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Morpho-facial variations in physical features of two tribal populations of Kargil (Ladakh, India): A bio-anthropological investigation

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Abstract: Somatoscopy is a systematic and cumulative visual examination of the morphological features of an individual. Physical anthropologists have classified humans into certain specific groups on the basis of specific morphoscopical features, and such variations have been widely studied. Fourteen somatoscopic traits of 800 adult (>25years) Ladakhi individuals, belonging to two Kargil (India) tribal groups (Brokpas and Purigpas) were examined; four hundred (N=400) healthy individuals from each tribe i.e., 221 males and 179 females of the Brokpa tribe and 210 males and 190 females of the Purigpa tribe, comprised the present study sample. Statistically significant differences were noticed between the Brokpas and the Purigpas with respect to the frequencies distribution of their skin colour, hair form, facial contour/profile, nasal types and presence/absence of epicanthic fold, prognathism, Darwin's tubercle, Adam's apple, scaphoid, attached ear lobe etc. The Brokpas exhibited significant sex differences in skin colour, eye colour, hair form, nasal septum, nasal tip, epicanthic fold, ear lobe, and Adam apple, whereas only skin colour, eye colour, ear lobe attachment, hair form, and prognathism were found significantly different in the two sexes of Purigpas. The morphological variation and sexual dimorphism in the human physical features of the two Ladakhi tribes will add to the existing knowledge regarding the anthropological characteristics of different ethnic groups of India. The differences in their morphological traits may be due to the differences in their genetic adaptations as the two tribal groups originated from two different ancient populations i.e., the Brokpas are of the European origin and the Purigpas are the descendants of the Tibeto-Mongoloids. The results of this study, however, need to be supplemented with a compressive investigation to confirm the heterogeneity in the morphological and genetic features of the two tribal groups of Ladakh (India) and the influence of differential ancestral migrations on the facial features of the individuals of the two tribal groups.

Key words: Morphological variations, somatoscopic features, Brokpas and Purigpas, Ladakh (India).

Original article

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Introduction

Human physical appearance is characterised by a substantial variation in different physiognomic facial features, such as height, skin colour as well as shape of nose, ear, lips. This variation has been explained in terms of biological adaptation, genetic fluctuation, environmental differences and sexual selection. Although it is widely believed that all humans belong to a single species, a considerable variation in various human physical features is observed among individuals belonging to different populations. Investigating this variation has remained a major topic in physical anthropology. Based on the differences and similarities in morphological and physical features, physical anthropologists have divided humans into distinctive ethnic groups. Documenting the vast range of human variability in the historical as well as present populations and identifying the evolutionary and environmental forces responsible for such variation among geographic populations has remained a major objective in many anthropological studies. Wade (2014) suggested that populations of each continent have adapted to their regional environment, and therefore, have developed independently of each other. Thus, human evolutionary history has led to a development of distinctive groups of people exhibiting diverse morphological features around the globe.

Human physiognomic variation, which varies from place to place due to the multiple geographical, genetic and nutritional factors (John 2003), has remained one of the most fascinating research topics in anthropology. The *Homo sapiens* species comprises several varieties of population groups that inhabit different geographical niches around the globe. Human morphology and its diversity is determined by a collective influence of different genetic processes (mutation, isolation, hybridization) as well as environmental, social, climate and nutritional factors. The adaptive fitness attributed to some genetic traits and their interactions with specific environmental factors determines, to a great extent, the morphological features of an individual. For example, skin colour is an adaptation to a specific environment. Human morphological variation helps to better understand an intricate relationship between structural forms and functions of various parts of the human body. The literature search has revealed that the interest in human biological and cultural variation arose in the 18th and 19th century, following colonial expansion during which Europeans intermixed with non-European populations around the globe (Sundquist 2008).

Somatoscopy is a visual, systematic and cumulative observation of the morphological features of an individual. The external physical characteristics are phenotypically visible variables which are mostly adaptive, but the internal physical characteristics are genotypic characters, which are strictly hereditary and non- adaptive (Jurmain et al. 2013). Historically, individuals of different world populations have been identified on the basis of pigmentation (such as colour of skin, hair, and eyes), shape of the skull, stature, nose and other morphological characteristics. The variation in the colour of hair, skin, and eyes is mainly due to differences in the amount and distribution of melanin pigment. The content of melanin pigment is comparatively more common amongst individuals inhabiting regions near the equator and less common among those living away from equator. The skin pigmentation shows a strong correlation with the ultraviolet radiations intensity, suggesting that the differences in skin pigmentation may result from an adaptation to a particular level of ultraviolet radiation through natural selection (Blum 1961; Loomis 1967; Walter 1971; Branda and Eaton 1978; Kollias et al. 1991; Jablonski and Chaplin 2000).

Skin colour is the primary characteristic that attributes individuals of different ethnic and geographical groups to a certain specific area. Skin colour is not uniform even all over the body of an individual due to various physiological (e.g., arterial or the venous blood supply) and environmental (e.g., exposure to the sunlight) factors that relate to the expression of skin colour. Human ear is also an amazing feature of the face and its size and shape are influenced by age, sex and ethnic origin of an individual (Kalla 1973; Aoki 2002). The shape, size, and orientation of the ear pinna are as unique as fingerprints, and it has been found that men tend to have a larger ear compared to women. The shape of an ear also varies with age as its size increases in both its length and breadth with advancing age; ear size also differs depending on the ethnic group that an individual belongs to (Alexander et al. 2011). Kalcioglu et al. (2003) studied the growth patterns of external ear in different ethnic groups and concluded that growth patterns of the ear are influenced by environmental, genetic and nutritional factors. An ear is an important feature that facilitates identification in forensic anthropology (Neimitz et al. 2007). Human ear is the most defining feature of a face as no two individuals are believed to have identical ear features (Bertillion 1893). Many studies that have used different ear landmarks, such as the length and the breath of ear, concha length, lobule height and attachment of the ear lobule to the external ear, have been quantified with varied accuracy levels and used for human identification (Snell 2004; Standing et al. 2008; Moore and Dalley 2006, 1999). Probably due to the unique variance among individuals, left ear prints are commonly found on the doors, window and glasses (Champod et al. 2001; Egan 1999; Hoogstrate et al. 2001; Lammi 2003; Moenssens 1995).

Ladakh is located at latitude of 32° 15′ to 36° 0′ North and 75° 15′ to 80° 15′ east longitude, and covers 2/3rd area of erstwhile state of Jammu and Kashmir. Kargil is a mountainous region of Ladakh, UT (India), situated at a height of 8780 feet above the sea level with a latitude of 34º33′N and 76º33′E longitude. Ladakh receives very low levels of rainfall, with an average rainfall of 15 inches, and it experiences severe cold in winters. Ladakh, the land of high passes and genetic heterogeneity, is a high altitude desert located at the western tip of Tibetan plateau (more than 3000 meters, on average), and is wedged between two mountain ranges i.e., the Kunlun in the north and the Himalayas in the south (Jina 1996). The Union Territory of Ladakh (in northernmost part of India) presents a unique geographic territory with a wide variety of landscapes ranging from plains to high altitudes. The Union Territory of Ladakh represents a conglomerate of multiple human physical features, cultures, languages and genetic diversity (Singh et al. 2020; Rowold et al. 2016). The population of Ladakh offers a unique human laboratory to study the demographic anthropological history as well as genetic diversity contemporary human population groups in India (Singh et al. 2020). Ladakh inhabitants are exposed to very harsh weather conditions throughout the year and it is considered one of the last region inhabited by prehistoric humans. Genetic studies (based on the patrilineal markers of Y-STRs and Y-SNPs) have found Ladakh population as a diverse genetic mosaic, owing to multiple contributors from the past migratory events. Singh et al. (2020) studied the mitochondrial genome of Ladhakis based on controlled region analysis and revealed that 'M9' haplogroup was the most abundant mitochondrial marker. Though both of the studied population groups (the Brokpa and the Purigpa) share a common geographic affinity/territory; their ancestral origins, migration history and settlement in India are vastly different, probably due to the differential demographic movements (Rowold et al. 2016). Because Ladakh is located in a strategic location in the Himalayan region, it has been used as a corridor for the historical trade routes, such as the ancient silk route (UNESCO 2010; Sharma et al. 2018). Nevertheless, Ladakh remains under-represented in the phylogenetic literature. Anthropologically, Ladakh is of a great importance and many scholars feel that Ladakh was a separate entity and was settled by the Mons and Dards of the Aryan race (Tsering 2012). The people of Ladakh consist of various ethnic groups, such as the Brokpa or Dard, the Balti, the Purigpa, the Beda, the Mon, the Garra, the Changpa and the Boto, which are mixture of Indo-Aryan and Tibetan-Mongoloid races (Jina 1996). These ethnics groups are considered as schedule tribes under Article 342 in The Constitution of India, and schedule tribe order of Jammu and Kashmir constitution (1982).

The Ladakhi language has both Mongolian and Indo-Iranian components (Jina 2001; Wirth et al. 2004). It has been argued that the original population of Ladakhi may have been Dard who colonised the western Himalayas via the Indus valley (Jina 2001). However, over 1000 years ago the Dard culture was overwhelmed by immigration of shepherds and nomads from Tibet. In the present day Ladakh, the majority of Muslims are the descendants of Sufi masters from Pakistan who settled in Ladakh after 14th century while the Buddhists are primarily the descendants of Mongolians who bear a close affinity with the Tibetans (Kaul and Kaul 1992; Srinivas 1998).

The main aim of this study is to determine differences and similarities in morpho-facial features of the two tribes that inhabit the same geographical region and are exposed to similar environmental conditions (since last many generations when their ancestors supposedly coming from different routes settled here). To our knowledge, so far there has been no study looking at the affinity in morphological features of the inhabitants. Therefore, this study aims to contribute to the demographic anthropological data for Indian populations.

Material and methods

The present study was conducted on 800 adults (>25years) belonging to two tribes of Kargil (Ladakh) namely the Brokpas and the Purigpas. Four hundred (N=400) healthy individuals from each tribe (221 males and 179 females of the Brokpa and 210 males and 190 females of the Purigpa) were examined and from whom 14 morphological features, using a pre-defined set of observations (Supplementary Table), were measured. Data was collected from two regions of Kargil namely the Suru valley (the Purigpas) and the Batalik sector (the Brokpas) using convenient and snowball sampling methods. The majority of study participants included in the study belonged to the age-group of 30–50 years. Somatoscopic data were collected based on observation by the naked eye with the help of a pre-defined proforma. The morphological observation techniques used in the study were based on the Fischer-Saller Scale (Sharma and Sharma 1997) and the Martin and Schultz eye colour chart (Glinka et al. 2008; Fitzpatrick 1988). Because no standards were available from the literature for other morphological features, the considered in the present study traits and the results were based on direct observations of the participants and were classified arbitrarily for convenience of the investigator. Because morphological features are affected by environment, nutrition and genetic factors, skin colour was measured on the forehead on each study participant, which was covered under the hairs and therefore not affected by sunlight (Fitzpatrick 1975). Before starting data collection, a well-informed written consent was obtained from each study participant, informing the participants about the aims, objectives, and purpose of the study. It was hypothesized that there were no differences in terms of the morphological features between the Brokpas and the Purigpas or between the two sexes of the two tribes independently or combinedly as they were inhabiting the same geographical inhabitation, environment, nutrition and occupations (though their ancestors came from different places/routes to settle here). To test these hypotheses, Pearson correlation coefficient was calculated using the IBM SPSS software (version 21.0) to find any correlation between the frequency distribution of occurrence of different facial features among the Brokpas and the Purigpas. The χ^2test was applied to estimate the level of significance of differences in frequency distribution of different studied features between the Brokpas and the Purigpas as well as between males and females of individual tribal groups or in combined (irrespective of sex) population of two groups, using non-parametric tests of the IBM SPSS software (version 21.0).

Ethical clearance was obtained to conduct this study from Panjab the University Institutional Ethics Committee (PUIEC) vide letter no. PUIEC/2017/66/R/06/02 dated: 21/02/2018, and "No Objection Certificate" (NOC) was obtained from the Directorate of Tribal Affairs, Government of Jammu and Kashmir, vide letter no: DTA/ESTT/2016/2043, Dated: 27/2/2017 and District Magistrate, Kargil (J&K) vide letter no: DMK/JC-Misc./2016, Dated: 03/08/2016

Results

The following observations were recorded regarding the morpho-facial physical features of two tribal groups of Kargil (Ladakh, India):

(i) Skin Colour

Individuals of the studied tribal groups (i.e., the Brokpas and the Purigpas) exhibited different skin colour; the dominant skin colour was yellowish (40.75%) and yellowish-white (38.5%) in the Brokpas and the Purigpas, respectively.

The differential distribution of various skin colour shades in the two studied groups have been graphically represented in Figure 1. The Brokpas had higher percentage of light-yellowish (19.5%) and light-brown (16.0%) and reddish-brown (14.5%) skin colouration whereas the Purigpas had more prevalence of light-brown (29.75%) or whitish-yellow (21.25%) skin colour pigmentations; such differences in different shades of skin colour between individuals of two groups were statistically significant (χ^2= 208.05; p<0.001), as well as between two sexes of the two tribes (p<0.001).

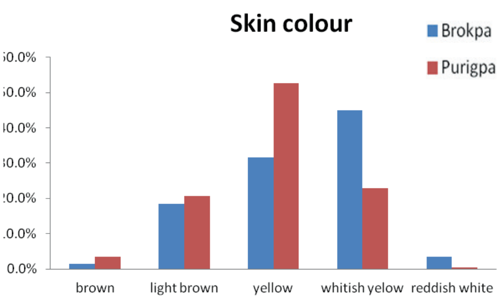

Fig. 1. Frequencies of skin colour shades among the studied Kargil population

(ii) Eye colour

Eye colour varied from black to many other shades among the studied population sub-groups of Kargil (Ladakh, UT). The most dominant eye colour was light-brown, with a frequency of 45. 5% in the Brokpas and 48.75% in the Purigpas. The next predominant eye colour was brown, with a frequency of 41.5% in the Brokpas and 26.2% in the Purigpas. In contrast, the black and the blue-brown eye colour were very rare in the Brokpa and the Purigpa individuals, respectively. The dark-brown eye colour was predominately the Purigpa eye feature whereas the blue-brown colour in the Brokpas iris feature (Figure 2). These differences in shades of eye colour were found statistically significant between individuals of the two sexes of the Brokpas (p<0.001) only. The light-brown colour was the most common (47%) among both the Brokpas and the Purigpas, followed by brown (33.8%) and dark-brown (11.4%) respectively.

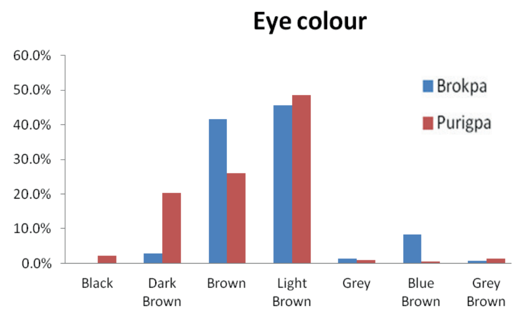

Fig. 2. Frequencies of eye colour among Brokpa and Purigpa

(iii) Hair Form

A substantial variation was found in hair forms among the Brokpas and the Purigpas. A low-waved hair was dominant in the Ladakhi individuals of both the studied population sub-groups (53.6%), followed by straight hair (33.0%), deep-waved (12.4%) and curly (1.0%) hair. The Brokpas had predominately deep-waved hairs (58.75%), followed by straight hair (31.5%) and deep-waved hair (9.75%); no curly hair has been found among the Brokpas. Hair among the Purigpas varied from straight to curly. More than 48.5% of the Purigpas had low-waved hair, followed by straight hair in 34.5%, deep-waved in 15.0% and curly in 2.0% (Figure 3). Thus, the Brokpas had predominately deep-waved hair whereas the Purigpas had more of low-waved hair while curly hair types were found only in the Purigpas. The differences in hair type were found highly significant (p<0.001) between individuals of two sexes of the studied Kargil tribes. The Brokpas had mostly thick beard and moustaches (69.9%), followed by medium (30.9.0%) or scanty beard and moustache. The majority of the Purigpas had sparsely distributed (57.6%) facial hair, followed by thick (23.3%) and scanty (19.1%) facial hair. When individuals of both tribes were considered together, it was revealed that the majority individuals had thick (46.2%) bread and moustaches, followed by medium (43.6%) and scanty (10.2%) facial hair outgrowths. The differences in beard and moustache frequencies among the Brokpas and the Purigpas were found to be statistically significant (p<0.001). The Brokpas had thickly distributed facial hairs compared to the medium facial hair among the Purigpas.

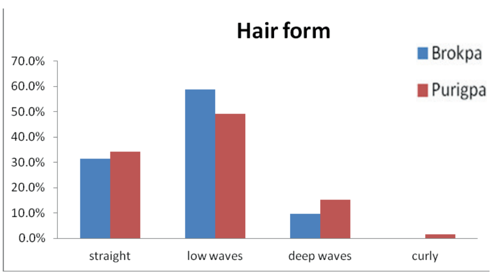

Fig. 3. Frequencies of hair forms among Brokpa and Purigpa

(iv) Nasal forms and septa

In the Brokpas, more than 47.5% participants had downward nasal septum, followed by the horizontal (44.75%) and upward 7.75%. Similarly, 65.3% of the Purigpas had a horizontal type of nasal septum, followed by the downwards (24.2%) or upwards nasal (10%) septa. These differences were statistically significant ($\chi^2 = 47.475^{**}$). Horizontal was the most predominant form of the nasal septum in the combined Kargil sample of the Brokpas and the Purigpas, followed by 35.8% individuals having downwards nasal septum (Figure 4). Only the Brokpas exhibited statistically significant sex differences in the shapes of nasal septum (p<0.002). The Brokpas had predominately sharper nasal tips (46.0%), compared to 31.2% sharp nasal tips of the Purigpas. When the two groups were combined, the majority of individuals had a medium form of nasal tip (52.6%) followed by the sharp (38.5%) nasal tips.

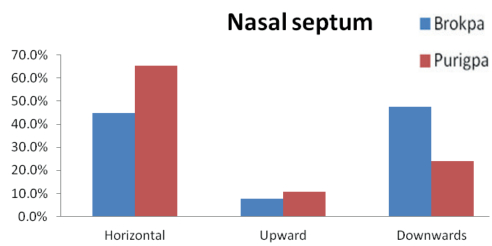

Fig. 4. Frequency distribution of nasal septum among Brokpa and Purigpa

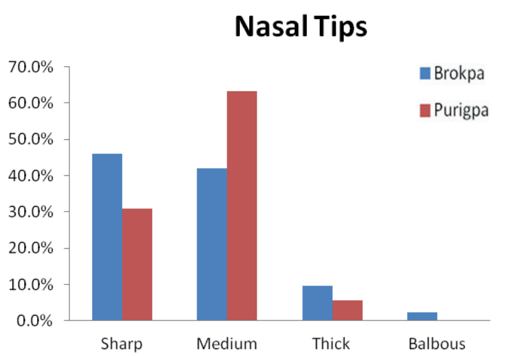

Fig. 5. Occurrence of nasal tips among Brokpa and Purigpa

(v) Lip and Chin forms

A variety of lip forms were observed in the studied population of Kargil. The thin lips were predominately noticed among the Brokpas (98.5%) and the Purigpas (73.25%) when both the males and females were considered collectively, the differences in lip forms between two tribes were found statistically significant (χ^2=103.007; p<0.001). Overall, 85.9% of the studied Kargil people had thin lips, followed by the medium (11.0%), everted (2.2%) and inverted (0.9%) lips (Figure 6).

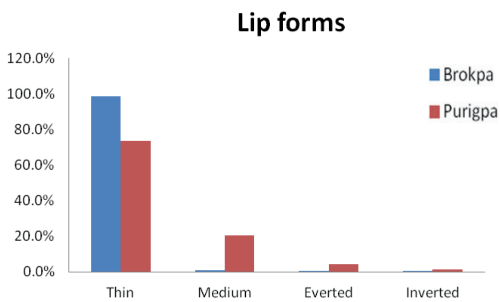

Fig. 6. Frequencies of lip forms among the studied Kargil population

When sex-specific differences were analysed among the individuals of the two tribal groups, no significant sex differences were observed in the lip forms. The chin forms were used to assess morphologic physiognomic facial features of the studied Kargil groups. When individuals of the two sexes were considered jointly, 32% of the Brokpas had a squarish chin, followed by roundish (29.75%), pointed or triangular (16.75%), oval (9.75%), elliptical (4.0%) or rectangular (7.75%) chin forms. Similarly, the majority of the Purigpas had a roundish chin shape (56.0%), followed by oblong (18.75%), oval (15.0%), squarish (9.5%) or rectangular (0.75%) chin forms. The differences in chin forms between the Brokpas and the Purigpas were statistically significant (p<0.001). Squarish chin shapes were found only in the Purigpas. In the combined studied sample, 42.9% of Ladakhis had a roundish chin, followed by squarish (20.7%), oval (12.4%), oblong (11.4%), triangular (8.4%) or rectangular (4.2%) chin forms**.**

(vi) Epicanthic Eye fold

The presence of the epicanthic eye fold varied greatly among the Brokpas and the Purigpas, probably due to admixture of different ethnic groups/elements in the Ladakh population. About half of the Brokpas (47.5%) had a partial epicanthic fold and 9.0% had a complete epicanthic fold, while the remaining had no presence of any such fold on their eyes. On the other hand, 43% of the Purigpas had a complete epicanthic fold, while 41.75% had a partial fold (Figure 7).

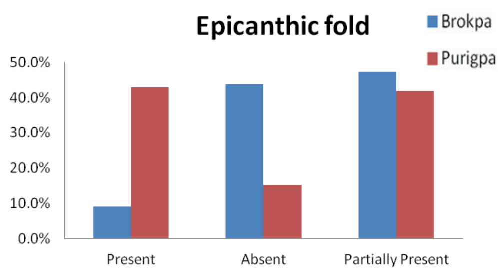

Fig. 7. Occurrence of epicanthic fold among Kargil population

Thus, the Brokpas had predominantly a partial skinfold whereas most of the Purigpas had a complete epicanthic fold. The differences in the frequency distribution of epicanthic fold between the Brokpas and the Purigpas were statistically significant (χ^2 = 145.043; p<0.001). When the individuals of two sexes of both the Brokpas and the Purigpas were considered jointly, a complete epicanthic fold was present only in 26.0% of the Kargil individuals, partial fold in 44.5%, and about 29.5% of study participants were lacking an epicanthic fold; thus a distinct predominance of partial epicanthic fold in the studied tribes. Sex-specific differences in the frequency of eye-folds were statistically significant (χ^2 = 13.230; $p<0.01$) in the Brokpas only.

(vii) Prognathism

The Brokpas and the Purigpas showed three stages of prognathism i.e., marked, medium or slight prognathism. Majority of the Brokpas had marked (57.5%) or medium (39.25%) form of prognathism; 54% prognathism in a lower jaw and 46% in an upper jaw. Similarly, 35.0% of the Purigpas had a marked protrusion of the jaw, followed by 57.75% having medium or slight (7.25%) degree of prognathism (Figure 8). The upper jaw was more prognathic (60.75%) in the Purigpas than the prevalence of the lower jaw (39.25%) in the Brokpas; thus, mandibular prognathism was a Brokpa and maxillary prognathism a Purigpa characteristic. The Brokpas had comparatively a more prognathic face compared to the Purigpas; the differences in the degrees of prognathism between two tribes were statistically significant (χ^2= 41.64; $p<0.001$). The differences in the degrees of prognathism relative to the jaw (upper and lower) were also statistically significant. In the combined sample (i.e., when the individuals of both sexes of two population groups were considered jointly), the majority of the Ladakhi had medium prognathism (48.5%), followed by marked (46.25%) prognathism. In addition, maxillary prognathism (53.4%) was more frequent than the mandibular prognathism (46.6%) in the combined population of two sexes of two tribes. Statistically significant sexual differences were found in prognathic features of the Brokpas and the Purigpas ($p<0.001$).

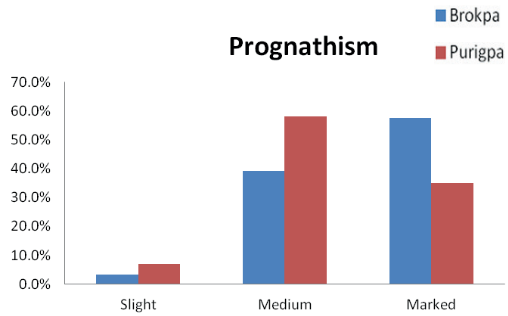

Fig. 8. Occurrence of prognathism among Kargil population

(viii) Ear Lobules

The Brokpas had a higher frequency of free ear lobule (72%) compared to the Purigpas (33.5%) and such differences were statistically significant ($p<0.001$). In the Brokpas, the predominant shape of the lobule was roundish (43.0%,) followed by tongue shaped (25.75%), arched (25.5%), triangular (5.25%) and rectangular (0.5%) ear lobes in combined sample of both the sexes. The tongue-shaped ear lobe was predominant in the Purigpas (38.25%,) followed by the arched (22.75%), triangular (19.75%) and round (19.25%) shaped ear lobes in both the Purigpas males and females (Figure 9). When all individuals of both the sub-population group were considered jointly, the majority of study participants exhibited tongue shaped ear lobes (32.0%), followed by round (31.13%), arched (24.12%), triangular (12.5%) and rectangular (0.25%). When chi-square test was applied to determine whether ear lobe shape differed between the two sexes of the Brokpas and the Purigpas, these differences were statistically insignificant (for Brokpas, χ^2=4.777; p=0.311, and for Purigpas, χ^2=2.028; p=0.731).

Ear attachment is also one of the defining features of the face and occurrence of this ethnic trait vary among different population groups. In this study, it was found that the majority of the Brokpa individuals had free or detached ear lobe (66.5%) and it was attached in the rest (33.5%) individuals of both the sexes. Similarly, 61.3% of the Purigpas had attached ear-lobe and in 38.7% individuals it was free or detached in the joint sample of both ethnic groups regardless of sex. When the individuals of both sexes of the Brokpas and the Purigpas were analysed together, it was found that 52.63% of study participants had free or detached and 47.37% had attached ear lobes in individuals of both the sexes. Statistically significant sexual differences were found in attachment or detachment of ear-lobe in the Purigpas and no such differences were found among the Brokpas.

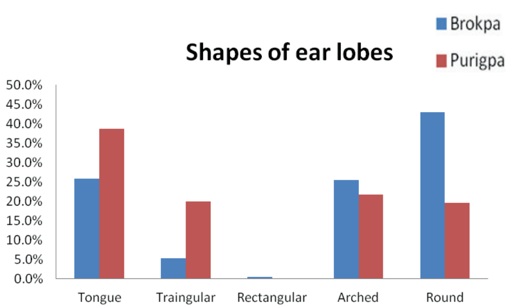

Fig. 9. Shapes of ear lobes among Kargil population

(ix) Darwin Tubercle and Adam's Apple

The majority of the Brokpas (94.3%) did not have the Darwin tubercle whereas the 34.9% of the Purigpas had this trait. Thus, more Purigpas were having Darwin tubercle than the Brokpas. In the combined population of two tribes, only 20.1% of individuals had this trait and remaining 79.9% were not having this trait and such differences in frequency distribution of Darwin tubercle between the two tribal groups were statistically significant (χ^2=104.828; p<0.001). The differences in frequency distribution of Darwin tubercle between two sexes of the Brokpas and the Purigpas were not statistically significant (Figure 10). The Adam apple was found distinctly visible in 40% of the Brokpas and 20.5% of the Purigpas, and it was mildly visible in 39% of the Brokpas and 32% of the Purigpas. A higher percentage of Purigpas had a complete absence of this morphological trait compared to the Brokpas. In the total population of both the tribes, it was distinctly visible among 30.25% individuals, mildly present among 35.5% and was absent in 34.25% individuals (Figure 11). Statistically significant differences were found in the occurrence of Adam's apple between the Brokpas and the Purigpas (χ^2 =70.214' p<0.001) as well as between two sexes of each Kargil tribe considered (χ^2=168.961 for the Brokpas and χ^2=76.964 for the Purigpas; p<0.001).

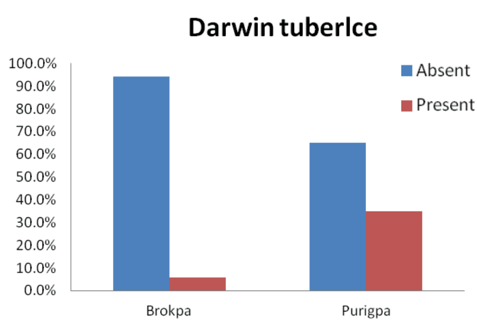

Fig. 10. Occurrence of Darwin tubercle among Brokpa and Purigpa

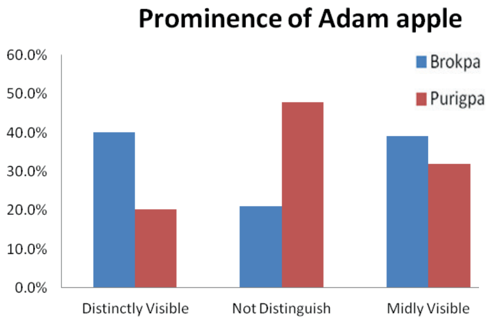

Fig. 11. Frequency distribution of Adam apple among Brokpa and Purigpa

(x) Auricular scaphoid shape

Our results show that the Brokpas and the Purigpas had different shapes and sizes of auricle (Figure 12). Majority of the Brokpa individuals (60%) had scaphoid shaped auricle, followed by long (36.8%) and knob shaped (3.3%) auricle when individuals of both sexes were considered jointly. Among the Purigpas, the majority of individuals had round-shaped auricle (60%), followed by long-shaped (28.8%) and knob-shaped (11.1%) auricular scaphoid in both sexes taken together. In total, the Kargil population of both the tribal groups, the 59.75% of study participants had round-shaped auricular scaphoid, followed by long 28.9% and knob-shaped 11.1% auricle in both the sexes (Figure 13). The differences in frequency distribution of shape of auricular scaphoid were found statistically significant between two population groups ($\chi^2=61.48$; $p<0.001$) and Brokpas males and females only ($\chi^2=9.338$; $p<0.009$).

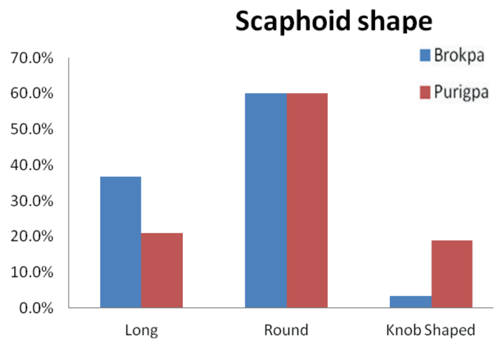

Fig. 13. Occurrence of auricle shape among Brokpa and Purigpa

(i) (ii) (a) (b)

Fig. 12. Shape of auricular scaphoid among Brokpas (i–ii) and Purigpas (a–b)

(xi) Facial shape

Our results show that different facial shapes exist among the Brokpas and the Purigpas (Figure 14). The majority of the Brokpas had long facial shape (60%), followed by oblong (36.5%), rectangular (2.25%) and oval (1.25%) profiles when both the sexes were considered jointly. Among the Purigpas, the majority of individuals had round facial shape (57.75%), followed by long (36.0%), rectangular (5%) and oval (1.25%) shapes when individuals of both the males and females were considered jointly. In total, the Kargil population of both the tribes, the majority of study participants had long facial profile (48.0%), followed by round facial profile (47.1%, rectangular (3.6%) and oval (1.3%) facial contour when both sexes were considered jointly (Figure 15). Highly significant statistical differences were found in the frequency distribution of facial shapes between the Brokpas and the Purigpas (χ^2=48.55; p<0.001) as well as between two sexes among the Brokpas (χ^2=65.139; p<0.001) and the Purigpas (χ^2=8.347; p<0.039).

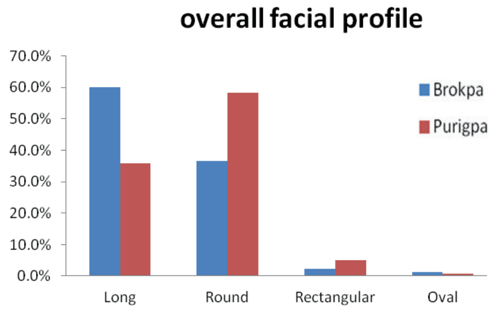

Fig. 15. Overall facial shape/profile among Brokpa and Purigpa

Table 1 shows the output of the Pearson correlation coefficients which highlight the degree of significance of differences in frequency distribution of various non metric variables in studied Kargil population.

(i) (ii)

Fig. 14. Facial shapes in (i) Brokpas and (ii) Purigpas

Table 1. Correlation between frequency distribution of various non-metric traits in the combined population of the Brokpas and the Purigpas

Non-metric traits	Correlation	Skin colour	Hair form	Eye colour	Epicanthic fold	Chin shape	Prognathism	Ear lobe	Ear size	Darwin's tubercle	Prominence of Adam's apple	Overall facial profile	Beard and moustache.
Skin colour	Pearson Correlation	1											
	Sig. (2-tailed)												
	N	800											
Hair form	Pearson Correlation	-.047	1										
	Sig. (2-tailed)	.182											
	N	800	800										
Eye colour	Pearson Correlation	.181**	-.078*	1									
	Sig. (2-tailed)	.000	.027										
	N	800	800	800									
Epicanthic fold	Pearson Correlation	.126**	-.001	.105**	1								
	Sig. (2-tailed)	.000	.972	.003									
	N	799	799	799	799								
Chin shape	Pearson Correlation	.130**	-.077*	.080*	.050	1							
	Sig. (2-tailed)	.000	.029	.024	.157								
	N	800	800	800	799	800							
Prognathism	Pearson Correlation	-.017	.011	-.008	-.018	-.054	1						
	Sig. (2-tailed)	.632	.764	.828	.606	.128							
	N	800	800	800	799	800	800						
Ear lobe	Pearson Correlation	-.032	.076*	-.116**	-.090*	-.109**	.106**	1					
	Sig. (2-tailed)	.360	.031	.001	.011	.002	.003						
	N	800	800	800	799	800	800	800					
Ear size	Pearson Correlation	-.082*	-.062	-.110**	-.061	-.047	.067	.087*	1				
	Sig. (2-tailed)	.021	.078	.002	.084	.185	.060	.014					
	N	799	799	799	798	799	799	799	799				
Darwin's tubercle	Pearson Correlation	-.051	.041	-.112**	-.079*	-.138**	.068	.322**	-.042	1			
	Sig. (2-tailed)	.153	.243	.001	.026	.000	.053	.000	.234				
	N	799	799	799	798	799	799	799	798	799			
Prominence of Adam's apple	Pearson Correlation	-.024	-.028	-.058	-.049	-.127**	-.143**	.020	.153**	.021	1		
	Sig. (2-tailed)	.500	.429	.100	.169	.000	.000	.572	.000	.561			
	N	800	800	800	799	800	800	800	799	799	800		
Overall facial profile	Pearson Correlation	-.109**	.056	-.124**	-.147**	-.162**	-.013	.024	.112**	.073*	.139**	1	
	Sig. (2-tailed)	.002	.111	.000	.000	.000	.723	.492	.001	.038	.000		
	N	800	800	800	799	800	800	800	799	799	800	800	
Beard and moustache	Pearson Correlation	-.019	.194**	.100**	.135**	.031	.194**	.032	-.146**	-.024	-.283**	-.262**	1
	Sig. (2-tailed)	.598	.000	.005	.000	.380	.000	.365	.000	.506	.000	.000	
	N	800	800	800	799	800	800	800	799	799	800	800	800

Discussions

Various waves of migration and invasions have resulted in a high cultural, social and genomic diversity observed in India's population (Bhasin and Nag 2002; Tamang and Thangaraj 2012). Owing to its geographical location, over many millenniums Ladakh has witnessed several ancient migrations and dispersals to and from the mainland India, Northeast Asia, Eurasia and Africa (Bhattacharyya and De 2009; Majumdar 2010; UNESCO 2010). The enormous diversity in cultural, religious and food practices in the Ladakh population may result from congruence of different ancestral groups (Rowold et al. 2016). It is believed that the earliest inhabitants of Ladakh were the 'Mons' of Indian descent and the 'Dards' from Iran resulting in a composite Indo-Aryan heritage (Jina 1996; Kuzmina et al. 2007). Researches have indicated that the paternal ancestry of Ladakh is genetically diverse and mosaic which have been laid down in multiple time intervals from different sources. (Rowold et al. 2016). The morpho-genetic heterogeneity of different sub-groups of Ladakh may be due to the multi-layered accumulation of demographic episodes (nomadic migration, foreign invasions, seasonal pastoral movements, military campaigns, refugee immigrations and trade) (Gayden et al. 2007). Each of these episodes have left its genetic imprints and somatoscopic signatures spread over the millennia of occupation (Rowold et al. 2016).

It is believed that a single species of *Homo sapiens* comprises of several varieties of ethnic groups inhabiting different geographical locations around the globe. Human morphology and its diverse characterization is a complex process and its manifestation is determined by the role of multiple genetic (mutation, isolation, hybridization), environmental, cultural, climatic and nutritional factors. The adaptive fitness of some genes/traits and their interactions with environmental factors determine the morphological features of an individual. Hence the population group, such as skin colour, are adaptive and have selected value. The amount of the melanin pigment determines the colour of human skin, eyes and hair. Skin colour in humans has resulted from biological adaptations in response to the environmental conditions in which our ancestors have lived. Fer example, people with fairer skin represent a lineage from ancestors who lived for many generations in colder climates. The processes underlying genetic influence in creating various phenotype/ morphological facial features, such as skin, hair and eye pigmentations have been explained by multiple studies (e.g. Kwon et al. 1987; Rinchik et al. 1993; Sturm et al. 2001). However, no such descriptions could be traced for the present study population. Our results show that in the study population the dark hair colour types vary from various shades of brown to black. Microscopic studies on the thickness of hair shaft have shown that coarse hair has the widest diameter while very fine hair has the narrowest. It has been shown that gene P2RY5 gene determines woolly hair (Shimomera et al. 2009). The white-skinned people have average hair shaft diameter less than 70μ, whereas people of Asian origin have thickness between 90 and 100 μ.

Skin colour is a complex biological trait characterized by different shades in diverse population groups inhabiting different parts of the globe. The variation of skin colour is due to the differential distribution of the melanin pigment among individuals of different populations, influenced by various biological, environmental, and genetic factors (Simon et al. 2009). People inhabiting near the equatorial region are exposed to more amount of sunlight and intense ultraviolet light rays producing blackish brown to yellowish-brown skin coloration as skin colour have some adaptive value (Blum 1961; Walter 1971). Hourblin et al. (2014) studied skin colouration patterns among different Indian ethnic groups and found that skin complexion ranges from whitish to brown and fairer in North Indians and, darker in South Indians, probably because heterogenic Indian population possesses different genetic polymorphism for skin colour. Indian studies revealed that measures of skin colour are based on certain classic methods, such as those based on visual observations or comparisons with chart having different shades of skin colour (Buchi 1957; Jaswal 1979). Skin pigmentation is also influenced by sex differences; North Indian Arora and Khatri females were found to have less facultative or inducible pigmentation compared to their male counterparts, probably due to the differences in tanning of skin colour (i.e., due to sun exposure or sunbathing) among the females (Kalla 1968). Lasker (1954) reported that skin colour also varies according to seasonal variations. In the present study, about 41% of the Purigpas had yellowish skin colour whereas 38.5% the Brokpas had whitish-yellow skin colour, followed by light-brown skin colour observed in 39.8% of the Purigpas, 19.5% of the Brokpas had a yellowish colour and about 10.5% of the Brokpas had reddish-brown skin colour. The studied area is located at a high altitude away from tropics with an intense cold season almost throughout the year. Therefore, Kargil individuals have yellowish or reddish-brown skin colour shades quite different from other Indian populations residing in plain areas.

Along with skin colour, hair and its structural features can contribute towards attribution of an individual to any of three major sub-divisions of mankind. The hair shape, texture, quantity, and colour have been used as reliable visual characteristics to classify an individual to a specific ethnic group by different researchers (Kirk 1940; Banerjee 1963). The hair forms may be straight (*leiotrichous*), wavy (*cymotrichous*) and curly (*ulotrichous*). In the present study, more than 56% males and about 62% females among the Brokpas and, 54.1% among the Purigpa males and 42.4% of the Purigpa females had low-waved hair, followed by the straight hair observed among 28.8% male and 34.8% female of the Brokpas in comparison to 23.9% males and 46.6% females of the Purigpa. No curly hair form was found among the Brokpa tribe; however, 2.9% of the Purigpa males had curly hairs, probably due to their biological affinity to the East Asians. Datta and Banerjee (1987) reported the hair form, texture, and hair quality among various population groups of North Eastern Indian population, and found that among the Purum hair varies from wide wavy (5.0%) to shallow-wavy (68.3%), texture varies from fine to coarse (1.7%–95%). They also found that quantity of hair varies from scanty to thick (1.7%–95%). In the Nagas, hair form varied from straight to curly while in the Kacharis from narrow-wavy (6.0%) to smooth (60%).

Eye colour is another useful human morphological trait used by the anthropologists for human differentiations and it varies from population to population due to certain genetic and environmental adaptive conditions affecting the amount of pigmentation present in the iris. It is well known that the European and the West Asians or the Americans have light-brown to blue-eye colour. Family pedigrees analyses have revealed that brown eye colour is dominant over blue among Europeans or White populations (Davenport and Gertrude 1910). Due to the higher amount of pigmentation in the iris of the Africans, their eyes appear to be brown in colour. The East Asians have a medium amount of melanin pigment which imparts light-brown to greyish-brown colour to their eyes. In the present study, eye colour in the studied population groups of Kargil varied from light-brown to blue-brown. The light-brown was the dominant eye colour present in both the studied ethnic groups. The Brokpas had a significantly higher frequency of brown eyes than the Purigpas. Gulati (1990) studied eye colour among six endogamous groups of Tamil Nadu and found that 90% of the Iyers had dark-brown eye colour, followed by 8% brown eye colouration, similarly 68% of the Naidus had dark-brown eye colour, followed by 19% individuals with brown eyes. Among present study individuals, although eye colour varied from light-blue to dark-brown in the combined population groups considered here, the brown eye colour was found dominant, which corresponds to results found among the Iyers and the Naidus of Tamilnadu (Gulati 1990). Among the tribal populations residing in the Himalayas of India, black eye colour was homogenously distributed, although it was heterogeneously distributed among non-tribal individuals of the area. In the present study, the Brokpas had a significantly higher frequency of brown eyes than the Purigpas. On the whole, the Kargil people had brown eye colours of different shades. Compared to darker eyes, blue eyes are better adapted for vision in regions where there is reduced light, as they let in more light. As such, the underlying genetic and environmental factors may be responsible for variations in the eye colour among individuals of the two studied tribal groups of Kargil (India).

We found that there is a significant positive correlation between skin colour and eye colour among both the two tribes. Skin colour also showed a significant correlation with epicanthic fold and chin shape. Another significant positive correlation was found between prominence of Adam apple and chin shape, as well as between beard and moustache with prognathism, beard and moustache with epicanthic fold in males of both tribes and over all facial profile with prominence of Adam apple. Negative correlation was found between eye colour and ear lobe as well as eye colour and Darwin tubercle (Table 1).

The physical features and the metric parameters of the external ear vary in different individuals of two sexes, different ages and ethnic groups (Meijerman et al. 2007). Various morphological characteristics of the human ear, such as size, attachment of lobes, shape of ear lobes, presence of Darwin tubercles, were observed in this study. Various studies have reported variation among the human external ears features and this variation has been used by anthropologists enabling human identification for forensic purposes (Feenstra and Lugi

2000). In some medico-legal context, the body of the deceased victim is recovered in dismembered conditions where the shape, size and morphological features of the ear/s may reveal the identity of the deceased. Also, ear prints left by the human ear on the doors, windows or other substrates can be lifted and developed using advanced techniques in forensic sciences. Ear lobe and its shape have been found to be associated with the geographical and genetic affiliation of a person and it has been found that the Africans have a small ear with little or no ear lobe when compared with other ethnic groups (Sharma 2017). Different shapes and sizes of ear lobes can be found among the different ethnic groups around the globe. The degree of attachment of the ear lobe differs from individual to individual. Many researchers have stated only two types of ear lobes: free and attached (Powell and Whitney 1937). Gabel (1958), on the other hand, classified ear lobe into intermediate, free and attached one. Many studies revealed that the free ear lobe is an autosomally dominant feature (Malhotra and Kanhere 1975; Winchester 1958; Guha 1935). Free ear lobes are mostly found among the European populations. Ear lobe attachment or detachments are features that can be used to investigate the variations among different ethnic groups and it is considered one of the important features of the human face used for recognition or forensic facial reconstruction purposes. Many researchers have reported that differences in ear morphology are due to certain genetic factors that are transmitted from parents to their offspring (Azaria et al. 2003). Some researchers have stressed that ear parameters can also be used for assessing familial relationships as the morphology of ears tends to be hereditary (Imhofer 1906).

Datta and Banerjee (1987) studied the ear lobe presence and its frequency among Indian population and revealed that frequency of free ear lobes varies from 70 to 95% and the free ear lobes tend to increase in Northern and North-eastern individuals, although it tends to decrease in Southern and Eastern Indians. When compared to Chattopadhyay (1968); Dutta and Ganguly (1965) studies the frequency of attached ear lobe in Delhi Jat (18.5%), Brahmins (63.9%) and Muslims (25.49%), and found comparatively much higher in the present study individuals of both the ethnic groups.

A study on North-west Indians found that the shape of the attached ear lobe was squarish in the majority of study participants, followed by the free pendulous ear lobe (Sharma et al. 2007). Similar findings were reported by Munir et al. (2015) on the Quetta population of Pakistan, which revealed that 58.2% of females and 41.8% of males had free ear lobe whereas 61.4% that 50.4% of Tibetans had attached ear lobe. These findings have been supported by the present study which revealed that the Purigpas had higher prevalence of the attached ear lobe compared to the Brokpas. The Brokpas are the Dard people who have an affinity to the Indo-Aryan population and belonged to European ancestry. On the other hand, the Purigpas are the descendants of the Tibeto-Mongoloid population (Rizvi 1996). Among the Newars of Nepal, the frequency of the attached ear lobe was 43.20% and 5.29% of the Newars did not have ear lobule (Bhasin 1969). The prevalence of free ear lobes is higher in the Brokpas than the Purigpas. Similarly, data collected in Manipur showed that individuals of a specific religion had higher frequencies of free ear lobe and they are thought to be European in origin (Shah et al. 2012). Among the Mongoloid-Japanese population, the frequency of the attached ear lobe was found higher (67.1%) in and (64.3%). Dharap and Than (1995) reported that 44.7% of Malaysians were having attached ear lobe which supported the notion that the frequency of attached ear lobes is comparatively higher among Asians compared to European and African populations. The findings of the present study also support the observations that the frequency of the attached ear lobe is highest among the East Asians. In the present study, about 43% of the Brokpas had roundish-oval ear lobe, followed by 25.8% tongue-shaped, 25.5% arched and the least frequency of ear lobe was rectangular (0.5%) in shape. Among the Purigpas, the tongue-shaped ear lobe was predominantly present in 38.7% of study participants, followed by the arched-shaped (22.8%) and the round-shaped (19.5%) ear lobes.

Darwin tubercle is also a unique heritable feature present on the posterior helix of the auricle (Millard and Pickard 1970; Donald 2011). It is thought to be a vestigial trait in modern humans. In the present study, only 8.1% of the Brokpa males and 6.2% of the Brokpas females had Darwin tubercle, whereas among the Purigpas, 37.8% males and 33.5% of females had Darwin tubercle. Thus, the Purigpas had much higher frequencies of Darwin tubercle than the Brokpas. A similar study conducted by Singh and Purkait (2009) on a central Indian population reported that 54–62% of people had Darwin tubercle present in node shape on helix of the auricle, although 54–60% of study participants of central Indian population lacked Darwin tubercle, such as in the present study 60–90% of the Kargil individuals did not have Darwin tubercle. The occurrence of Darwin tubercle varies among different population groups (Loh and Cohen 2016; Tiffany and Cohen 2016). The presence and absence of Darwin tubercle have some evolutionary and anthropological significance regarding studying different morphological features in different ethnic groups (Loh and Cohen 2016; Tiffany and Cohen 2016). Darwin's tubercle is a benign but unique helical feature which may contribute to the individuality of human ears and may help in personal identification (Tiffany and Cohen 2016). Dharap and Then (1995) reported that among Malaysian males, 17.5% exhibited Darwin tubercle in the right ear and 17.2% in the left ear. In females, on the other hand, 5.5% exhibited Darwin tubercle in the right and 4.1% in the left ear, although no explanation has been given for the right/left asymmetry in the occurrence of Darwin tubercle. Overall, frequency has been reported and an individual having this trait in both side ears was considered as a single case. Among the tribal population of Taiwan, the incidence of Darwin tubercle was 12.8% in females, and it was lesser in males (Chai 1967). The frequency of Darwin tubercle among the Malaysian population is comparatively lower than in the present study of the Purigpas and other Indian studies.

The nose shape and size is also considered as one of the useful criteria used for attributions of individuals to a specific group as it varies among different population groups, between two sexes, and among different age-groups. A long and narrow nose, by facilitating more efficient counter current systems, helps in heat conservation in cold climates. On the other hand, in warmer climates a short and broad nose, by discharging of water vapours, reduces body temperature. People living in cold but dry climates generally have smaller, longer and narrower noses that helps moisten and warm the incoming cold air. The nasal septum of the nose may be upwards, horizontal and downwards. The nasal root may be depressed or otherwise shallow, medium or deep. It has been found that these nasal features depicting the shape and size of the nose show significant sex differences due to significant natural selection pressures and the varied climatic conditions (Van Doom et al. 2009; Zaidi et al. 2018). Nasal tip is also a useful character for the somatoscopic observation of nose, commonly used for classification of major subdivisions in humans. Different types of nose tips have been reported in different populations which are adapted to different and varied climatic conditions (Zaidi et al. 2018). Therefore, the observed differences in nose shapes among individuals of different populations may be due to climatic adaptations and not simply due to genetic drift. Among the Indo-European population, sharp nasal tips are prominent as they have narrower noses compared to Africans and East Asians. Africans mostly has thick and bulbous nasal tip whereas, in East Asians, the nasal tip is medium shaped. A nasal tip among different populations varies and in the present study, 47.5% of the Brokpas had downwards nasal septum, followed by 44.8% horizontal and the remaining 7.8% had upwards nasal tips. In the Purigpas, 65.3% of study participants had a horizontal nasal septum, followed by 24.2% downwards, and 10.6% as downwards nasal tips. Among the Iranians population, horizontal nasal tips were prominently present in 94.3% of individuals, followed by 3.1% downwards and the rest 2.5% downwards nasal tips (Zolbin et al. 2015). Thus, the Brokpas and Purigpas were having different nasal shapes (Figure 16).

(i) (ii) (a) (b)

Fig. 16. Nasal shapes among (i–ii) Brokpas and (a–b) Purigpas

Lip form, which refers to the thickness of lips, ranges from thin, thick, inverted and diverted or puffy types. It is an important morphoscopic characteristic used in classifying individuals to certain groups. Individuals adapted to different environmental conditions have different lip forms to help facilitate heat balance in a particular environment. In a hot environment, thick or everted forms of lips help in heat loss due to its increased surface area, such as in people of African origin. Thin lips help to conserve body heat due to its decreased surface area in the colder environment, such as among Europeans. In the present study, 98.6% of males and 98.3% of females of the Purigpas, and 72.2% of males and 74.9% of females of the Brokpas, had thin lips, thus showing their affinity toward western Europeans. Bindal et al. (2015) reported that 40% of males and 40% of North Indians females had medium lip from, followed by 24% and 36.67% thin lip forms in males and females respectively; no puffy lips were reported in the present study. The shape of lips has become a famous beautification characteristic and lip enhancement has become a common practice in many western countries.

Epicanthic fold, i.e., a deposition of fat around the eyeball, is a characteristic of Siberian Asians, Inuits, and others those living at the arctic. Epicanthic fold is one of the biological characteristics of the Asian population in which the skin fold hangs over the free edges of the entire upper lid and conceals it thoroughly and extends from the outer corner to the inner corner (Haddon 1924). In East Asians, the shape of the epicanthic fold varies from partial to complete fold. This fold helps in adaptation to cold climates and fats deposited around the eye give insulation to eyeball and sinuses as well as prevent eyeball from sharp glare of ultraviolet radiations reflected from snow blindness, and freezing winds (Coon et al. 1950; Blackburn 2000). In the present study, 43% of the Purigpas had distinct epicanthic fold; however, majority of the Brokpas had partial epicanthic fold (47.3%). A similar study on the double eyelid in Malays and Chinese by Lu et al. (2017) reported that 81.3% of Chinese and 70.1% of Malays exhibited double eyelids, which is also called Mongolian-fold or epicanthic fold. These findings are supported by our study showing that among Mongolian fold was predominantly present at the Purigpas, which is considered as Tibetan descendent tribal group; while lower in the Brokpas whose ethnicity has been traced from Indo-Aryan Western European descendants (Francke 2008). The epicanthic fold is an adaptation for protecting the eyes from the hard driving snow or snow glare.

Pattern, density, and distribution of beard and body hairs vary in different ethnic groups (Loussouarn et al. 2005). It is well known that the Western Europeans and the West Asians have abounded beards on their faces, and the East Asians have very scanty or no beards on their faces which might have been affected by their genetic as well as geographical conditions or androgenic factors. In the present study, 68.4% of the Brokpa males had thick beards and moustaches, followed by medium 30.2% beards. However, 57.8% of the Purigpa males had medium beards and moustaches on their face, followed by thick beard types among 22.9% individuals; thus, the Brokpas had more thick facial hairs.

Conclusions

Studying human physical and genetical variations along with their environmental influences have remained the major focus in anthropological research. People inhabiting high mountains show a variety of biological adaptations in response to the adverse environmental conditions. Though human physical characteristics are determined by genetics, sufficiently long periods of inhabiting specific climatic conditions provided selective pressure to develop particular characteristics within populations inhabiting different geographic parts of the world. In present study, statistically significant differences occurred between the Brokpas and the Purigpas of Kargil (UT, India) with respect to the frequencies of their skin colour, hair form, facial contour/profile, nasal types and presence/absence of epicanthic fold, prognathism, Darwin's tubercle, Adam's apple, scaphoid, attached ear lobe. The whitish yellow skin colour, thick beards and moustache, downwards nasal septum, sharp nasal tip, squarish facial contour, partial epicanthic fold, distinctly marked mandibular prognathism, free ear lobe, round scaphoid, prominent Adam's apple, were the Brokpa morphological traits. On the other hand, the rounded facial form, complete epicanthic fold, maxillary prognathism, and attached ear lobe were the most predominant morphological traits among the Purigpa. Significant sexual differences were found regarding skin colour, eye colour, hair form, nasal septum, nasal tip, epicanthic fold, ear lobe, and Adam apple among the Brokpas and for skin colour, eye colour, ear lobe attachment, hair form, and prognathism among the Purigpas. Individuals of two tribes occupy almost similar geographical terrain (mountainous region) and have been exposed to similar climatic and nutritional factors since many generations. Therefore, the reasons behind the observed morphological differences may reflect differences in genetics as they trace their affinity origins separately to two different ancient populations. The Brokpas are considered Dard people who link their affinity to the Indo-Aryan population of Western European ethnicity whereas the Purigpas are believed to be the descendants of the Tibeto-Mongoloid population (Rizvi 1996). The majority of the morphological traits of the Brokpas and the Purigpas endorse the Western European and East Asian ethnic relationships respectively. which needs to be supplemented with a larger and compressive study with regards to their genetic characteristics. Our study results of the morphological features of Ladakhi individuals will add to the existing literature about the anthropological characteristics of different ethnic groups of India.

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Conflict of interests

There is/are no potential conflicts of interest for publication of this manuscript.

Authors' contribution

JSS conceptualized the concept, analysed and interpreted the data/results, drafted, prepared and edited the manuscript, responded to and incorporated the reviewers' and Editor comments/suggestions in final manuscript, communicated with the journal.

MA collected raw data from the field, tabulated data, got statistics applied to data, helped in drafting and preparing preliminary version of manuscript.

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Supplementary Table

Morphological Features considered in present study

Skin colour:				
	(a) Greyish-black	(b) Blackish-brown	(c) Reddish-brown	
	(d) Brown	(e) Light-brown	(f) Yellowish	
	(g) Whitish-yellow	(h) Reddish-whitish	(I) Whitish.	

These were observed on inner side of arms, cheeks and forehead.

Hair form:	(a) Straight	(b) Low waves	(c) Deep waves	
	(d) Curly	(e) Frizzly	(f) Woolly.	
Beard and moustache:	(a) Scanty	(b) Medium	(c) Thick	
Eye colour:	(a) Black	(b) Dark brown	(c) Brown	(d) Light brown
	(e) Grey	(f) Blue brown	(g) Grey	(h) Brown.
Nasal septum:	(a) Horizontal	(b) Upwards	(c) Downwards	
Nasal tips:	(a) Sharp	(b) Medium	(c) Thick	(d) Bulbous
Lips:	(a) Thin	(b) Thick	(c) Averted	(d) Inverted
Chin:	**(i) Prominence**:	(a) Prominent	(b) Medium	(c) Round
		(d) Square	(e) Pointed	(f) Oval.
	(ii) **Shape** :	(a) Rectangular	(b) Elliptical	(c) Round
Prognathism:	(a) Slight	(b) Medium	(c) Marked	
Ear lobe attachment:	(a) free	(b) attached		
Shape of Darwin's tubercle:	(a) tongue	(b) Triangular	(c) Rectangular	
	(d) Arched	(e) Round		
Scaphoid:	Size:	(a) Small	(b) Weak	(c) Medium
	Shape:	(a) long	(b) round	(c) knob-shaped
Prominence of Adam's apple:	a) Distinctly visible	b) not distinct	c) mildly visible	
Prognathism:	(a) Slight	(b) Medium	(c) Marked	
Epicanthic fold:	(a) Present	(b)absent		

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Low 2D:4D is associated with delayed age at menarche among women of Sikkim, India

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Abstract: The index-finger and ring-finger ratio (2D:4D) is a potential biomarker that reflects prenatal hormonal exposure and thus has a long-term impact on reproductive health. The present study aims to determine the relationship between the 2D:4D (representing the prenatal hormonal environment, i.e., early androgen exposure) and early or delayed age at menarche among women in Sikkim, India. A total of one hundred nineteen Sherpa tribal women, ages 18–49, from the Soreng district of Sikkim, India, were included in the study using a stratified random sampling method. To calculate the 2D:4D ratio, the lengths of the index and ring fingers (2D and 4D) were measured using standard procedures. The category-wise mean comparison revealed that women with a more feminine 2D:4D ratio (in both left and right hand) had significantly earlier age at menarche (in years) compared to women with a more masculine 2D:4D ratio. The linear regression analysis revealed that the left 2D:4D was significantly related to menarche age. There is an association between the 2D:4D and menarche age, indicating a link between women's reproduction patterns and the influence of the prenatal hormonal environment as an important factor in attaining an early or delayed menarcheal age.

Key words: Menarche, 2D:4D, Reproductive pattern, prenatal hormone.

Original article

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Introduction

The index-finger and ring-finger ratio (2D:4D) is a retrospective biomarker that may indicate prenatal testosterone and estrogen exposure and have long-term effects on physiology, behaviour, fertility, disease risks, pubertal development, and reproductive success (Manning et al. 2000; Manning 2002; Matchock 2008; Manning and Fink 2011; Kalichman et al. 2013; Klimek et al. 2016; Kirchengast et al. 2020). The 2D:4D appears to be sexually dimorphic, with men typically having lower ratios than women due to earlier hormonal exposure to testosterone (Manning et al. 2000; Manning 2002; McIntyre 2006). A more masculine (low: 2D<4D) and more feminine (high; 2D>4D) digit ratios are clearly the result of increased prenatal testosterone and estrogen exposure, respectively (e.g. Manning et al. 1998; Klimek et al. 2016). Furthermore, 2D:4D may represent individual susceptibility to certain chronic diseases and hormonal disorders (Luijken et al. 2017; Tabachnik et al. 2020). The 2D:4D has been linked to higher levels of putatively androgenic outcomes, such as foetal and pubertal development and left-handedness (Manning et al. 2000; Manning 2002; Li et al. 2019), improved athletic ability, and an increased risk of autism (Manning et al. 2001; Mackus et al. 2017). It is also believed that the 2D:4D serves as a proxy indicator of intrauterine sex hormone levels because it significantly correlates with a number of somatic and behavioral characteristics as well as fertility measures (Manning et al. 1998; Fink et al. 2004; Klimek et al. 2016; Kirchengast et al. 2020). An explanation for the association between 2D:4D and prenatal sex hormone has been put forth as the action of the HOX A and HOX B genes, which regulate digit and toe differentiation and are implicated in sex determination, morphogenesis of the urino-genital system, appendicular skeleton, and fertility (Manning et al. 1998; Manning 2002; Manning et al. 2003; Zhang et al. 2013). The 2D:4D has also been connected to facial asymmetry (Fink et al. 2004), reproductive success and a longer reproductive period (Klimek et al. 2016), breast cancer (Muller et al. 2012), age at menarche (Matchock, 2008), age at menopause (Kirchengast et al. 2020), reproductive and general health (Tabachnik et al. 2020), and cardiovascular disease risk (Luijken et al. 2017). It has been recently reported that the 2D:4D ratio could predict an early or late onset of menarche (Matchock 2008; Manning and Fink 2011). Several studies have found that masculinized hands (low 2D<4D) are significantly related to delayed age at menarche (e.g., Matchock 2008; Manning and Fink 2011; Kalichman et al. 2013; Kirchengast et al. 2020). Therefore, more research is needed to ascertain whether there is a relationship between the 2D:4D and age at menarche in various populations in order to evaluate the population specific data on such a biological trait. The present study aims to determine the associations between the 2D:4D, which represents the prenatal hormonal environment (i.e., early androgen exposure) and the age at menarche of Sherpa tribal women living in Sikkim, India. Specifically, the aim of this study is to investigate the long-term influence of the prenatal hormonal environment as reflected in the 2D:4D on the onset of menarche in one of the indigenous populations in Sikkim, India.

Material and methods

The present community-based cross-sectional study was conducted among 119 Sherpa tribal women in the reproductive age group of 18–49 years in the Soreng district of Sikkim, North-East India. According to the 2011 National Census of India (2011), Sikkim had a population of 610,577 lakh (male: 323,070; female: 287,507) with a sex ratio of 890 (women to every 1000 men), which was lower than the national average of 940. Sikkim's literacy rate was reported to be 81.42 percent (86.55% for men and 75.61% for women). Sherpa is an indigenous community in Sikkim that belongs to the Nepali ethnic group. According to the Denzong Sherpa Association, there are around 65,000 Sherpas in the population, with 32,000 of them speaking their mother tongue in their native environment. According to the 2011 National Census of India, a total of 13922 residents identified as Sherpa speakers in Sikkim. The Bodish or Bodic group organizes the Sino-Tibetan-Burman family, which includes the Sherpa language. The study participants were an ethnically homogeneous ethnic group of the Sherpa tribe of Sikkim, geographically situated in intermediate altitude locations (~7600 ft. and above). Study participants were selected using stratified random sampling methods. The Sherpa community was chosen for the study due to its vast distribution in the Soreng district of Sikkim. The homogeneity of the selected participants is determined in terms of their ethnicity, language, cultural practices, geographic distribution, history of ancestry, and shared beliefs in the community. The present study was conducted in the Sherpa-dominated village of Okhrey, located in the Soreng district of Sikkim, India. Okhrey village, with a population of 1,683 people, is mostly inhabited by Sherpa tribal populations and located in the Soreng subdivision of Sikkim's west region (Male 821; Female 862). The covered community is located roughly 110 kilometres from Gangtok's headquarters, the Daramdin BAC Block Administrative Center of Soreng district of Sikkim. The Soreng district is estimated to have 85,483 people (in 2021). According to the 2011 Indian census, this district has 64,760 residents (33,061 males and 31,699 women). Among the 44,921-literate people, there are 20,254 women and 24,667 men. Agriculture, the principal economy, employs 11,908 cultivators (4,171 women and 7,737 men respectively). A total of 1,949 individuals are estimated to work as agricultural labourers in Soreng, including 1,281 men and 668 women. The nearest town from Okhrey, about 20 km away, is Sombaria. A formula for calculating the sample size for a single percentage population was used to determine the estimated minimum sample size (n=96). With an anticipated proportion of 50% ($p=0.5$) based on the response distribution, a desired precision of 10% (E=margin of error) at the 95% confidence interval was used. Present study has used the following formula to calculate the sample size for a single proportion: $n=Z^2\times p\times(1-p)/E^2$. In total, a sample size of 119 Sherpa tribal women was collected for the present study. The fieldwork was completed between February and April 2022. Before collecting data, study participants who willingly participated in the study provided informed consent. The participants' ages and ethnicities were verified using birth certificates and official documents. The participants' average age was 27.81±8.65 years. The objectives and scope of the present study were explained to the participants. Pre-structured schedules and household survey methods were used to collect data on age at menarche, anthropometric measures, and demographic factors, which was subsequently used to calculate the respondents' age at menarche. Study participants were asked to describe the entire year when they first experienced menstrual bleeding. The participation in this examination was entirely voluntary, and the overall response rate was relatively high, with all eligible persons who met any excluding criteria taking part in the present study. The present study was carried out in accordance with the Helsinki Declaration's ethical guidelines for human research (Portaluppi et al. 2010).

Collection of Anthropometric Measurements

The standard methods for obtaining finger length measurements were used (Manning et al. 1998). A single experienced observer measured the finger lengths from the proximal crease at the base of the finger to the tip of the finger in the midline on the palmar aspect of the hand using a vernier calliper nearest to 0.1 mm without exerting pressure; protruding fingernails were excluded. The participant's hand was placed on a plastic board, palm up, to obtain the digit lengths [i.e., index finger (2D) and ring finger (4D)]. This measurement has been reported to be highly repeatable (Manning et al. 1998). Participants with digit/hand abnormalities/fractures, injuries, or surgical episodes were excluded from participating in the study. However, the participants in this study were also measured precisely to avoid any possible systematic errors and to establish landmarks in the process of anthropometric data collection. The mean values of two consecutive measurements were reported. The mean differences in repeated measurements were found to be statistically insignificant ($p>0.05$). The technical errors of measurement $TEM=(D^2/2N)$, where "D" represents the difference between anthropometric measurements and "N" represents the number of individuals measured, is an accuracy index that evaluates the standard deviation between repeated measures used to determine the consistency of anthropometric measurements. The ideal approach involves calculating relative TEM followed by calculating the reliability coefficient [$R=1-(TEM)^2/SD^2$], (SD=the standard deviation of all measurements) (Goto and Mascie-Taylor 2007). The TEM was determined using numerous measurements taken from 30 randomly chosen Sherpa women. In TEM analysis, very high reliability (R) ($R>0.975$) was achieved in both left and right 2D and 4D lengths, and values were found to be within the permissible range of $R=0.95$ (Goto and Mascie-Taylor 2007).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS; Version 16.0) was used to analyse the data. In descriptive statistics, continuous variables were represented by mean and standard deviation (SD), and the Kolmogorov-Smirnov (K-S) non-parametric test was used to assess whether the data distribution's normality significantly deviated from the predetermined theoretical distribution. The length of the 2D was divided by the length of the 4D to calculate the 2D:4D digit ratio. Further, the study participants were divided into two groups based on the 2D:4D values for both hands: more feminine (2D:4D≥1) and more masculine (2D:4D<1), in accordance with previously described methodologies (e.g., Klimek et al. 2016). In particular, this study compared groups of reproductive women with more masculine (2D:4D<1) and more feminine (2D:4D≥1) characteristics to examine the relationship between 2D:4D and the age of menarche. A One-Way Analysis of Variance (ANOVA) was used to compare the category-wise means of the finger length variables and age at menarche. The linear association between two or more continuous variables, such as digit lengths, 2D:4D, and the age of menarche, was determined using Pearson's correlation coefficient as well as linear and multiple regression models. A p-value < 0.05 was considered statistically significant.

Results

Table 1 depicts the descriptive statistics (mean ±SD) of 2D and 4D lengths and 2D:4D and age at menarche among Sherpa tribal women. Sherpa women had a mean menarche age of 13.17 (±1.23) years. The mean and SD for the right hand 2D:4D were 0.99±0.05, and the mean SD for the left hand 2D:4D was 0.99±0.06. The results of K-S non-parametric test indicate that age at menarche (K-S value: 0.23; p<0.01), left 2D:4D (K-S value: 0.11; p<0.01) and right 2D:4D (K-S value: 0.12; p<0.01) p-values are small, which show significant deviations from the normal distribution, thus indicate nonlinearity in the data distribution. The Boxplot and Q-Q plots of age at menarche, 2D:4D distribution, and deviation from normality of the data are depicted in Fig. 1. Using ANOVA, the mean differences in finger lengths, 2D:4D, and age of menarche were shown to be statistically significant between the feminine and masculine categories (p<0.01) (Table 1). A Pearson correlation analysis plot of the 2D and 4D lengths and 2D:4D with age at menarche among Sherpal reproductive women is presented in Fig. 2.

Table 1. Differences between groups with low and high left and right -hand digit 2D:4D and age at menarche among Sherpa tribal women of Sikkim, India

Variables	Mean ±SD (N=119)	95% CI of Mean	Left: Masculine (Low 2D:4D <1.00) (N=81)	Left: Feminine (High 2D:4D ≥1.00) (N=38)	Left: F-value p-value	Right: Masculine (Low 2D:4D <1.00) (N=77)	Right: Feminine (High 2D:4D ≥1.00) (N=42)	Right: F-value p-value
2DL mm	61.96 ±4.32	61.18–62.75	60.91±4.25	64.23±3.60	17.15**	61.51±4.61	62.79±3.65	2.43
2DR mm	62.33 ±4.71	61.48–63.19	62.13±4.93	62.76±4.25	0.45	61.04±4.59	64.70±4.00	18.84*
4DL mm	62.81 ±4.31	62.03–63.60	63.45±4.59	61.46±3.28	5.78*	62.89±4.51	62.68±3.94	0.06
4DR mm	63.20 ±4.63	62.36–64.04	63.57±4.76	62.41±4.29	1.64*	63.84±4.74	62.04±4.24	4.23*
2D:4D (Left)	0.99 ±0.05	0.98–1.00	0.96±0.03	1.05±0.03	184.71**	0.98±0.05	1.00±0.06	6.78*
2D:4D (Right)	0.99 ±0.06	0.98–1.00	0.98±0.04	1.01±0.07	7.79**	0.96±0.03	1.04±0.05	153.52**
Age at Menarche (years)	13.17 ±1.23	12.94–13.39	13.36±1.23	12.75±1.16	6.66*	13.36±1.27	12.82±1.14	5.26*

*p<0.05;**p<0.01

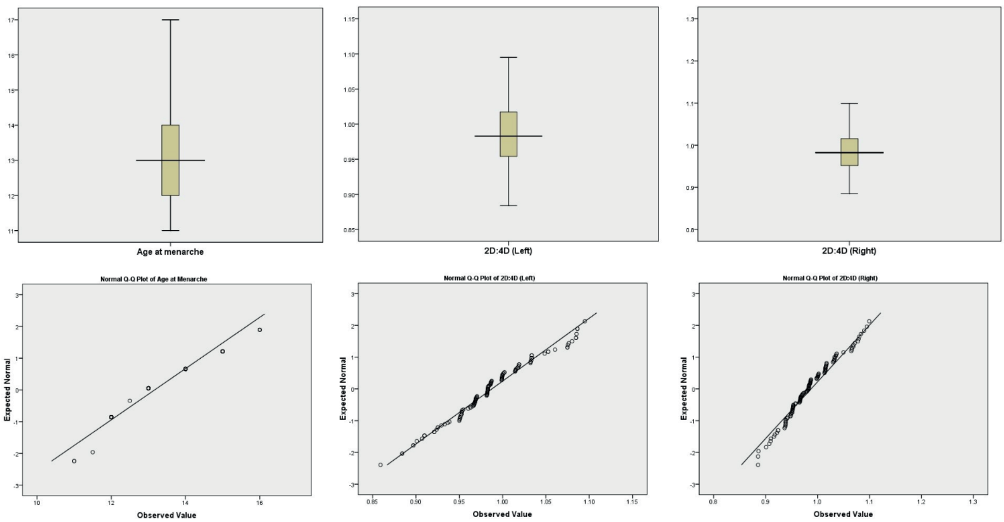

Fig. 1. The distribution and deviation of age at menarche, 2D:4D (left) and 2D:4D (right) using Box-plot and Q-Q plot among Sherpa tribal women of Sikkim, India

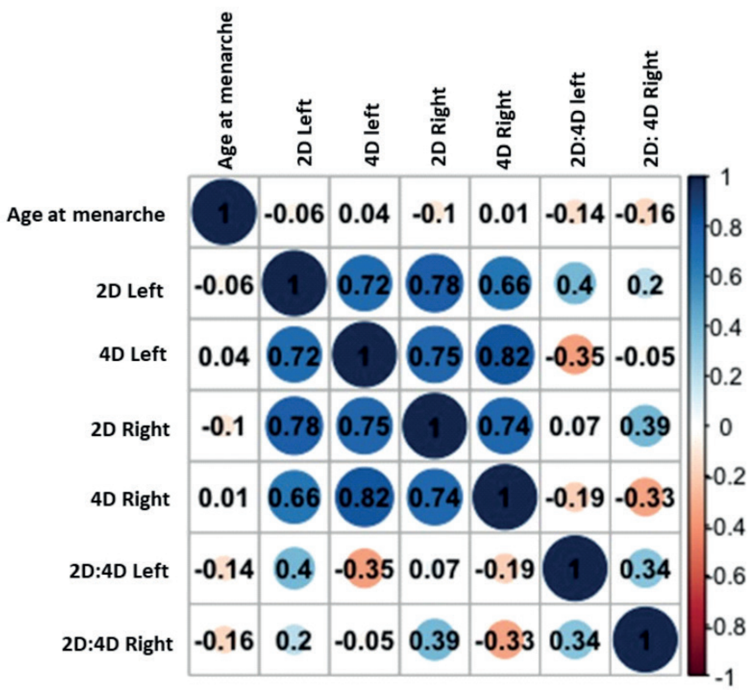

Fig. 2. Pearson correlation analysis plot of the 2D and 4D lengths and 2D:4D with age at menarche among Sherpa tribal women of Sikkim, India

Pearson correlation coefficient analysis revealed that age at menarche is significantly ($p<0.01$) and negatively correlated with the 2DL (r=−0.06) and 2DR (r=−0.10) but positively correlated with the 4DL (r=0.04) and 4DR (r=0.01) ($p>0.05$). A negative coefficient implies that a lower 2D:4D ratio is associated with an earlier age at menarche, whereas a positive coefficient indicates a later age at menarche. On the other hand, age at menarche was found to be inversely linked with 2D:4D left (r=−0.14) and 2D:4D right (r=−0.16) (Fig.2). Thus, the correlation analysis of 2D:4D does not show a strong and statistically significant relationship with the age at menarche ($p>0.05$).

The more masculine 2D:4D (<1.00) was found to be more frequent in both the left (63.70%) and right (61.30%) hands of participants, whereas only 36.52% (in the left hand) and 38.70% (in the right hand) demonstrated a more feminine 2D:4D (≥1.00). The category-wise mean comparison revealed that Sherpa women with a more feminine 2D:4D (≥1.00) had a substantially earlier attainment of menarche (in years) in both the left (12.75 vs. 13.36; $p<0.05$) and right (12.82 vs. 13.36; $p<0.05$) hands compared to those exhibiting a more masculine 2D:4D (<1.00). The linear and multiple regression analysis revealed that digit length (2DL, 2DR, 4DL, and 4DR) and 2D:4D left and right were strongly related to age at menarche ($p<0.01$). The regression coefficient for the age at menarche on 2D:4D left was 0.14 (t-value= 7.41, $p<0.01$), while the regression coefficient for age at menarche on 2D:4D right was 0.16 (t-value= 8.36; $p<0.01$), indicating that the right 2D:4D was more significantly associated with age at menarche ($p<0.01$). Multiple regression analysis for age at menarche on left and right 2D:4D was statistically significant ($p<0.01$) (Table 2). Regression analysis confirmed a significant association between 2D:4D and the age of menarche. Furthermore, 2D:4D appears to influence the timing of menarche in Sherpa women, with more masculine 2D:4D (<1.00) associated with later attainment of menarche.

Table 2. Linear/multiple regression analysis and age at menarche with digit lengths and ratio (2D:4D) among Sherpa tribal women of Sikkim, India

Variables	Linear Regression Model	R	R^2	SEE	t-value	*p*-value
2DL (mm)	14.246 −0.017	0.061	0.004	1.242	8.673	<0.001
2DR (mm)	14.885−0.028	0.105	0.011	1.237	9.884	<0.001
4DL (mm)	12.527 +0.010	0.035	0.001	1.243	7.483	<0.001
4DR (mm)	14.885−0.028	0.105	0.011	1.237	9.854	<0.001
2D:4D (Left)	16.459+3.333	0.136	0.018	1.232	7.410	<0.001
2D:4D (Right)	16.693−3.569	0.161	0.026	1.227	8.357	<0.001
2DL+4DL (mm)	13.377−0.051 (2DL) +0.047 (4DL)	0.129	0.017	1.239	7.499	<0.001
2DR+4DR (mm)	13.933−0.065 (2DR) +0.052 (4DR)	0.169	0.029	1.231	8.494	<0.001
2D:4D (Left) + 2D:4D (Right)	18.236 + 2.251 (2D:4D L) −2.881 (2D:4D R)	0.183	0.033	1.228	7.069	<0.001

Discussion

Over the last two decades it has been strongly suggested that 2D:4D is an indirect biomarker of intrauterine steroid levels, such as testosterone (Manning et al. 1998; Manning et al. 2000; Manning 2002; McIntyre 2006; de Sanctis et al. 2017). The present study advances our knowledge of the association between prenatal sex hormone exposure indicated by the 2D:4D ratio, and age at menarche among Sherpa tribal women in Sikkim, India (Table 1). The 2D:4D values have been used to predict reproductive capacity and success, fertility measures, natural menopause, and age at menarche (Manning 2002; Klimek et al. 2016; Kirchengast et al. 2020; Tabachnik et al. 2020). Furthermore, biological and cultural factors, such as age at menarche, age at marriage, breast feeding, nutrition, postpartum ovarian function, illness prevalence, early age at natural menopause, and reproductive period, have been found to have a considerable impact on population reproductive performance (Matchock 2008; Klimek et al. 2016; Kirchengast et al. 2020). This is interesting because numerous studies have discovered that women exhibiting a feminine 2D:4D (≥1.00) tend to marry at a younger age, prefer marriage at a younger age, have more successful pregnancies and children (Manning and Fink 2008; Klimek et al. 2016; Eresheim et al. 2020; Kirchengast et al. 2020) and experience menopause at a later age (Kirchengast et al. 2020). This study found that women with high 2D:4D (≥1.00) had marginally earlier age at menarche compared to those with low 2D:4D (1.00) ($p>0.01$) (Table 1). Women having a feminine 2D:4D (≥1.00) were more likely to reach menarche early than women with masculine 2D:4D (<1.00) (Matchock 2008; Manning and Fink 2011; Eresheim et al. 2020; Tabachnik et al. 2020).

Several researchers have hypothesized that prenatal testosterone levels and 2D:4D are negatively correlated (Manning et al. 1998, 2000, 2003; de Sanctis et al. 2017). The possible mechanisms for this correlation are supported in part by lower 2D:4D with congenital adrenal hyperplasia and higher 2D:4D with complete androgen insensitivity syndrome, as well as a relationship between 2D:4D and polymorphisms in the androgen receptor. However, a few contradictory findings has also been reported in order to establish a possible mechanism linking 2D:4D or testosterone secretion reduction with genetic disorders, such as Klinefelter syndrome (Manning et al. 2013), offspring birth sex ratio (Helle and Lilley 2008), age at menarche (Helle 2010; Muller et al. 2012), and salivary testosterone change under acute exercise (Kowal et al. 2020). For instance, Helle (2010) revealed no indication that the 2D:4D of the right or left hand is related to menarche age in Finnish women while Gooding and Chambers (2018) identified no statistically significant link between digit ratios and menarche age in women ($p>0.05$).

Several studies have shown that the 2D:4D is strongly and inversely related to menarcheal age in the population (Matchock 2008; Manning and Fink 2011; Oberg and Villamor 2012; Kalichman et al. 2013; Tabachnik et al. 2020; Eresheim et al. 2020). Research on the relationship between 2D:4D and age at menarche (Manning et al. 2003; McIntyre 2006; Matchock 2008) supports a delayed menarcheal association with more androgen exposure during the intrauterine period. Hence, greater exposure to androgens in the womb is thought to have an impact on the timing of pubertal development, including the onset of menarche. In addition, women who delay menarche may have a less than ideal reproductive profile, which increases their risk of sub-fecundity and infertility (Guldbrandsen et al. 2014; Tabachnik et al. 2020). According to several studies, early menarche is associated with a higher risk of diseases like cancer, type 2 diabetes, hypertension, cardiovascular disease, and metabolic disorders manifesting later in life (Muller et al. 2012; Luijken et al. 2017; Wang et al. 2018). The present study also shows a significant negative correlation between the left hand 2D:4D and menarche age. Some researchers have proposed that the left 2D:4D is a reliable indicator of prenatal hormone exposure, and that the left side of the body may significantly reflect female-typical factors, puberty traits and development, and the prevalence of specific diseases (Manning 2002; Wang et al. 2018; Li et al. 2019).

The results of the present study showed that left 2D:4D was significantly inversely related to women's age at menarche. Several studies have reported the associations between left hand 2D:4D and biological factors, habits, and disease in females (e.g., Mackus et al. 2017; Wang et al. 2018). It is believed that the left-hand 2D:4D is a useful predictor of a variety of reproductive features, such as age of menarche and reproductive success and thus it is biologically appropriate for reproductive women to examine the 2D:4D in both hands (Manning and Fink 2008; Klimek et al. 2016; Tabachnik et al. 2020). However, Manning and Fink (2011) reported a negative relationship between right 2D:4D and age of menarche in a large population group of women. Overall, while the present study adds to our understanding of the association between 2D:4D and menarche age, its limitations must be mentioned when extrapolating its results to other populations or contexts. To further understand the association between the 2D:4D and age at menarche in adults, future research might benefit from larger sample sizes, longitudinal designs, and comprehensive confounding factor control.

Overall, the results of this study showed that a low 2D:4D as a proxy measure for prenatal hormone exposure may be associated with delayed menarche and therefore reproduction in women. This study also shows that prenatal hormonal exposure, indicated by the 2D:4D, is a significant factor determining age at menarche among Sherpa tribal women; hence, women with high prenatal testosterone and low prenatal estrogen tend to have delayed menarche.

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Conflict of interest

The authors do not have conflict of interest to declare.

Authors' contribution

NM: Conceived the idea, carried out the literature search and analysed the data, and wrote the manuscript. RR: Conducted the fieldwork and collected the data, carefully revised the draft manuscript,

and contributed important intellectual content to the final manuscript. All the listed authors have contributed substantially to finalisation of the manuscript.

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Linear Morphometric Analysis of Auricular Measurements of a Nigerian Igbo population

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Abstract: The human ear (auricle) is an important body component in biometric studies and forensic identification. The purpose of this study was to evaluate linear auricular morphometric measurements of a Nigerian Igbo population.

300 participants (142 males to 158 females) of at least 16 years of age were randomly recruited from settlements in the South-Eastern states of Nigeria. The participants were made to sit in a Frankfort horizontal position and auricular measurements such as total ear height (TEH), ear width (EW), lobule height (LH) and lobule width (LW) were obtained using a digital vernier caliper (validated by calibrating to 0.01mm). The ear index (EI) and lobule index (LI) were calculated using the measurements. Data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS version 23.0).

The following mean values were recorded for males: TEH [Right (R) = 47.01±6.30, Left (L) = 46.24±5.85], EW (R = 25.06±4.14, L = 24.68±4.32), LH (R = 13.92±2.61, L = 13.99±2.18), LW (R = 12.99±2.30, L = 13.30±1.94), EI (R = 53.47±7.23, L = 53.28±6.69) and LI (R = 95.17±18.12, L = 96.80±18.56); for females, TEH (R = 48.25±6.83, L = 48.25±6.24), EW (R = 25.19±3.93, L = 24.57±4.21), LH (R = 14.44±2.67, L = 14.89±2.55), LW (R = 13.37±2.26, L = 13.78±2.04), EI (R = 52.50±6.85, L = 50.93±7.05) and LI (R = 94.60±19.59, L = 94.30±16.63). Gender differences were highly significant regarding total ear height, lobular length and width, as well as the ear index of the left auricle.

This study provides a baseline data for auricular morphometrics of adult Nigerian Igbos and could be used as an ergonomic guide towards the creation of hearing aids in the future and to solve possible forensic issues associated with identification of remains.

Key words: Auricle, morphometrics, ergonomic guide, forensic identification, Igbo.

Original article

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Introduction

Anthropometry often uses a collection of human bodily measurements in order to understand human physical variation as it plays an important role in plastic surgery, prosthetics, personal identification, biometrics and forensic profiling (Purkait and Singh 2007; Ekanem et al. 2010; Deopa et al. 2013). The ear, or pinna, is an important but under-studied feature of the face which shape and size conveys information about age and sex (Brucker et al. 2003). The shape, size and orientation of each pinna are as unique as fingerprints although it is possible to make some generalizations (Alexander et al. 2011; Osunwoke et al. 2018).

Currently, anthropometric studies have shown that the morphological variation of the external ear depends on age, sex and race and side (i.e., side-to-side variation) (Meijerman et al. 2007; Purkait and Singh 2007; Murgod et al. 2013). Jung and Jung (2003) surveyed the dimensions and characteristics of Korean ears and found that age, sex, and specific ethnic group were contributing factors of auricular dimensions. Sharma et al (2007) carried out a morphometric study in India where it was observed that North-West Indians have smaller ear lobules compared to Caucasian and Japanese populations but similar to those observed among the Onge tribe of Andhra (India) and Newars of Nepal. A study done by Murgod et al (2013) that assessed ear shape and earlobe metric dimensions of 300 Indian adult subjects concluded that identifying males was about 69% accurate while that of females was 72%. Several Nigerian studies have been conducted on the morphological and morphometric variation of an external ear (Ekanem et al. 2010; Eboh 2013; Taura et al. 2013). The aim of this study was to examine the accuracy of prediction of age and sex of a selected Nigerian Igbo population using auricular morphometrics for forensic applications.

Materials and Methods

A cross-sectional, descriptive study design was applied to randomly select Igbo heterogeneous indigenes residing in the South-Eastern states of Nigeria (Abia, Anambra, Ebonyi, Enugu and Imo). Using the Cochran formula, the calculated sample size was 300 adult subjects, comprised of 142 males and 158 females of at least 16 years of age. Study participants showed no past records of ear abnormalities or surgical operations and were selected in line with the Declaration of Helsinki research ethics protocol for human research. Both primary and secondary data were obtained from study participants. Biological profile, such as age and sex, made up the primary data while ear measurements the secondary data.

The participants were made to sit in a Frankfort horizontal position and with the aid of a digital vernier caliper (validated by calibrating to 0.01mm), the ear measurements obtained from participants included the following:

i. total ear height (TEH) – The length measured from the most superior part of the ear to the most inferior part of the earlobe.

ii. ear width (EW) – The length measured from the most anterior to the most posterior parts of the ear.

iii. lobule height (LH) – The distance between the most inferior point of the earlobe, and the deepest point of the intertragic notch

iv. lobule width (LW) – The distance between the most anterior and most posterior points of the earlobe

To test the reliability of the instrument, the above measurements were obtained twice and the average score was calculated.

In addition to these parameters, two indices defining the proportion of the ear were also calculated: the ear index (EI) [ear width / ear height x 100] and the lobule index (LI) [lobule height / lobule width x 100].

Statistical Analyses: Raw data obtained from participants were recorded into Microsoft Excel 2019 version and analyzed using the Statistical Package for the Social Sciences (SPSS version 23.0). Sexual dimorphism was determined using the Independent Sample t-test, while side (left and right) differences were determined using the Paired sample t-test. Differences in auricular dimensions between age categories were determined using Analysis of Variance (ANOVA). Confidence interval was set at 95% and therefore $P<0.05$ was considered significant.

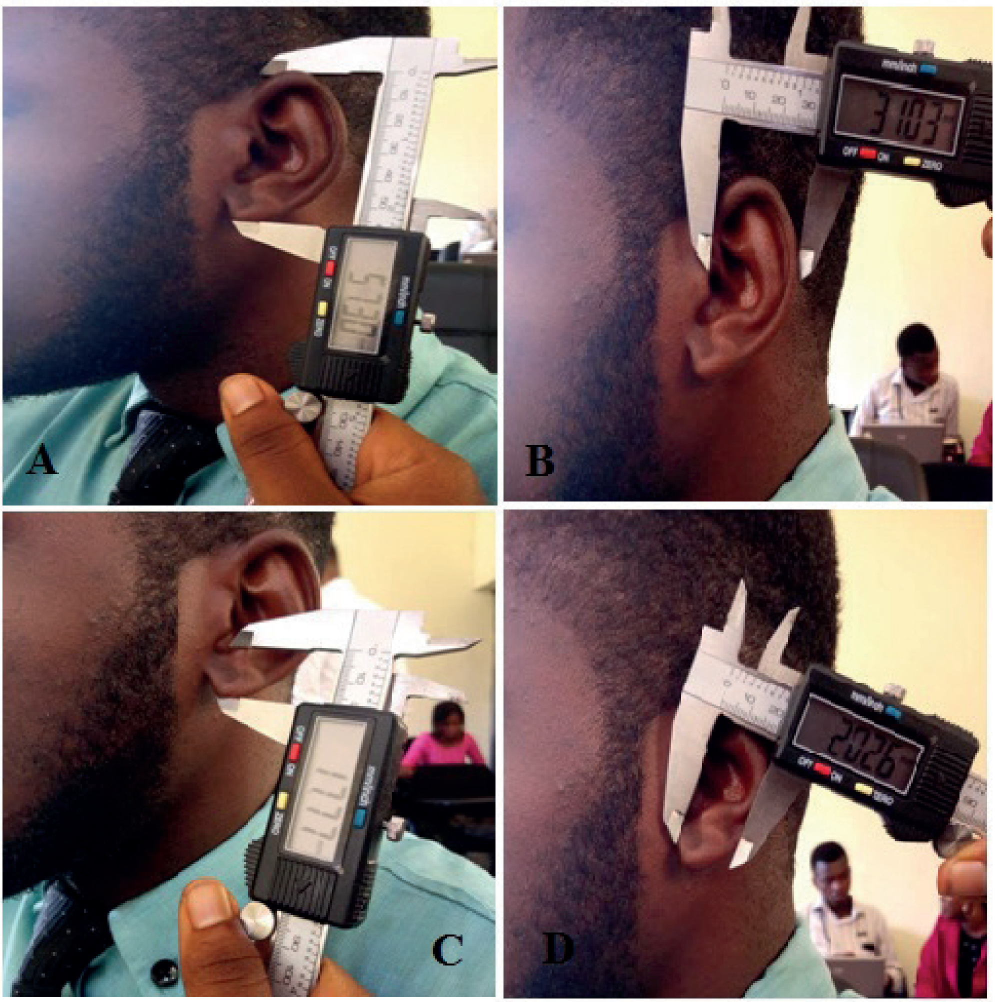

Fig. 1. Ear morphometric dimensions. (A) Total ear height (B) Ear width (C) Lobule height (D) Lobule width

Results

Males had a mean age of 35.34, SD=12.04, while for female subjects, the mean age was 35.21, SD=12.90 (Table 1). The minimum age for males was 16, while the maximum was 62, while for females; it was 16 and 63 respectively (Table 1). There was no significant difference between the mean age of male and female subjects (Table 1).

Gender differences between auricular dimensions are shown in Table 2. Significant differences were observed in left ear height, left lobular height, left lobular width and left ear index (Table 2).

Significant differences in symmetry (i.e., side differences) in male Igbo auricular dimensions were only observed in total ear height (Table 3).

Significant differences regarding asymmetry in female Igbo auricular dimensions were observed in ear width, lobular height, lobular width and ear index (Table 4).

Table 1. Descriptive statistics of age of Igbo ethnic group

Sex	Age in years					T-test		
	N	Min	Max	Mean	S.D	t-value	Df	p-value
Male	142	16	62	35.44	12.04	0.09	298	>0.05
Female	158	16	63	35.32	12.90			
Total	300	16	63	35.38	12.48			

Min = *Minimum*, Max = *Maximum*, S.D = *Standard Deviation*, df = *degree of freedom*

Table 2. Descriptive statistics of the auricular dimensions of the Igbo ethnic group

Auricular Dimensions (mm)	Male [142]			Female [158]		
	Mean (SD)	Min	Max	Mean (SD)	Min	Max
Right Auricle						
Total Ear Height	47.01 (6.30)	24.06	60.84	48.25 (6.83)	24.06	65.14
Ear Width	25.06 (4.14)	11.16	33.22	25.19 (3.93)	13.45	33.51
Lobular Height	13.92 (2.61)	10.03	20.54	14.44 (2.67)	10.19	21.57
Lobular Width	12.99 (2.30)	10.00	22.15	13.37 (2.26)	10.00	20.25
Ear Index	53.47 (7.23)	30.97	70.45	52.50 (6.85)	30.96	69.39
Lobular Index	95.17 (18.12)	55.89	186.26	94.60 (19.59)	53.48	193.44
Left Auricle						
Total Ear Height	46.24 (5.85)	25.67	58.29	48.25 (6.24)	25.67	61.66
Ear Width	24.68 (4.32)	10.21	33.38	24.57 (4.21)	10.12	34.05
Lobular Height	13.99 (2.18)	10.21	21.24	14.89 (2.55)	10.19	21.24
Lobular Width	13.30 (1.94)	10.11	19.75	13.78 (2.04)	10.21	19.26
Ear Index	53.28 (6.69)	32.48	75.12	50.93 (7.05)	29.20	72.06
Lobular Index	96.80 (18.56)	61.29	193.44	94.30 (16.63)	61.80	153.45

SD = *Standard Deviation*, M.D = *Mean Difference*, S.E.M = *Standard Error of Mean*

Table 3. Paired t-test results comparing the right and left auricular dimensions of Igbo males

Auricular Dimensions (mm)	M.D	S.E.M	95% C.I of the Difference		t-value	df	p-value
			Lower	Upper			
Right TEH - Left TEH	0.76	0.27	0.23	1.30	2.84	141	<0.05
Right EW - Left EW	0.38	0.21	-0.04	0.80	1.77	141	>0.05
Right LH - Left LH	-0.07	0.22	-0.51	0.36	-0.34	141	>0.05
Right LW - Left LW	-0.31	0.18	-0.67	0.06	-1.66	141	>0.05
Right EI - Left EI	0.19	0.57	-0.93	1.30	0.33	141	>0.05
Right LI - Left LI	-1.63	1.96	-5.50	2.24	-0.83	141	>0.05

Table 4. Paired T-test comparing the Right and Left auricular dimensions of Igbo females

Auricular Dimensions (mm)	M.D	S.E.M	95% C.I of the Difference		t-value	df	p-value
			Lower	Upper			
Right TEH - Left TEH	0.00	0.27	-0.53	0.53	-0.01	157	>0.05
Right EW - Left EW	0.62	0.21	0.20	1.05	2.91	157	<0.001
Right LH - Left LH	-0.44	0.19	-0.82	-0.07	-2.32	157	<0.05
Right LW - Left LW	-0.40	0.18	-0.75	-0.06	-2.30	157	<0.05
Right EI - Left EI	1.57	0.57	0.45	2.70	2.76	157	<0.05
Right LI - Left LI	0.30	1.86	-3.38	3.98	0.16	157	>0.05

Age related differences were observed in right ear index, left total ear height, and left ear width as well as left ear index (Table 5).

Table 5. Distribution of ear dimensions by age category in Igbo ethnic group along with ANOVA results

Auricular Dimensions (mm)	Age (years)	Descriptive Statistics					ANOVA		
		N	Min	Max	Mean	S.D	df	F-value	p-value
Right Auricle									
Total Ear Height	16 - 25	90	32.15	59.95	48.28	5.73	4	2.17	>0.05
	26 - 35	61	24.06	65.14	46.27	7.87			
	36 - 45	78	24.06	62.84	46.86	6.36			
	46 - 55	52	32.68	61.81	49.47	6.68			
	56 and above	19	39.48	60.84	47.53	5.98			
Ear Width	16 - 25	90	11.16	33.22	25.15	3.93	4	1.30	>0.05
	26 - 35	61	13.45	32.81	24.19	4.73			
	36 - 45	78	13.50	33.51	25.69	4.29			
	46 - 55	52	15.98	30.99	25.42	3.19			
	56 and above	19	19.95	29.51	24.95	2.45			

Auricular Dimensions (mm)	Age (years)	Descriptive Statistics					ANOVA		
		N	Min	Max	Mean	S.D	df	F-value	p-value
Lobular Height	16 - 25	90	10.19	20.85	14.64	2.58	4	1.22	>0.05
	26 - 35	61	10.03	20.54	14.25	2.60			
	36 - 45	78	10.20	21.41	13.82	2.68			
	46 - 55	52	10.19	21.57	14.12	2.95			
	56 and above	19	10.84	18.03	13.66	2.08			
Lobular Width	16 - 25	90	10.11	20.25	13.68	2.40	4	1.64	>0.05
	26 - 35	61	10.11	18.59	13.07	1.76			
	36 - 45	78	10.01	19.75	12.85	2.38			
	46 - 55	52	10.00	22.15	13.12	2.48			
	56 and above	19	10.00	17.15	12.89	2.14			
Ear Index	16 - 25	90	32.56	65.22	52.12	6.29	4	2.46	<0.05
	26 - 35	61	33.11	69.11	52.50	7.59			
	36 - 45	78	30.96	70.45	55.03	7.64			
	46 - 55	52	40.91	66.49	51.83	6.51			
	56 and above	19	41.02	61.68	52.99	6.19			
Lobular Index	16 - 25	90	55.89	186.26	94.97	17.27	4	0.13	>0.05
	26 - 35	61	53.48	120.20	93.57	14.10			
	36 - 45	78	62.33	193.44	94.84	19.19			
	46 - 55	52	65.18	193.44	96.17	26.52			
	56 and above	19	75.95	124.46	95.08	14.30			
Left Auricle									
Total Ear Height	16 - 25	90	32.89	57.25	47.70	5.54	4	2.67	<0.05
	26 - 35	61	25.67	58.29	45.62	7.24			
	36 - 45	78	25.67	61.66	46.86	5.85			
	46 - 55	52	31.43	61.45	49.23	6.10			
	56 and above	19	41.39	56.30	47.37	4.94			
Ear Width	16 - 25	90	11.13	34.05	24.79	4.22	4	2.84	<0.05
	26 - 35	61	10.12	31.27	23.05	5.28			
	36 - 45	78	10.65	33.38	25.31	3.97			
	46 - 55	52	10.12	29.92	25.02	3.44			
	56 and above	19	19.73	28.40	24.98	2.78			
Lobular Height	16 - 25	90	10.21	21.05	14.58	2.34	4	2.40	=0.05
	26 - 35	61	10.19	19.97	13.74	2.29			
	36 - 45	78	10.71	20.36	14.46	2.20			
	46 - 55	52	10.33	21.24	15.11	2.82			
	56 and above	19	10.84	21.24	14.48	2.56			

Auricular Dimensions (mm)	Age (years)	Descriptive Statistics					ANOVA		
		N	Min	Max	Mean	S.D	df	F-value	p-value
Lobular Width	16 - 25	90	10.11	19.75	13.89	2.03	4	1.11	>0.05
	26 - 35	61	10.21	17.00	13.48	1.76			
	36 - 45	78	10.31	18.89	13.28	2.11			
	46 - 55	52	10.35	18.36	13.54	2.08			
	56 and above	19	10.59	18.36	13.27	1.91			
Ear Index	16 - 25	90	29.20	66.06	51.91	6.43	4	3.21	<0.05
	26 - 35	61	30.77	75.12	50.22	8.11			
	36 - 45	78	35.14	72.06	54.11	7.11			
	46 - 55	52	32.20	63.95	51.00	6.20			
	56 and above	19	45.52	61.82	52.90	4.93			
Lobular Index	16 - 25	90	66.74	193.44	97.03	18.71	4	2.08	>0.05
	26 - 35	61	70.81	134.73	99.70	14.96			
	36 - 45	78	61.29	147.86	93.78	19.93			
	46 - 55	52	61.80	127.37	91.33	15.00			
	56 and above	19	73.74	118.45	93.01	13.56			

Discussion

The racial distributions of ear (auricular) morphological variation and morphometric dimensions have been extensively studied in recent times, providing a relevant knowledge to the fields of biological and forensic anthropology, surgical anatomy and prosthetics. However, certain precautions need to be taken to ensure reliability and reproducibility of anthropometric data. The estimation of age and sex from anthropometric measures of an ear has been studied worldwide across plenty populations (Meijerman et al. 2007; Sforza et al. 2009; Murgod et al. 2013; Eboh 2013; Ahmed and Omer 2015; Sharma 2016). The purpose of this study was to determine the accuracy of prediction of age and sex of selected Nigerian Igbo population using auricular morphometrics for forensic applications.

Studies show that men tend to have larger ears than women, ears increase in both length and width with increasing age, and overall ear size differs according to ethnic groups. In this study, there was no significant difference between the mean age of males and females. Regarding the sex differences of the right auricle between sexes, males had slightly lower values in total ear height and lobule height compared to females, leading to a slight increase in their ear and lobule indices. In contrast, this study does not support the notion that ear dimensions increased in size with age as they were relatively similar to one another across all age groups. Hence, the morphometric values of right and left auricle of males and females were comparatively very similar. A study done by Kumar and Selvi (2016) showed that the Malaysian female total ear length, width and ear lobe height were not significantly different on both right and left side. They showed that in the Indian population, the morphometry of ear was found to be more pronounced on the right ear in both sexes.

Faakuu et al. (2020) data based on Ghanaian population showed that right ear height was higher compared to the left auricle and similar pattern was observed in this study. In contrast, Barut and Aktunc (2006) had larger ear measurements in the left auricle. This could be influenced by age and geographical differences.

Gender differences were highly significant in the total ear height, lobular height, width, and the ear index of the left auricle of this present study, which could be due to the wide age group in the study population. This is consistent with several other studies which showed similar significant differences in auricular measurements (Alexander et al. 2011; Ahmed and Omer 2015). In a similar study, sexual differences were reported in the total ear height of Americans from Rhode Island only (Brucker et al. 2003). Furthermore, Taura et al. (2013) showed sexually dimorphic ear length and width on the right side and only in the width on the left side, which corresponds to the findings of our study. In addition, significant side differences were observed only in total ear height of males while side symmetry was observed in all parameters for females except the total ear height and lobular index. Meijerman et al. (2007) argued that these differences might be due to the differences in body maturity levels between sexes.

Studies have reported that the earlobe height increases with age (Alexander et al. 2011; Deopa et al. 2013). Age-related differences were observed in right ear index, left total ear height, and left ear width as well as left ear index in this study.

Table 6. Comparison of auricular dimensions (measured in mm) of the present study with related literature

Author(s)	Year	Mean TEH		Mean EW		Mean LH		Mean LW	
Tatlisumak et al.	2015	65.49 (L) 64.47 (R) (males)	61.33 (L) 60.30 (R) (females)	33.96 (L) 35.23 (R) (males)	32.29 (L) 32.97 (R) (females)	18.37 (L) 18.40 (R) (males)	17.31 (L) 17.33 (R) (females)	17.33 (L) 19.22 (R) (males)	17.08 (L) 18.73 (R) (females)
Elyasi et al.	2020	59.86 (males)	60.12 (females)	30.71 (males)	31.36 (females)	N.A		N.A	
Rani et al.	2021	60.40 (L) 61.20 (R) (males)	57.60 (L) 57.90 (R) (females)	32.60 (L) 33.20 (R) (males)	30.50 (L) 30.40 (R) (females)	16.80 (L) 16.50 (R) (males)	16.90 (L) 16.70 (R) (females)	20.10 (L) 18.30 (R) (males)	18.20 (L) 17.70 (R) (females)
Singh et al.	2022	62.30 (L) 62.90 (R) (males)	59.10 (L) 59.90 (R) (females)	32.80 (L) 33.10 (R) (males)	30.50 (L) 30.40 (R) (females)	17.70 (L) 17.60 (R) (males)	17.50 (L) 16.70 (R) (females)	20.20 (L) 19.00 (R) (males)	19.80 (L) 18.20 (R) (females)
Present study	2023	46.24 (L) 47.01 (R) (males)	48.25 (L) 48.25 (R) (females)	24.68 (L) 25.06 (R) (males)	24.57 (L) 25.19 (R) (females)	13.99 (L) 13.92 (R) (males)	14.89 (L) 14.44 (R) (females)	13.30 (L) 12.99 (R) (males)	13.78 (L) 13.37 (R) (females)

L = Left, R = Right, N.A = Not Available

Conclusions

Overall, this study provides a baseline data for auricular morphometrics of adult Nigerian Igbos. In both sexes, the total ear height and ear width values were slightly higher on the right sides compared to the left side while the lobule height and lobule width values of the right side were slightly lower compared to the left side. However, the total ear height and lobular width values in females were much higher than that of the males. The values obtained from this study might play a significant role in forensic identification and surgical operations, as well as serve as ergonomic guide for the production of prosthetics and hearing aids for correction of ear deformities.

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Conflicts of interest

All authors declare that there is no form of competing interests.

Authors' contributions

GSO was the lead researcher, conceived study concept and design, critical revision of the article for important intellectual content, and provision of study materials; OMA performed the data collection, statistical analysis, and wrote the manuscript. JNE performed data collection and compilation, and revised the manuscript. All authors discussed the results and contributed to the final manuscript for publication.

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Associations between birth season and lumbar spine bone mineral density in perimenopausal Polish women

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Abstract: In European populations, the birth season significantly correlates with many biological features. It is thus possible that the observed clinical effects of bone metabolism disorders are a partial consequence of bone mineral density (BMD), modified by the season of prenatal development (the birth season). The aim of this study was to evaluate the relationship between the birth season and BMD among Polish women in perimenopausal age.

A total of 653 Polish women aged 50.0–59.9 years were included in the study. BMDs of lumbar vertebrae were measured by dual-energy x-ray absorptiometry. Statistical analyses were based on measured lumbar BMD values, age, and body mass index (BMI). The analysis of variance (ANOVA) was applied to evaluate the season-related differentiation of mineral density of lumbar vertebrae. BMDs of lumbar vertebrae negatively correlated with age and positively with BMI. We regressed BMD on age and BMI and used the residuals as a measure of age- and BMI-independent lumbar BMD values.

The ANOVA results showed that women born in summer had significantly lower BMD of the L1 vertebra compared to those born in autumn, regardless of age and BMI.

The results of our study indicate the need to extend the group of risk factors for osteoporosis in Central Europeans with the season of woman's birth.

Key words: season of birth, prenatal development, BMD, BMI, osteoporosis.

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Introduction

Decreasing mineral density of skeleton bones is a natural involutionary process, which may lead to osteoporosis (i.e., excessively low bone mass with a simultaneously maintained proportion between the organic and the mineral fraction) and, ultimately, to enhanced bone fragility. The main factors that significantly increase the risk of osteoporosis among postmenopausal women include low peak bone mass (PBM, achieved, on the average, at the age of 18 years; Roy et al. 2005), late menarche age (McKay et al. 1998) and menopause with accompanying declines in oestrogen levels. The most common environmental determinants of osteoporosis are low body mass index (BMI), irregular nutrition, diet poor in calcium and vitamin D, sedentary lifestyle, alcohol abuse and tobacco smoking (Cooper et al. 2006; Sinaki 2007; Wilsgaard et al. 2009; Özbaş et al. 2012). The body weight and BMI are important factors affecting BMD of women at the age of peak bone mass (Henderson et al. 1995; Al Rassy et al. 2018) and in postmenopausal females (Ravn et al. 1999; Wu & Du 2016). An increased BMI (and the resulting higher fat mass) has a protective impact on bone density (Barrera et al. 2004), and, depending on the fat distribution in pre- and postmenopausal females (Fu et al. 2011), correlates with endocrine alterations. The latter positively influences bone metabolism (Zhao et al. 2008), predisposing to higher BMD, thicker and denser cortices, and higher trabecular number (Evans et al. 2015). At a population level, high BMI remains a protective factor for most sites of fragility fracture (Johansson et al. 2014). Thinness (low percentage of body fat, low BMI, or low body weight) predisposes postmenopausal females to rapid bone loss, low bone mass, osteoporosis and related fracture risk (Ravn et al. 1999; Kanis et al. 2011; Prieto-Alhambra et al. 2012), which is mediated by the interaction between BMI and BMD (Johansson et al. 2014).

Genetic factors are also significant. The genes, whose polymorphisms significantly correlate with an increased risk of osteoporosis, include, among others: oestrogen receptor (*ER*), transforming growth factor beta 1 (*TGF-β1*), interleukin 10 (*IL-10*), interleukin 6 (*IL6*), interleukin 17-F (*IL17F*), vitamin D receptor (*VDR*), cytochrome P-450c17alpha (*CYP17*), plasminogen activator inhibitor-1 (*PAI-1*), collagen type I alpha 1 (*COL1A-1*) and calcitonin receptor (*CALCR*) (Chen et al. 2005; Bustamante et al. 2007; Seremak-Mrozikiewicz et al. 2009; Oishi et al. 2012; Tural et al. 2013).

Some studies also indicate prenatal risk factors of osteoporosis, as well as osteoporosis-related, higher incidence of femoral bone fractures (Cooper et al. 2009). There is some evidence suggesting that the peak bone mass might be heritable although the current genetic markers are able to account only for a small proportion of individual bone mass variation or fracture risks. The mechanism of this relation reveals an intrauterine control mechanism of neonatal skeletal growth and mineralization. This mechanism appears to be mediated by modulation of the set-point for basal activity of a pituitary-dependent endocrine system, such as the hypothalamic-pituitary-adrenal (HPA) and the growth hormone/insulin-like growth factor-1 (GH/IGH-1) axes (Godfrey et al. 2001; Javaid et al. 2006; Cooper et al. 2009). According to Godfrey et al. (2001), the neonatal bone mass is significantly and positively correlated with childbirth parameters (body weight and length), standardised for sex and pregnancy duration and by weight of the placenta alone. Maternal factors, which significantly and negatively correlate with offspring bone mass content (BMC), include maternal smoking and maternal energy intake at 18 weeks of gestation (Godfrey et al. 2001), as well as reduced maternal height, lower pre-conception maternal weight, reduced maternal fat stores during late pregnancy and lower maternal social class (Javaid et al. 2006).

Birth season is a derivative of the prenatal development season. In European populations, the birth season significantly correlates with many biological features that is observed both at the population and individual level, including fecundity, conception and birth (Lam et al. 1994), sex ratio at birth (Nonaka et al. 1999), childbirth parameters (Chodick et al. 2009), infant and adult mortality (Doblhammer & Vaupel 2001), body height and body weight in later life (Krenz-Niedbała et al. 2011), cardiovascular conditions in adulthood (Doblhammer & Vaupel 2001), life expectancy and the probability of death at older ages (Doblhammer & Vaupel 2001; Doblhammer et al. 2005), the incidence of certain neurodegenerative diseases, such as multiple sclerosis (Salzer et al. 2010) and Parkinson's disease (Gardener et al. 2010), as well as the incidence rates of certain mental diseases, such as schizophrenia and psychotic-like experiences (Tochigi et al. 2013).

Studies on Polish population demonstrate a significant relationship of the birth season with biological features, such as birth body length (Siniarska & Kozieł 2010), birth body height and body weight in later life (Krenz-Niedbała et al. 2011), the width of enamel layer of deciduous teeth (Żądzińska et al. 2013), as well as the incidence of the nervous system diseases, including cerebral palsy (Kulak & Sobaniec 2005).

Although the number of studies analysing the relationship between the prenatal development environment (including the birth season) with neonatal bone mineral density (BMD) and neonatal bone mineral content is fairly high (Namgung et al. 1998; Godfrey et al. 2001; Javaid et al. 2006), the number of reports, indicating "maintenance" of this relationship in adult life is rather low. Some notable exceptions from the mentioned above relationship include studies regarding birth season to significantly increase the risk of osteoporosis-related femoral bone fractures in Danish men and women at the age above 65 (Abrahamsen et al. 2012), and a study conducted on the Norwegian population that indicated that the month of birth significantly correlates with radiographically diagnosed bilateral hip and knee arthrosis (Følnnebø 1995). It is thus possible that the observed clinical effects of bone metabolism disorders are partly a consequence of bone mineral density modified by the season of prenatal development (the birth season). The study concerns women at the perimenopausal age in whom, while ageing, the natural phenomenon of gradual bone density loss in the lumbar section of the spine occurs. The aim of this study was to evaluate a relationship between the birth season and the level of bone mineral density in Polish women in perimenopausal age.

Material and methods

Study participants

A total of 653 Polish women at the age of 50.0–59.9 years were included in the study, all of them being first-time patients, attending the Outpatient Clinic of Osteoporosis at the Medical University Hospital in Łódź (Poland) during the years 2002–2015. All the patients were at that time residents of Łódź – a city located in central Poland with the population of 722 thousand inhabitants. The study was approved by the Institutional Bioethical Committee of the University of Łódź. A written informed consent was obtained from all study participants.

The mean age of examined women was 55.80±2.55 years, the mean weight: 67.37±12.07 kg, the mean height: 161.28±5.67 cm, and the mean body mass index (BMI): 25.88±4.29 kg/m^2. Birth season was defined as follows: spring – women, born from the 1st of March through the 31st of May; summer – women, born from the 1st of June through the 31st of August; autumn – women, born from the 1st of September through the 30th of November; winter – women, born from the 1st of December through 29th of February.

Bone mineral density measurements

BMD measurements were performed at the Outpatient Clinic of Osteoporosis, Medical University Hospital of Łódź. BMDs of the lumbar spine were measured by dual-energy x-ray absorptiometry (Lunar Prodigy, GE Lunar, Madison, WI, USA) at medium 750 μA scan mode. Lumbar spine scans were obtained with patient on table in supine position, adhering to the manufacturer's protocols. Quality control scans, carried out during a 4-year follow-up period, indicated no gear-related shifts in BMD levels. Statistical analyses were based on measured lumbar BMD values (g/cm^2) for L1, L2, L3, L4, L1-L2, L1-L3, L1-L4, L2-L3, L2-L4 and L3-L4.

Statistical analysis

All studied variables were evaluated for normality using the Shapiro-Wilk test and for equality of variance using the Levene's test. The associations of the studied variables (age, BMI, and BMD measurements of the lumbar section of the spine) were assessed with a non-parametric correlation test (Spearman's R). Age and BMI of the examined women by season of birth were compared using non-parametric equivalent of ANOVA (Kruskal-Wallis test). To eliminate the influence of age and BMI on BMD, we used the multiple regression-dependent variable: BMD (g/cm^2) measurements of lumbar section of the spine; the independent variables: age (years) and BMI (kg/m^2). Thus, we considered the residuals as age- and BMI-independent measures of BMD. The residuals were calculated separately for measurement of lumbar section of the spine (*res*L1, *res*L2, etc.). They were used as dependent variables to assess the diversity of the BMD values according to the season of birth of women using the analysis of one-way variance (ANOVA) with the Bonferroni post-hoc test. All the statistical analyses were performed using the *STATISTICA* software (TIBCO Software Inc., version 13).

Results

Age of female study participants was not significantly correlated with their BMI ($R=0.034$; $p=0.392$). However, the BMD values of the lumbar spine showed significant and negative correlation with age ($p<0.001$) and positive correlation with BMI ($p<0.001$) – Table 1.

The birth season did not differentiate the examined women by age ($H=2.43$; $p=0.488$) and BMI ($H=0.75$; $p=0.861$) – Table 2. Over half of the women were born in spring and summer (56.2%).

Table 1. Characteristics and comparison of the analyzed variables of examined women (N=653)

BMD (g/cm^2) measurements of the lumbar section of the spine			Age (years) & BMD (g/cm^2)		BMI (kg/m^2) & BMD (g/cm^2)	
	Mean	SD	R	p value	R	p value
L1	0.923	0.151	-0.213	<0.001	0.262	<0.001
L2	0.981	0.165	-0.213	<0.001	0.282	<0.001
L3	1.041	0.176	-0.183	<0.001	0.263	<0.001
L4	1.024	0.195	-0.136	<0.001	0.262	<0.001
L1-L2	0.953	0.154	-0.218	<0.001	0.283	<0.001
L1-L3	0.985	0.159	-0.209	<0.001	0.282	<0.001
L1-L4	0.996	0.164	-0.191	<0.001	0.283	<0.001
L2-L3	1.013	0.167	-0.201	<0.001	0.278	<0.001
L2-L4	1.017	0.172	-0.181	<0.001	0.279	<0.001
L3-L4	1.032	0.180	-0.162	<0.001	0.269	<0.001

BMI, body mass index; BMD, bone mineral density; SD, standard deviation; R and p value, non-parametric correlation test (Spearman's R).

Table 2. Descriptive statistics of age and BMI of examined women (N=653) according to season of birth

Season of birth	n	%	Age (years)			BMI (kg/m^2)		
			Median	Q1	Q3	Median	Q1	Q3
Spring	182	27.9	56.21	54.18	58.25	25.84	23.06	28.63
Summer	185	28.3	55.99	53.39	57.69	25.08	22.83	28.63
Autumn	152	23.3	55.93	53.92	57.97	25.23	22.86	28.16
Winter	134	20.5	56.03	54.00	57.78	25.45	23.23	28.44
Kruskal-Wallis test			H = 2.43; p = 0.488			H = 0.75; p = 0.861		

n, sample size; BMI, body mass index; Q1, lower quartile; Q3, upper quartile.

The results of multiple regression show that the BMI values have a larger share in the estimation of lumbar spine BMD variability than the age of women (Table 3). BMI, along with age, explained about 9.9-14.2% of the total BMD variability (according to adjusted R^2).

The one-way analysis of variance to assess the variability of age- and BMI-independent measures of BMD (*res*L1, *res*L2, etc.) in the seasons of the women's birth indicated a statistically significant relationship - for the first lumbar vertebra (*res*L1: F=2.37; p=0.043). Women born in summer have a lower *res*L1 as compared to those born in autumn (according to Bonferroni post-hoc test: p=0.046) – Table 4, Fig. 1. For the remaining 9 measurements of the lumbar spine, the observations of the lowest *res*BMD values in women born during summer do not exceed the threshold of statistical significance (p>0.05) – Fig. 2–3.

Table 3. Multiple regression results

BMD (g/cm^2)		Independent variables	B	SE	t	p value	partial corr.	Adj. R^2	F	p value
L1	Age (years)	-0.225	0.037	-6.18	<0.001	-0.235	0.132	50.55	<0.001
	BMI (kg/m^2)	0.297	0.037	8.15	<0.001	0.304			
L2	Age (years)	-0.231	0.036	-6.35	<0.001	-0.242	0.136	52.21	<0.001
	BMI (kg/m^2)	0.300	0.036	8.22	<0.001	0.307			
L3	Age (years)	-0.200	0.037	-5.42	<0.001	-0.208	0.115	43.23	<0.001
	BMI (kg/m^2)	0.285	0.037	7.74	<0.001	0.291			
L4	Age (years)	-0.158	0.037	-4.25	<0.001	-0.164	0.099	36.83	<0.001
	BMI (kg/m^2)	0.283	0.037	7.60	<0.001	0.286			
L1-L2	Age (years)	-0.235	0.036	-6.48	<0.001	-0.246	0.142	55.00	<0.001
	BMI (kg/m^2)	0.307	0.036	8.47	<0.001	0.315			
L1-L3	Age (years)	-0.226	0.036	-6.22	<0.001	-0.237	0.137	52.67	<0.001
	BMI (kg/m^2)	0.305	0.036	8.38	<0.001	0.312			
L1-L4	Age (years)	-0.209	0.037	-5.72	<0.001	-0.219	0.131	50.15	<0.001
	BMI (kg/m^2)	0.308	0.037	8.42	<0.001	0.314			
L2-L3	Age (years)	-0.219	0.037	-6.00	<0.001	-0.229	0.130	49.54	<0.001
	BMI (kg/m^2)	0.298	0.037	8.15	<0.001	0.305			
L2-L4	Age (years)	-0.200	0.037	-5.47	<0.001	-0.210	0.124	47.21	<0.001
	BMI (kg/m^2)	0.301	0.037	8.22	<0.001	0.307			
L3-L4	Age (years)	-0.182	0.037	-4.93	<0.001	-0.190	0.113	42.53	<0.001
	BMI (kg/m^2)	0.294	0.037	7.96	<0.001	0.298			

BMD, bone mineral density; BMI, body mass index; Beta, standardized regression coefficient; SE, standard error of standardized regression coefficient; t, t-Student test value; R^2, coefficient of determination; F and p value for model.

Table 4. The one-way ANOVA output

Dependent variables	Season of birth								F	p value
	Spring		Summer		Autumn		Winter			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
*res*L1	0.003	0.152	-0.024	0.136	0.018	0.133	0.009	0.137	2.73	0.043*
*res*L2	0.007	0.161	-0.023	0.155	0.014	0.140	0.006	0.150	1.98	0.115
*res*L3	0.004	0.175	-0.022	0.160	0.015	0.155	0.007	0.168	1.67	0.172
*res*L4	-0.004	0.189	-0.020	0.173	0.016	0.185	0.014	0.192	1.39	0.246
*res*L1-L2	0.005	0.152	-0.023	0.140	0.016	0.132	0.007	0.139	2.47	0.061
*res*L1-L3	0.005	0.157	-0.023	0.144	0.015	0.137	0.008	0.146	2.21	0.085
*res*L1-L4	0.002	0.161	-0.022	0.145	0.016	0.145	0.010	0.155	1.98	0.116
*res*L2-L3	0.002	0.169	-0.021	0.153	0.015	0.153	0.010	0.164	1.90	0.129
*res*L3-L4	0.000	0.177	-0.021	0.158	0.016	0.165	0.011	0.175	1.73	0.160

SD, standard deviation; F and p value, one-way analysis of variance (ANOVA) for season of birth on dependent variables: age-and BMI-independent measures of bone mineral density (BMD) by regressing BMD on age and BMI.

* The Bonferroni post-hoc test: Spring = Summer (p=0.441); Spring = Autumn (p=1.000); Spring = Winter (p=1.000); Summer ≠ Autumn (p=0.046); Summer = Winter (p=0.251); Autumn = Winter (p=1.000).

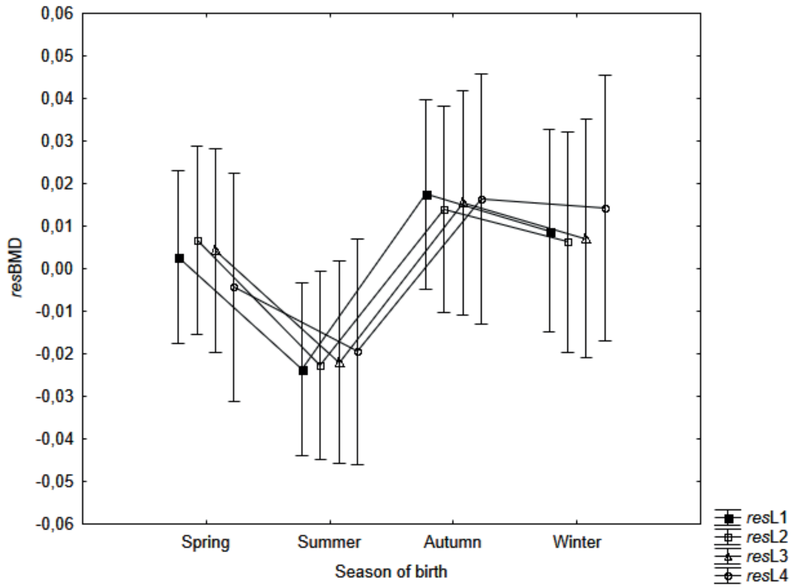

Fig. 1. Mean *res*BMD measurements of L1, L2, L3 and L4 according to the season of birth of examined women. Statistically significant differences in measurement of *res*L1 (F=2.37; p=0.043). According to the post-hoc Bonferroni test: significant difference (p=0.046) between women born in Spring (March-May) vs Autumn (September-November). Vertical bars indicate 0.95 confidence intervals

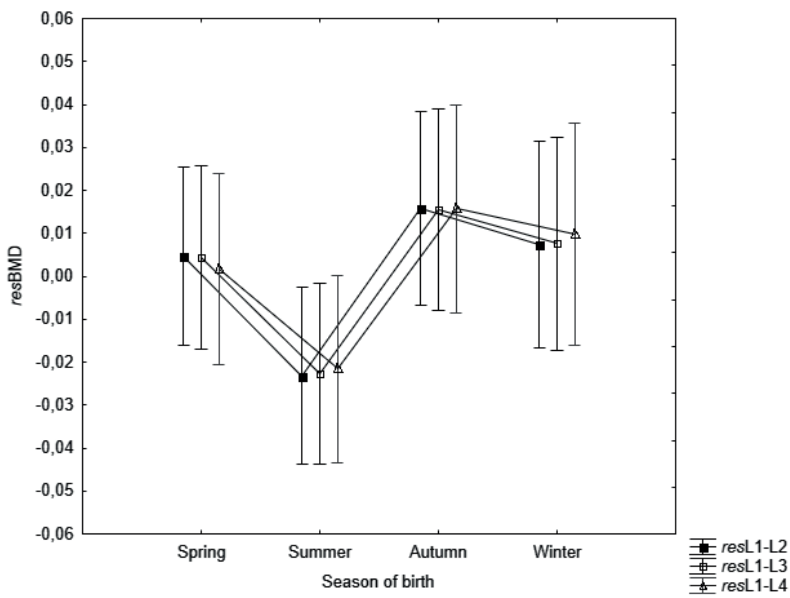

Fig. 2. Mean *res*BMD measurements of L1-L2, L1-L3 and L1-L4 according to the season of birth of examined women. Vertical bars indicate 0.95 confidence intervals

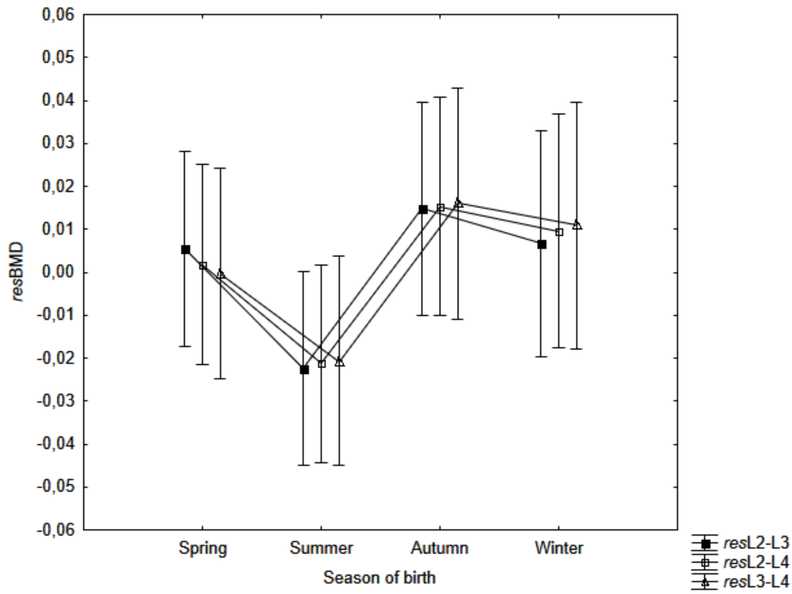

Fig. 3. Mean *res*BMD measurements of L2-L3, L2-L4 and L3-L4 according to the season for birth of examined women. Vertical bars indicate 0.95 confidence intervals

Discussion

The main result of the study is the indication of the significant relationship mineral density of lumbar vertebrae with the season of birth of women in the perimenopausal age. Women born in summer (June-August) were characterised by the lowest bone mineral density in the first vertebrae of the lumbar spine compared to women born in the autumn months (September-November). And this relation concerns the part of the BMD variability which is independent of age and BMI of the examined women.

The birth season is a direct consequence of the season of prenatal development, an environment, which is a multi-factor modifier of the human development during the first (most important) months of life. With regards to full-term children (gestation of 37–42 weeks), born in summer months (i.e., from the 1st of June through the 31st of August), the first two trimesters of gestation occur in autumn and winter months. Regarding the Central Europe, this part of the year is characterised by the lowest sun activity (insolation) (for the territory of Poland, the mean insolation level, measured by the number of sunny hours per month, is the highest from May through August – 243.17 and the lowest from November through February – 50.42). The autumn and winter months in Poland are also characterised by the lowest air temperature (the mean temperature level in summer months varies between 16.5 and 20°C, while it is only -6 to 0°C in winter), a limited availability of fresh vegetables and fruits, and a high incidence of infections (in Europe, the seasonal peak of influenza infections is usually between January and March). The insolation level significantly determines the synthesis of vitamin D which is delivered to the foetus exclusively from the mother's body (Salle et al. 2000). Vitamin D deficits in pregnant women are significantly more frequently observed during winter months and in countries, where food stuffs are not routinely supplemented with vitamin D, also in members of the ethnic groups, in which women cover their bodies regardless of the season of the year and among persons with high pigmentation level of their skin (Specker 2004). Insufficient vitamin D levels in mother's body compromise skeletal structure development and strength in the foetus, including, among others, lower bone mineral density, observed already in newborns (Tobias et al. 2005; Javaid et al. 2006; Cooper et al. 2009).

Maternal factors, which form the environment of prenatal development, "programming" the earliest stages of human skeleton formation, play a significant role in the epidemiology of osteoporosis (Cooper et al. 2009). These factors may include maternal nutrition (particularly deficient in vitamin D), maternal smoking and/or alcohol consumption during pregnancy. Low birth weight and body length as well as a low placental weight (an effect of vitamin D deficits in the prenatal environment) significantly correlate, both with low neonatal bone mineral (Dennison et al. 2001) and low neonatal bone mineral content (Godfrey et al. 2001). Reduced neonatal bone mass density leads to decreased adult bone mass density and, in consequence, to osteoporosis and an increased risk of hip fracture (Abrahamsen et al. 2012). Studies based on databases for European (UK and the Netherlands), US, Asian (Japan, Korea) and New Zealand population as well as the studies based on meta-analysis confirm this significant, one-way relationship of birth weight with lumbar bone mineral content (BMC) (Namgung et al. 1998; Baird et al. 2011). For example, a meta-analysis demonstrated that a 1000 g increase in birth weight was associated with a 1.49 g increase in lumbar spine BMC (95% CI 0.77-2.21) (Baird et al. 2011).

The season of birth is a significant newborn's body weight regulatory factor. In European populations, (e.g., those in the Northern Ireland, Greece, Poland) the peak in childbirths with low body weight is recorded in spring and summer months (Murray et al. 2000; Flouris et al. 2009; Siniarska & Kozieł 2010). According to Murray et al. (2000), Irish children, born in July, have, on the average, lower (by 31.6 g) birth weight compared to children born in January (95% CI 35.2, 28.0). Polish children, born in April and May, exhibit the lowest average birth (Siniarska & Kozieł 2010).

Seasonal variation in maternal serum vitamin D levels is among the major causes of the observed relationship between birth season and biological features of man, including BMD and BMC, indicated by most researchers. In European populations, especially those inhabiting the Northern part of the continent, vitamin D synthesis is limited to 5-6 months during the year (Brot et al. 2001). This limitation, while determining the maternal vitamin D level, may significantly control vitamin D levels in child's body, depending on the season of the year, during which prenatal development took place (characterised – at a given altitude – by specific conditions of exposure to sunlight, air temperature, the availability of fresh vegetable nutrition and the incidence of infections).

The link between maternal vitamin D status and child bone mineral density was observed by Javaid et al. (2006), who measured BMD by DXA in 198 9-year-old children whose mothers had their serum 25 OH-vitamin D levels measured in the last weeks of the third trimester of pregnancy. Children of mothers with low vitamin D levels were characterised by much lower BMD values, measured both at the spine and in total body, as well as by lower levels of calcium ions in umbilical blood.

The prenatal and neonatal „programming" of disorders in mineral density of skeletal bones and, in consequence, of osteoporotic changes, is, in part, underlain by an epigenetic mechanism (Holroyd et al. 2012). Modification of *PMCA3* (placental calcium transporter) gene expression level, which determines the neonatal whole-body BMC (Martin et al. 2007), may be of key significance in the control of vitamin D transport by the placenta, which, in turn, controls the prenatal level of ionized calcium concentration and, eventually, regulates skeletal growth and mineralization (Javaid et al. 2006).

The prenatal environment conditions, in which spine mineralisation processes take place, are thus of key importance in human BMC formation. It is possible that the prenatal environment conditions affect the peak bone mass attained at the age of 18 years, the low values of which are among the major risk factors of osteoporosis (Roy et al. 2005) and, in consequence, determine the rate of involutionary changes in the skeleton. According to Noback and Robertson (1951), the ossification of the spine spreads from two basic regions: the cervical and the lower thoracic/upper lumbar regions. Ossification in the lower spine region begins simultaneously at 3 points: within L11 and L12 vertebrae of thoracic spine and L1 vertebra of the lumbar spine. Only in further sequence do ossification centres occur in other vertebrae as well, both in cephalic and caudal directions (Bagnall et al. 1977). The ossification process in L1 begins on the 9th week of gestation and attains the L5 level at the end of the 3rd month. According to Scheuer and Black (Scheuer & Black 2000) the lumbar vertebrae are readily identifiable from the end of the fourth foetal month.

It is possible that the strength of the prenatal conditions depends on sex. According to Siniarska and Kozieł (2010), for instance, the influence of birth season on body length of the Polish newborns is characterised by distinctive "sex dimorphism". In boys, the highest correlation between the average values of atmospheric characteristics and the neonatal body length was observed for the second trimester of prenatal growth, whereas in girls the highest correlation occurred for the first trimester.

The occurrence of ossification centres in L1 during the 2nd month of foetal life and, in subsequent lumbar spine vertebrae by the end of the 3rd month of foetal life, only begins the entire process of ossification. Fusion of the primary centres of ossification in the lumbar spine begins from L1 on the 1st year of postnatal life and continues in distal direction, attaining L5 at, approximately, the 5th year of child's life (Scheuer & Black 2000).

It is therefore possible that the critical period for L1 vertebra development in children born in summer season (thus beginning the 2nd trimester of prenatal development in winter months, of the lowest exposure to sun light) is slightly more sensitive to the limitation of vitamin D levels and normal transport of calcium ions, thus being most susceptible to the consequential BMD reduction. According to Fønnebø (1995) and Abrahamsen et al. (2012), a low sunlight exposure prior to the crucial period in skeletal development should be considered as a risk factor of hip fracture in Northern European populations.

In conclusion, a significant diversity of bone density can be observed with respect to both the season of birth of the Polish women, and thus with respect to the season in which their prenatal development occurred. The analysis of variability of age- and BMI-independent measures of BMD (*res*L1, *res*L2, etc.) allowed us to indicate a statistically significant relationship for the first lumbar vertebra. Women born in summer have a lower BMD of the L1 vertebra compared to those born in autumn, regardless of the rate of bone density loss with age and a positive correlation with BMI.

The obtained results indicate the need to extend the group of risk factors for osteoporosis with the season of woman's birth. The results of this study also suggest that women in Central Europe similarly and countries of the Northern Europe should be encompassed by special prophylactic care against osteoporosis of pregnant women, especially if the term of delivery is planned for summer months.

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Conflict of interests

Authors declare no conflict of interests.

Authors' contributions

EŻ and IR analyzed the data and drafted the manuscript. AES and MS collected the data. EŻ, IR and ES edited the manuscript for intellectual content and provided critical comments on the manuscript.

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The assessment of the relationship between the traits of temporal muscle and the massiveness of the supraorbital region of the Homo sapiens crania including the influence of the neurocranial shape and size of the occlusal surface of the upper molars – preliminary study

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Abstract: The knowledge about the pattern of the relationship between the morphological variability of the supraorbital region of human skulls and the features of the temporal muscle is far from complete. The main aim of this study was to determine the relationships between the traits of human temporal muscle (i.e., its relative height and length) and the relative massiveness of the supraorbital region of the frontal bone with taking into account the potential influence of the neurocranial shape and the relative area of the occlusal surface of the upper molars.

Skulls of African and Australian males (n = 44) exhibiting high variability in the massiveness of the supraorbital region, the presence of two types of the upper molars (first and second, n = 88) and a good state of the preservation of the braincase with the clearly visible inferior temporal line were examined.

The qualitative scales were used to assess the degrees of the massiveness of the supraorbital regions. Metric traits of temporal muscle and that used to calculate the index of the neurocranial shape, size of the braincase and the facial skeleton were collected. Values of the occlusal areas of the molars were obtained using the ImageJ software.

Spearman's rank correlation and partial rank correlation analyses were performed.

The results of our study showed the relationships between the traits of the temporal muscle and only the degree of the robusticity of most lateral part of the supraorbital area (trigonum). However, when the influences of the neurocranial shape and the relative occlusal area of molars were excluded, these relationships disappeared. The greatest importance of the neurocranial shape for the formation of the morphology of the trigonum was indicated. The results of the study were discussed from the perspective of the potential role of the temporal muscle as the part of the mastication apparatus for the development of the robusticity of the cranial supraorbital region.

Key words: cranial robusticity, masticatory stress, masticatory muscles, human skulls, cranial shape.

Original article

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Introduction

The temporal muscle is the largest and strongest muscle of the mastication apparatus, placed in the temporal fossa. Its main role is to elevate the jaw associated with the chewing and biting process. This muscle is fan-shaped, consisting of numerous fibers running in three different directions (the anterior fibers run almost vertically, the medial fibers run obliquely, and the posterior fibers run horizontally). These fibers start at the inferior temporal line and descend inferiorly into the tendon, which includes the coronoid process of the mandible (also attaches to the retro-molar fossa) (e.g., Tolhurst et al. 1991). After birth, this muscle grows only through the hypertrophy of its fibers (Rowe and Goldspink1969; Pearson 1990). Based on data obtained from the 3D CT scans of their skulls, it has been established that in human children occurs an age-related expansion of the area of the attachment of the temporal muscle (the bony origin) in two directions (vertical and horizontal) which is not explained only by the covariation of the growth of the temporal muscle and braincase (Moltoni et al. 2021). It has been suggested that the main cause of this phenomenon might be a response to a more solid diet through the age-related changes in the chewing pattern (e.g., Kamegai et al. 2005) that requires an increase in the strength of this muscle (Moltoni et al. 2021). Given the above, it could be hypothesized that individuals who consumed harder food during childhood should have a more developed and stronger temporal muscle in relation to the size of their braincase. They should also exhibit more massive supraorbital area of the frontal bone, especially its most lateral region located closest to the anterior area of attachment of this muscle. The stresses generated during functioning of the temporal muscles acting during the growth of the skulls could affect the morphology of the skulls and the formation of the massiveness of the supraorbital area of the frontal bone.

So far, the reasons for the variability in the massiveness of the supraorbital area of the frontal bone observed in representatives of *Homo sapiens* populations derived from different geographical areas of the world have not been fully understood (e.g., Lahr 1996; Lahr and Wright 1996; Baab et al. 2010; Nowaczewska et al. 2015). This variability concerns the degree of development of the massiveness of the following regions of the frontal bone, such as the glabella (the region located between the two superciliary arches), the superciliary arches and the so-called trigonum (the most lateral structural element of the supraorbital region) (Figure 1a) (Cunningham 1908). These three structural areas may show a very different degree of expression from extremely high, often occurring in, for instance, Patagonians, Australians or former inhabitants of Tasmania, through a moderate degree of development in European populations, to weak, often occurring in Africans (Lahr 1996; Lahr and Wright 1996). Among various factors potentially influencing the massiveness of the above-mentioned regions of the skull (apart from the basic sex factor related to the influence of sex hormones (e.g., Buikstra and Ubelaker 1994; White and Folkens 2000)), the biomechanical impact on the growing facial skeleton through the stresses generated during the functioning of the mastication apparatus has been discussed (e.g. Endo 1970; Russell 1985). In the case of this factor, significant developmental plasticity and epigenetic influence have been suggested as mechanisms explaining this relationship (Lieberman 2011; Von Cramon-Taubadel 2014; Katz et al. 2017). According to the localized masticatory stress hypothesis, greater masticatory stress can contribute to greater facial skeleton massiveness during the period of its formation in the regions influenced by its action (Lahr 1996; Lahr and Wright 1996). Based on the results of experiments carried out on mammals, the formation of stronger stresses caused by eating harder food, the chewing of which required generation of greater forces by the mastication apparatus, has been suggested to be associated with the development of a larger size of facial skeleton and its massiveness in humans (Lieberman et al. 2004; Ravosa et al. 2010; Lieberman 2011). This has been further supported by the experimental studies concerning the reduction in the functioning of the mastication apparatus (e.g., in rats Watt and Williams 1951; Beecher and Corruccini 1981; Bresin et al. 1994), along with the latest study on the consequences of the soft food consumption (short-term and multi-generational) reflected in the changes in the craniofacial morphology of mice (Hassan et al. 2020).

The results of the examinations of the skulls of archaeological human populations concerning the analysis of their morphology indicated differences in their massiveness and shape between populations (maintaining regional and genetic continuity) that changed their lifestyle from a hunter-gatherer to a sedentary (agricultural) lifestyle, associated with a change to a more processed and soft diet – farmers' skulls showed a reduction in the massiveness and size of the facial skeleton and a more short and rounded braincase compared to hunter-gatherer skulls (Carlson and Van Gerven 1977; Larsen 1995; Sardi et al. 2006). However, this phenomenon has not yet been fully explained. The occurrence of larger sizes (e.g., height, length) of the attachment area of the temporal muscles as elements of the mastication apparatus have been suggested to be associated with the generation of greater biomechanical stresses during the mastication (chewing) process acting on the human skull (Lahr 1996; Noback and Harvati 2015; Toro-Ibacachea et al. 2016).

It has been also suggested that one of the reasons for the differences observed in the neurocranial shape (its breadth and length) between human populations could be changes in the type of diet associated with reduction of the size and strength of the temporal muscles causing reduction of the stress acting on the lateral parts of the neurocranium (more globular braincases exhibited less robust temporal muscles) (e.g. Hylander 1977; Perez and Monteiro 2009; Paschetta et al. 2010; Perez et al. 2011). It is worth pointing out that the reduction of the size of the temporal muscle is not considered as the basic factor explaining the observed variation in the neurocranial shape as the main importance of genetic factors has been commonly suggested (Roseman 2004; Harvati and Weaver 2006; Smith et al. 2007; Von Cramon-Taubadel 2011; Matsumura et al. 2022). However, in the light of the presented above information, the role of the temporal muscle activity as an additional factor influencing the shape of the braincase during its growth can be considered as probable. A relationship between the traits of the temporal muscle and the shape of the braincase has been also suggested based on the fact that, due to the location of this muscle attachment on the lateral wall of the vault of the braincase, the development and growth of this muscle (a large part of it) cooccurs with the growth of the braincase. Its size is also largely determined by the capacity of the braincase (Lieberman 2011; Noback and Harvati 2015). The importance of the shape of the skull for the formation of the massiveness of the human facial skeleton (including the supraorbital area) was suggested by Lahr and Wright (1996) and Nowaczewska et al. (2015). Baab et al. (2010) showed a significant relationship between the shape of the skull and inter-population differences in cranial robusticity.

Only a few studies have focused on the assessment of the meaning of the traits of the temporal muscle for the formation of the massiveness of the supraorbital region of the facial skeleton. For instance, the relationship between these features and the massiveness of this area was suggested by Lahr (1996), Lahr and Wright (1996). Lahr (1996), based on the results of a study concerning a sample of adult human skulls from different geographical areas, suggested a relationship between the metric traits of the mastication apparatus (including temporal muscles) with the massiveness of the supraorbital areas. However, in her study, the differences in the values of these features related to the differences in the size of the facial skeleton of the examined skulls and the potential influence of the shape of the braincase on the features of the temporal muscle were not considered. Thus, further studies exploring this topic are needed.

The first aim of this study is to determine whether in the examined sample of adult male skulls there are relationships between the features of the temporal muscle (its height and length) standardized to the size of the braincase and the degree of massiveness of the glabella, superciliary arch and trigonum standardized to the size of the facial skeleton. It is worth noting that in this study the male skulls of Africans and Australians were examined together as one sample of male *Homo sapiens* skulls. Australian skulls are commonly considered as the most robust and the African skulls as more gracile compared to other adult human skulls (e.g., Lahr 1996). By including Australian and African crania in one sample, we obtained a greater variability of the examined traits and, therefore, a greater chance of detecting the relationship between the examined traits compared to samples that include crania exhibiting a low degree of diversity of the examined traits.

Given the potential influence of the neurocranial shape on the analyzed traits and the meaning of the biomechanical stresses transmitted by the permanent upper molars during mastication into the facial bones influencing the development of the massiveness of the supraorbital region during facial growth (see Nowaczewska et al. 2023), the second (main) aim of this study is to determine whether there is a significant relationship between analyzed traits of the temporal muscle and the degree of the massiveness of the supraorbital region with the exclusion of the influence of the neurocranial shape and the size of the occlusal surface of the permanent upper molars (first and second). The last of these traits was included because the results of the study of Nowaczewska et al. (2023) regarding the *Homo sapiens* cranial sample indicated the presence of a positive relationship between the area of occlusal surface of permanent upper molars and the degree of massiveness of trigonum independent of the influence of the size of the facial skeleton.

The second stage of our research (i.e., on the exclusion of the potential influence of the above-mentioned traits on the assessed relationships) has been carried out only for the degree of the massiveness of the supraorbital area related to the traits of the temporal muscle. The supraorbital area, which is located closest to the place of the temporal muscle attachment, has been suggested to be considerably predisposed during the growth of the skull to the impact of mastication stress generated by the working temporal muscle (Mitteroecker et al. 2012; Noback and Harvati 2015).

The results of these analyses will allow to establish which of the features (including the relative height and length of the temporal muscle, the shape of the braincase or the relative size of the first or second molar occlusal area) show the strongest relationship with the degree of the massiveness of the supraorbital region of the human skulls.

Material and methods

Sample

The sample of adult male skulls (from the 19th century) including 29 belonging to Africans and 15 belonging to Australians were used (Milicerowa 1955; Górny 1957). The African and Australian skulls were parts of two human cranial collections – first housed at the Department of Anthropology of the Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław, Poland) and the second housed at the Department of Human Biology of the University of Wrocław (Poland). To exclude the influence of sex on the development of the cranial traits (degree of the robusticity of the areas of the supraorbital part of the facial skeleton (Rosas and Bastir 2002; Garvin and Ruff 2012)) and the size of the crown of the upper molars (Hillson 1996; Dempsey and Townsend 2001), only male crania were examined. Data regarding sex of the Australian specimens was obtained from literature (Milicerowa 1955). Sex of the African specimens was established based on the assessment of the expression of the qualitative cranial traits according to the methodology described by Ferembach et al. (1980), with the exclusion of the supraorbital part of the facial skeleton (to avoid the problem of circularity). There was a lack of postcranial bones for most of the examined African specimens, thus their traits could not be included in the assessment of sex. The adult specimens were selected based on the standard criteria including the presence of the fully fused spheno-occipital synchondrosis, the third molar and/or advanced degree of obliteration of the cranial sutures (Buikstra and Ubelaker 1994; White and Folkens 2000).

Traits

All traits used in this study are described in Table 1. The criterion of the presence of the data for all of the analysed traits for each of the skulls substantially affected the size of the finally selected cranial sample (n = 44), all of these skulls exhibited the preserved two types of upper molars (first – M^1 and second – M^2) embedded in the alveolar process (n = 88).

Two traits of the temporal muscle attachment area were used in this study: height (H-TM) and length (L-TM) (Figure 1b). Both of these traits were collected from the left side of the cranial vaults (exceptionally on the right side only when the external surface of the cranial bones in the region of the inferior temporal line presence did not exhibit a sufficiently good state of preservation – the inferior temporal line was not visible) and indicated appropriate repeatability (Table 2). The metric traits of the braincase and facial skeleton were collected by one of the authors (WN) using a standard sliding calliper and spreading calliper with an accuracy of 0.5 mm, except for the height and length of the temporal muscle, which were collected using a digital sliding calliper with an accuracy of 0.01 mm. The raw data on metric and qualitative traits of facial skeleton was used as the part of database (prepared earlier by WN) that was also used in the other study indicating their high repeatability (Nowaczewska et al. 2023). The traits of the temporal muscle were standardized to the neurocranial size (NC-S) to eliminate the problem of the differences in their values caused by differences in the neurocranial size in examined skulls. By analogy, the degree of the massiveness of the three supraorbital areas of the frontal bone including the glabella (G), supraorbital ridge (S) and trigonum (T) (Figure 1a) were standardized to the size of the facial skeleton (FS-S). The metric traits of the mandibles were not included in the measure of the facial size because of the lack of mandibles in a good state of preservation in most of the examined crania. The geometric mean of the chosen metric facial and neurocranial traits was used as the measure of facial size and neurocranial size, respectively. This method of size assessment of the cranial modules has been commonly used in other studies (e.g., Lieberman et al. 2002; Sardi et al. 2006; Nowaczewska et al. 2023).

Table 1. A description of the traits used in this study

Trait (abbreviation) [units]	Definition
Height of the temporal muscle (H-TM) [mm]	The linear distance between the highestmost point on the inferior temporal line (on the parietal bone) and the point defined as localised – cite: "(…) above porion on the surface of the temporal bone, at the root of the zygomatic arch anteriorly and supramastoid crest posteriorly, to mark the lowermost point of the temporal muscle" (Lahr 1996: p. 355) (Figure 1b)
Length of the temporal muscle (L-TM) [mm]	The linear distance from the point localised on inferior temporal line (on the parietal bone) as distal-most in relation to anterior-most point localised on the temporal crest (on the frontal bone) to this point (Lahr 1996: p. 355) (Figure 1b)
Degree of the robusticity of glabella (G)	The degree of massiveness of the glabellar (G) region of the frontal bone – the area in central part of the frontal bone between left and right arcus superciliaris (Figure 1a) The scale proposed by Buikstra and Ubelaker (1994) including five degrees of its massiveness was used (from first degree recognised when this region is flat or projects minimally to fifth degree when this region is strongly convex)
Degree of the robusticity of supraorbital ridge (arcus superciliaris) (S)	The degree of massiveness of the arcus superciliaris (S) localised between the glabella and trigonum (Figure 1a). The scale including four degrees of its massiveness described by Nowaczewska et al. (2015) – from first degree when minimal prominence of this region is observed (or its external surface is flat) to fourth degree when the strong projection of this region is present

Trait (abbreviation) [units]	Definition
Degree of the robusticity of trigonum (T)	The degree of massiveness of the most lateral part of the supraorbital area of frontal bone described as trigonum (T) (Cunningham 1908) (Figure 1a) The modified scale proposed originally by Lahr (1996) was used without the assessment of the area of the frontal process of the zygomatic bone. Four degrees of this trait were distinguished (first – when this region is flat or exhibits slightly salient surface, second – when this area is raised and prolonged in anterior direction; third – when this region is prominent, wide and convex and fourth – strongest development of this region with well-rounded surface is observed) (Figure 2)
Size of the braincase (NC-S*) [mm]	The measure of the size of the braincase calculated as the geometric mean of three measurements: maximum length (GOL); maximum breadth (XCB) and cranial height (BBH)
Relative height of the temporal muscle (H-TM/NC-S) [without units]	Height of the temporal muscle standardized to size of the braincase
Relative length of the temporal muscle (L-TM/NC-S) [without units]	Length of the temporal muscle standardized to size of the braincase
Size of the facial skeleton (FS-S*) [mm]	The measure of the size of the splanchnocranium calculated as the geometric mean of the following five measurements: nasal height (M55 = n-ns); orbital height (M52); outer biorbital width (M43 = fmt-fmt); bimaxillary width (M46 = zm-zm); length of the facial roof in midsagittal plane (n-ho)
Relative degrees of robusticity of the glabella (G/FS-S) [1/mm]; supraorbital ridge (S/FS-S) [1/mm] and trigonum (T/FS-S) [1/mm]	The grades of expression of the massiveness of the examined in this study supraorbital regions standardized to the size of the facial skeleton
Breadth-length index of the braincase (XCB/GOL × 100) [without units]	The maximal breadth of the cranium – XCB (M8 = eu-eu)/maximal length of the cranium – GOL (M1 = g-op) x 100; this index describes the breadth of the braincase in relation to its length; the higher value of this trait means the wider and shorter cranial vault
Total occlusal area of the permanent upper first and second molar (TOA M^1) [mm^2] (TOA M^2) [mm^2]	The area of the occlusal surface of examined molars taken according to the methodology described by Górka et al. (2015, 2016) (Figure 3)
Relative total occlusal area of the permanent upper first molar and second molar (TOA M^1/FS-S) [mm]; (TOA M^2/FS-S) [mm]	The total occlusal area of the permanent upper first M^1 and second M^2 molar standardized to the size of the facial skeleton

*These traits were not analysed separately: FS-S and NC-S were used only to calculate the other traits included in the statistical analyses; the measurements used to calculate two traits above-mentioned were taken according to Martin's definitions (Bräuer, 1988) except the one in the case of which the point hormion (ho) was localised on the sphenoid bone in the point of the intersection of midsagittal line of the cranium (at right angle) with line passing through the most posterior point on the ala vomeris; the abbreviations of the anthropometric points presented in this Table: eu – euryon; g – glabella; op – opisthocranion; b – bregma; ba – basion; n – nasion; ns – nasospinale; fmt - frontomalare temporale; zm – zygomaxillare.

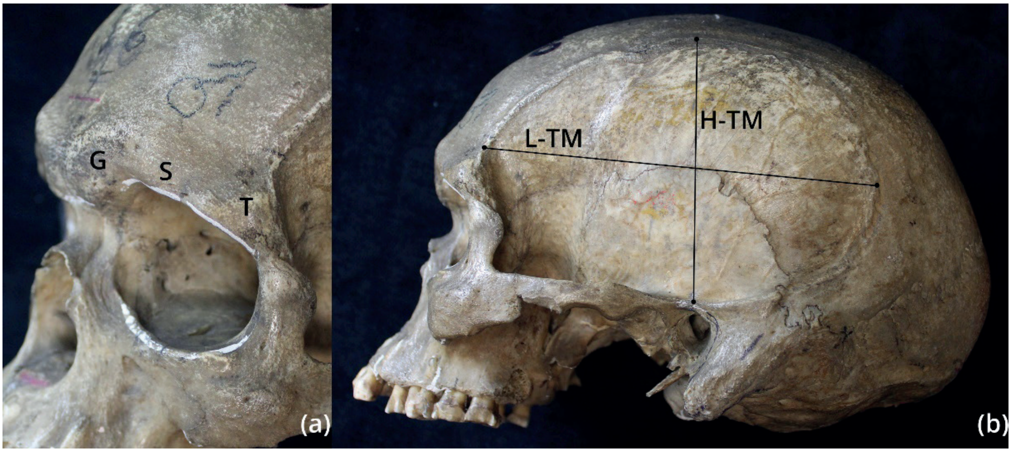

Fig. 1. The three regions of the supraorbital part of the frontal bone for which the degree of the massiveness was assessed (a: G – glabella; S – supraorbital ridge; T – trigonum) and the two measurements of the temporal muscle size (b: L-TM – length of the temporal muscle, H-TM – height of the temporal muscle)

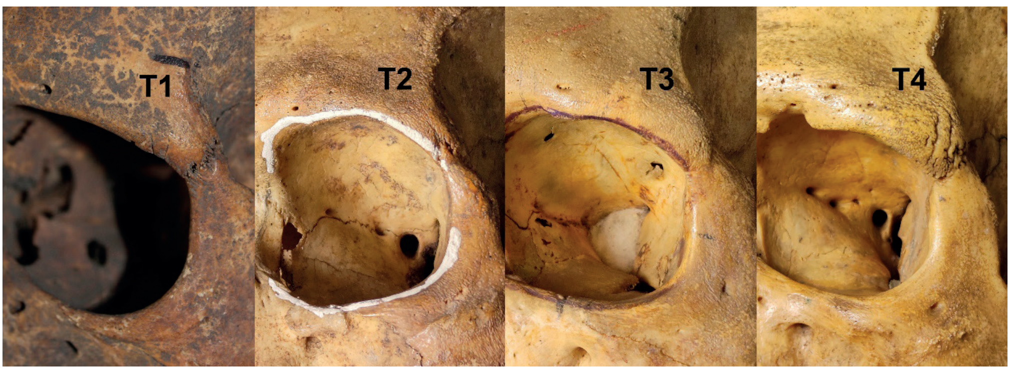

Fig. 2. Four-stage scale of development of the trigonum (T) massiveness: from the weakest formation (T1) to the strongest (T4)

Table 2. Results of the statistical analyses showing the intra-observer error

Measurements of temporal muscle	T-test (T, paired-test)/or Wilcoxon (W) test* statistic	*p* – value
H-TM	W = 0.019	0.985
L-TM	T = 1.738	0.089

$p < 0.05$; W* - non-parametric equivalent of T-test (paired test) using when the condition of normality of the distribution of the differences between the first measurement of H-TM and the second measurement of H-TM is not met.

To obtain data on the size of the occlusal surface of the examined molars (M^1s and M^2s) the methodology described by Górka et al. (2015, 2016) was used. The values of this trait were collected by one of the authors (KG) from photos of the occlusal surface of their crown (300 dpi). These photos were taken using the Canon EOS 600 D camera. The camera was attached to a stand (the focal distance = 0.5 m). A linear scale for later calibration was placed parallel to the camera lens and at the same height as the upper (occlusal) surface of each examined tooth. Images were calibrated and processed with the use of the ImageJ software (Abràmoff et al. 2004). The total occlusal area (TOA) was calculated from the outline of the perimeter of the occlusal surface. It was possible by using the polygon tool. A minimum of 30 points were recorded for the crown outline (Figure 3). The examined teeth were always orientated in such a way that the occlusal surface of each tooth was placed parallel to the camera lens. The right molars were examined only when the left molars were not present or did not exhibit a good state of crown preservation. The molars with a severe degree of crown wear were not included in the analysis. The raw data of the TOA of M^1s and M^2s was used in this study as a part of the database including these traits of the African and Australian males and females (collected by KG) which was used earlier in other research indicating that the method of the TOA collection was reliable and precise (Nowaczewska et al. 2023).

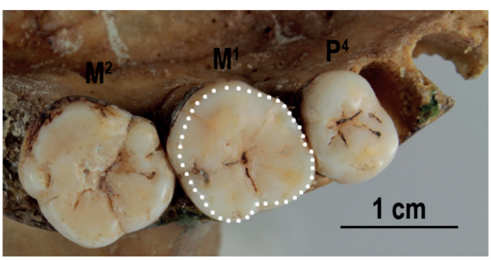

Fig. 3. The total occlusal area of the examined M^1 marked with white points according to the methodology used in this study (cranium of adult Australian male)

Statistical analyses

Statistical analysis was performed using the "Paleontological Statistics software package for education and data analysis" - PAST version 4.10–4.12 (Hammer et al. 2001). In the first stage of the analysis, to establish whether significant correlations occurred between the metric traits of the temporal muscle (its height, H-TM and length, L-TM) and the degrees of the massiveness of the three supraorbital areas of the frontal bone (G, S and T), Spearman's rank correlation analysis was performed. To determine whether the cranial size influenced the analysed relationships, Spearman's rank correlation analyses were performed to establish the presence of a significant correlations between two traits of the temporal muscle standardized to the neurocranial size (relative height H-TM/NC-S and relative length L-TM/NC-S) and the degrees of massiveness of the three supraorbital areas standardized to facial size (G/FS-S, S/FS-S, T/FS-S). In the second stage of the analysis regarding the degree of the relative massiveness of the examined supraorbital area, for which a significant correlation with the relative length and/or relative height of the temporal muscle was established, a partial rank correlation was performed. These types of analyses included the models encompassing two additional features (the shape of the braincase - XCB/GOL x 100) and the relative size of the occlusal area of the upper molars (the TOA of M^1s/FS-S or the TOA of M^2s/FS-S). Due to the differences in the time of development of the crown of the permanent first molar in relation to the second (Hillson 1996), the influence of various factors on these two types of teeth and other structural conditions of the skull during their development the analyses including the occlusal area of these teeth was conducted separately. Two types of models were used: first included, among the other traits, the TOA of M^1s/FS-S and the second included the TOA of M^2s/FS-S. A partial rank correlation analysis was used to determine the relationship between the examined traits with the exclusion of the potential influence of the other traits included in the models. These analyses were performed to determine whether there was a significant relationship, independent of neurocranial shape and relative size of the occlusal area of upper molars, between the examined trait/traits of the supraorbital region of the frontal bone and the traits of the temporal muscle. The results of the correlation analyses were considered significant when $p < 0.05$.

Results

The summary statistics of the quantitative traits used in this study and the percentage of the observed degrees of massiveness of the examined supraorbital areas of the facial skeleton are presented in Table 3 and Table 4, respectively.

