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Traits Selection in Created Personal Ads and Sociosexuality: A New Method to Assess Sexual Strategy in Humans

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Abstract: Sociosexuality (SO) refers to preferring sexual interactions with or without commitment. Those who prefer long-term relationships have restricted SO, and those who pursue short-term relationships have an unrestricted SO. Sociosexuality may be assessed by Sociosexual Orientation Inventory (SOI-R). Here, we test a new method to assess SO. Respondents are asked to create a personal ad by selecting six out of 10 suggested preferred traits in a partner. Among these 10 traits, there were two traits in each of five evolutionary relevant categories i.e., attractiveness, commitment, resources, cognitive and social skills. We hypothesize that seeking attractiveness/sensuality in a potential partner is related to concentrating on mating investments (higher SOI-R) and to commitment to parental investment (lower SOI-R). Out of 416 subjects who participated in the study, 299 (188 women) were included in the analysis. We found that choosing two traits of attractiveness is related to a less restricted SO, while preference for two commitment traits category characterizes those with a more restrictive SO. No relationship between SOI-R and the preference for cognitive skills or resources was found. Women with more and men with less restricted SO sought partners with better social skills. The proposed new method could be used to assess reproductive strategy.

Keywords: sociosexuality, preferred traits, attractiveness, commitment, social skills, sexual strategy

Original article

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Introduction

Sociosexual orientation refers to an individual's willingness to have sexual encounters with or without commitment and intimacy (Simpson and Gangestad 1991). At one extreme, are those with a Restricted Sexual Orientation (RSO), who want more commitment and emotional intimacy before deciding to have sex with someone. Therefore, they generally report fewer sexual partners and rarely engage in casual or 'one-night stand' sex (Simpson and Gangestad 1991). On the other side, are those with an Unrestricted Sexual Orientation (USO), who more frequently engage in short-term relationships based on uncommitted sex and have little or no need for an emotional bond with their partner. Such individuals quickly initiate sexual intercourse in romantic relationships, more often engage in 'one-night stands' and have more sexual partners (Simpson and Gangestad 1991). Despite many studies indicating that men are more permissive toward casual sex and more likely to engage in non-restrictive sociosexual behavior than women (Jurich and Jurich 1974; Mercer and Kohn 1979; Hendrick et al. 1985; Buss and Schmitt 1993; Barta and Kiene 2005), some research has shown more variation within than between genders (Kinsey et al. 1948; Eysneck1976; Hendrick et al. 1985; Schmitt 2005).

From an evolutionary point of view, the most important trait categories, which are decisive when choosing a partner are (1) physical attractiveness, (2) commitment, (3) social skills, (4) resources, and (5) cognitive skills (Buss 1989; Kenrick et al. 1993; Regan et al. 2000; Li at al. 2002; Sprecher and Regan 2002; Buss 2015; Li and Meltzer 2015; Fales and al. 2016; Jonanson et al. 2017; Thomas et al. 2019; Walter et al. 2020). Each trait would have played at least some role in successful mating and reproduction in the ancestral past. Physical attractiveness likely indicated fertility, and offspring with an attractive partner would have had a higher chance of becoming desirable mates themselves (Cornwell and Perrett 2008; Pflüger et al. 2012; Rosenthal 2017; Bovet et al. 2018). