth (Fig. 4). There appeared to be an inflammatory process as well.

Vertebral fusion resulted not only from osteomalacia of the bodies, but also from the overgrowth of the intervertebral joints and fusion of the arches. The spinous processes are practically intact due to the development of pathology, partially destroyed only when in burial. Interestingly, the ribs were still attached to the vertebrae. Anyway, there were upper and lower rib fossae on the vertebrae.

The upper (L2) and lower (L5) body surfaces are deformed, including at the edges, and there are signs of osteochondrosis and osteoporosis. A recessed area was formed in front of the bone conglomerate with a height of 26.2 mm, width of 23 mm, and depth of 12 mm. The surface itself is rough and porous (Fig. 3). Convergence of the spinous processes to the contact between the L4–L5 spinous processes is revealed. Convergence of the spinous processes is due to lumbar hyperlordosis, which inevitably occurs to compensate for kyphotic deformity and humping of the spine.

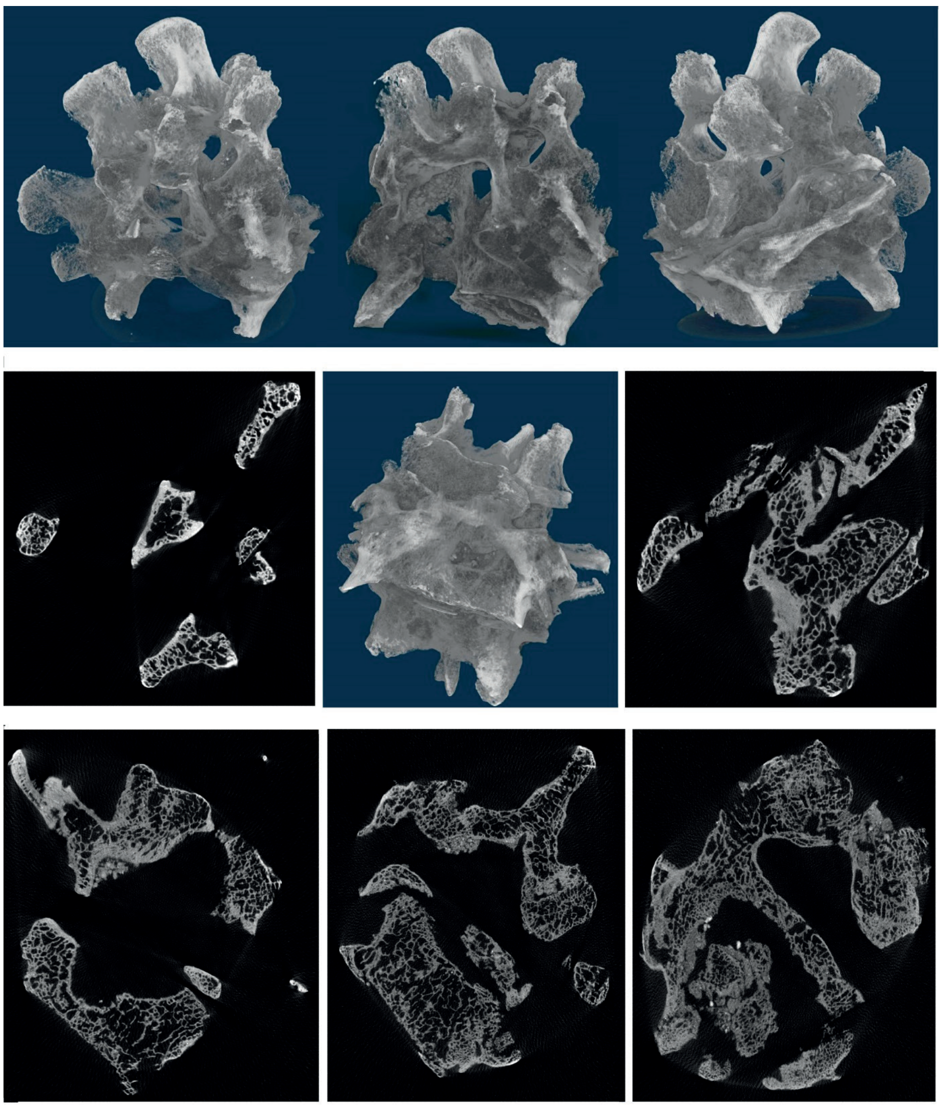

Fig. 4. Computed tomography of the fused vertebrae

The body length reconstructed from femur measurements calculated using Pearson and Lee's formulae could be 166 cm. However, in terms of the deformity of the spine, the length of the body was rather unusual.