Table 3. Descriptive statistics of the quantitative traits[a] used in this study

Traits[a] (n = 44)	Mean	Minimum	Maximum	SD
H-TM [mm]	77.23	64.12	91.54	6.35
L-TM [mm]	112.19	90.72	127.23	8.56
NC-S [mm]	145.98	137.40	153.44	4.14
L-TM/NC-S [without units]	0.77	0.65	0.87	0.05
H-TM/NC-S [without units]	0.53	0.45	0.63	0.04
XCB/GOL × 100 [without units]	71.35	62.90	77.84	3.30
TOA M^1 [mm^2]	99.92	75.22	126.52	11.73
TOA M^2 [mm^2]	92.43	65.13	121.33	12.49
FS-S [mm]	64.68	58.15	68.36	2.52
TOA M^1/FS-S [mm]	1.54	1.20	1.99	0.16
TOA M^2/FS-S [mm]	1.43	1.09	1.80	0.17

[a] abbreviations of all of these traits are explained in Table 1.

Table 4. The percentage of examined male crania showing the distinguished degrees of massiveness in three supraorbital regions: glabella (G) – from first to fifth, supraorbital ridge (S) and trigonum (T) – from first to fourth

Qualitative trait	Degree 1	Degree 2	Degree 3	Degree 4	Degree 5
G	36.4%	31.8%	18.2%	6.8%	6.8%
S	22.7%	36.4%	18.2%	22.7%	
T	50%	27.3%	18.2%	4.5%	

Note: examined cranial sample includes 44 crania = 100%

The obtained results of Spearman's rank correlation analyses concerning the traits non-standardized to the cranial size indicated the presence of a significant and positive correlation between the two traits of the temporal muscle (H-TM and L-TM) and the degree of the massiveness of S (weak in the case of both metric traits) and T (moderate and weak, respectively) (Table 5). In the case of the standardized cranial traits, the results showed the occurrence of a significant (positive, weak) correlation between the traits of the temporal muscle (H-TM/NC-S and L-TM/NC-S) and T/FS-S (Table 5). There were no other relationships between these traits and other traits of the supraorbital region (Table 5).

Taking into account the results presented above, the partial rank correlations (PRCs) analyses included only T/FS-S from all three standardized traits of supraorbital robusticity. There were no significant partial correlations between the two traits of the temporal muscle (H-TM/NC-S and L-TM/NC-S) and T/FS-S in both types of models (Tables 6 and 7). The results of the PRCs concerning the first model indicated the presence of a significant (negative, moderate) partial correlation of T/FS-S with only the index of the neurocranial shape (XCB/GOL x 100) (Table 6). In the case of the second model the first of these traits showed a significant (negative, moderate) partial correlation with the same trait and a significant (positive, weak) partial correlation with the TOA of M^2s/FS-S (Table 7).

It is worth noting that the results of the additional Spearman's rank correlation analyses concerning the traits included in the two above-mentioned models indicated a significant correlation between T/FS-S and three traits: the index of the neurocranial shape (negative, strong), the TOA of M^1s/FS-S (positive, weak) and the TOA of M^2s/FS-S (positive, moderate) (Tables 6 and 7).

Table 5. Results of Spearman's rank correlations between the two traits of the temporal muscle: height (H-TM) and length (L-TM) without and with their standardization to the size of neurocranium (NC-S) and the examined traits[a] of the supraorbital part of the frontal bone

Traits[a]	H-TM rs	*p*	L-TM rs	*p*
G	rs = 0.228	0.137	rs = 0.164	0.286
S	rs = 0.373	0.013*	rs = 0.317	0.036*
T	rs = 0.400	0.007*	rs = 0.354	0.018*
Traits[a]	**H-TM/NC-S rs**	***p***	**L-TM/NC-S rs**	***p***
G/FS-S	rs = 0.147	0.342	rs = 0.143	0.350
S/FS-S	rs = 0.271	0.075	rs = 0.196	0.202
T/FS-S	rs = 0.335	0.026*	rs = 0.304	0.045*

[a] abbreviations of all these traits are explained in Table 1; rs – Spearman's rank correlation coefficient; * $p < 0.05$.

Table 6. Results of Spearman rank correlations and partial rank correlation (bottom) – model with relative total occlusal area of the permanent upper first molars

Traits[a]	T/FS-S (p)	H-TM/NC-S (p)	L-TM/NC-S (p)	XCB/GOL x 100 (p)	TOA M^1/FS-S (p)
T/FS-S	-			-0.603 (0.000)*	0.363 (0.016)*
H-TM/NC-S	0.092 (0.568)	-	0.642 (0.000)*	-0.470 (0.001)*	0.076 (0.625)
L-TM/NC-S	-0.106 (0.510)	0.575 (0.000)*	-	-0.489 (0.001)*	0.187 (0.224)
XCB/GOL x 100	-0.588 (0.000)*	-0.209 (0.190)	-0.231 (0.146)	-	-0.302 (0.046)*
TOA M^1/FS-S	0.301 (0.056)	-0.119 (0.458)	0.060 (0.708)	-0.015 (0.926)	-

[a],abbreviations of all these traits are explained in Table 1; * $p < 0.05$.

Table 7. Results of Spearman rank correlations (top) and partial rank correlation (bottom) - model with relative total occlusal area of the permanent upper second molars

Traits[a]	T/FS-S (p)	H-TM/NC-S (p)	L-TM/NC-S (p)	XCB/GOL x 100 (p)	TOA M^2/FS-S (p)
T/FS-S	-				0.412 (0.006)*
H-TM/NC-S	0.076 (0.635)	-			0.164 (0.289)
L-TM/NC-S	-0.126 (0.432)	0.576 (0.000)*	-		0.233 (0.128)
XCB/GOL x 100	-0.568 (0.000)*	-0.212 (0.183)	-0.221 (0.164)	-	-0.354 (0.019)*
TOA M^2/FS-S	0.312 (0.048)*	-0.065 (0.686)	0.127 (0.430)	-0.069 (0.669)	-

[a],abbreviations of all these traits are explained in Table 1; * $p < 0.05$.

Discussion

The results of the first stage of this study showed a positive relationship between the analyzed traits of the temporal muscle (its height and length) and the degree of massiveness of two supraorbital areas (the superciliary arch and the trigonum). It shows that the examined crania with the higher and longer area of attachment of this muscle exhibited a more robust superciliary arch and trigonum compared to that with a lower and shorter area of the attachment of this muscle. However, the obtained results of the analysis of the correlations between the traits above-mentioned standardized to the neurocranial size and the facial skeleton size (respectively) showed that only the degree of massiveness of the trigonum was significantly correlated to the features of the temporal muscle independently of the size of the examined cranial modules. This shows that the greater the relative height and relative length of the temporal muscle, the higher the relative degree of trigonum robusticity. It suggests that the relationship between the two traits of the temporal muscle and the degree of superciliary arch robusticity described above was only a by-product of the influence of the cranial size. According to the authors' prediction, the obtained results indicated a lack of importance of the examined features of the temporal muscle for the formation of the massiveness of the glabella as the trait concerning the area located at the greatest distance in relation to these muscles in comparison to other examined supraorbital traits. The strong covariation of the cranial traits that are closely located to each other was also suggested by other authors (e.g., Mitteroecker et al. 2012; Noback and Harvati 2015).

It has been shown by Lahr (1996) that the development of the massiveness of the supraorbital region of the human facial skeleton is related to the activity of the temporal muscle. It has been suggested by her that the development of the superstructures of this region (such as the supraorbital ridge/torus and the zygomatic trigone) is at least partially dependent on the functional mechanisms concerning the masticatory apparatus based on the presence of a significant relationship between the degree of development of these superstructures and the traits of this apparatus (Lahr 1996). It has been indicated by her that the crania of adult representatives of *Homo sapiens* with larger dimensions of the temporal muscle (e.g., its height, and length) exhibited more pronounced robusticity of the supraorbital ridge (Lahr 1996). The Lahr (1996) study also demonstrated the significance of the height of the temporal muscle and the length of the temporal fossa in predicting the degree of development of the zygomatic trigone. It is worth stressing that the results obtained in our study are only partially congruent with those presented by Lahr (1996) mostly because of the lack of a significant correlation between the relative degree of the superciliary arch massiveness and the relative height and length of the temporal muscle in the examined cranial sample. However, Lahr (1996) analyzed supraorbital traits which, as opposed to our study, were not standardized to cranial size, and examined skulls of adult males and females together. The definitions of the traits of the supraorbital region used by Lahr (1996) were also different from those used in our study (i.e., the degree of the expression of the supraorbital ridge encompassed the glabella and superciliary arch, and the zygomatic trigone included the trigonum and the upper part of the zygomatic bone). These differences in the methodology used by Lahr (1996) in comparison to that used in this study can explain the incongruence described above.

The second stage of our study (concerning the partial rank correlation) showed a lack of a relationship between the relative degree of massiveness of the trigonum and the relative height and length of the temporal muscle, when the influence of the other analysed traits (such as the index of neurocranial shape and the relative occlusal area of the molars – M^1s and M^2s) on these relationships was excluded. It suggests that the correlations established in the earlier stage of this study between the above-mentioned traits could result from the association of these traits (or one of them) with the shape of the braincase and/or the relative occlusal area of the molars, or with other features that were not included in the analysis. Thus, the relationship between the two traits of the temporal muscle (i.e., standardized to neurocranial size) and the relative degree of the robusticity of the trigonum (i.e., standardized to the size of the facial skeleton) in the examined male cranial sample can be interpreted as examples of covariances of these traits – not as the "true relationship" in the sense of cause and effect. This can be interpreted as a lack of a direct effect of the first type of these traits on the trait concerning the trigonum in the sense of the influence of the forces generated by the temporal muscle with greater height and length of the area of its attachment during the mastication of food on the formation of the robusticity of the trigonum area during the period of facial growth. Further research is needed using a larger sample of adult human crania or living individuals (e.g. using cone beam computed tomography – see Maskos et al. 2022; Merema et al. 2022) including also other features of the temporal muscle such as the assessment of the cross-sectional area of this muscle and the total area of the attachment of this muscle to confirm the results obtained in our study and also to establish if there is no true relationship (in the sense described above) between these traits and the degree of the robusticity of the lateral supraorbital areas of the facial skeleton. Among these traits, the size of the infratemporal fossa was suggested as a good measure of the cross-sectional area of this muscle (Demes and Creel 1988; Noback and Harvati 2015). Taking into account the observation that the force of the muscle is proportional to its whole cross-sectional area (including all fibers) (e.g., Maughan et al. 1983; Weijs and Hillen 1984) inclusion of this trait in future analysis would be very important.

Although there was no significant partial correlation between the index of the shape of the braincase and the relative height and length of the temporal muscle, there were significant, negative and moderate Spearman's rank correlations between these traits (i.e., the relatively higher and longer temporal muscle was observed in skulls with a narrower and longer neurocranium in comparison to those with a wider and shorter braincase). These correlations could probably have resulted from the presence of the influence of other features that were not included in the models analyzed in this study. It should be stressed that the pattern of interactions of individual structural cranial modules (including structural parts as the sub-modules) during their development and growth has not been fully understood so far, therefore further research is needed that involve a larger number of human skulls and features (Bastir and Rosas 2005; Singh et al. 2012). Noback and Harvati (2015) indicated the presence of covariation between the shape of the temporal muscle and the general shape of the human skulls, explaining this as a result of the localization of the main attachment area of this muscle on the lateral surface of the braincase, suggesting an influence of the shape of the neurocranium on this muscle and vice versa. Thus, this explanation is also most probable in the case of the presence of the above-mentioned negative correlation concerning the analyzed traits of the temporal muscle.

In the case of the index of the shape of the braincase, our results indicated the presence of the strongest partial correlation of this trait with the relative degree of the massiveness of the trigonum (i.e., the narrower the braincase in relation to its length, the greater the relative degree of trigonum robusticity when the influence of the other traits included in the analysis was excluded). The meaning of the shape of the human neurocranium for the formation of the robusticity of the cranial superstructures was suggested by Lahr and Wright (1996), Baab et al. (2010), Nowaczewska et al. (2015, 2022). Thus, the results of this study are congruent with those obtained in other studies. However, Nowaczewska et al. (2015, 2022) included in their analysis only the degree of the massiveness of the glabella and supraorbital arch, not the trigonum, while Baab et al. (2010) and Lahr and Wright (1996) assessed the degree of development of the trigonum with the upper part of the zygomatic bone as the zygomatic trigone. The relationship between cranial robusticity and cranial vault breadth was shown by Lahr and Wright (1996) in the case of Australian crania – the more robust crania indicated the narrower vaults. Thus, our results are congruent with those obtained by them.

In addition, the obtained results showed also a positive and weak correlation between the relative degree of the massiveness of the trigonum and the relative occlusal area of M^1s and M^2s (i.e., the greater the relative robusticity of the trigonum occurred in the skulls with the larger occlusal surface of the above-mentioned molars, standardized to the size of the facial skeleton, than in those with lower values of these traits). However, there was a significant partial rank correlation between the relative degree of robusticity of the trigonum and only the relative occlusal area of one of these two types of molars – M^2s. This suggests that there was a positive relationship between these traits also when the influence of the other traits analyzed in this study, such as the shape of the braincase and the relative height and length of the temporal muscle, was excluded. It also suggests the presence of a "true relationship" between these traits, supporting the localized stress hypothesis concerning, in this case, the meaning of the mastication stresses transmitted through the upper second molars towards the most lateral part of the supraorbital area for the formation of its robusticity. However, it is worth noting that the above-mentioned relationship was weak, and the *p*-value was close to a non-significance level ($p = 0.048$). Thus, further study is needed, encompassing a larger sample of human skulls with permanent molars, to confirm this interpretation.

According to the currently dominant view based mainly on the results of studies of living primates (Hylander et al. 1991) regarding the action of the stresses generated during mastication on various areas of their facial skeletons, a weak impact of these forces on the upper (supraorbital) area of their facial skeleton was found. In our study the type of diet, in terms of hardness and composition of the food, such as the amount of animal proteins, was not taken into account as one of the potential factors (Demes and Creel 1988; Von Cramon-Taubadel 2014; Noback and Harvati 2015; Katz et al. 2017). The latter could influence the size of the facial skeleton, the size of the temporal muscles and the degree of massiveness of the trigonum. Although the examined traits of the facial skeleton were standardized to its size and the above-mentioned factor could be more important in terms of the cross-sectional area of temporal muscle compared to the examined traits, the type of diet of the examined groups of humans to which belonged the examined crania should be included in any further studies including more human populations.

Given that the shape of the human braincase and the size of the molar crowns are strongly genetically determined (as was indicated in the case of the first of these traits in other studies concerning cranial dimensions and those concerning the presence of the differences in craniofacial shape between human populations at the early stages of cranial ontogeny (see Gonzalez et al. 2010; Viðarsdóttir et al. 2002; Viðarsdóttir and Cobb 2004)) and in the case of the second of these traits (see e.g. Hillson 1996; Dempsey and Townsend 2001; Townsend et al. 2012), the obtained results suggest a greater importance of genetic factors for the formation of the massiveness of the trigonum in the examined sample of adult male skulls compared to masticatory stress. However, the results of this study suggest also that biomechanical forces concerning the function of the mastication apparatus could influence the massiveness of the trigonum, but to a much lesser degree.

Conclusions

The positive and weak relationships established in this study of the relative height and length of the temporal muscle with only the relative massiveness of the trigonum (from all three examined traits of the supraorbital cranial region) are most probably an example of covariance of these traits, not a "cause-effect" relationship. The obtained results also indicated the greatest importance of the shape of the braincase for the formation of the relative massiveness of the trigonum among the other analyzed features - independent of their potential influence. However, further research is needed to confirm these interpretations in a larger sample of human skulls or leaving individuals using the cone beam computed tomography including other features of the temporal muscle.

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Conflict of interest

The authors declare no conflict of interest.

Author's contributions

WN: conceptualization, methodology, investigation, formal analysis, software, validation, interpretation of the data and results, writing of the original draft, project administration, data curation; KG: methodology, investigation, software, writing of the original draft; AC: interpretation of the data; MP: interpretation of the data; UZ-D: interpretation of the data; all authors reviewed and edited the manuscript.

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

Economic stress affects the human sex ratio: A retest of the Trivers–Willard hypothesis in Poland

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ABSTRACT: This paper contributes to the verification of the Trivers–Willard hypothesis (TWH). Based on the TWH, observed sex (male-to-female) ratio at birth in a population is presumed to decline when parents experience economic stress. The empirical evidence so far is mixed.

The research material consisted of data on the total numbers of live male and female births in the Polish population in the years 1995–2020. The data were used to produce semiannual time series for secondary sex ratio (SSR), and the sex ratio at birth (SRB). The variable describing the economic stress of households was percentage change in private consumption. The statistical method proposed by Catalano and Bruckner – expanded to include additional statistical analyses – was applied to retest the economic stress hypothesis. The analysis led to complementary ARMAX models, explaining SSR or SRB variance based on autoregression and the moving average process, as well as private consumption. The results indicate that periods of decreasing consumption favored mothers having daughters, while periods of increasing consumption favored mothers having sons. The number of "additional" male births resulting from improved economic conditions was calculated for the period of study. Consequently, the economic factor was shown to have a positive effect on the human sex ratio. At the same time, it should be noted that SSR and SRB reaction lags to consumption changes were different for Łódzkie Province (one year) and for Poland (two years).

The obtained results led to the "correct" verification of the Trivers–Willard hypothesis (TWH): economic stress affected the SSR and SRB in Poland. Both SSR and SRB were useful in analyzing economic stress (a dual solution). The use of a greater density of data points was shown to improve analysis effects and increase the likelihood of a "positive" verification of the economic stress hypothesis.

KEY WORDS: secondary sex ratio, sex ratio at birth, ARMAX model, dual solution.

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Introduction

The Trivers–Willard hypothesis (TWH) posits that the maximization of reproductive success in mammals is associated with modifying the sex ratio of offspring depending on maternal condition (Trivers and Willard 1973). As indicated by research on human populations, natural selection mechanisms decrease the male birth rate in stressful periods by reducing the number of conceptions and increasing the proportion of spontaneous abortions of male fetuses (James and Grech 2017). This implies that a long-term deterioration of living conditions (e.g., economic stress) in the human population may lead to a lower sex ratio at birth. However, research on the verification of the so-called economic stress hypothesis initiated by Catalano and Bruckner (2005) has not led to conclusive results.

That research has been based on the secondary sex ratio (SSR), which is the ratio of male to female live births (Catalano 2003; Catalano and Bruckner 2005; Żądzińska et al. 2007; 2011) or the human sex ratio measured as the sex ratio at birth (SRB) calculated as the proportion of male births in relation to all births (Helle et al. 2009; Wu 2021). Analysis has also included the annual number of stillbirths by sex, accounting for part of the overall number of prenatal deaths. Findings on the mortality of male and female fetuses may shed light on the mechanisms underlying the Trivers–Willard hypothesis (Catalano et al. 2005a; 2005b; Helle et al. 2009)

Research in this area is usually based on the annual number of live births by sex. The availability of birth registers and metrics describing changes in economic conditions over time in a given country have made it possible to design and conduct analyses for different periods and different human populations: for 1862–1991 and 1749–1991 in Sweden (Catalano and Bruckner 2005; Wu 2021), for 1865–2003 in Finland (Helle et al. 2009), for 1946–1999 in East and West Germany (Catalano 2003), and for 1956–2005 (Żądzińska et al. 2007) and 1995–2007 (Żądzińska et al. 2011) in Poland (with quarterly data in the latter case).

Previous research, using different measures of changes in economic conditions, has partially confirmed that an economic recession may reduce the proportion of male births. Catalano (2003), who applied time series analysis to data from East and West Germany for 1946–1999, found the SSR in East Germany in the turbulent year 1991 (1.044) to be lower than the ratio of 1.059 expected from history and from the ratio in West Germany.

Analyzing monthly data from January 1989 to December 2001, Catalano et al. (2005a) reported that the fetal death sex ratio increased in months when the unemployment rate also increased. Using demographic and economic data spanning 129 years for Sweden, Catalano and Bruckner (2005) found a positive correlation between percentage change in private consumption and the SSR in that country in the period 1862–1991.

A few subsequent studies did not report conclusive results, which indicates the need to further verify the relationship between economic stress and the sex ratio at birth (Margerison Zilko 2010). A Polish study following Catalano and Bruckner's methodology (2005) using annual data for 1956–2005 did not reveal a correlation between consumption and the SSR (Żądzińska et al. 2007). Similarly, findings from another study using a dynamic regression model did not bear out an association between the SRB and changes in real GDP in Finland between 1865 and 2003 (Helle et al. 2009). Catalano and Bruckner's analysis (2005), involving data from 1862–1991, did confirm an association between private consumption and the Swedish SSR over 129 years, while an extended research by Wu (2021), encompassing time series from almost two and a half centuries (1749–1991), was inconclusive as to the effects of economic stress on changes in the Swedish SRB.

The verification of the economic stress hypothesis requires long study periods, which may not always be possible due to the absence of data or inaccurate records of live births. In addition, in many places around the world there are no records of prenatal deaths. In the case of Poland, data on births in the interwar period (1918–1939) may be distorted as some parents may have consciously delayed birth registration or failed to register (Szukalski 2010). As was mentioned above, attempts to explain changes in the SSR in Poland by economic stress (deterioration of living conditions) over a period of 50 years (1956–2005) did not bring the expected results (Żądzińska et al. 2007). According to some Polish demographers, this may be partially attributed to the choice of indicator of living conditions in the study i.e. the private consumption of households, which may have overestimated the actual living conditions in the years of market shortages (Szukalski 2010). On the other hand, the results of another study, limited to the years following the period of political and economic transformation in Poland (1995–2007) did show a positive correlation between percentage change in private consumption and the SSR using quarterly data for Łódzkie Province (Żądzińska et al. 2011): the SSR was found to decrease four quarters after the occurrence of an economic stressor. Thus, the results may suggest that SSR fluctuations could be used as an indicator of economic stress at the population level.

Testing the Trivers–Willard hypothesis

The hypothesis posited by Trivers and Willard (TWH) initiated research on the effects of economic stress (consumption) on the number of male births in relation to that of female births. To verify the influence of the economic stress under the "incorrect" null hypothesis (H_0) it is assumed that when consumption increases over its expected value, then the sex ratio should fall below its expected value (it is an assumption that consumption does not matter). In turn, the alternative "correct" hypothesis (H_1) assumes that lower-than-expected consumption of goods and services may cause economic stress leading to a lower SSR (it is an assumption about a positive dependence between consumption and the secondary sex ratio). Firstly, from hypothesis H_1 it follows that the observed SSR for live births should increase (as a result of a higher number of male births and/or a lower number of female births) when households consume more goods and services than they actually need. Secondly, this hypothesis suggests that the observed SSR for live births should decrease (due to a higher number of female births and/or a lower number of male births) when households consume fewer goods and services than they need.

Even though the literature does not provide a test that can directly verify this hypothesis, the authors believe that the method proposed by Catalano and

Bruckner (2005) – based on an econometric model – is correct. However, the method requires time series of sufficient length and temporal resolution, proper data preparation, and appropriate time series analysis. Therefore, it is crucial to have an in-depth understanding of the procedures used to verify the posited hypothesis (see: Statistical analysis methods). In the classical theory of hypothesis verification, null and alternative hypotheses are not treated in the same way. H_0 enjoys a certain advantage over H_1 as it can be rejected only on the basis of very substantial evidence against it, usually at a 0.05 or 0.01 alpha level.

The TWH was previously confirmed for a region in central Poland (Łódzkie Province) based on quarterly data for the years 1995–2007, following a period of political and economic transformation (Żądzińska et al. 2011). The objective of the current study is to retest the TWH, this time using semiannual time series for Łódzkie Province and a new dataset for Poland. In addition, two different measures of the human sex ratio (the SSR and SRB) are analyzed in the study. The statistical method proposed by Catalano and Bruckner (2005), expanded to include additional statistical analyses, is applied to retest the economic stress hypothesis in Łódzkie Province and in Poland for the years 1995–2020.

Material and methods

Research material

Research material consisted of semiannual data on the total numbers of live male births (M) and live female births (F) in the Polish population in the years 1995–2020 (52 semiannual data points) derived from balance tables of population, natural movements, and migrations. The authors had access to data for Poland and for individual regions (provinces) of the country at the NUTS-2 level (Statistics Poland). The available data were used to produce semiannual time series for the secondary sex ratio (SSR), which is the proportion of male to female births [M/F], and the sex ratio at birth (SRB), which is the proportion of male births to all births [M/(M+F)]. Fig. 1 presents SSR results for Poland and for Łódzkie Province.

In 1995–2020, the M/F ratio for Poland ranged from 1.0507 to 1.0814, with a mean of 1.0598, a standard deviation (SD) of 0.006, and a coefficient of variation (CV) of 0.58% (CV, expressed as a percentage, is the ratio of the standard deviation to the mean times 100). In the case of Łódzkie Province, the corresponding values for the M/F ratio in 1995–2020 were: 1.0198 (min), 1.1217 (max), 1.0600 (mean), 0.018 (SD), and a CV of 1.73% (Fig. 1). Diagrams plotted for the M/(M+F) ratio – despite using a different unit (%) – appeared very similar. In the years 1995–2020, SRB values ranged from 51.2% to 52.0% for Poland and from 50.5% to 52.9% for Łódzkie Province; the results for Łódzkie Province also revealed greater variation.

According to the methodology described in previous research (Catalano and Bruckner 2005; Żądzińska et al. 2011), the economic stress experienced by the population in Poland and in Łódzkie Province was expressed by means of private consumption, that is, the final consumption expenditure of households (in million units of national currency at constant 2015 prices) was based on seasonally and calendar adjusted data. In statistical analysis (ARMA models), the variable describing the economic condition of households was percentage change in private consumption [$\Delta C/C$] (Fig. 2).

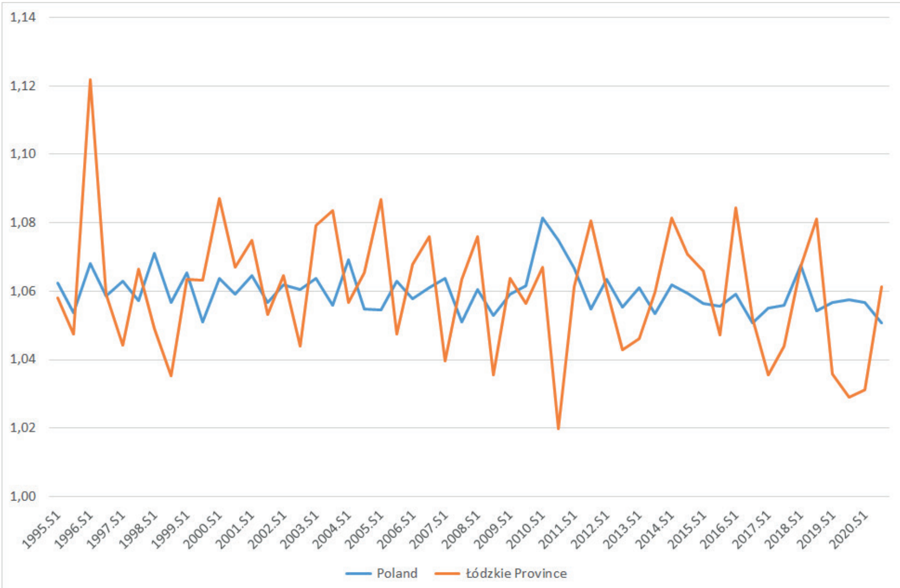

Fig. 1. M/F ratio (semiannual data) for Poland and for Łódzkie Province in 1995–2020. Authors' calculation based on data obtained from https://demografia.stat.gov.pl/BazaDemografia/StartIntro.aspx (Statistics Poland)

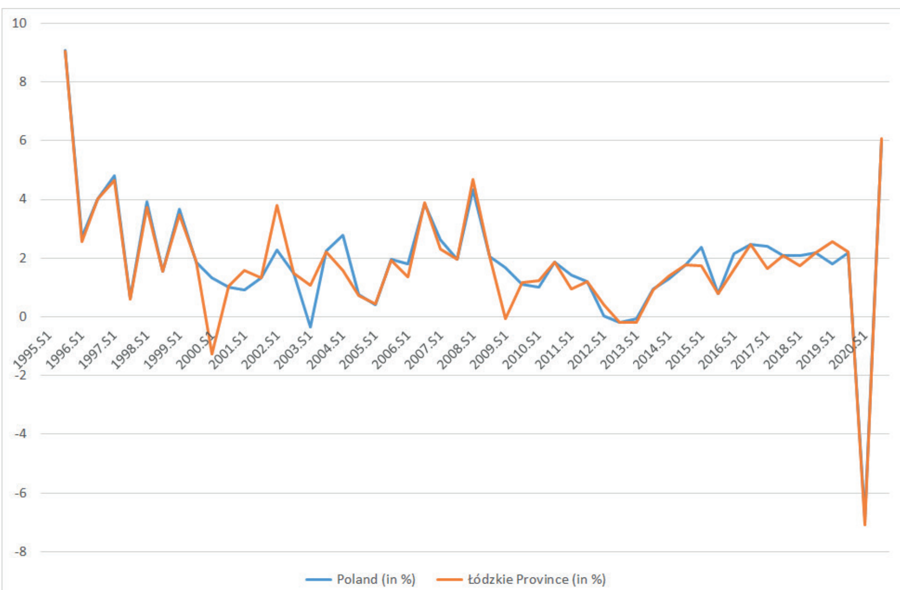

Fig. 2. Percentage change in private consumption [ΔC/C] for Poland and for Łódzkie Province in 1995–2020. Authors' calculation based on data obtained from https://ec.europa.eu/eurostat/data/database (Economy and Finance / National accounts (ESA 2010) / Quarterly national accounts / Main GDP aggregates, Eurostat)

Percentage change in private consumption for Poland and for Łódzkie Province in the years 1995–2020 (semiannual data) was similar. The greatest one-off drop in consumption in the study period (by approx. 7.0%) was found for the first half of 2020, at the height of the COVID-19 pandemic (Fig. 2).

Statistical analysis methods

The effects of economic stress on the SSR and SRB were analyzed using the method proposed by Catalano and Bruckner (2005) supplemented by the authors with additional calculations, marked with an asterisk in the scheme below (*).

Step 1: Testing the stationarity of the [ΔC/C], M/F, and M/(M+F) time series. The trend line slope coefficient was determined and evaluated. We used the augmented Dickey–Fuller (ADF) test and Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test to evaluate stationarity of the analyzed processes. Moreover, we examined fractional integration of the time series by means of Geweke, Porter-Hudak (GPH) test (*).

Step 2: Construction of a private consumption model [ΔC/C]. Percentage change in private consumption was decomposed into statistically expected and unexpected components. The Box and Jenkins method (Box and Jenkins 1983) was applied to identify the order of the autoregressive process (AR), the moving average process (MA), and the autoregressive moving average process (ARMA) in this step and in Steps 3 and 4. The estimated values of the best-fitting Box–Jenkins model can be thought of as the expected component of the modeled series while the differences between the observed and estimated values are the unexpected component.

Step 3: Construction of the M/F model. The SSR ratio was decomposed into statistically expected and unexpected components (see: Step 2). The unexpected component of private consumption (eΔC/C – residuals) was added to the M/F model.

Step 4: Construction of the M/(M+F) model. The SRB ratio was decomposed into statistically expected and unexpected components (see: Step 2). The unexpected component of private consumption (eΔC/C – residuals) was added to the M/(M+F) model.

Step 5: Choosing the final M/F and M/(M+F) equations. The final models of the effects of economic stress on the sex ratio were selected according to the following criteria: (1) all parameters of the model were statistically different from zero, (2) the model exhibited the lowest Akaike Information Criterion (AIC), (3) the coefficient of the lagged explanatory variable (residual from the consumption model [eΔC/C(.)] was positive, and (4) the model enabled a dual solution – obtaining the same specification and (statistically different from zero) model parameters for both the SSR and SRB. This was an additional analysis measuring the stability of the association (*).

Step 6: Analysis of residuals from the model. Residuals from the final equation (stationarity, normal distribution) were evaluated and the equation was re-estimated.

Step 7: Decision making (TWH). In constructing a model explaining the SSR and SRB, the null hypothesis (H_0) is deliberately incorrect. It assumes that an improvement in economic conditions reflected in increased consumption causes a decline in the SSR and SRB (an assumption about the absence of a consumption effect), versus an alternative hypothesis (H_1), that the economic factor has a positive effect on the SSR and SRB. In subsequent steps of the analysis, efforts are made to reject the null hypothesis in favor of the alternative one. Thus, verification is carried out as follows (three variants): if the value of the eΔC/C(.) parameter estimated is at least two times greater than its standard error, then the estimation is deemed "stable" and H_0 (no effect) is rejected in favor of H_1 (economic effect on the SSR).

Further interpretation concerning the direction of the effect of economic conditions (consumption) on the SSR and SRB is made on the basis of the sign of the coefficient and the lag order of the eΔC/C(.) variable: 1) If the estimated coefficient is positive, then increased consumption leads to an increase in the SSR and SRB with the lag (.) (decreased consumption leads to a decrease in that ratio with the lag (.)), which provides a "correct" verification of the economic stress hypothesis. 2) If the estimated coefficient is negative, then increased consumption leads to a decrease in the SSR and SRB with the lag (.), which means a failure to confirm the hypothesis posited by Trivers and Willard as to the relationship between decreasing consumption and decreasing numbers of male births (increasing numbers of female births).

Finally, 3), if the value of the estimated parameter is not at least two times greater than its standard error, then the solution is deemed "unstable", which does not allow to reject hypothesis H_0 that consumption does not affect the SSR (the hypothesized association was not found).

Step 8: Additional analyses. Optional procedures to evaluate the stability of the association: 1) checking additional lags in the final model (*), 2) checking for outliers, 3) transforming the secondary sex ratio into its natural logarithm to determine if systematic variability in the series could have induced the association, 4) conversion of the final M/F and M/(M+F) equation into a new equation (model) in which the dependent variable is the number of males born (M) and the independent variables include the number of females born (F) – in order to estimate how many boys in the population were born "additionally" due to good economic conditions and/or how many boys in the population were not born due to economic stress (*).

The analysis led to complementary ARMAX models (ARMA with eXogenous variables) for Poland and for Łódzkie Province, explaining SSR or SRB variance based on autoregression and the moving average process, as well as private consumption defined as the final consumption expenditure of households.

The parameters of the models were estimated using the statistical program GRETL (GNU Regression Econometric and Time-Series Library) ver. 2021d (Kufel 2013; Cottrell and Lucchetti 2016).

Results

The results of time series analysis for both variables are presented according to the successive steps laid out in the section Material and methods: Statistical analysis methods.

Step 1: Testing the stationarity of the ΔC/C, M/F, and M/(M+F) time series.

Analysis of individual time series, that is, ΔC/C, M/F, and M/(M+F), revealed that the mean value of the process was constant over time (the trend line slope coefficient statistically did not differ from zero) and that it was not integrated (ADF). Additional analyses confirmed the stationarity of the process (KPSS) and the absence of evidence for a "long memory" effect (GPH). All three time series, both for Poland and for Łódzkie Province, were deemed stationary (Table 1).

Table 1. Evaluation of the stationarity of time series

	Specification	Poland			Łódzkie Province		
		ΔC/C	M/F	M/(M+F)	ΔC/C	M/F	M/(M+F)
Trend line	Slope coefficient	-0.0406	-0.0001	0.0000	-0.0407	-0.0003	-0.0001
	p-value	0.0352	0.1082	0.1070	0.0407	0.1039	0.1039
Augmented Dickey-Fuller (ADF) test with a constant term	Value (*a*)	-1.0851	-0.9752	-0.9772	-1.0981	-1.0116	-1.0087
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test	Statistics	0.3531	0.2646	0.2657	0.3865	0.3679	0.3653
	p-value	0.0990	p>0.10	p>0.10	0.0840	0.0920	0.0940
Geweke, Porter-Hudak (GPH) test	Fractional integration (*d*)	0.1358	0.0682	0.0309	0.2616	0.0183	0.0191
	p-value	0.4145	0.7738	0.8529	0.6517	0.9272	0.9222

Step 2: Construction of the private consumption model [ΔC/C].

The parameters of the private consumption model [ΔC/C] for Poland and for Łódzkie Province were identified on the basis of the autocorrelation function (ACF), partial autocorrelation function (PACF), Ljung–Box autocorrelation test, and plot (correlogram) analysis. The authors reviewed numerous models with different combinations of AR and/or MA lags. The results of parameter estimation for the private consumption model for Poland (Eq. 1) and for Łódzkie Province (Eq. 2) are given below in their final versions.

Eq. 1. ARMAX(3,3) estimation with restrictions[a] for 51 observations (1995.S2–2020.S2), dependent variable – ΔC/C (Poland)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	2.878	0.681	4.226	<0.0001
AR(3)	0.531	0.166	3.199	0.0014
MA(2)	0.792	0.117	6.742	<0.0001
MA(3)	0.447	0.123	3.638	0.0003
Z_2020.S1	−9.312	1.141	−8.161	<0.0001
Z_2005.S1	−3.792	0.571	−6.638	<0.0001
Z_2009.S1	−1.974	0.572	−3.454	0.0006
Z_2000.S2	−3.082	0.586	−5.257	<0.0001
Z_2012.S2	−1.575	0.460	−3.426	0.0006
Z_2015.S2	−1.163	0.558	−2.083	0.0372

Mean value of the dependent variable = 1.898
Standard deviation of the dependent variable = 2.042
Average random disturbances = −0.100

Standard deviation of random disturbances = 1.034
Log-likelihood = −78.161
Akaike Information Criterion (AIC) = 178.322
Schwarz Bayesian Information Criterion (BIC) = 199.572
Hannan–Quinn Information Criterion (HQC) = 186.442

Dependent variable ΔC/C – percentage change in private consumption (final consumption expenditure of households at constant 2015 prices); AR(3) – autoregressive parameter of the consumption equation representing relative change in consumption with three semiannual lags (1.5 years); MA(2) and MA(3) – two parameters of the moving average representing the estimated random term of the ΔC/C equation with two and three semiannual lags (1 year and 1.5 years), respectively; Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with all coefficients at lag 1 restricted to 0 and the coefficient at lag 2 (AR) restricted to 0.

Eq. 2. ARMAX(3,3) estimation with restrictions[a] for 51 observations (1995.S2–2020.S2), dependent variable – ΔC/C (Łódzkie Province)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	2.589	0.637	4.062	<0.0001
AR(3)	0.505	0.181	2.795	0.0052
MA(2)	0.835	0.101	8.299	<0.0001
MA(3)	0.401	0.103	3.883	0.0001
Z_2020.S1	−10.555	1.100	−9.597	<0.0001
Z_2000.S1	−4.071	0.614	−6.629	<0.0001
Z_2008.S1	3.171	0.464	6.833	<0.0001
Z_2002.S1	2.662	0.586	4.541	<0.0001
Z_2005.S1	−3.008	0.493	−6.101	<0.0001
Z_2013.S1	−1.530	0.484	−3.161	0.0016

Mean value of the dependent variable = 1.806

Standard deviation of the dependent variable = 2.103

Average random disturbances = −0.096

Standard deviation of random disturbances = 1.009

Log-likelihood = −76.887

Akaike Information Criterion (AIC) = 175.775

Schwarz Bayesian Information Criterion (BIC) = 197.025

Hannan–Quinn Information Criterion (HQC) = 183.895

Dependent variable ΔC/C – percentage change in private consumption (final consumption expenditure of households at constant 2015 prices); AR(3) – autoregressive parameter of the consumption equation representing relative change in consumption with three semiannual lags (1.5 years); MA(2) and MA(3) – two parameters of the moving average representing the estimated random term of the ΔC/C equation with two and three semiannual lags (1 year and 1.5 years), respectively; Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with all the coefficients at lag 1 restricted to 0 and the coefficient at lag 2 (AR) restricted to 0.

The above ΔC/C models previously passed the verification step according to Box and Jenkins's approach. All the estimated parameters were found to be statistically different from zero, and the models exhibited the lowest AIC values in their classes (178.3 and 175.8, respectively). In addition, a lack of autocorrelation of the error terms was found (Maddala and Lahiri 2009; Maddala 2013; Kufel 2013) based on the Lagrange multiplier (LM), which is used for checking autoregressive order correctness in ARMA models. In all analyzed cases with lags of 4–10, the value of the LM test statistic with a chi-squared distribution was smaller than the critical value, and so the null hypothesis was not rejected (the residual process had the nature of white noise).

Residuals from the studied models of consumption were recorded separately as eΔC/C and used as an explanatory variable in the M/F and M/(M+F) models, for Poland and for Łódzkie Province, respectively.