Discussion

Tuberculous spondylitis accounts for about 40–50% of all bone tuberculosis cases. The thoracic vertebrae are affected most often (60%), while the lumbar vertebrae are less frequently affected (30%). The cervical region accounts for about 5–9%. The lower thoracic and upper lumbar regions are particularly susceptible to tuberculosis (Krasnobaev 1950; Kornev 1953; Talantov 1961; Mushkin et al. 2012; Ratobylsky et al. 2012). Men and people of young age are more prone to getting the disease. Among individuals with tuberculous spondylitis, 66% are children under 10 years of age, and 83.3% are individuals under 20 years of age (Kornev 1959; Gratsiansky and Khokhlov 1966).

The earliest case of tuberculous spondylitis dates back to the Neolithic (Bartels 1907; Baker et al. 2015, 2017). Palaeopathological lesions reminiscent of skeletal tuberculous spondylitis have been observed in northern Syria (Dja'de el Mughara, 8800–8300 BC). Typical lesions are found on the 9th and 10th thoracic vertebrae (Dja'de el Mughara). They are mainly lytic. The lower part of the 9th thoracic vertebra is completely destroyed, and the upper part of the 10th thoracic vertebra shows cystic rounded cavitations extending to the vertebral body, with a space-occupying mass aspect (Baker et al. 2015). In the site of Atlit Yam, the remains of an adult female and an immature individual presented paleopathological evidence of TB, confirmed by lipid biomarkers and aDNA analyses (Hershkovitz et al. 2008). In the same geographic area, paleopathological evidences of tuberculosis were previously mentioned for contemporaneous site (Ain Ghazal, ca. 7250 BC) (El-Najjar et al. 1996). In Egypt, in excavations of burials near the Nile River, 4 out of 10 skeletons (3000 BC) had signs of tuberculous spondylitis. E.G. Smith and W.R. Dawson (1924) provide a photograph of a mummy with tuberculous spondylitis and marked kyphosis of the thoracic spine. This individual lived in Egypt in the 11th to 10th century BC. D.G. Rokhlin and V.S. Maikova-Stroganova describe 5 cases of spinal tuberculosis in adults whose skeletons were found in Russia in burials from the last centuries BC and early AD (Rokhlin 1965; Galinskaya 2013). A skeleton of a man with tuberculous spondylitis was found in burial No. 2 of the Vaskovichi cemetery, Belarus (late 19th century) during the archaeological excavations (Vasiliev et al. 2022). The young man was lying on his back with his arms visibly slumped down and his spine unnaturally curved around his lower back. The case of subligamentous tuberculous spondylitis, a rare form of extrapulmonary tuberculosis, was diagnosed in the skeleton of a middle-aged man found in the ossuary of the Franciscan crypt of the church of Saints Anthony and Eusebius in north-western Italy (Larentis et al. 2020). The skeleton can be dated between the 17th and 19th centuries. Frequent cases of tuberculous spondylitis have been found in Armenia (Khudaverdyan 2005), Georgia (Pirpilashvili 1956), the Baltics (Darums 1970; Jankauskas 1998), Hungary (Nemeskery and Harsanyi 1959; Hlavenková et al. 2015), and Slovakia (Hanakova and Stloukal 1966; Kyselicová et al. 2015). The case of tuberculous spondylitis was present in Transylvania (Romania) during the 12th and 13th centuries. The paleopathological diagnosis was supported by M. tuberculosis analysis for complex ancient DNA (Hajdu et al. 2012).

Tuberculous spondylitis, found in an individual from Nor Armavir Monument, is a long, drawn-out disease that last for years. The rapidity of the development of this process may have depended on the local and general immunity of the individual, lack of treatment and physical stress on the spine. Due to the lack of any changes in the facet joints, it does not seem that vertebral collapse did occur, in spite of the important lytic destruction of the 4 vertebral bodies. This pattern is not commonly described in modern clinical practice but can be seen by medical imaging on living patients (Baba et al. 2013), and on skeletal materials (Pálfi et al. 2012; Baker et al. 2015). A typical sign of tuberculous spondylitis is deep, often subtotal contact destruction of the closure plates, which is an important differential diagnostic criterion. As we know, patients with tuberculous spondylitis stop walking because of pain, and with neurological insufficiency – because of weakness in the legs. They are characterized by sharp pain on percussion (latin percussio) of the spinous processes of the affected vertebrae, and on loading along the axis of the spine, there is the restriction of mobility in the damaged spine, its deformation. Under the skin, a thickening is formed, and then a bulge, the skin over which at first is not changed, but then as a result of the inflammatory process fuses with it, the abscess bursts outwards with the formation of a fistula, through which pus and necrotic masses are discharged. Individuals experience a change in gait: when walking, there is a slight leaning forward and towards the localization of the abscess. The development of the hump is caused by the destruction of vertebral bodies, accompanied by their flattening with wedge-shaped deformation of the destroyed vertebral bodies; the spinous process of the destroyed vertebrae protrudes most sharply and is located at the top of the hump, which has the shape of an angle. The size of the hump depends on the number of vertebrae destroyed, and its presence is the most characteristic sign of tuberculous spondylitis.