Steps 3–6: Construction and choice of the final equations, and analysis of residuals from the M/F and M/(M+F) models.

Parameters of the M/F and the M/(M+F) models for Poland and for Łódzkie Province were identified (pursuant to Box and Jenkins's approach) on the basis of ACF, PACF, the Ljung–Box autocorrelation test, and plot (correlogram) analysis. The authors reviewed multiple models with different combinations of AR and/or MA lags. The results of parameter estimation for the SSR models for Poland (Eq. 3) and for Łódzkie Province (Eq. 4) are presented below in their final version. All the estimated parameters were found to be statistically different from zero, and the models exhibited the lowest AIC values in their classes (−379.9 and −280.5, respectively).

Eq. 3. ARMAX(3,2) estimation with restriction[a] for 47 observations (1997.S2–2020.S2), dependent variable – M/F (Poland)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	1.059	0.002	701.900	<0.0001
AR(2)	0.342	0.147	2.326	0.0200
AR(3)	0.436	0.125	3.485	0.0005
MA(1)	−1.066	0.150	−7.099	<0.0001
MA(2)	1.000	0.088	11.400	<0.0001
eΔC/C(4)[b]	<0.001	<0.001	2.255	0.0242
Z_ 2010.S1	0.041	0.002	19.150	<0.0001
Z_ 2011.S1	0.018	0.002	11.220	<0.0001
Z_ 2005.S1	−0.012	0.001	−11.330	<0.0001
Z_ 1999.S2	−0.009	0.001	−6.307	<0.0001
Z_ 2016.S2	−0.007	0.002	−3.290	0.0010
Z_ 2018.S1	0.009	0.002	3.895	<0.0001

Mean value of the dependent variable = 1.060
Standard deviation of the dependent variable = 0.006
Average random disturbances < −0,001
Standard deviation of random disturbances = 0.003
Log-likelihood = 202.892
Akaike Information Criterion (AIC) = −379.784
Schwarz Bayesian Information Criterion (BIC) = −355.732
Hannan–Quinn Information Criterion (HQC) = −370.733

Dependent variable M/F – secondary sex ratio (SSR); AR(2), AR(3) – two autoregressive parameters of the M/F equation with two and three semiannual lags (1 year and 1.5 years), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M/F equation with one and two semiannual lags (0.5 year and 1 year), respectively; eΔC/C(4) – residuals from the consumption model with four semiannual lags (2 years); Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with the coefficient at lag 1 (AR) restricted to 0.

[b] The exact value of the coefficient of eΔC/C(4) was 0.000519 with a standard error of 0.000230.

Eq. 4. ARMAX(2,2)[a] estimation for 49 observations (1996.S2–2020.S2), dependent variable – M/F (Łódzkie Province)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	1.061	0.002	462.500	<0.0001
AR(1)	0.357	0.118	3.025	0.0025
AR(2)	−0.820	0.108	−7.597	<0.0001
MA(1)	0.285	0.116	2.461	0.0139
MA(2)	1.000	0.094	10.590	<0.0001
eΔC/C(2)[b]	0.009	0.002	5.316	<0.0001
Z_ 2010.S2	−0.048	0.008	−5.991	<0.0001
Z_ 2005.S1	0.031	0.009	3.546	0.0004
Z_ 2008.S2	−0.037	0.008	−4.853	<0.0001
Z_ 2002.S2	−0.019	0.008	−2.360	0.0183
Z_ 2006.S2	0.027	0.008	3.314	0.0009

Mean value of the dependent variable = 1.059

Standard deviation of the dependent variable = 0.017

Average random disturbances < 0.001

Standard deviation of random disturbances = 0.010

Log-likelihood = 152.237

Akaike Information Criterion (AIC) = −280.474

Schwarz Bayesian Information Criterion (BIC) = −257.773

Hannan–Quinn Information Criterion (HQC) = −271.861

Dependent variable M/F – secondary sex ratio (SSR); AR(1), AR(2) – two autoregressive parameters of the M/F equation with one and two semiannual lags (0.5 year and 1 year), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M/F equation with one and two semiannual lags (0.5 year and 1 year), respectively; eΔC/C(2) – residuals from the consumption model with two semiannual lags (1 year); Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with no restrictions.

[b] The exact value of the coefficient of eΔC/C(2) was 0.008914 with a standard error of 0.001677.

In the SSR models (Eq. 3–4), a lack of autocorrelation of the error terms was found (Maddala and Lahiri 2009; Maddala 2013; Kufel 2013) based on a test involving the Lagrange multiplier (LM), which is used for checking autoregressive order correctness in ARMA models. In all the analyzed cases with lags of 5–10 the value of the LM test statistic with a chi-squared distribution was smaller than the critical value, and so the null hypothesis was not rejected (the residual process had the nature of white noise). Moreover, the Doornik–Hansen (DH) test was used to verify that the residuals had a normal distribution. In the M/F model, the following results were obtained for Poland: (p=0.1676), and for Łódzkie Province: (p=0.8631). In both cases, at the alpha level of 0.05

there was no reason to reject the null hypothesis that the empirical distribution of residuals was normal (Doornik and Hansen 2008).

The results of parameter estimation for SRB models, that is, the M/(M+F) model for Poland in the ARMAX(3,2) version with restriction and for Łódzkie Province in the ARMAX(2,2) version, given in Eq. 5 and Eq. 6, also passed verification. LM tests confirmed autoregression order correctness in the ARMA models (the residual process had the nature of white noise). The residual distribution was normal (DH test). The following results were obtained for the M/(M+F) models: (p=0.1631) for Poland and (p=0.8734) for Łódzkie Province.

Eq. 5. ARMAX(3,2) estimation with restriction[a] for 47 observations (1997.S2–2020.S2), dependent variable – M/(M+F) (Poland)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	0.514	<0.001	1463.000	<0.0001
AR(2)	0.340	0.148	2.289	0.0221
AR(3)	0.434	0.126	3.456	0.0005
MA(1)	−1.064	0.152	−6.987	<0.0001
MA(2)	1.000	0.088	11.360	<0.0001
eΔC/C(4)[b]	<0.001	<0.001	2.297	0.0216
Z_ 2010.S1	0.010	0.001	18.420	<0.0001
Z_ 2011.S1	0.004	<0.001	10.860	<0.0001
Z_ 2005.S1	−0.003	<0.001	−11.270	<0.0001
Z_ 1999.S2	−0.002	<0.001	−6.253	<0.0001
Z_ 2016.S2	−0.002	<0.001	−3.288	0.0010
Z_ 2018.S1	0.002	0.001	3.856	0.0001

Mean value of the dependent variable = 0.514

Standard deviation of the dependent variable = 0.002

Average random disturbances < −0,001

Standard deviation of random disturbances = 0.001

Log-likelihood = 270.777

Akaike information Criterion (AIC) = −515.554

Schwarz Bayesian Criterion (BIC) = −491.502

Hannan–Quinn information Criterion (HQC) = −506.503

Dependent variable M/(M+F) – sex ratio at birth (SRB); AR(2), AR(3) – two autoregressive parameters of the M/(M+F) equation with two and three semiannual lags (1 year and 1.5 years), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M/(M+F) equation with one and two semiannual lags (0.5 year and 1 year), respectively; eΔC/C(4) – residuals from the consumption model with four semiannual lags (2 years); Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with the coefficient at lag 1 (AR) restricted to 0.

[b] The exact value of the coefficient of eΔC/C(4) was 0.000126 with a standard error of 0.000055.

Eq. 6. ARMAX(2,2)[a] estimation for 49 observations (1996.S2–2020.S2), dependent variable – M/(M+F) (Łódzkie Province)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
Const	0.515	0.001	952.600	<0.0001
AR(1)	0.358	0.117	3.046	0.0023
AR(2)	−0.823	0.107	−7.708	<0.0001
MA(1)	0.282	0.116	2.435	0.0149
MA(2)	1.000	0.095	10.560	<0.0001
eΔC/C(2) [b]	0.002	<0.001	5.268	<0.0001
Z_ 2010.S2	−0.011	0.002	−6.080	<0.0001
Z_ 2005.S1	0.007	0.002	3.501	0.0005
Z_ 2008.S2	−0.009	0.002	−4.857	<0.0001
Z_ 2002.S2	−0.004	0.002	−2.324	0.0201
Z_ 2006.S2	0.006	0.002	3.295	0.0010

Mean value of the dependent variable = 0.514
Standard deviation of the dependent variable = 0.004
Average random disturbances < 0.001
Standard deviation of random disturbances = 0.002
Log-likelihood = 222.944
Akaike Information Criterion (AIC) = −421.887
Schwarz Bayesian information Criterion (BIC) = −399.185
Hannan–Quinn information Criterion (HQC) = −413.274

Dependent variable M/(M+F) – sex ratio at birth (SRB); AR(1), AR(2) – two autoregressive parameters of the M/(M+F) equation with one and two semiannual lags (0.5 year and 1 year), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M/(M+F) equation with one and two semiannual lags (0.5 year and 1 year), respectively; eΔC/C(2) – residuals from the consumption model with two semiannual lags (1 year); Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model.

[b] The exact value of the coefficient of eΔC/C(2) was 0.002097 with a standard error of 0.000398.

Step 7: Decision making (TWH).

The obtained results led to the "correct" verification of the TWH based on the SSR. In the M/F model for Poland (Eq. 3), the coefficient of eΔC/C(4) – the lagged explanatory variable described as "residuals from the consumption model" – was positive: 0.000519 with a standard error of 0.000230. In the M/F model for Łódzkie Province (Eq. 4), the coefficient of eΔC/C(2) was also positive: 0.008914 with a standard error of 0.001677.

The evaluation of coefficients and standard errors of eΔC/C variables indicated that both models were stable. For Poland, the value of the eΔC/C(4) parameter was more than two times greater than its standard error (in other words, the standard deviation of the estimated parameter amounted to 44% of its value), while the corresponding parameter eΔC/C(2) for Łódzkie Province was more than five times greater (19% of the value, respectively). This implies that hypothesis H_0 should be rejected in favor of the alternative hypothesis H_1 that the economic factor had an effect on the SSR both in Poland as a whole and in Łódzkie Province.

In turn, the positive values of the estimated parameters revealed appropriate one-way relationships, thus corroborating the hypothesis about the direction of changes, that is, a decrease in the M/F ratio as a result of an increase in female births and/or a decrease in male births. For Poland, a decrement in M/F in period *t* was caused by a decline in private consumption in period *t–4* (with a lag of two years), while the lag for Łódzkie Province was 1 year (a decrement in M/F in period *t* was caused by a decline in private consumption in *t–2*).

The above results (including lag orders) were also confirmed for the SRB (Eq. 5 and Eq. 6).

Step 8: Additional analyses.

An additional analysis, combining results for the models ΔC/C and M/F or M/(M+F), involved the conversion of the final equations for SSR or SRB into new equations (models) in which the dependent variable was the number of males born (M) and the independent variables included the number of females born (F). These converted models (Eq. 7–8) do not include constant terms as the constant terms present in the M/F models (Eq. 3–4) became parameters estimated for the variable F.

Eq. 7. ARMAX(3,2) estimation with restriction[a] for 47 observations (1997.S1–2020.S2), dependent variable – M (Poland)

Variable	Coefficient	Standard error	*t*-Statistic	*p*-Value
AR(2)	0.297	0.156	1.906	0.0566
AR(3)	0.440	0.127	3.469	0.0005
MA(1)	−0.998	0.166	−6.024	<0.0001
MA(2)	1.000	0.086	11.650	<0.0001
eΔC/C(4)	42.334	21.959	1.928	0.0539
F	1.059	0.001	752.200	<0.0001
Z_ 2010.S1	3988.210	209.369	19.050	<0.0001
Z_ 2011.S1	1612.480	194.010	8.311	<0.0001
Z_ 2005.S1	−1100.100	105.296	−10.450	<0.0001
Z_ 1999.S2	−897.709	145.425	−6.173	<0.0001
Z_ 2016.S2	−598.946	212.751	−2.815	0.0049
Z_ 2018.S1	840.882	228.012	3.688	0.0002

Mean value of the dependent variable = 97961.890

Standard deviation of the dependent variable = 5234.665

Average random disturbances = −23.788

Standard deviation of random disturbances = 276.285

Log-likelihood = −335.649

Akaike Information Criterion (AIC) = 697.298

Schwarz Bayesian Information Criterion (BIC) = 721.350

Hannan–Quinn information Criterion (HQC) = 706.349

Dependent variable M – number of male live births; AR(2), AR(3) – two autoregressive parameters of the M equation with two and three semiannual lags (1 year and 1.5 years), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M equation with one and two semiannual lags, respectively (0.5 year and 1 year); eΔC/C(4) – residuals from the consumption model with four semiannual lags(2 years); F – number of female live births; Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with the coefficient at lag 1 (AR) restricted to 0.

Eq. 8. ARMAX(2,2)[a] estimation for 49 observations (1996.S2–2020.S2), dependent variable – M (Łódzkie Province)

Variable	Coefficient	Standard error	*t*-Statistics	*p*-value
AR(1)	0.357	0.114	3.132	0.0017
AR(2)	−0.840	0.098	−8.540	<0.0001
MA(1)	0.263	0.105	2.494	0.0126
MA(2)	1.000	0.095	10.510	<0.0001
eΔC/C(2)	50.480	9.588	5.265	<0.0001
F	1.061	0.002	480.000	<0.0001
Z_ 2010.S2	−302.506	43.312	−6.984	<0.0001
Z_ 2005.S1	168.364	48.592	3.465	0.0005
Z_ 2008.S2	−230.279	43.357	−5.311	<0.0001
Z_ 2002.S2	−97.346	46.246	−2.105	0.0353
Z_ 2006.S2	151.097	46.167	3.273	0.0011

Mean value of the dependent variable = 6022.306
Standard deviation of the dependent variable = 384.618
Average random disturbances = 0.998
Standard deviation of random disturbances = 56.487
Log-likelihood = −270.718
Akaike Information Criterion (AIC) = 565.437
Schwarz Bayesian Information Criterion (BIC) = 588.138
Hannan–Quinn Information Criterion (HQC) = 574.050

Dependent variable M – number of male live births; AR(1), AR(2) – two autoregressive parameters of the M equation with one and two semiannual lags (0.5 year and 1 year), respectively; MA(1), MA(2) – two parameters of the moving average representing the estimated random term of the M equation, with one and two semiannual (0.5 year and 1 year) lags, respectively; eΔC/C(2) – residuals from the consumption model with two semiannual lags (1 year); F – number of female live births; Z_... – binary variables used to explain outliers in the time series and increase the fit of the model.

[a] ARMAX model with no restrictions.

Analysis of results for Poland indicates that with each 1% increment in private consumption over its expected value (mean=1.898, Eq. 1), the number of male births increased at the expense of female births on average by 42–43 (eΔC/C(4), Eq. 7). Conversely, with each 1% decrement in private consumption below its expected value the number of male births decreased in favor of female births on average by 42–43. Surplus consumption from periods in which consumption increases were higher than expected (i.e., greater than the mean) in 1995.S2–2020.S2 adds up to 30.7 percentage points. By multiplying this figure by 42–43 male births, one can estimate that the number of "additional" male births attributable to an "improvement of economic conditions" (as a result of greater-than-expected consumption) was 1290 to 1320 in Poland over the study period with a lag of 2 years.

In turn, analysis for Łódzkie Province indicates that with each 1% increment in private consumption over its expected value (mean=1.806, Eq. 2) the number of male births increased at the expense of female births on average by 50–51 (eΔC/C(2), Eq. 8). Conversely, with each 1% decrement in private consumption below its expected value the number of male births decreased in favor of female births on average by 50–51. Surplus consumption from periods in which consumption increases were higher than expected in 1995.S2–2020.S2 adds up to a total surplus of 31.8 percentage points. This translates into 1592–1624 "additional" male births over the study period due to improved economic conditions with a 1 year lag.

Discussion

The first study, which tested the Trivers–Willard hypothesis among a large contemporary Polish sample using first birth interval and extent of breastfeeding as measures of parental investment, and economic status and level of parental education as measures of parental condition, provided evidence of greater investment in female offspring at the lower extremes of income, and greater investment in males at higher levels of income (Koziel and Ulijaszek 2001).

In the present paper, the statistical method proposed by Catalano and Bruckner (2005) was expanded and re-applied to verify the economic stress hypothesis in Poland as a whole and in one of its regions (Łódzkie Province) for the years 1995–2020. Repeated research after an interval of more than 10 years, using new, longer, and modified time series (semiannual data) as well as employing additional analyses to expand the study concept has resulted in an important contribution to the discussion about TWH verification.

In the authors' view, appropriate preparation of data for analysis (economic data in constant prices) is of the essence, and making sure that all the assumptions of applicability of time series analysis models (process stationarity, correct evaluation of lags) are met. The basic characteristics of a stationary process are determined on the basis of its mean, variation, and autocovariance (overall and partial autocorrelation). It is necessary to verify hypotheses about the trend line slope coefficient (that the mean value is constant over time), as well as hypotheses about series nonstationarity arising from the trend, "long memory" effect (nonstationary variance), and the autocorrelation of the error terms (Maddala and Lahiri 2009; Kufel 2013; Maddala 2013).

In the present study (see: Step 1) analysis of individual time series [ΔC/C, M/F, and M/(M+F)] gave no reason to reject H_0 that the trend line slope coefficient was equal to zero (Table 1). The coefficient values confirm that the mean value of the process was constant over time for data both for Poland and for Łódzkie Province. In a further step, the variance of the series was analyzed for nonstationarity, which would be unfavorable (heteroscedasticity). The nonstationarity of variance was evaluated with the ADF test with the null hypothesis being that the process was integrated of order $I(1)$ versus an alternative hypothesis that the process was not integrated $I(0)$. The value of the parameter a obtained in the ADF test with a constant term for each of the series was statistically different from zero i.e. negative ($p<0.0001$), which indicates that the process was not integrated. In other words, each time the null hypothesis (H_0) that the process was integrated of order one ($d=1$) was rejected in favor of the alternative hypothesis (H_1) that it was integrated of order zero ($d=0$). The analyzed series did not require differentiation.

The above results were then verified in confirmatory analysis, applying the Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test, where the null hypothesis assumes process stationarity and the alternative hypothesis – non-stationarity. The *p*-values obtained for each of the series were greater than 0.08, at the alpha level of 0.05 there was no reason to reject H_0. Furthermore, in recent years it has been noted that some processes could be fractionally integrated ($0.5<d<1.0$), and so the authors also verified such a hypothesis by means of the Geweke, Porter-Hudak (GPH) test (Kufel 2013). The high *p*-values obtained from that test ($p>0.4$) indicate that the estimated fractional values of the *d* parameter for the studied time series were not statistically different from zero. These results in fact indicate integration of order zero, corroborating the findings from the ADF test. Consequently, no "long memory" effects were present.

In the study (see: Steps 3–6) models with all parameters statistically different from zero were developed for two types of the human sex ratio (the SSR and SRB) for Poland and for one of its regions – Łódzkie Province. Comparative analysis of the obtained results led to new, interesting conclusions relevant for this kind of research, both in terms of statistics (model construction) and interpretation of results. In the authors' view, the "dual solution" assumption (the same models used for the SSR and SRB) appears to be highly meaningful from both theoretical and practical standpoints. First, it should be noted that SSR and SRB series offer different ways of presenting one concept of the human sex ratio – they provide similar information and they are designed on the basis of the same time series for male and female births. Second, time series analysis is multi-faceted and employs advanced statistical methods. In addition, stationary ARMA processes are reversible, which means that the moving average equation can be written in an autoregressive form (of an infinite order). Due to these considerations, it is very difficult, complicated, and time consuming to identify the right model and arrive at one acceptable final solution. This points to yet another problem – the authors believe that such a complicated model (ARMAX) cannot be identified using an automatic lag selection method; rather, this requires knowledge and experience. Therefore, the additional "dual solution" assumption adopted in this work may be treated as a stop criterion in choosing the optimal solution. The dual results obtained in this way *de facto* confirm the stability of the solution, i.e., of changes in the sex ratio relative to private consumption. Given the above, the dispute about the superiority of one human sex ratio over the other (the SSR vs. SRB) becomes futile, as here both of them are equally important.

In the case of the discussed M/F and M/(M+F) models, the other lags for the "residual from the consumption model" variable (see: Step 8) was also checked bearing in mind that different lags for Poland and for Łódzkie Province were used in the final models (eΔC/C(4) and eΔC/C(2), respectively). It should be noted that the model for Poland with a 1 year lag (the same as for Łódzkie Province) exhibited a differ from zero but negative coefficient of eΔC/C(2). In turn, in the case of Łódzkie Province the M/F and M/(M+F) models with all parameters statistically different from zero were also identified for the simultaneous use of eΔC/C(1) and eΔC/C(2) lags as well as eΔC/C(0), eΔC/C(1), and eΔC/C(2) lags. However, while in those models the coefficient of eΔC/C(2) remained positive, the coefficients of the other lagged variables, that is, eΔC/C(0) and/or eΔC/C(1), were negative. In the authors' view, while such results should be deemed incorrect they lend support to the correctness and stability of the previously identified final models (Eq. 3–4, Eq. 5–6).

In determining the number of "additional" male births attributable to improved economic conditions, one should pay particular attention to the reference point of analysis, that is, the expected values of private consumption. If the ΔC/C model does not contain autoregressive parameters, then the expected value of the constant term will be equal to the mean value of the time series (*const=mean*). On the other hand, if the series does contain autoregressive parameters, then the constant term will not correspond to the mean value of the series (*const≠mean*). Then, as in the presented case, the expected value should be identified in the additional characteristics of the model. If the time series was previously differentiated, the situation becomes much more complicated; then the constant term of the model may correspond to either the mean or the constant term of the differentiated time series.

The current results based on semiannual data from 1995–2020 are consistent with the previously obtained SSR results based on quarterly data from the years 1995–2007 for Łódzkie Province (Żądzińska et al. 2011). In the present work the occurrence of economic stress was followed by a decline in the SSR (and also in the SRB) at a 1 year interval. Relationships between M/F or M/(M+F) and percentage change in private consumption were also examined on the basis of semiannual data for Poland. In this study, analysis of a completely new dataset, which was nevertheless defined in the same way as in the prior study, confirmed the presence of a one-way relationship consistent with the TWH between M/F or M/(M+F) and ΔC/C. However, the lag for the whole country was as much as 2 years.

The question thus arises as to the difference in SSR and also SRB reaction time to changes in private consumption between Łódzkie Province (shorter lag) and the whole country (longer lag). A detailed analysis of the time series for Poland revealed that within the time of study, 27 semiannual periods exhibited decreases in consumption below the mean value (max 8.79 percentage points) while 24 semiannual periods revealed increases above the average (max 7.17 percentage points). In comparison, in the case of Łódzkie Province: 29 semiannual periods exhibited decreases in consumption below the mean value (max 8.88 percentage points) while 22 semiannual periods revealed increases above the average (max 7.25 percentage points). Of course, the tested TWH was supported both by periods of "good conditions" and "bad conditions." Periods of reduced consumption favored mothers having daughters (leading to lower M/F and M/(M+F)), while periods of growing consumption favored mothers having sons (leading to higher M/F and M/(M+F)). However, it should be noted that the effects of change in private consumption were stronger in Łódzkie Province as compared to the whole country, and so the population's reaction time (lag) to the presence or absence of economic stress was shorter. This situation is also attributable to the variability of the human sex ratios.

It should be borne in mind that one of the goals of time series analysis is to identify the nature of the phenomenon represented by the sequence of observations contained in the explained variable. As the M/F and M/(M+F) time series for Łódzkie Province exhibited greater variability than those for Poland (Fig. 1), they could be more readily "modeled" taking into consideration the advantages and shortcomings of ARMA models. In statistical terms, the regional models for Łódzkie Province were found to be better, less complicated, and contained a lower number of autoregressive lags. A similar situation is also true of other regions in the country (the authors have access to SSR and SRB semiannual data from the years 2000–2020 for all 16 Polish provinces). Preliminary calculations indicated that fluctuations in the sex ratios by province as measured by the coefficient of variation ranged from 1.45% and 0.70% in Wielkopolskie Province to 4.39% and 2.09% in Opolskie Province for M/F and M/(M+F), respectively, while the CV for the whole country was much lower, at 0.58% and 0.28%, respectively. According to the authors, the greater variation of the ratios in individual regions translates into shorter lags (faster reaction times) in regional models, ceteris paribus. Consequently, it seems that the strength (value) of the reaction and differences in reaction time of human sex ratios to economic stress may also be considered in the context of the biological resistance of a given population to stress (depending both on the homeorhetic mechanisms and economic conditions).

Also greater data resolution improves the effectiveness of analysis and increases the likelihood of a "positive" verification of the economic stress hypothesis. In their previous study using annual data for Poland (Żądzińska et al. 2007), the present authors were unable to confirm that hypothesis. In addition, the time series required differentiation (to ensure stationarity), which made it more difficult to interpret the results. In turn, models using semiannual and quarterly data exhibit greater accuracy and have been successfully employed in verifying the effects of change in private consumption on the SSR and SRB in line with the TWH for the population of Łódzkie Province as well as for the Polish population as a whole.

For obvious reasons, the above considerations cannot explain the immediate causes of the 2 year lag in SSR and SRB increments following increases in private consumption above the expected levels for Poland; those causes should be sought outside the discussed models. In this context it should be noted that there exist substantial differences between Polish regions in terms of both changes in the sex ratios (Szukalski 2010; 2021a) and in living conditions (Domański 2020). According to the literature, these variables could be affected by the general decline in the birth rate, local depopulation including the migration of young women in pursuit of better living conditions (Szukalski 2020), as well as the study period, including the outbreak of the COVID-19 pandemic characterized by a major contraction of private consumption.

The demographic consequences of the COVID-19 epidemic include a sharp year-on-year decline in the birth rate in Poland in 2020 (from 375.0 thousand to 355.3 thousand) as well as a drop in the total fertility rate (TFR) (Szukalski 2021b). While the TFR decreased from 1.419 to 1.378 children, according to Szukalski (2021b) almost half of that change is attributable to structural factors, that is, a reduction in the birth rate which occurred two to three decades previously and which has led to a smaller number of women at the peak of reproductive age nowadays.

The decreased tendency to have children during the COVID-19 pandemic should be treated as population adjustment to new conditions at a time of a social crisis. In the case of procreation, this may take two forms – delayed childbearing or the decision to remain childless, for instance due to increased job insecurity and reduced availability of healthcare services (Szukalski 2021b). In a study of the Norwegian population, it was found that pandemic influenza virus infections increased the risk of fetal death (Håberg et al. 2013). In turn, the latest research shows that children born in the first year of the current pandemic, even those whose mothers did not contract COVID-19, achieved inferior results in social and motor ability tests as compared to children born before the pandemic (Shuffrey et al. 2022). According to the authors of that study, a potential mechanism behind the observed decrements in neurodevelopment was COVID-19–related stress, with the reported stressors being job loss, food insecurity, and loss of housing. The pandemic also exacerbated symptoms of anxiety and depression.

In summary, the obtained results led to the "correct" verification of the TWH: economic stress affected the secondary sex ratio in Poland in a period following the country's political and economic transformation. However, in the opinion of the present authors, it is necessary to further verify the economic stress hypothesis for Poland taking into account factors such as maternal stress, interpopulation differences in terms of SSR (and SRB) reaction time to stress, and inter-regional comparisons (economic differences), as well as using longer time series (extending after the COVID-19 pandemic).

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Conflict of interests

Authors declare no conflict of interests.

Authors' contributions

AM: data curation, methodology, resources, formal analysis, writing – original draft, visualization, writing – review & editing. IR: conceptualization, writing – original draft, writing – review & editing. EŻ: conceptualization, writing – review & editing, project administration.

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Factors affecting stunting among 3–12 years old girls of Purba Medinipur, West Bengal, India

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ABSTRACT: Stunting is the impaired growth and development in children caused due to poor nutrition, recurrent infections and lack of psychosocial stimulus. Because stunting affects physical development and inhibits the child from attaining his full cognitive development as an adult, children may never regain the height lost due to stunting. Stunting (Low height-for-age) in infants and young children is a simple, well-known indicator of undernutrition. The current study sought to determine the effects of socio-economic and demographic factors on the frequency of stunting among Bengalee girls. The study was carried out in the Deshopran block and Haldia municipality area (West Bengal, India). The total number of participants included in the study was 530 Bengalee girls. 53.39% (283) of study participants were urban residents while 46.61% (247) were rural residents. Stunted growth in children was defined as the height for a given age in children less than two standard deviations of the WHO Child Growth Standards. The prevalence of stunting among girls in our study was 11.13%. The predictor variables with substantial associations with stunting in the binary logistic regression (BLR) analyses were further used in the multiple binary logistic regression (MBLR) analyses. Odds ratios with 95% confidence intervals were used to calculate the stunting risk. Results showed that the risk of stunting was significantly associated with low birth weight, presence of younger brothers, a large number of family members and place of delivery. Improving maternal and child access to nutrient-rich food, decreasing infectious disease, and promoting women empowerment initiatives are the main recommendations for resolving the issue.

KEY WORDS: Girls, Stunting, India, West Bengal.

Original article

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Introduction

Internationally, child growth is recognized as a significant measure of a population's nutritional health and status. One of the three anthropometric indicators that are frequently used to assess a child's growth is stunting. Stunting, generally referred to as low height for age, is considered to be the outcome of chronic undernutrition often associated with poverty, poor maternal nutrition and health, recurrent sickness, and/or inappropriate feeding and care for young children. Stunting prevents children from developing to their full physical and intellectual potential (WHO 1995a). Due to its great prevalence and significant negative effects on development and health, stunted linear growth has emerged as the primary measure of childhood undernutrition (Black et al. 2013a). Stunting is characterized as having a height for age Z (HAZ) score that is less than 2 in the WHO growth reference standard (WHO 1995b). Height for age Z-score measures linear growth that occurs before and after birth; deficiencies show long-term, cumulative impacts of poor health, food quality and care. It is a significant predictor of human capital and social advancement since it is linked to higher illness and mortality, delayed mental development, low educational attainment, and diminished intellectual capacity (Victora et al. 2008; Srivastava et al. 2012; De Onis et al. 2012; Prendergast et al. 2014). During maturity, stunting can lead to decreased labour capacity, weakened social skills, behavioural issues, and metabolic illnesses (Grantham-McGregor 2014; Rengma et al. 2016; Akseer et al. 2017).

Stunting is one of the biggest and most difficult public health issues in today's world. Although it is invisible in many nations, it affects 165 million children worldwide, 90% of whom reside in Asia and Africa (UNICEF 2012a) and is a significant cause of concern in developing nations. This makes it a bigger problem than being wasted or underweight. The incidence of stunting, linked to several determinant variables along with prolonged malnutrition, is a serious issue among those living in resource-poor nations. WHO estimates that among school-age children aged 5 to 18 in Africa, the prevalence of stunting was 37% in 2015, whereas Asia had the second-highest prevalence rate at 23% (De Onis 2012). Numerous studies have already reported the prevalence of stunting in West Bengal (India) boys and girls (Mondal and Sen 2010; Soumyajit et al. 2011; McGuir 2015; Bishwajit 2015; Rengma et al. 2016; Akseer et al. 2017; Kwon and Kim 2017). Stunting among early adolescent girls is associated with different factors. For instance, several studies have reported significant associations between poor socio-economic, demographic and environmental conditions and chronic nutritional deficiencies (WHO 2006; Bishwajit 2015; McGuire 2015; Akseer et al. 2017).

Due to their customary early marriage and pregnancy, teenage girls may be more susceptible to stunting (Black et al. 2013a; Prentice et al. 2013). Importantly, because of fetal programming, stunted adolescent girls are more likely to deliver underweight and stunted babies (Prentice et al. 2013; Kwon and Kim 2017). NFHS-5 (National Family Health Survey) has reported that 6.8% of teenage girls were pregnant in India. The proportion of women who have started childbearing rises sharply from 15% at the age of 17 years to

24% among women aged 18, indicating a very high proportion compared to NFHS- 4, which is a very crucial stage for child and maternal nutrition in West Bengal. The proportion of women who have started childbearing is much higher among those who have no schooling (IIPS 2021). Several causes of malnutrition among children include poverty, living in a rural area and family size (UNICEF WHO and World Bank 2012; Bhutta and Salam 2012; Black et al. 2013b). Improving maternal and child access to nutrient-rich food, decreasing infectious disease, and empowering maternal empowerment initiatives are the main recommendations for resolving the issue (WHA 2012). However, reducing the differential vulnerability of girls is also necessary, at least in the context of South Asia, where sons is preferred over daughters (Baqui et al. 2001; Bhuiya et al. 2003; Jain et al. 2004; Koenig et al. 2006; Silverman et al. 2007; Silverman et al. 2011). Gender discrimination among siblings may play a major role in high rates of child malnutrition observed in South Asia, a region that exhibits higher rates of under-5 mortality for girls than boys (UNICEF 2012b; Basu et al. 2018; Mumtaz et al. 2019).

A girl's risk of acute malnutrition (wasting) is increased by having brothers, whereas her risk of chronic malnutrition (stunting/underweight) is increased by having many sisters. In contrast, siblings have less of an impact on boys malnutrition (Raj et al. 2015). According to previous studies, stunting affects teenage girls at a rate of 48% in Bangladesh and 47% in Nepal (WHO 2006; Bishwajit 2015). Stunting prevalence among teenage girls in Ethiopia ranges from 26.5% to 41.8% (Mulugeta et al. 2009; Wassie et al. 2015). The percentage of stunted children increased marginally from 33% to 34% between NFHS- 4 to NFHS- 5 which suggests that higher levels of undernutrition are still a major problem in West Bengal (IIPS 2021). According to the fourth National Family Health Survey (NFHS) 2015–2016, 38.4% of children in India were found to be stunted (IIPS 2017). According to numerous research (Chirande et al. 2015; Rengma et al. 2016; Dubey et al. 2018; Abbasi et al. 2018; Mazengia and Biks 2018; Titaley et al. 2019), the prevalence of stunting in India ranges from 10.9% to 55.9% in boys and 18% to 58.4% in girls. In West Bengal the prevalence of stunting has been reported to be above 12% in both sexes (Bisai et al. 2008; Bisai and Mallick 2011; Sarkar 2016; Giri et al. 2017; Pal et al. 2017; Khanra et al. 2022). The objective of the current study was to determine the effects of socio-economic and demographic factors on the frequency of stunting among Bengalee girls.

Methodology

Participants and settings

Haldia Municipality and Deshopran Block (Rural Areas) in Purba Medinipur District of West Bengal, India, were selected for this study. This cross-sectional study was conducted from December 2014 to April 2016. Of those, 628 (urban: 313; rural: 315) participated in the survey, and 530 (84.39%) provided complete information. Of them, 283 (53.39%) were urban residents, and 247 (46.60%) were rural Bengalee girls aged 3–12 years. In the state's public education system, children go to daycare for half a day's meals and some pre-school education around the age of 3. Children start attending childcare facilities at the age of 3 years for a mid-day meal and introductory pre-nursery education. According to Indian government regulations, every child enrolled and attending school in classes from one to eight between the ages of six and fourteen must get a free lunch every day, except from school holidays. On the other hand, since the purpose of the study was to limit the pre-adolescent age of girls, the upper age of study participants was limited to 12 years. A detailed description of the sample recruitment procedures has been described by Khanra et al. (2020; 2021). Data were collected from one rural and one urban area of Purba Medinipur District (PMD). Rural girls were recruited from the following villages (n=3): Kultalia, Sikdarchak and Uttar Amtalia, PMD Desopra Block of Contai subdivision and urban girls from three settlement colonies (CPT, IOC and HREL) and Rairarchak district under Haldia municipality. The study followed the ethical guidelines of the 2000 Declaration of Helsinki (Touitou et al. 2000).

Demographic, socio-economic and birth-related information

Demographic, socio-economic, maternal health and childbirth-related data were collected directly from the parents, usually from the mothers, through a structured questionnaire. The information included social category (general or scheduled caste), place of residence (urban or rural), number of family members, number of elder and/or younger sisters and brothers, number of living rooms, house ownership, family income and expenditure, parental education, type of cooking fuel and sanitary system. Information regarding the mother's age at childbirth, height and nutritional status were also recorded. The place of delivery and birth weight of the child were also recorded. Information about birth weight was obtained from the mothers. Low birth weight was defined as less than 2,500 grams of body weight (WHO 2001).

Anthropometry

One researcher (PK) recorded all anthropometric measurements from the studied children. Height (in cm) was measured from all children, following standard procedures (Lohman et al. 1988). Height-for-age 'Z Score' (HAZ) was computed to determine stunting among the children. The WHO Anthro 3.2.2 and Anthro Plus 1.0.4 software was used to calculate the Z score. Stunting was defined as HAZ less than two standard deviations (WHO 1995b).

Statistics

Percentages were used to report the distribution of the population according to categories of different variables. Mean, and standard deviation (SD) statistics were used to describe continuous variables. Binary logistic regression (BLR) analyses (univariate model) were performed for each independent factor to assess whether it is significantly associated with stunting. In each BLR, odds ratio (OR) with 95% confidence interval (CI) was calculated to show the magnitude of association of a particular predictor category with stunting relative to the other category of the variables. To assess the effects of the factors relative to one another and identify the most potent predictor variables, the factors significantly associated with the bivariate analyses were also included in stepwise multivariate logistic regression analyses (enter method). In the regression models the dependent variable (i.e., stunting), was coded as 1 for "stunted" and 0 for "non-stunted". Social category (general or scheduled castes), place of residence (urban or rural), family size (≥ 5 members), number of elder or younger siblings (Nil vs. either or both present), number of living rooms (≤2 or >2 rooms), house ownership (own or rental), monthly family income per capita (Rs. ≤2000 or Rs. >2000), parental education (both above secondary level or not), and type of cooking fuel (smoky or smokeless) were the categories used to group the predictor variables in the current study. The better possibilities (like smokeless fuel) or higher values (like birth weight 2,500 grams) for each of these predictors were coded as 0, whereas the corresponding worse conditions or qualities (such as smoky fuel) or lower values (such as birth weight 2,500 grams) were coded as 1, respectively.

According to their relative 50th%iles, family size, the number of living rooms, the number of younger and elder sisters and brothers, and parity data were all categorized. The vaccination document verified the mother's age at delivery, birth weight, place of delivery, and date of birth. Birth weight data were categorized according to the relevant standards (WHO 2001). Body mass index (BMI), which is computed as weight in kilograms (kg) divided by height in meters (m) squared (kg/m^2), was used to determine the mother's nutritional condition. The nutritional status of the mother was categorized as undernourished (BMI <18.5 kg/m^2) or normal (>18.5 kg/m^2) based on BMI values. Statistical significance is defined as a p-value < 0.05. SPSS-16 for Microsoft Windows was used to conduct all statistical analyses.

Result

Overall, 11.13% of the study's girl participants had stunting. The% distribution of the subjects for each category of independent factor is shown in Table 1, along with the significance of the relationship between each independent factor and stunting as determined by the outcomes of univariate BLR analyses. The results of BLR indicated that the risk of stunting was significantly higher among the girls whose parents were less educated. Girls whose mothers were homemakers exhibited better nutritional indices compared to those born to working mothers. The higher risk of stunting (ORs = 2.22, p<0.01) was found in girls who were living with more than four family members. The risk of stunting was significantly higher in girls living in homes with a smaller number rooms (up to two) and with no proper sanitary system. Poor household income (ORs = 2.15, p<0.01) and expenditure were also significantly associated with higher prevalence of stunting (ORs = 1.95, p<0.05). Girls who had a very low weight at birth were significantly (ORs = 10.44, p<0.001) more likely to be stunted. A higher prevalence of stunting (ORs = 1.41) was found in the category of the undernourished mother. Girls who were nutritionally stunted their mothers had low height (ORs = 1.21). Girls who had younger brothers were significantly more likely to be stunted (ORs = 2.53, p<0.001). Girls delivered at home were more likely to be stunted compared to those delivered at health institutions (ORs= 3.36, p<0.01). The risk of stunting was higher in girls in the category of mother age at childbirth, and mothers who have started childbearing at the age of below 20 years (ORs = 1.66).

Table 2 presents the results of MBLR analysis to identify independent risk factors predicting stunting. MBLR analyses were performed on those predictor variables that showed significant associations with stunting in the univariate BLR analyses. Girls who had a large family (above four members) were more likely to be stunted (ORs = 2.28, $p<0.01$) compared to those who belonged to a small-sized family (up to four members). The risk of stunting was significantly higher in those girls who had younger brothers than those who had no younger brothers (ORs = 1.99, $p<0.05$). Girls who exhibited low weight at birth were significantly more likely to be stunted compared to girls who had a normal or healthy weight ($p<0.001$). The girls delivered at home were more likely to be stunted (ORs = 2.00, $p<0.05$) than those delivered at health institutions.

Table 1. Logistic regression of associated factors with stunting among the girls

Variables	Categories	Stunting (n)	Total	Stunting (%)	B	Wald	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
									Lower	Upper
Place of residence	Haldia®	25	283	8.83						
	Rosulpur	34	247	13.76	0.45	3.19	0.74	1.65	0.95	2.84
Social category	General®	37	387	9.56				1		
	Others	22	143	15.38	0.54	3.52	0.06	1.72	0.98	3.03
Fathers education	Above upper primary®	17	237	7.17				1		
	Upto upper primary	42	293	14.33	0.77	6.55	0.01	2.17	1.2	3.91
Mothers education	Above upper primary®	17	228	7.46				1		
	Upto upper primary	42	302	13.91	0.7	5.3	0.02	2	1.11	3.62
Fathers occupation	Nonmanual®	20	242	8.26				1		
	Manual	39	288	13.54	0.55	3.63	0.06	1.74	0.98	3.07
Mothers occupation	Home-maker®	45	458	9.83				1		
	Working mothers	14	72	19.44	0.8	5.58	0.02	2.22	1.15	4.28
Numbers of family members	Upto 4 members®	20	272	7.35				1		
	Above 4 members	39	258	15.12	0.81	7.76	0.01	2.24	1.27	3.96
Numbers of employed persons	Above 1 persons®	15	111	13.51				1		
	1 person	44	419	10.5	-0.29	0.8	0.37	0.75	0.4	1.4
Parity	1st parity®	37	311	11.9				1		
	2nd and others parity	22	219	10.05	-0.19	0.44	0.51	0.83	0.47	1.44
Numbers of elder brothers	Have no elder brothers®	49	414	11.84				1		
	Have elder brothers	10	116	8.62	-0.35	0.94	0.33	0.7	0.34	1.43

Variables	Categories	Stunting (n)	Total	Stunting (%)	B	Wald	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
									Lower	Upper
Numbers of elder sisters	Have no elder sisters®	44	414	10.63				1		
	Have elder sisters	15	116	12.93	0.22	0.48	0.49	1.25	0.67	2.33
Numbers of younger brothers	Have no younger brothers®	34	399	8.52				1		
	Have younger brothers	25	131	19.08	0.93	10.58	0	2.53	1.45	4.43
Numbers of younger sisters	Have no younger sisters®	43	433	9.93				1		
	Have younger sisters	16	97	16.49	0.58	3.38	0.07	1.79	0.96	3.34
House ownership	Own®	51	405	12.59				1		
	Rental	8	125	6.4	-0.75	3.56	0.06	0.47	0.22	1.03
Numbers of living rooms	Above 2 rooms®	5	100	5				1		
	Upto 2 rooms	54	430	12.56	1	4.35	0.04	2.73	1.06	7.01
Sanitary system	Have proper sanitary system®	46	458	10.04				1		
	Have no proper sanitary system	13	72	18.06	0.68	3.91	0.05	1.97	1.01	3.87
Cooking system	Non smoking®	21	251	8.37				1		
	Smokey	38	279	13.62	0.55	3.62	0.06	1.73	0.98	3.03
Income	≥ Rs. 10001®	17	236	7.2				1		
	≤ Rs. 10000	42	294	14.29	0.76	6.4	0.01	2.15	1.19	3.88
Expenditure	≥ Rs. 8001®	18	235	7.66				1		
	≤ Rs. 8000	41	295	13.9	0.67	5.01	0.03	1.95	1.09	3.48
Birth weight	2.500gm & Above®	27	450	6				1		
	Below 2.500gm	32	80	40	2.35	60.16	0	10.44	5.77	18.89
Mother's height	149.05cm & Above®	27	265	10.19				1		
	Below 149.04cm	32	265	12.08	0.19	0.48	0.49	1.21	0.7	2.08
Place of delivery	Institutional®	22	336	6.55				1		
	Home	37	194	19.07	1.21	17.94	0	3.36	1.92	5.89
Mother's age at child birth	20years & Above®	32	344	9.3				1		
	Below 20years	27	186	14.52	0.5	3.27	0.07	1.66	0.96	2.86
Mother's nutrition	Normal®	53	489	10.84				1		
	Undernutrition	6	41	14.63	0.34	0.55	0.46	1.41	0.57	3.51

® - reference category, Binary logistic regression analysis (univariate model) considering effect of one predictor variables, significant variables are marked in bold.

Table 2. Results of a multivariate logistic regression model (enter method) to predict stunting

Variables	B	S.E.	Wald	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
						Lower	Upper
Numbers of younger brothers	0.69	0.34	4.13	0.04	1.99	1.02	3.84
Fathers education	0.11	0.47	0.05	0.82	1.11	0.45	2.78
Mothers education	-0.19	0.46	0.17	0.68	0.83	0.33	2.04
Mothers occupation	0.66	0.41	2.56	0.11	1.93	0.86	4.31
Number of family members	0.82	0.35	5.54	0.01	2.28	1.14	4.52
Numbers of living rooms	0.73	0.6	1.5	0.22	2.09	0.64	6.76
Sanitary system	0.1	0.46	0.04	0.84	1.1	0.44	2.72
Income	0.52	0.58	0.81	0.37	1.69	0.54	5.26
Expenditure	0.07	0.58	0.02	0.90	1.08	0.34	3.37
Birth weight	2.25	0.33	46.49	0.00	9.48	4.97	18.04
Place of delivery	0.69	0.35	3.98	0.05	2.00	1.01	3.94

CI – confidence interval, significant variables are marked in bold.

Discussion

By lowering productivity, diminishing learning capacity, and raising dangers to maternal and reproductive health, poor diet is exacerbating gender disparities and causes intergenerational cycles of hunger and inequality to persist. Stunting, cognitive deficits, decreased immunity, and a higher risk of illness and death are all more common among offspring born to undernourished mothers. However, the workload is disproportionately heavier on women as they make up approximately 40% of formal labour force around the world, despite being more likely than men to work as an unpaid household laborer or in an unorganized sector. Still, women continue to provide the majority of childcare and feeding practically everywhere (UNICEF 2019a). Lack of nutritional diversity, skipping meals, exposure to a contaminated environment and poor hygiene (Wolde et al. 2015) are all linked to an increased risk of stunting (Prendergast et al. 2014). Indian society has prioritized males over females in terms of education, occupation, and provision of better household and dietary habits. Generally, women eat meals after all family members have finished and skip meals daily (Rao et al. 2010; Mumtaz et al. 2019; UNICEF 2019b), which is a common scenario of dietary habits of Indian women. In West Bengal, there is a strong preference for sons. For instance, 10% of women and 19% of men have been reported to prefer sons over daughters (As per NFHS-5 reports). 86% of men and 81% of women desire to have at least one or two sons. The parental desire to have more children is strongly affected by their current number of living children, particularly sons (IIPS 2021).

The present study showed a significant association of stunting with parental education, mother's occupation, family income and expenditure when their effects were assessed separately. In contrast to their higher levels, lower parental income and educational levels were associated with a higher prevalence of stunting. The higher risk of stunting was found in the girls who were living with above four family members. When they had younger brothers, they were also to be stunted. The risk of stunting was significantly higher in girls living in up to two rooms and with no proper sanitary system. Previous studies on the same dataset showed that the degree of education of mothers of rural children and family economic circumstances in urban counterparts were the most significant independent factors of undernutrition among 3–12-year-old children (Khanra et al. 2020; 2021). However, these studies used a composite index of anthropometric failure (CIAF), but not stunting as the measure of undernourishment. A recent study on the same dataset revealed that low birth weight is significantly associated with the prevalence of stunting among boys (Khanra et al. 2022). Previous researchers have shown that various measures of socio-economic status, such as income, education and family assets were associated with nutritional status in children (Victora et al. 2008; Nguyen et al. 2013; Mohammed et al. 2014). There is plenty of evidence showing a positive association between low income and the prevalence of stunting (Keino et al. 2014; Mondal et al. 2015; Sharma and Katoch 2016; Pal et al. 2017; Kirk et al. 2018).

The results of our study show that children who live in homes with two or fewer rooms had a higher prevalence of stunting. Other studies (Owoaje et al. 2014), including those conducted in the same Indian state, have reported similar findings (Biswas et al. 2013). This would suggest that having better housing circumstances, such as a greater space, is associated with more income and, consequently, with a reduced prevalence of undernourishment. Poor living conditions, unclean living standards, unsanitary conditions, dangerous drinking water, and a low-calorie diet are all directly related to low monthly income (Rahaman et al. 2016).

Child nutrition could benefit from income, a key socio-economic factor, and household consumption decisions only depend on production outcomes through total wages; cash from any source will be helpful (Svedberg 2000; Kirk et al. 2018). Children's respiratory illnesses, asthma, and mental health are all correlated with their living situations (Krieger and Higgins 2002; Harker 2006; Oudin 2017). Previous research has found a strong correlation between the number of living rooms and children's undernutrition (Biswas et al. 2011; Biswas et al. 2013).

As mentioned above, the present study also revealed a strong link between stunting and having younger brothers. This could, however, be the result of relatively increased attention towards the younger children, especially boys, by the parents in a resource-constrained setting, particularly regarding food distribution and health care (Pande 2003). Indeed, previous studies in similar populations in the same Indian state showed that a higher risk of stunting was associated with the presence of younger brothers (Biswas et al. 2013; Mondal et al. 2015). Large families (above four members) significantly impacted the prevalence of stunting. The risk of stunting was higher in girls in the category of maternal age at childbirth and mothers who have started childbearing at the age of below 20 years (ORs = 1.66). NFHS- 5 (2019–2020) has reported that among young women aged 15–19 in West Bengal, 16% have already begun childbearing. The proportion of women who had started childbearing is much higher among young women (33%) who had no schooling (IIPS 2021). A previous study reported that low level of education among mothers is significantly associated with higher prevalence of undernutrition (Khanra et al. 2020). A higher risk of stunting was found in the category of low-height mothers and nutritionally undernourished mothers. Gender discrimination regarding food distribution, health care and other facilities in the household are a major problem in society. The psychosocial mind and beliefs (male preference) are solely responsible for this unequal food distribution during food distribution among family members (Ahmed 2018). The present study shows that the risk of stunting was higher among children with low weight at birth. In this study, the direction of the relationship between birth weight and childhood undernutrition was in line with the results of other studies showing that low birth weight had a significantly higher risk of stunting in childhood (Rahman et al. 2016; Ntenda and Chuang 2017; Khanra et al. 2022). The present study also shows that place of delivery was a significant predictor of stunting, as reported in another study in Malawi (Chirande et al. 2015).

However, all the above associations faded out in the multiple regression analyses. In contrast, the number of younger brothers, number of family members, birth weight, and place of delivery showed a significant independent association with the prevalence of stunting, allowing for all other potential factors. Girls with younger brothers were more likely to be stunted compared to those with no younger brothers. Girls who had a large family (above four members) were more likely to be stunted compared to those who belonged to a small-sized family (up to four members). Several previous studies have reported that the presence of younger brothers and large family size is associated with a poorer child nutrition (Biswas et al. 2013; Zelellw and Gebreigziabher 2014; Mondal et al. 2015).

According to the patriarchal system, the practice of favouring sons over daughters in India is becoming more common (Marcoux 2002). A family without a boy is seen as inadequate and embarrassing in public. Daughters, in contrast, are viewed as a responsibility and it is commonly regarded that it is a waste of time and money to raise girls. After paying the obligatory dowry and entering her new family, a girl will leave her parents in a precarious financial situation. Daughters are consequently equivalent to low-yield investments (Basu et al. 2018).

Since the beginning, our culture has given more preference to sons over daughters in all spheres, including healthcare and household food distribution. During infancy there are established gender variations in nutritional status along with prejudiced nursing and supplementing patterns (Pande 2003). Unlike boys, infant girls are breastfed less frequently and for shorter periods of time (Barcellos et al. 2014). No matter how wealthy or how poor the household is, girls tend to consume less nutritious food compared to boys (Marcoux 2002). Gender discrimination regarding food distribution in the household is a major problem that prevails in society. Psychosocial causes for this unequal food distribution are commonly related to the mind and beliefs of society about male preference while giving food to family members (Basu et al. 2018; Ahmed 2018; Mumtaz et al. 2019). Lack of education and awareness might be a cause of a greater trend of offering good and nutritious food preferentially to males while female members are considered second priority and, thus, insufficient and inadequate meals (Alam 2012). Moreover, with greater awareness and educational improvement observed in recent years, people all over the world have become more rational. However, the discrimination problem still exists particularly in the lower socio-economic community (Alesina et al. 2013; Berti 2012).

This study shows that girls with lower birth weight showed higher chances of being stunted at 3–12 years. Girls born at home were more likely to be stunted compared to those born at health institutions. Institutional childbirth is defined as childbirth that occurs in a hospital setting that is technologically advanced and is also supervised by trained birth attendants. Institutional childbirth is one of the most effective strategies for lowering mother and neonatal morbidity and mortality. In institutional deliveries, various medical devices and technology are employed to ensure the newborns' health (WHO 2018; UNICEF 2019a). A recent survey has reported that the percentage of births in a health facility increased in the four years, from 75% (NFHS -4) to 92% (NFHS- 5) although 8% of births still occur at home. Children who were delivered at home did not receive any vaccine. A recent study reported that stunting was frequently observed in under-vaccinated children in four countries (Solis-Soto et al. 2020). Children who were delivered at the hospital receive superior care for both the mother and the child's postpartum complications. Future maternal and fetal health may benefit from this. India alone supplied 40% of the world's LBW (Low Birth Weight) population, with an estimated 33% of all babies weighing less than 2,500 grams at birth (Jain and Singhal 2012). The prevalence of low birth weight in India was 21.4% in 2017 (ICMR 2019). As per NFHS-3, the prevalence of LBW in West Bengal was 22% (IIPS 2008). The WHO has set a goal of reducing LBW and stunted children aged 5 years by 40% between 2010 and 2025 (WHO 2014). Many different factors contribute to LBW. It is dependent on intricate interactions between a number of variables, including those related to genetics, reproduction, socio-demographics, culture, politics, and the immediate physical environment (Aries et al. 2012) and regional factors (Mazengia and Biks 2018; Titaley et al. 2019; Pal et al. 2019). The aetiology of LBW is maximally related to maternal risk factors (Dasgupta and Basu 2011; Golestan et al. 2011; Mumbare et al. 2011; Demelash et al. 2015) and socio-economic and psychological factors (Agarwal et al. 2012; Meshram et al. 2016). A recent study conducted in India found that women suffering from anemia are at higher risk of poor birth outcomes, such as preterm birth and low birth weight due to weak intrauterine growth (Sunuwar et al. 2020). A study has documented that half of the expectant mothers, children, and adolescent girls in India suffer from anemia (Singh et al. 2018). Teenage girls are more vulnerable to stunting due to early marriage and pregnancy and are more likely to deliver underweight babies. NFHS-5 has reported that 6.8% of teenage girls were pregnant in India. That is a very vulnerable condition for future generations (IIPS 2021). At least half of the burden of anemia is due to iron deficiency, and both folic acid and iron deficiency during pregnancy are important factors for preterm delivery, anemia, low birth weight, and increased stunting among children (Shah 2016; Halli et al. 2022). Indeed, stunting has long-lasting effects on future generations. In addition, a high occurrence of anemia among women causes them to enter pregnancy in an anemic stage, which has well-documented adverse effects on fetal growth, birth weight and the mother's health (Sheila and Shoba 2021). These newborns' malnutrition and poor health tend to get worse after birth due to not getting enough nutrients, especially in the first 24 months of life. According to a recent study, about 68% of under-five child mortality in India is linked to malnutrition (Swaminathan et al. 2019). Stunting is generally regarded as an expression of chronic deprivation from nutritional requirements at the population level. According to our study, there is a link between low birth weight and childhood undernutrition that is in line with findings reported in other studies (Rahman et al. 2016; Ntenda and Chuang 2017; Khanra et al. 2022).

As many studies involving natural human populations, the present study also had some inherent limitations. For instance, it failed to reveal the sex-difference in the effects of living conditions and socioeconomic status on the nutritional status, as it included only female children. It also did not measure nutrient intake patterns or energy storage in terms of body fat deposition, such as skin fold measurements. Moreover, data were collected from parents of Bengalee girls aged between 3 and 12 year old. A limited number of girls at the upper end of the age group plausibly experienced menarche. The growth pattern of those pubertal girls could have been different from those who did not have their menarche yet during the study. However, the present study did not take into consideration this biological phenomenon. The WHO also does not provide separate reference values for pre- and post-menarcheal children.

Conclusion

The present study suggests that having a large number of family members and younger brothers is among high risk for being stunted among the girls between 3 and 12 years of age. Similarly, the study shows that the risk of stunting is significantly higher in children who were delivered at home and had low weight at birth. If the Indian government is to fulfil the World Health Organization's goal of reducing stunting by 40% by 2025, these primary risk factors for stunting among the children in India must be addressed. The present study emphasizes the necessity of increasing community involvement in various developmental programmes to eradicate poverty and raise female literacy rates. To enhance the nutritional status of mothers and children, particularly girls, it is necessary to strengthen health and nutrition education. More micro-level research should be undertaken to broaden our knowledge regarding the relative role of socioeconomic and demographic factors in determining the prevalence and magnitude of undernutrition among Indian children. This would enable a proper implementation of development projects aiming at eradication of undernutrition and achievement of the targeted outcome.

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Conflict of interests

The Authors declare no conflicts of interests.

Authors' contributions

PK collected and analyzed the data and prepared the draft manuscript. KB designed and supervised the study, analyzed the data and provided intellectual inputs to the manuscript. RC designed the study and prepared the final manuscript.

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POLSKIE TOWARZYSTWO ANTROPOLOGICZNE

A new perspectives on breastfeeding practice reconstruction in bioarchaeology – an oxygen isotopes study in an animal model

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Abstract: Research using stable isotopes for the reconstruction of breastfeeding strategies are based on assumptions that have not yet been verified by experimental studies. Interpreting the results of isotope analysis is associated with a certain degree of uncertainty, mainly due to the lack of information on how isotopes are distributed in mothers, breast-fed and weaned offspring. Culinary practices also can affect the interpretation of isotope results.

Considering positive correlation between oxygen isotope composition of drinking water and bone phosphates, experimental studies were carried out using rats as an animal model.

The experiment showed that apatites of breast-fed offspring were enriched 1.6‰ in comparison to the values observed in their mothers. In the boiled water model, the difference was 1.8‰. On the basis of the animal model, it was estimated that the difference in ^{18}O between mother and child in the human species may amount to approximately 2.7‰, and long-term intake of boiled liquid food and beverages will not compensate the difference.

The experiment allowed observation of the effect of changes in isotope ratios to a change in trophic levels during breastfeeding and weaning, as well as the additional effect associated with the consumption of isotope enriched water during thermal treatment.

Keywords: oxygen isotopes, breastfeeding, weaning of ancient population, animal model, bioarchaeology.

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Introduction

Within the last three decades, breastfeeding and infant feeding strategies have become a one of the most important fields in anthropology, archaeology, evolutionary biology, and historical demography (e.g., Burt 2013; Britton et al. 2015; Tsutaya and Yoneda 2015; Cienkosz-Stepańczak et al. 2017; Crowder et al. 2019; Miller et al. 2020; Stantis et al. 2020; Loponte and Mazza 2021; Tomczyk et al. 2021; Chinique de Armas et al. 2022). Based on the connection between breastfeeding and signs of skeletal stress, weaning is assumed to exert an impact on individuals' health later in their lives as well as population dynamics (King et al. 2018). This type of studies allows us to verify the hypotheses on the effect of parental investment (Kwok et al. 2018) and the structure of a group or family (Scharlotta et al. 2018).

Studying breastfeeding and weaning practices (BWPs) helps us understand how varied breastfeeding strategies were over the centuries and by how many factors (both biological and cultural) they were shaped. Cultural factors which are held to be the most critical socio-economic determinants of breastfeeding, supplementation or weaning practices include religious and social beliefs, type of economy and related food strategies described as subsistence economy (WHO 1998; Dettwyler 2004; Tsutaya and Yoneda 2015; Chinique de Armas and Pestle 2018). The last of the aforementioned factors is of particular interest to bioarchaeologists. Our research helped us determine that human groups dominated by specialised craftsmen, and traders were ones in which children were fed with mother's milk longer and that breastfed children's tombs were better equipped (Scharlotta et al. 2018). For example, the weaning process started earlier in hunter-fisher-gatherer groups than in agricultural and horticultural populations (Chinique de Armas and Pestle 2018). However, variations in breastfeeding duration are also observed within agricultural communities, which may stem from the type/species of plants grown and the source of protein consumed by the group (marine versus terrestial protein) (Chinique de Armas and Pestle 2018). It appears that the variability concerns not only the duration of the breastfeeding period but also the type of food fed to children: variation in diets immediately post-weaning, with increasing homogeneity in diet thereafter (Eerkens 2018). In this context, the availability of cultigens (for example *zea mays*) is not insignificant (Chinique de Armas and Pestle 2018; Schurr 2018). Going forward, factors influencing BWPs are also traced in studies involving diachronic comparisons of weaning age in the same populations. These studies demonstrate that reformation changes and growing urban environments may lead to changes in breastfeeding strategies (Britton et al. 2018). Moreover, research in context of the length of mother's milk feeding includes cases of children buried in unusual ways. Researchers highlight that funeral rituals did not always result from the young age of the individual at the time of death, but it would be related to the loss of mother during birth or the first few days after birth (Craig-Atkins et al. 2018).

In conclusion, assessment of diet provides a valuable supplement to studies on origin and mobility. Of interest is the analysis of dietary practices pertaining to children after birth, including breastfeeding and weaning process.

This subject is seen as important from the perspective of a child's physical and psychological development, parental investment in offspring, and a reproductive strategies of a population (Lee 1996; Tsutaya and Yoneda 2015). Therefore, findings concerning breast-fed infants in archaeological populations may improve our current knowledge, not only in the cultural attitudes towards nutritional choices, but also women's and children's health, birth spacing, and the social and demographic structure of the analysed population.

Our knowledge about the diet of adults and children has significantly expanded thanks to the research on stable isotopes of carbon, nitrogen and oxygen. Stable isotope ratios of nitrogen and carbon in bone and dentine collagen have been used for over three decades to estimate palaeodiet, life strategies or breastfeeding duration of our ancestors (Szostek 2009; Arnay-De-La-Rosa et al. 2010; Schwarcz and Schoeninger 2012; McClure et al. 2020; Tomczyk et al. 2020a; Tomczyk et al. 2020b; Tomczyk et al. 2021).

In the studies of BWPs the most commonly used are nitrogen isotopes which reflect the general trophic level of consumed foods (Schoeninger 1985; Britton et al. 2018). Research performed on the hair of new-born infants and their mothers revealed no significant differences in isotope delta between the woman and her child (Fuller et al. 2006; De Luca et al. 2012; Dailey-Chwalibóg et al. 2020). In infants breast-fed over the first weeks of life, $\delta^{15}N$ values in hair keratin rise until a 2–3‰ difference is reached, relative to the value of the parameter in their mother's hair (Fogel et al. 1989). This difference in isotope proportions maintained until the first non-maternal food is introduced in the child's diet. When weaning begins, the child starts consuming protein sources other than mother's milk, the isotope delta of nitrogen gradually decreases and eventually fall in line with $\delta^{15}N$ of the mother and other adults from that population (Katzenberg 1996; Lewis 2007). The improvement of the method is the Weaning Age Reconstruction with Nitrogen isotope analysis (WARN) model for analyzing cross-sectional $\delta^{15}N$ data of subadult bone collagen, which is used for reconstructing precise weaning ages (Tsutaya and Yoneda 2013).

Longitudinal analysis of stable carbon isotopes in the keratin of hair and nails of infants and their mothers show that during breastfeeding, keratin in infants is enriched with heavy carbon isotopes (^{13}C) and that the average difference between the offspring and their mother is c.a. 1‰ (Fuller et al. 2006). In other studies of proteins isolated from hair, the carbon isotope ratio differed between mothers and newborn infants by 0.9 ‰, and in the case of nitrogen the difference was 0.4 ‰ (De Luca et al. 2012). The process of carbon incorporation into bone occurs in balance with carbonates bound in the blood and such carbonates are products of nutrient metabolism. Authors concluded that $\delta^{13}C$ analysis allowed them to retrace the moment when solid food, with different isotopic proportions from mother's milk, was introduced into the diet, and what could be the proportion of C3 and C4 plants in the diet (e.g., Eerkens et al. 2018) whereas $\delta^{15}N$ values were the record of the duration of milk consumption (Fogel et al. 1989; Fuller et al. 2006; Tsutaya and Yoneda 2015; Cienkosz-Stepanczak et al. 2017; Stantis et al. 2020). In all of the cited studies, the isotope values of breastfed infants were higher than those of the mothers. However, despite the hair tests set a certain model of changes in isotope values between mother and offspring, it should be emphasized that short-term changes in $\delta^{15}N$ hair values may not reflect dietary shifts, but may be the result of the changes in an individual's metabolic balance owing to specific physiological events (D'Ortenzio et al. 2015).

Isotope analysis of the weaning process typically involves the comparison of isotope ratios in the tissues of children weaned at different ages and isotope levels in women of reproductive age. The start of the weaning process is defined as the moment in which isotope levels in the child drop and approaches those of the isotope levels in the mother. The weaning age is the moment when the isotope delta of those two groups equalize (Dupras & Tocheri 2007; Jay et al. 2008; Choy and Richards 2009). This approach was improved through the application of mathematical models proposed by Schurr (1997) and Tsutaya and Yoneda (2013). Nevertheless, it should be noted that the relationship between adult/child diet and carbon and nitrogen isotope delta has yet to be fully explored. C and N isotope ratios in collagen may be affected by a specific fragment of the skeleton (bone/tooth type and its section) characterised by different bone turnover and remineralisation rate, and even short-lived individual physiological changes during bone growth, pregnancy, and breastfeeding, and, importantly, nutritional, and physiological stress, as well as diseases caused by diet modifications (Katzenberg 1999; Lewis 2007; Beaumont et al. 2015; Britton et al. 2018; Beaumont 2020). Interpretation of C and N isotope analysis results may also be hindered if children experienced non-standard breastfeeding or weaning practices (Tsutaya and Yoneda 2015).

Currently, isotopic proportions taken from microscopic enamel or dentine incremental layers is considered to be the most precise research tool for capturing the most likely moment of a change in the diet of a child from exclusive breastfeeding to complete weaning (Humphrey et al. 2004; Fuller et al. 2003 as cited in Lewis 2007). However, all methods have limitations, in this case there are also a few to mention.

In addition to analyses of carbon and nitrogen isotopes derived from collagen, which is more susceptible to diagenetic processes than enamel, analysis of the weaning process may also be performed using a method based on stable oxygen isotopes in a mineral fractions of enamel and dentin (Montgomery 2010; Budd et al. 2000; Dudás et al. 2016; Simpson et al. 2021). Wright and Schwarcz (1998; 1999) made an attempt at using apatite from the teeth of analysed individuals, which currently seems the optimum solution to the issue of reconstructing the weaning age based on skeletal minerals. This solution uses knowledge of the duration and sequence of mineralization in each tissue (enamel, dentine) for various types of teeth. In practice, researchers compare the isotope composition of enamel or dentine for various types of teeth in an individual (Wright and Schwarcz 1998; Wright and Schwarcz 1999; White et al. 2004b) or isotope levels in a child's teeth with corresponding values obtained for the teeth of women of reproductive age (White et al. 2004b). However, in many studies it is impossible to carry out the reconstruction of weaning based on odontological material because of limitations such as the absence of odontological material in the uncovered skeleton, insufficient amount of teeth to conduct isotope analyses, the presence of significant carious lesions, or no possibility of sacrificing teeth for isotope analysis (irreversible damage to the tooth's structure in the preparation procedure).

With time, around 2000, weaning studies started analysing relationships between nitrogen and oxygen isotopes. It is assumed that stable isotopes of both elements reflect changes in breastfeeding but involve different physiological mechanisms (Britton et al. 2018). While nitrogen isotope methodology is based on protein metabolism, oxygen isotope ratios are indicative of isotope fractionation in body water. Body water is characterised by a higher oxygen isotope level than environmental water due to fractionation in metabolic processes (Luz et al. 1985; Bryant and Froelich 1995). On the basis of previous studies on archaeological dental material, it may be concluded that children not fed with mother's milk have lower isotope proportions compared to breastfed children, similarly to weaned children, because after weaning, when their bodies start absorbing water from food (oxygen source) rather than water contained in milk, there should be a drop in their $\delta^{18}O$ level (Wright and Schwarcz 1999; Lewis 2007).

Stable oxygen isotope analyses were first applied in research on the paleoclimate (Cormie et al. 1994; Iacumin et al. 1996; Tütken et al. 2006) and are currently used in bioarchaeology to investigate multiple aspects of life in ancient populations (e.g., Evans et al. 2006; Lee-Thorp and Sponheimer 2006; Knudson and Torres-Rouff 2009; Price et al. 2010, 2019; Perry et al. 2020). Reconstruction of the place of origin and movement of populations may help retrace the dynamics of mobility, possible exchange of commodities, contacts, and cultural diffusion of human groups. Isotope analyses are commonly conducted in the context of origin and migration and concern an increasing number of diverse archaeological sites (e.g., Dupras and Schwarcz 2001; Depaermentier et al. 2020; Leach et al. 2010; Price et al. 2010; Lisowska-Gaczorek et al. 2016; Harris et at. 2017; Osipowicz et al. 2017; Turner 2021).

Isotopic approaches such as bone phosphate oxygen may be useful in confirmation and further characterizing trends observed in an analysis of bulk bone collagen or/and enamel/dentin incremental layers. This subject requires further analysis, and the potential for application appears significant in certain cases, e.g., if collagen is poorly preserved and has undergone extensive diagenesis. Bone mineral, particularly enamel, is the tissue most resistant to diagenetic processes (Montgomery 2010; Cienkosz-Stepańczak et al. 2021).

The selection of research method/technique depends on the availability of the material and the condition in which it is preserved. When collecting samples from bones, areas displaying signs of lesions or fractures are omitted. In addition, an analysis of diagenesis is performed. The condition of odontological material is assessed for the extent of diagenetic alterations, the presence of fractures and noticeable *intra vitam* tooth degradation (crown wear, dental caries). In the context of diagenesis, of high significance are tissue properties such as collagen content, histological integrity, porosity (water absorption potential), crystallinity (Hedges 2002), and on the other hand, the presence and extent of the effect of specific chemical factors in the grave environment, exerting a degrading influence on skeletal material (diagenetic factors) (Kendall et al. 2018).

In general, dental tissues are better preserved *post mortem* due to the resistance of enamel and a less porous structure than in bones. However, certain exceptions still apply (Hollund et al. 2015).

Similarly to collagen, bone hydroxyapatite is exposed to diagenetic factors but less susceptible to its effects. One of the reasons for its increased resistance to diagenesis is the fact that bone mineral is protected in the initial stages of decay by collagen. A substantial collagen loss in bones is correlated with microbial activity (Hedges 2002). It should be emphasised that tooth enamel has larger, neatly ordered bioapatite crystallites, which contain less carbonate and more fluorine than bones (Wopenka and Pasteris 2005). In addition, enamel has lower organic content (<1% by volume), as opposed to bone (32–44% by volume) (Olszta et al. 2007), and the presence of organic compounds plays a key role in diagenesis.

For teeth, larger crystallites, lower carbonate proportions, low collagen content, and the presence of F ions in *in vivo* enamel results in a more thermodynamically resistant material in comparison to bone (e.g., Wopenka and Pasteris 2005). In the event of insufficient odontological material and/or diagenesis of a part of the dental tissue, the solution is to choose the bone component which is more stable in the harsh environment of the tomb. In the light of the above juxtaposition of tooth and bone tissues, we decided that phosphates rather than collagen should be the subject of our study.

Oxygen isotopes in bone and tooth apatites as a source of information on the weaning process

Due to the isotope fractionation process, mother's body water and produced milk may contain increased oxygen isotope ratios compared to local water drunk by the mother (Wright and Schwarcz 1998). The research by Kornexl et al. (1997) shows that the oxygen isotope of cow's milk was on average about 2–6 ‰ higher compared to drinking water. The experiment of Lin et al. (2003) showed that cow's milk was on average about 4‰ heavier isotope than water, while in analogous studies by Camin and colleagues (2008) found a similar regularity of about 2 ‰. The child's total water intake includes water from the oxidation of human milk proteins, lipids and carbohydrates, and water from sources other than human milk. Exclusive breastfeeding means that the infant receives human milk without any additional food or drink, not even water. For an exclusively breastfed infant, the mother's milk is the main source of oxygen (the proportion of oxygen in the bone tissue from the atmospheric air is insignificant). Due to the fractionation between the oxygen contained in milk and body water, the breastfed offspring builds into its tissues a pool of oxygen, the isotope composition of which is potentially even more enriched in heavy isotopes of oxygen ^{18}O (Figure 1) (Wright and Schwarcz 1998). We may expect that tissues mineralized during this period (bones or teeth) will display this kind of enrichment in heavier isotopes (^{18}O) until the start of the weaning process. If mother's milk is excluded from the diet, local drinking water becomes the main source of oxygen and $\delta^{18}O$ level in tissues of a young individual should drop to the level of the mother's isotope delta (Figure 1). It should be noted that after weaning, the isotopic values of nitrogen and carbon in a child may differ from that of the mother due to eating foods with higher or lower trophic levels, however, after weaning, the children were fed only porridges and plant food, for example, in other populations animal milk (e.g., Dupras et al. 2001). Weaning studies using hydrogen isotopes show that both the cooking water and the partially-soluble barley fraction become elevated in ^{2}H as a result of cooking (Ryan et al. 2020). It seems that in the case of oxygen isotopes, the isotopic proportions in sceletel tissues of children may also differ after complete weaning from mother's milk, for example, due to thermal processing of food or consumption of animal milk.

However, it should be emphasized that despite the noticeable tendency for oxygen isotope ratios to increase in the teeth of individuals who were probably breast-fed, the exact course of this phenomenon could not be retraced, since it is not possible to perform relevant research on living individuals (the necessity to collect bone fragments and teeth from an individual at several stages of ontogenetic development). Researchers acknowledged the problem and it was presumably for this reason that relatively few of them decided to study breastfeeding practices and determine the weaning age using stable oxygen isotopes, although stressing that such an approach has considerable potential (Britton et al. 2015).

Research using tissues collected from animals in a controlled experiment could be a solution to this problem. This type of experimental study allows us to avoid the so-called "osteological paradox", as well as additional variation resulting from the introduction of water with diverse isotope content into the body or the effect of taphonomic factors on the skeleton or the effect of previous illnesses on isotope levels measured in bones.

Another important aspect of oxygen isotope analysis that may pertain to reconstruction of the breastfeeding and weaning process is the influence of culinary practices on the isotope composition of beverages and liquid food on mineralizing bone tissue. Isotope ratios in consumed beverages may diverge from "local" ones due to boiling, brewing, or stewing of meals, which has only recently been explored in experimental studies (Brettell et al. 2012; Royer et al. 2017; Lisowska-Gaczorek et al. 2020). The literature emphasizes that culinary practices involving thermal treatment of water may lead to a substantial depletion of the isotope pool of 16O in body water, which is reflected in isotope ratios of bone tissue (Daux et al. 2008; Brettell et al. 2012) and increased oxygen isotope ratios in the body. Apparently, drinking water that has been thermally processed for an extended period (2.5 h) increases its isotope value by 6.1 ‰; an increase in isotope delta of phosphatic apatites by approx. 4 ‰ was reported in animals drinking isotopically enriched water (Lisowska-Gaczorek et al. 2020).

To date, however, it has not been investigated whether drinking mother's milk (a source of oxygen enriched by isotope fractionation in the metabolic process of mother's milk production) influences oxygen isotope levels in bone tissue of offspring, and consequently, the interpretation of results of such studies.

Considering that the effect of changes in oxygen isotope level in bones related to a change in the trophic level and during weaning has not been investigated, this study is an attempt to evaluate the influence of the replacement of mother's milk with another source of oxygen on the isotope ratios of weaned individuals.

The goal of this work was to ascertain whether thermal processing of potable water is related to a change in the oxygen isotope ratio in breast-fed and weaned individuals. Additionally, the isotopic effect of the change in trophic level of the boiled water model was analysed. It should be noted that due to the analysis of fresh teeth and bones, diagenesis does not affect the isotopic proportions obtained in this study and does not affect the interpretation in any way.

Our hypotheses concern primarily the isotope effect related to a change in the trophic level. We assume that isotope fractionation in a physiological process like milk production leads to a higher oxygen isotope level in the tissues of breastfed individuals than in their mother's skeletons. Besides, weaning and the introduction of another source of oxygen isotopes to its diet means a change in the trophic level of the young organism. Our experiment, carried out on livestock in uniform conditions, may allow us to determine if and to what extent extra-dietary factors influence oxygen isotope variation.

Material and methods

Analyses were performed on an animal model of the Wistar Cmd:(WI)WU rat strain. Since rodents, such as rats and mice, are used in model studies on human metabolism, genetics, and physiology, including skeleton development and mineralization (Smith and Warshawsky 1975; Jheon et al. 2013), we decided to use this model in the present work. All procedures involving animals were approved by the Ethical Committee of The Jagiellonian University in Krakow, Poland (no. 122/2011) in accordance with international standards.

A total of 8 females, divided into two groups, were used in the experiment. The number of animals that were used in the experiment resulted from applying good practices in animal studies. The first group drank tap water from the water supply network, while the second group drank boiled water. Tap water was collected from the network over the period for which the rats were kept, i.e., less than 3 months to exclude seasonal variation in the isotope levels in environmental water. The method of preparing boiled water has been described in detail in the publication on the first part of the experiment (Lisowska-Gaczorek et al. 2020). Rats were kept at a stable humidity (55%), lighting (artificial light 60lx, 12 h/12 h), temperature (22°C), and with *ad libitum* availability of feed and water. The only differentiating factor was the type of consumed water. Pregnant females (8 rats), who were already drinking water appropriate for their group three weeks prior to mating, gave birth to offspring that were subsequently placed in cages for female rats and divided according to the type of water provided. The group of offspring of the mother drinking tap water included 19 rats, while the group of offspring of the mother drinking thermally processed water included 33 rats. All young offspring had access to mother's milk until the 21st day of life, with a few of them fed in this way several days longer. After this period, mothers and half of the offspring from each cage were put down. Instead of milk, the remaining rats went on to drink the same type of water as that given to their mothers, until the 69th day after weaning.

Afterwards, they were put down. Each mother was kept in a separate cage with its offspring, which enabled separate/individual analysis for each subgroup, with the exception of those subgroups in which the offspring did not survive.

Teeth and femurs were collected from each animal. The analysis took into account long bone apatite isotope ratios. Meanwhile, in a situation where isolating bone phosphates was not possible for technical reasons, we analysed teeth to supplement the material. Rat teeth mineralize and grow over the entire lifetime like bones, additionally high bone turnover increases the amount of tooth movement (Verna et al. 2003) therefore it was possible after checking isotope differences between the two types of tissue.

Material cleansed of soft tissues was dried, ground, and weighed into 0.4 g portions. Next, apatite phosphates were isolated from bones and teeth in accordance to the procedure designed by O'Neil and collaborators (1994) and as described by Vennemann (2002).

The isotope composition of oxygen in the extracted phosphates was determined at the Department of Radioisotopes, Institute of Physics-CSE, Silesian University of Technology, Gliwice, Poland using continuous-flow isotope ratio mass spectrometry. The results of isotope analysis are presented in delta notation $\delta^{18}O_{sample}=[(R_{sample}-R_{standard})/R_{standard}]*1000$; $R=[^{18}O]/[^{16}O]$. VSMOW was used as a reference for the NBS120C standard (Lécuyer et al. 1996). Pre-treatment of the NBS 120C samples was identical for all apatite samples. A NBS 120C standard sample was also analysed ($\delta^{18}O=21.7‰$).

The isotope ratio in rats is presented in delta notation ($\delta^{18}O$). In several places, the following markings were used: the subscript indicates the individual's age class: "f" – females/mothers, "y" – young breastfed offspring, and "o" – older weaned offspring. For example, $\delta^{18}O_o$ represents older offspring weaned off mother's milk.

Comparisons of isotope results in groups differing in the type of analysed tissue, as well as the age and type of consumed water were carried out using the Mann-Whitney test. The normality of distributions was tested with the Kolmogorov-Smirnov test. We assumed the significance level of $p < 0.05$.