We have used diagnostic criteria derived from both the paleopathological and clinical literature.

A) The probable cause of this pathology was fractures of the bodies of the 2nd, 3rd, 4th and 5th lumbar vertebrae, fusion of the resulting bone mass, leading to a kyphosis bone conglomerate in the lumbar region. If there was trauma, which is the most likely scenario, the spinal cord and spinal nerves would have been damaged as well. In the case of trauma, this is determined by the migration of bone fragments into the spinal canal, canal occlusion, and in the case of purulent-inflammatory lesions – the development of an epidural abscess or myelitis (inflammation of the spinal cord). The formation of this pathology (resulting from a fracture) in the individual from Nor Armavir would be incompatible with life, primarily due to the formation of bone fragments and damage to the main vessels. Similarly, fractures caused by traumatic events rarely involve two or more vertebrae and, most importantly, they do not usually lead to osteoclastic activity, which is clearly evident in our individual case. In fact, osteoclastic activity is usually associated with the necrosis of isolated bone elements in a complex trauma, such as a comminuted fracture, which is not visible on a CT scan (Fig. 4). Because the processes leading to callus formation result in osteoblastic activity, the presence of mild and severe areas of lytic lesions in our individual allows us to easily rule out trauma as the cause of our findings (Lovell 1997).

B) Among inflammatory diseases, haematogenous osteomyelitis shares the greatest similarity to tuberculous spondylitis of the spine (Musaev 2000; Tikhodeev and Vishnevsky 2004; Nene and Bhojraj 2005). The most common causative agent of osteomyelitis is *Staphylococcus aureus*. In most cases, non-specific spondylitis is formed due to the haematogenous spread of the pathogen from the infectious focus, complicated by bacteremia. Common sources of infection include genitourinary tract, leg thrombophlebitis with chronic shin ulcers, pneumonia, bronchiectatic disease, streptoderma, furunculosis, tonsillitis, odontogenic infection (Mwachaka et al. 2011).

In addition to the haematogenous spread of infection, the venous route through the pelvic and vertebral plexus to the sacrum is well-known lumbar and lower thoracic vertebrae – for thrombophlebitis, paraproctitis, haemorrhoids, purulent processes in the pelvis and along the pharyngovertebral venous plexuses of the posterior surface of the pharynx to the upper and middle cervical vertebrae – in case of oral cavity infection, tonsillitis (Guseva et al. 2006). Pre-existing traumatic or dystrophic lesions of discs, joints, vertebral bodies, or processes may contribute to osteomyelitis at a particular level. That is, osteomyelitis of the spine is characterized by association with a past inflammatory disease, trauma, etc. (Ivanov et al. 2003). It is worth noting that sequestrations and abscesses in osteomyelitis are less common than in tuberculosis. Spinal deformity is less pronounced or absent in osteomyelitis. Unlike tuberculous spondylitis, osteomyelitis more often affects the posterior aspect of the vertebrae: the wishbones and transverse processes. Therefore, the diagnosis made for the individual from Nor Armavir is correct.

C) Syphilitic spondylitis results from the passage of syphilitic ulceration of the mucous membrane of the mouth and pharynx to the upper cervical vertebrae, where this lesion occurs predominantly. On radiological examination, bone sclerosis is typical. An individual from Nor Armavir has affected lumbar vertebrae.

D) Brucella spondylitis and tuberculous spondylitis, caused initially by bacteremia, are the two leading types of granulomatous spinal infections (Cordero and Sanchez 1991). Brucella spondylitis is easy to miss or may be misdiagnosed as tuberculous spondylitis. There was a significantly lower severity of vertebral destruction, vertebral posterior convex deformity, dead bone, and abscess scope in brucella spondylitis when compared to tuberculous spondylitis. Through the analysis of vertebra and intervertebral space, it was significantly lower in the severe vertebral destruction, vertebral posterior convex deformity, dead bone, and narrow – disappear change of intervertebral space in the patients with brucella spondylitis than those in tuberculous spondylitis (Yang et al. 2014; Liu et al. 2018; Guo et al. 2021).This widespread destruction in tuberculous spondylitis may result from the rapid involvement of the endplate (inflammatory reaction). With the progress of tuberculous spondylitis, gradually the vertebrae were severely destroyed. Our study found that the vertebral destruction in the individual from Nor Armavir was more severe. The vertebral erosion in tuberculous spondylitis was caseating granulomas and dead bone without new bone formation (Sapico and Montgomerie 1979).

E) Post-typhoid spondylitis usually occurs about a month after the end of typhoid fever and is localized most commonly in the lumbar spine. This disease is characterized by early, noticeable disc changes after only a few weeks. The intervertebral gap narrows and then completely disappears. The periosteum of the vertebral bodies is thickened and overgrown, so the lateral contours of the vertebrae also appear altered. It is not uncommon for bony brackets to form, fusing vertebral bodies and ossifying ligaments. The process is usually limited to 2 vertebrae and ends in bony ankylosis. In the person from Nor Armavir, post-typhoid spondylitis was also ruled out.

F) Actinomycosis of the spine is, as a rule, a secondary lesion and occurs when the fungus passes from the primary focus, localized in the submandibular region, in the lungs, oesophagus, intestine. Quite quickly, fistulas are formed, which are preceded by a dense ("like a board") infiltrate against the background of this infiltrate and open the external fistula openings. The course of actinomycosis of the spine is long, resembling the course of tuberculous spondylitis with fistula. In radiological examination, focal destruction in the form of small patterns combined with sclerotic areas, moderate narrowing of the intervertebral gap corresponding to the lesion of the vertebral bodies, and development of ossification of ligaments in the form of "brackets" are typical. Spinal actinomycosis has also been ruled out in an individual from Nor Armavir.

G) Among the many non-tuberculous lesions, malformations, acquired deformities, neuralgia, lumbalgia, dystrophic and oncological diseases may be present. The malformations that may give rise to suspicion of tuberculous spondylitis are manifested mainly externally in the form of scoliosis, kyphoscoliosis and other spinal deformities associated with changes in the number and shape of the vertebrae and their relationship to each other and to other bones such as the skull, pelvis and ribs. These malformations are noticed in early childhood as soon as the child begins to sit up. In the beginning, they do not give any painful sensation, or any disturbance in the mobility of the spine, but later there is pain due to disturbance of the normal statics. Wedge-shaped vertebrae, or rather semi-vertebrae, are the most common developmental anomaly and cause of congenital scoliosis with a slight angular curvature, but without rotation around the axis (torsio), as in normal scoliosis. Non-inflammatory spinal lesions were also excluded in the individual from Nor Armavir.

H) Infectious arthritis is an acute or chronic inflammation that occurs in the joint bag due to bacterial or viral infection. The cause of arthritis is usually echinococcosis, which affects the bone tissue of the vertebrae, pelvic bones, and long bones of the limbs. Rheumatoid arthritis typically affects the periarticular area of the hands and joints (Grassi et al. 1998; Tesi et al. 2019). Reactive arthritis is characterized by peripheral arthritis, enthesopathy and asymmetric involvement of the sacroiliac joint, feet, heels, knees and ankles (Rogers and Waldron 1995; Carter and Hudson 2009). Psoriatic arthritis usually affects asymmetrically hands and feet, leading to a characteristic appearance of the disease in the phalanges of the hands and feet, known as "pencil-in-cup" (Rogers et al. 1987; Gladman 2005). Infectious arthritis, rheumatoid arthritis, reactive arthritis, psoriatic arthritis have also been excluded in the individual from Nor Armavir.

Conclusions

In this study, we presented a new probable case of tuberculous spondylitis. The pathology studied occurred long before the death of the individual who had been alive for years with that fused spine. Most probably, this deformity occurred because of chronic disease lasting for months or years, as evidenced by the vertebral fusion and bone callus formation. Such course of the disease is characteristic of tuberculous spondylitis. With such pathology of the spine, the man, of course, could not live a full life.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

The study was designed and conceived by AYK1. Fieldwork was carried out by SGH1, NGT1 and MSH1. 3D MicroCT was carried out by APA4 and VRK4. Analysis and interpretation were conducted by AYK1, AAY2, SAV3. The manuscript was written by AYK1 and VRK4.

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