Application of the experiment in bioarchaeological studies

One of the key outcomes of the present experiment, in addition to the isotope effect observed in the form of the change in the trophic level, was an attempt to study the dynamics of changes in ^{18}O level as a result of weaning in the human species. For this purpose, we employed regression equations describing the relationship between the isotope composition of drinking water and phosphates for rats (Luz and Kolodny 1985) and for humans (Daux et al. 2008; Equation 6).

In the tap water model, phosphate isotope values for rat females/mothers were converted to isotope levels for water (Equation A: $\delta^{18}O_p= 0.49x\delta^{18}O_w+ 17.88$ designed by Luz and Kolodny 1985) to determine the common baseline value for organisms that potentially drank the same water. Equation B: $\delta^{18}O_w= 1.54\ (\pm0.09)x\ \delta^{18}O_p - 33.72\ (\pm1.51)$ was employed to estimate isotope levels which we expect to be characteristic of individuals who drank water of a similar isotope composition to the water ingested by rats (Daux et al. 2008; Equation 6). In this way we estimated the isotope ratio for mothers/women whilst being able to link the data to the experiment. First, in order to estimate isotope proportions in children who drank milk from the aforementioned mothers, we attempted to determine the approximate value of the isotope ratio for milk consumed by the offspring. Based on earlier findings, according to which body fluids such as urine, milk, saliva, sweat, and blood have similar oxygen isotope ratios (Bryant and Froelich 1995), we applied a regression equation for the relationship between drinking water ($\delta^{18}O_{dw}$) and body water ($\delta^{18}O_{bw}$) isotope ratios – Equation A': $\delta^{18}O_{bw} = 0{,}5x\ \delta^{18}O_{dw}$ -0,68 ‰ (Valenzuela et al. 2021; Equation 3). Another assumption concerned young rats. Milk drank by rats (or, specifically, water in the milk) was the only potable source of oxygen. Hence, for small children, we assumed that $\delta^{18}O$ for milk equals $\delta^{18}O$ for drinking water. Then, as previously for women, we used Equation B (Luz and Kolodny 1985) to estimate the isotope ratio in children who consumed milk with the isotope proportion calculated in the previous step. In this way, we obtained estimated $\delta^{18}O_p$ values for breastfeeding mothers and children fed exclusively with milk.

Results

It was necessary to verify any statistically significant differences between isotope delta for oxygen collected rat tissues in each age class, considering the division into the tap water and boiled water group. The results of the Mann-Whitney test for each group indicated that the isotope composition of bones and teeth did not vary significantly in either group (Table 1).

Due to the absence of differences, when for a given animal it was not possible to isolate phosphates from bones but only from teeth, the results of isotope analyses were interpreted together, in order not to minimize the research group.

Oxygen isotope data provided the basis for subsequent stages of the presentation of results and are shown in Table 2.

Table 1. The results of the Mann-Whitney test comparing the oxygen isotopic values of bones and teeth in different age groups

Age	tap water group			boiled water group		
	N		p	N		p
	bone samples	teeth samples		bone samples	teeth samples	
Mothers	4	3	0.38	3	3	0.19
Breast-fed rats	10	10	0.34	16	16	0.98
Weaned rats	6	9	0.22	12	10	0.34

since the p-value is greater than or equal to 0.05, there is not a statistically significant difference between the medians at the 95.0% confidence level.

Table 2. The results of oxygen isotope analysis grouped according to age class and the type of water (tap/ boiled water)

	Age	Skeletal fragment	N	$\bar{x}$	S	V [%]	Me	min	max
Tap water	Females/ mothers*	bone	4	13.0	0.1	1.1	13.1	12.8	13.1
	Breast-fed rats about 3 weeks	bone	10	14.8	1.2	8.0	14.7	12.8	16.6
	Weaned rats about 3 months*	bone/tooth	9	14.2	1.0	7.0	14.2	12.6	16.0
Boiled water	Females/ mothers*	bone/tooth	4	16.9	1.9	11.7	16.6	15.1	19.2
	Breast-fed rats about 3 weeks	bone/tooth	20	18.4	1.1	5.9	18.4	16.0	19.9
	Weaned rats about 3 months*	bone/tooth	13	17.6	1.4	8.1	18.2	14.9	19.4

where; n - numbers
$\bar{x}$ - mean average
Me - median
s - standard deviation
min - the lowest value in the range
max - the highest value in the range
V - coefficient of variation
* Lisowska-Gaczorek et al. 2020

The theoretical model of the isotopic effect related to a change in the trophic level suggests observable changes in the isotope ratios of biological apatite as a response to the introduction of a different oxygen source into the body (Figure 2). Because of this, the oxygen isotope variation for apatite phosphates in females/ mothers ($\delta^{18}O_f$), young offspring aged up to approx. 3 weeks ($\delta^{18}O_y$), and older offspring ($\delta^{18}O_o$) was traced. The analysis was performed separately for the tap water and boiled water model groups. The results are presented in Table 2 and Figure 1. In both models, females who drank water throughout the experiment had the lowest isotope values. Meanwhile, the highest isotope delta values were observed in individuals fed with mother's milk and intermediate values for rats who were weaned and had not drunk mother's milk for 69 days.

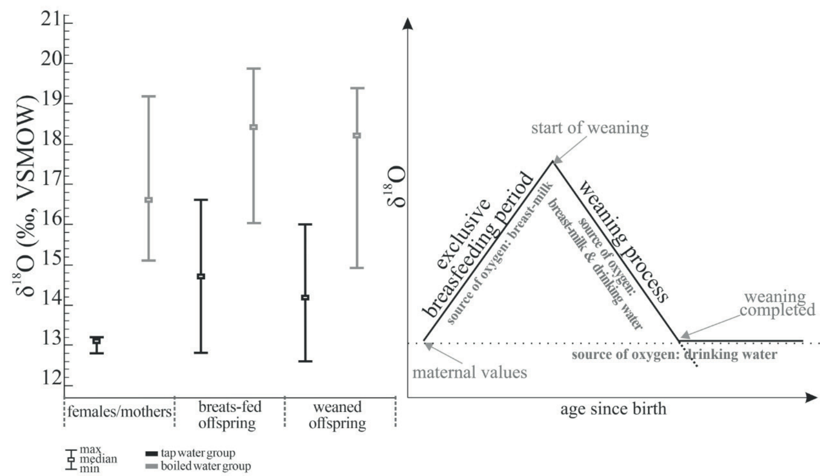

Fig. 1. Oxygen isotope diversity in apatite phosphates for mothers/breastfeeding females, children drinking mother's milk and weaned individuals in the tap water model (black line), boiled water model (grey line) in juxtaposition with the theoretical diagram, in which the dotted line stands for the mother's oxygen level and the solid line indicates the offspring's changing oxygen isotope level

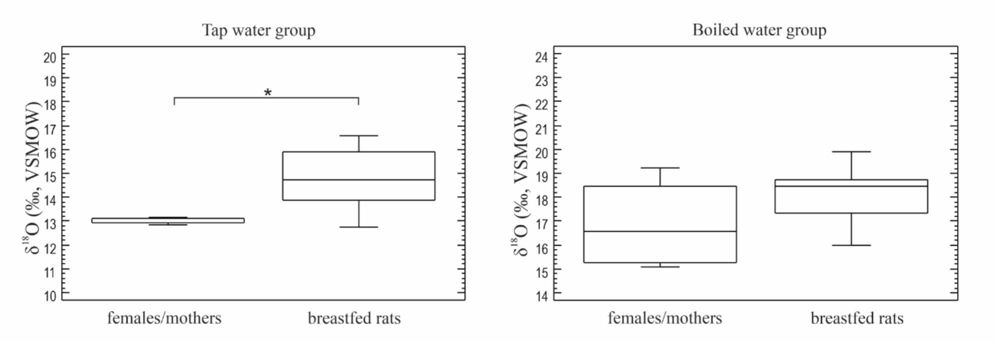

Fig. 2. Comparison of isotope values of mothers and milk-fed offspring by type of water consumed by female / mothers

To determine the effect of mother's milk intake on the isotope ratio of bone apatite, isotope data obtained for mothers were juxtaposed with isotope ratios for breast-fed offspring. In the group of animals with access to tap water, the isotope ratio for breast-fed individuals was 1.6‰ higher than for their mothers, while the stable oxygen isotope ratio for breast-fed young rats in the boiled water model was 1.8‰ greater than the same parameter for their mothers (Table 2, Figures 1 and 2).

A comparison of isotope values for mothers and their breastfed offspring using the Mann–Whitney nonparametric test shows that the difference between the medians in the group in which mothers drank tap water, is statistically significant ($W=36.0$; $p=0.0283$), whereas there is no statistically significant difference in the boiled water group ($W=56.0$; $p=0.2298$) (Figure 2).

After 69 days, which passed from the moment in which the offspring was denied any access to mother's milk, $\delta^{18}O$ values in both tap water and boiled water models dropped. In some of the offspring the isotope level became equal to that of mothers, while in others the effect of weaning manifested itself more slowly (Figure 1). In the tap water model, oxygen isotope delta for weaned individuals dropped 0.5‰ in comparison to breast-fed ones (median). In the boiled water model, the reported decrease in the isotope ratio was less noticeable and equalled 0.2‰.

The next step involved testing the dispersion of $\delta^{18}O$ values for females, offspring fed with their milk, and weaned offspring in each subgroup (e.g., female 1 vs offspring breastfed by female 1 vs completely weaned offspring of female 1). Table 3 and Figure 3 present an analysis of the variation in oxygen isotope proportions among the offspring of each female in the tap water model; Table 4 and Figure 4 show groups from the boiled water model. Groups from the tap water model are marked by the letter Z, while groups from the boiled water model are marked by the letter G.

Groups of breastfed young rats which were varied in terms of the type of water drank by their mothers had different isotope ratios. The 3.7‰ difference between median values for the two groups of breastfed rats (Table 2) referred to above was statistically significant, as confirmed by the results of the Mann-Whitney test ($W= 6.0$; $p<0.001$).

Table 3. Analysis of mothers and their offspring (tap water). Differences between group medians, minimum and maximum values from the ranges within each group are highlighted in the table

	N		breastfed vs females			weaned vs females			breastfed vs weaned		
	breastfed	weaned	Me	min	max	Me	min	max	Me	min	max
female Z1	2	3	1.7	1.6	1.7	1.3	1.3	1.6	0.4	0.3	0.1
female Z3	4	4	2.5	0.7	2.9	1.7	1.1	2.8	0.8	-0.3	0.1
female Z4	4	2	1.2	-0.4	3.4	-0.3	-0.5	-0.1	1.5	0.2	3.5

Z – a particular group of rats: a mother drinking tap water and her offspring
1,3,4 – individual group numbers

Table 4. Analysis of mothers and their offspring (boiled water). Differences between group medians, minimum and maximum values from the ranges within each group are highlighted in the table

	N		breastfed vs females			weaned vs females			breastfed vs weaned		
	breastfed	weaned	Me	min	Max	Me	min	max	Me	min	max
female G1	4	5	3.2	1.4	4.8	0.3	1.4	1.5	0.3	1.4	1.5
female G2	4	4	-0.3	-0.1	-3.2	2.2	1.1	0.7	2.2	1.1	0.7
female G3	5	0	1.7	0.7	3.1	-	-	-	-	-	-
female G4	5	4	0.9	-0.2	1.6	-0.2	0.7	-0.1	-0.2	0.7	-0.1

G – a particular group of rats: a mother drinking boiled water and her offspring
1–4 – individual group numbers

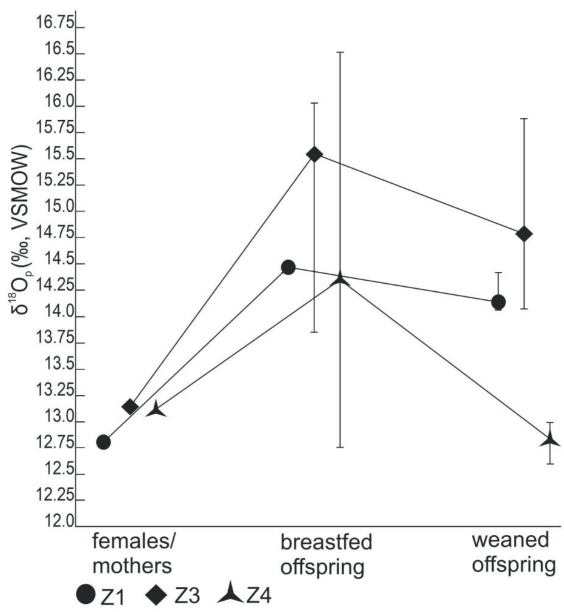

Fig. 3. Analysis of individual mother-offspring groups- tap water model. Z – a particular group of rats: a mother drinking tap water and her offspring; 1,3,4 – individual group numbers

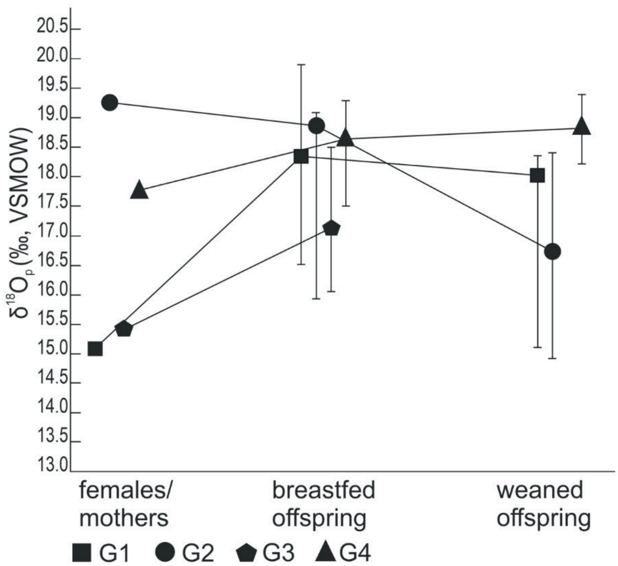

Fig. 4. Analysis of individual mother-offspring groups- boiled water model. G- a particular group of rats: a mother drinking boiled water and her offspring; 1–4 – individual group numbers

In addition, variation in the isotope effect caused by the change in the source of oxygen introduced in the rat's body at different development stages/ages was observed in both models (Table 3, 4, Figure 3, 4). In the tap water model (Table 3, Figure 3), the greatest difference between the isotope ratio in the mother's and her suckling's bone tissue was reported for female Z3 (Δ=2.5‰), and the smallest for female Z4 (Δ=1.2‰). In contrast, a comparison between isotope ratios for breastfed and weaned rats indicates that in group Z4 (Δ=1.5‰) the difference is the most prominent, whereas the smallest difference is present in group Z1 (Δ=0.4‰).

In the boiled water model (Table 4, Figure 4), the most salient difference in $\delta^{18}O$ for females and breastfed offspring was in group G1 (Δ=3.2‰), while in group G2 oxygen isotope level in the apatite from breastfed rats was slightly lower than in their mothers (Δ=-0.3‰). In G2, rats weaned off mother's milk were the quickest to lower their isotope ratio post-weaning (Δ=2.2‰) and for G4 the median for weaned rats was higher than one for breastfed individuals (Δ=-0.2‰). Note, however, that in the latter group no weaned individual attained an apatite isotope value significantly higher than the highest level of $\delta^{18}O$ reported for an individual breastfed by a mother from group G4.

Application of our models in bioarchaeological studies

Tap water

One of the aims of this study was to determine the way in which the results could be used in the context/interpretation of bioarchaeological research. In an attempt to establish a common denominator for humans and rats, the isotope ratio for apatite phosphates in female rats who had only drunk tap water ($\delta^{18}O_p$=13.1‰) (Table 2) was calculated based on Equation A, designed by Luz and Kolodny (1985), as the $\delta^{18}O_w$ value of potential drinking water. As a result, the value of -9.8‰ was obtained (Step I, Figure 5).

In order to verify what isotope ratio in bone apatites a human drinking water with a value of –9.8‰ would have, an equation proposed by Daux et al. (2008; Equation 6) was used, returning 15.6‰ as the result (Step II, Figure 5).

A further step was to establish (approximate) values of oxygen isotope ratio for a human child breastfed by a mother who is assumed to ingest water with an oxygen isotope ratio of -9.8‰. For humans, there is a commonly applied regression equation describing the relationship between oxygen isotope ratios in drinking water and body water. Water's isotope value (potential source of water for the population of the Krakow area) of -9.8‰ was converted to body water values (Step III, Figure 5). Assuming

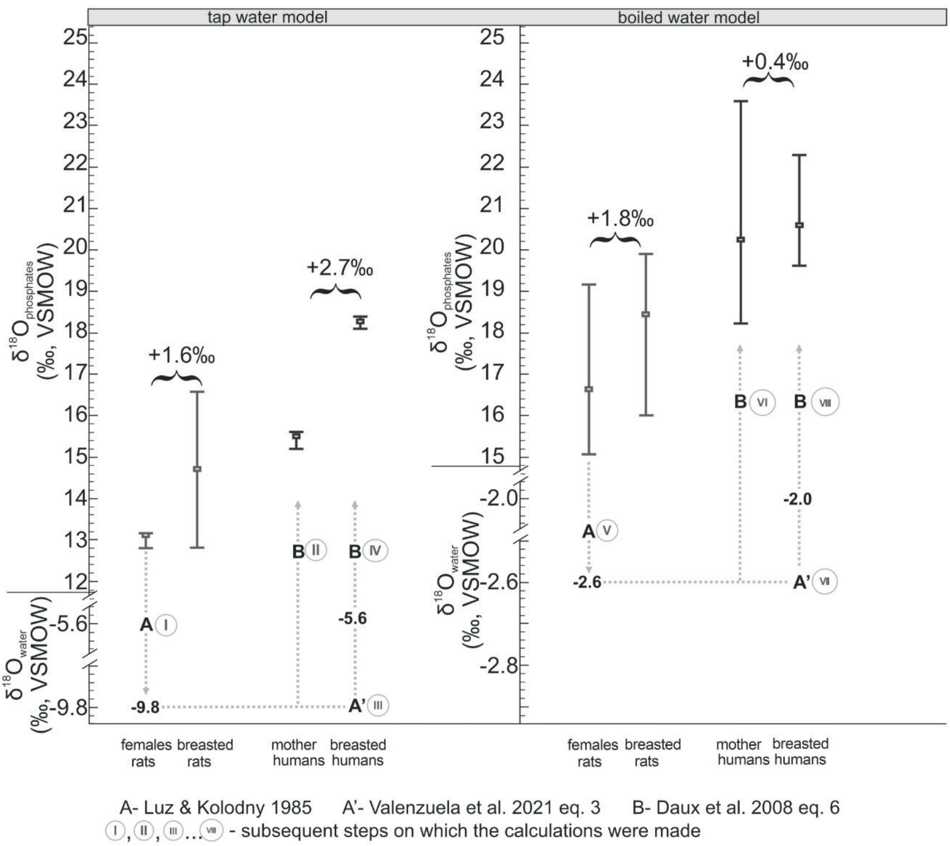

Fig. 5. Estimated differences in the isotope composition of oxygen in human bones, from mother to child, based on the results of the experiment and theoretical assumptions for the tap water and boiled water models. Roman numerals I–VIII were used to mark references to the text: steps according to which the conversions were made

that mother's milk contains isotopes in proportions similar to those of body water, whose $\delta^{18}O$ level was -5.6‰, we employed Equation B to calculate the value for bone phosphates expected in children fed from an oxygen source having the aforementioned isotope proportion (Step IV, Figure 5). It was estimated that children breastfed with mother's milk with an isotope value of approximately -5.6‰, resulting from the regression linking the drinking water isotope ratio ($\delta^{18}O_{dw}$) and the body water isotope ratio ($\delta^{18}O_{bw}$), could have an isotope value for bone phosphates equal to 18.3‰.

Boiled water

The same analysis was performed separately for the boiled water model (Figure 5). As in the model presented above, Equation A (Luz and Kolodny 1985) was used to verify the isotope ratio of the oxygen source ingested by females/mothers in the form of (boiled) water. The isotope ratio for water calculated by converting Equation A is -2.6‰ (Step V, Figure 5). Applying an equation developed by Daux et al. (2008; Equation 6) (Step VI, Figure 5), we were able to estimate the isotope ratio for bone phosphates in a (human) mother at 20.2‰, i.e., 4.6‰ higher than in a woman drinking water without any thermal processing. The value for female body water (and, by extension, also for milk) (Step VII, Figure 5) was calculated as approximately -2‰, whereas the isotope ratio for bone phosphates in breastfed children was 20.6‰ (Step VIII).

As shown in Table 2 and Figure 5, the difference between mothers and offspring in the case of tap water equalled 1.6‰, while for boiled water it was 1.8‰. For the estimated human ratios, $\delta^{18}O$ level in breast-fed children can be enriched by 2.7‰ and if their mothers ate long-boiled meals and drank long-boiled beverages, the difference was smaller and amounted to 0.4‰ (Figure 5).

Discussion

Biochemical tests on skeletal material are increasingly often applied in bioarchaeology since they may provide plenty of valuable knowledge on the lives of our ancestors. Yet, such tests are beset with certain limitations due to poorly preserved biogenic structure of the material as well as incomplete knowledge of the phenomena determining the quantitative and qualitative incorporation of dietary components into bone structure.

Providing a full explanation of many of those phenomena would require a long-term observation of isotope ratios in the bone tissue in contemporary individuals, taking into account multiple internal and external factors which would potentially affect those values. For this reason, based on biological knowledge, researchers take advantage of various model studies, including ones based on experimental research (Fogel et al. 1989; Fuller et al. 2003; IAEA 2010; Brettel et al. 2012; Herrscher et al. 2017; Tuross et al. 2017; Leichliter et al. 2021). One of them involves the creation of an animal model (e.g., Leichliter et al. 2021), as in the present study. As already mentioned, rats are very frequently used as model animals in studying multiple aspects of the functioning of the human body (Smith and Warshawsky 1975; Jheon et al. 2013).

In the first part of the analysis of the results it was found that the oxygen isotope composition for teeth and bones in the rat groups did not differ in a statistically significant way (Table 1), which is probably due to the fact that rat teeth are elodontic, i.e., they grow continually (which makes them chemically changeable/dynamic, like bones) (Verna et al. 2003). Since no differences were shown, in certain groups a fusion of bone and tooth samples was used, yet the test group size was not increased, as only one bone or tooth sample was collected from each individual.

Breastfeeding

The effect of isotope fractionation during lactation raises the question of the extent to which oxygen isotope ratios for apatites in mothers and children with access to mother's milk vary. As an extension of this subject, the present study verified if the mother's intake of isotopically heavier water due to the application of specific culinary practices influenced the intensity of the process.

The analysis of isotope ratios in mothers and their breast-fed offspring in both models (tap water and boiled water) demonstrated that apatites in offspring were enriched with heavy oxygen isotopes in comparison to the values observed in their mothers. In the group of animals with access to tap water, $\delta^{18}O$ values were 1.6‰ higher in breast-fed offspring than in females/mothers (median values), while for the group in which mothers drank boiled water the difference was 1.8‰. The different results from isotope fractionation, based on the assumption that mother's milk had a higher oxygen isotope ratios in comparison to drinking water, led to an increase in the oxygen isotope value in infant tissues. This is a consequence of the fact that body water is enriched in heavier isotopes of oxygen in relation to drinking water, as lighter isotopes of ^{16}O (Bryant and Froelich 1995) are excreted to a greater extent than heavier ones. Since milk is produced from water present in the body, it contains heavier isotopes than drinking water. Numerous studies have reported that isotope ratios of mother's milk are higher than $\delta^{18}O$ of water drank by mothers due to the presence of isotope fractionation in the physiological process of milk production. For example, in a study by Kornexl et al. (1997) the isotope ratio for cow milk was on average 2–6‰ higher than for drinking water made available to the animals. An experiment by Lin et al. (2003) showed that cow milk was on average 4% isotopically heavier than water, while in a study by Camin et al. (2008) a similar tendency corresponding to roughly 2‰ was observed. Until now, isotope analysis had not been performed for rodent milk. However, the findings of this study indicated that $\delta^{18}O_p$ in females/mothers and their offspring differed by approximately 1,6‰ and this difference was observed between maternal and offspring apatite. It is similar to differences observed in the literature between water and milk water (Kornexl et al. 1997).

An analysis of the isotope effect as a response to the change in the trophic level was reported both in the tap water model and the boiled water model, and its extent was similar (1.6‰ in the tap water model vs 1.8‰ in the boiled water model). Please note that for the former the difference is statistically significant, which is not the case with the latter. We may suspect that this is related to the fractionation factor (kinetic fractionation, which happens regardless of temperature) (Ustrzycka 2019) between mother's milk and child's body fluids. At birth, the isotope level for the offspring of a female who had drunk boiled water, due to the contact with mother's bodily fluids throughout the gestation, was significantly higher (3.7‰ relative to the medians) than the isotope level for individuals from the same age group but born from a mother in the tap water model (not enriched to such extent in ^{18}O). It seems that fluids/ tissues enriched in a heavy isotope will be characterized by a lower fractionation level, being already saturated with a less reactive, heavier oxygen isotope.

Accordingly, in spite of the proved larger difference in isotope level between mothers and breastfed offspring in the boiled water group, the difference is not statistically significant, since the intervals in the upper part of dispersion overlap with the values reported for females drinking boiled water.

A detailed analysis performed separately for each female and her offspring from two groups established based on water type (Figures 3 and 4) demonstrates relatively large variability in terms of the quantitative difference in the isotope delta for individual females and breastfed rats. The maximum difference between a mother and her offspring was 2.5‰ in the tap water model and 3.2‰ in the boiled water model. Meanwhile, some young rats had less significantly elevated values, e.g., in the tap water model by a mere 1.2‰, albeit in the boiled water model there was a particular case in which the value dropped by 0.3‰ relative to the mother's level. Studies on the teeth of various primates, including humans, also reveal a distinct fluctuation in the oxygen isotope ratio (~1-2‰) in the offspring over the period of several weeks following birth, whereas heightened ^{18}O values in tooth enamel in certain individuals were not observed altogether (Smith et al. 2022). Our study also reported cases in which the isotope ratio was lower in offspring than in their mother/female. This could be explained by the fact that several hours after birth the body of the newborn preferentially loses lighter oxygen isotopes (^{16}O) as it starts to breathe on its own, transpire water through skin as well as lose liquids; moreover, body mass also decreases in the first days following birth (DiTomasso and Paiva 2018; Smith et al. 2022). Depending on the intensity of the aforementioned processes, for several weeks immediately after birth certain young individuals may compensate for the perinatal isotope loses.

Weaning process

Weaning is a process whereby mother's milk is gradually replaced with food obtained from the environment (Dettwyler and Fishman 1992). The term "weaning age" signifies the moment in the life of a child when mother's milk has been eliminated from the child's diet (Williams et al. 2005). Stable isotopes of some elements (C, N, O) can detect the stages of the process. In the context of oxygen isotope analysis, we can consider weaning as a process of a gradual shift from the source of oxygen introduced into the body from the pool of oxygen present in mother's milk to oxygen contained in weaning foods and drinks (containing environmental water). Accordingly, weaning age is the moment in which mother's milk is entirely replaced by external water, which at the same time becomes the main source of oxygen incorporated in the individual's bone tissue. In the case of the oxygen stable isotopes, it has the power of detecting the start of the weaning process because typical weaning foods such as paps, cereals, soups or juices and many others are prepared using external/ environmental/ drinking water.

In the group of animals with access to tap water and boiled water, we observed a drop in isotope ratios between breastfed and completely weaned individuals (Figure 1). In this way, we observed the isotopic effect of oxygen related to the individual's trophic level. The drop in the isotope composition after the start of weaning was also observed in oxygen isotopic studies of teeth from individuals from ancient populations in which isotopic proportions in teeth that are formed and mineralized asynchronously were compared (Wright and Schwarcz 1999; White et al. 2004a).

To date, there have been two publications that used analysis of oxygen isotope composition in bones to reconstruct weaning in ancient populations. In 2015, Britton et al. conducted research (White et al. 2004a; Britton et al. 2015) that outlined the need to verify whether analysis of stable oxygen isotopes in bone apatite may be used for the reconstruction of weaning age. Researchers analysed $\delta^{18}O_p$ in the bones of children and adults from a mediaeval archaeological site in Wharram Percy (England). In these publications, in children whose age was estimated at 3 years, the oxygen isotope ratio was higher by 1‰ on average compared to the bones of adult individuals and lower than the isotope delta for bone apatites in younger individuals. Based on archaeological material, these studies reported a drop in the oxygen isotope ratio in older children, which in the light of the present experiment confirms the hypothesis that this observation was an effect of weaning. The authors concluded that isotope analysis of bone tissue may be used to determine weaning age, considering that the interpretation of results needs to allow for the application of culture-specific routines pertaining to thermal processing. The second issue, i.e., the effect of thermal processing, was also analysed in this study.

The intensity of the effect of changes in the trophic level in the group of animals with access to tap and boiled water were analysed to determine the influence of isotope fractionation due to drinking water heating on the rate of change in isotope ratios of bone tissue in weaned individuals. With regard to the difference between the median isotope ratio in mothers and older weaned offspring (69 days after the start of weaning), we noticed that in the boiled water model, the difference (calculated on the basis of data from Table 2) was 1.1‰, which is lower than that of the tap water model (1.6‰). The results indicated that weaned individuals are close to attaining their mother's isotope ratio, and hence, the level characteristic of the environmental water that they drank. This, however, does not mean that isotope proportions in tissues of rats that drank boiled water and were weaned, equalize more quickly with environmental water; an analysis of isotope ratios for breast-fed and weaned individuals showed that the difference in the tap water model was 0.5‰, while in the boiled water model it equaled 0.2‰ (Table 2). This means that the intake of thermally processed water after weaning did not affect oxygen isotope ratio in the offspring's bone tissue – although in both models the isotope effect related to transition to extra-maternal oxygen source was noticeable.

A detailed analysis of individual "female vs offspring" groups indicates that the isotope ratio in the tissues of rats whose apatite was slightly enriched in heavy isotopes during breastfeeding lowered more rapidly and intensively after weaning. In other words, the greater the disproportion in $\delta^{18}O_p$ between mothers and their breastfed offspring, the less pronounced weaning-related isotope effect, which was observed independently of the type of water drunk by the female and her offspring after weaning.

An analysis of the possible application of the model to human populations

In isotope studies concerning humans, there is no exact knowledge of the discrepancy between the isotope composition of the apatites of the mother and breastfed offspring.

So far, anthropological and bioarcheological studies have not suggested what would be the sufficient extent of the differences in the isotope composition of oxygen in bone apatites of mothers and children to determine that it is either caused by breastfeeding or indicates another phenomenon, e.g., related to variability across individuals. To date, no isotope relationship between $\delta^{18}O$ in mother's milk, her body fluids, and the newborn's tissue has been investigated. Little is yet known about the fractionation process of the physiological production of milk, the effect of hormones on isotope ratios in body fluids, and the tissues of individuals of various ages. Breastfeeding strategies and food preparation techniques in humans are so diverse that any generalisations of the phenomena of breastfeeding and weaning are impossible. The assumptions made in this study with regard to the model expected to translate the results of the experiment into information referring to humans was necessary due to the subject being insufficiently covered in research.

The literature offers tools, such as regression equations, describing the relationship between isotope composition of apatite phosphates and drinking water for various mammal species. Notably, values describing the isotope composition of bone phosphates are strongly correlated to the isotope ratio of drinking water, and although this relationship does not differ significantly, it is to a slight extent species-dependent; therefore, there are different regression equations for different animal species and humans (e.g., D'Angela and Longinelli 1990). Another key point in the context of the present study was the analysis of the relationship between the isotope composition of drinking and body water. It is commonly held that animal physiological water balance is reflected in the isotope signature of oxygen (Vander Zanden et al. 2016; Gregorčič et al. 2020). Oxygen isotope ratio in water contained in milk, as in environmental water, depends on temperature, humidity, and geographic coordinates of the location in which the animal lives, e.g., altitude above sea level (Abeni et al. 2015; Gregorčič et al. 2020).

The relationship between the isotope composition of oxygen in drinking water, body water, and bone phosphates relied upon in processing the results implies that human children fed only with the milk of their mother (who drinks thermally unprocessed water and eats thermally unprocessed food) may raise oxygen isotope proportions in their bones by approximately 2.7‰ (Figure 5). If the mother drinks only thermally processed liquids, the difference between the mother and her child, estimated for humans, may equal 0.4‰ (Figure 5). It appears that if the difference in oxygen delta between the mother and her offspring was smaller than in the tap water model, the fractionation had to "decelerate", i.e., it must have been less intensive in locations where the environment was saturated with heavy isotopes. This suggests that differences between isotope values of mothers and breast-fed offspring may vary due to the intake of thermally processed liquids. Estimated for the human species, a reduced isotope effect in breastfed offspring (an increase by 0.4‰) related to the consumption of thermally processed, heavy isotope food by the mother (which translates into milk with heavier isotopes) differs from the results obtained for rats (an increase by 1.8‰) and requires additional research.

Scientific research show that human and rat milk have a similar qualitative composition. Comparative chromatography of human, cow, rat, and pig milk proved that human and rat milk displayed the smallest differences (Roberts et al. 1954). In the same study, it was reported that apart from lactose, which was obviously present in the milk of all species concerned, glucose was detected only in humans and rats. It appears, however, that the discrepancies observed in the experiment and in the estimation for humans (tap water vs boiled water model) arise from different relationships between the isotope values for drinking and body water, including rat milk and human milk, but also from various other factors, such as physical activity (e.g., Bryant and Froelich 1985). The authors would like to stress that any attempts to use animal models for interpretation in bioarchaeological studies are always highly challenging and tainted by uncertainty. Therefore, the results of the analysis concerning the application of the results of the experiment to human population should be approached with considerable caution.

It is rather difficult to discuss the results in reference to literature because it is the first research of this type on fragments of fresh skeletons. Variability may only be evaluated by contrasting the resulting data with data available in literature on BWP studies carried out on other-archaeological skeletal material (Table 3).

The rise in oxygen isotope ratios in tissues of offspring in relation to adult individuals has been studied in many archaeological studies (e.g., Williams et al. 2005; Britton et al. 2015; Smith et al. 2022). In studies on archaeological material, disproportions in the isotope composition of oxygen between bone apatite in children and women or adult individuals varied. For example, in a study on the skeletons of adults and children under the age of 3 years, White et al. (2004a) reported a 2‰ difference in isotope delta. Research by Wright and Schwarcz (1998) involved a comparison of the value of isotope delta in the tissue of first molars that mineralize from birth to the age of 3, premolars that mineralize between the age of 2 and 6, and M3s that start to mineralize around age 9 (Wright and Schwarcz 1998). However, in this case, findings reported by researchers show that the difference between $\delta^{18}O$ for tooth phosphates at the age when children were breast-fed and the period when their diet was likely similar to the one typical of adults was only 0.6 to 0.7‰. Other studies performed on archaeological dental material revealed that the oxygen isotope delta for hydroxyapatite carbonate groups differed by 0.5‰ across deciduous and permanent dentition (Dupras and Tocheri 2007). Perhaps this is where we may observe the effect of boiling liquid food and beverages on the variation of isotope values between adult and child tissues. It seems that a reliable method of studying child diets is the method using incremental dentine collagen samples, which to a substantial extent allows us to avoid uncertainties related to changes in nitrogen isotope values due to, e.g., perinatal stress or the mother's non-standard diet. However, even this method is not perfect. Research on deciduous teeth in contemporary populations of children from Bradford (UK) proved that the dentine of children fed with modified milk until 9 months of age did not reveal any nitrogen isotope variability, whereas breastfed children had a wider range of nitrogen isotope values, reflecting their mothers' diverse diet. In addition, some children breastfed until 6 months of age still showed increased nitrogen isotope values after the introduction of supplementary food (Beaumont 2020).

Table 5 contains data from available studies that analysed the weaning and breastfeeding process in human and nonhuman individuals with the use of oxygen isotopes. In archaeological studies of human populations, the difference between oxygen isotope ratios for potentially breastfed offspring and adult individuals is a rather extensive range from 0.5‰ to 2‰ (Table 5). This range includes also other species of primates (Smith et al. 2022), and even the results of analysis for the extinct woolly mammoth (Metcalfe et al. 2010). The maximum difference between oxygen isotope ratio in literature is 2‰ but we are aware that in uncontrolled studies we should include various factors which may affect interpretation.

We must emphasize that increased isotope values in offspring that we may suspect as having been weaned does not necessarily mean that they continued to drink mother's milk. Higher isotope ratios may result from feeding thermally processed food to children, especially if we consider that paps, gruels, or soups were prepared by boiling cereal flakes and grains in water. Indian sources from 6th century BC to 1st century AD suggest that weaning started by feeding the child with boiled rice (Fildes 1986). In the 15th century, doctors recommended that children weaned before the end of the first year of life should be fed with boiled goat milk with water and a pap made of boiled water, bread, and honey (Fildes 1986). In analysis of the prevalence of scurvy in medieval and early modern populations, Krenz-Niedbała (2016) concluded that weaned children and their parents ate mostly meals based on cereals and boiled vegetables. The author observed that scurvy was not such a severe problem in the area of today's Poland as suggested in historical sources, and explains that this

Table 5. Changes in oxygen isotope delta for the skeletal tissues of a child as a result of breastfeeding

Increase of $\delta^{18}O$ child vs mother	Material	Species	Authors, year
~0.7‰	enamel carbonates	humans	Roberts et al.1988
~0.7‰	enamel carbonates	humans	Wright & Schwarcz 1999
~1.2‰	enamel phosphates	humans	White et al. 2004a
~2‰	carbonates of molar teeth enamel	woolly mammoth	Metcalfe et al. 2010
~0.5-0.7‰	bone carbonates	humans	Williams et al. 2005
~1.2‰	bone phosphates	humans	Britton et al. 2015
~1-2‰	prenatal vs postnatal enamel of M1	humans, other primates	Smith et al. 2022
~1.6‰	bone and teeth phosphates	rats	this research, tap water model
~2.7‰	bone phosphates	humans	estimation-this research, tap water model

was thanks to a technique used by historical populations that involved boiling meals slowly and for an extended period of time. This method enabled them to preserve, in an unchanged form, at least 50% of the vitamins contained in vegetables. Water used in making meals for weaned children could additionally be isotopically enriched not only by boiling, but also by being stored by human groups inhabiting dry areas (Perry et al. 2020).

Thus, many factors may modify the breastfeeding effect by enriching or depleting offspring's tissues in the heavier isotope ^{18}O. However, as demonstrated in the experiment, oxygen isotope value in the apatites of the offspring fed by milk from female rats who had drunk thermally processed water was as much as 3.7‰ higher than in rats fed by females from the tap water group. This finding proves that the intake of liquids which have been boiled or otherwise culturally enriched in heavy oxygen isotopes has a substantial impact on the isotope ratios in the bones of both mothers and their breastfed offspring and that it is highly probable that this holds true not only for bones but may be of significance for isotope studies analysing teeth.

Conclusions

The high complexity of the breastfeeding and weaning processes prompts researchers to ask many questions. How do cultural practices involving thermal processing of food and drink influence the interpretation of results? Are human groups which less often prepared food with the use of fire characterized by a greater difference between $\delta^{18}O$ in mothers and breastfed children than populations whose diet is strictly based on boiling and other thermal processing methods? Could this be correlated with the environmental conditions in which a given community functioned (latitude, climate)? Does the inaccessibility of odontological material combined with diagenesis of collagen preclude the reconstruction of infant feeding strategies? On the basis of this study, it may be argued that, despite multiple unfavourable issues, in exceptional cases oxygen isotopes analysed in bone phosphates can be used in BWP studies and effectively supplement other methods. Caused by breastfeeding and weaning, the change in the oxygen isotope level in bone phosphates is noticeable, and thermal processing is a factor which bolsters the effect. It was estimated that breastfeeding can raise oxygen isotope level in a human child's bones by 2.7‰ relative to its mother's level. If the mother consumes only thermally processed food, the difference may be smaller, approximating 0.4‰.

In the light of many assumptions which have been made in this study, our work does not constitute a ready-made solution to be applied in research on children's diet, yet, undoubtedly its experimental section emphasises the need to find more information on the basis of the BWP phenomenon so that isotope analyses (including oxygen isotope analyses) could be employed as broadly as possible and provide research opportunities in the absence of odontological material, with results that could be reliably interpreted.

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Conflict of interests

All Authors declare no conflict of interests.

Authors' contribution

AL-G: conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, wrote the paper, review and editing the paper; BC-S: involved in planning the work, review and editing the paper; MF: review and editing the paper; KS: involved in planning and supervised the project, aided in interpreting the results, review and editing the paper.

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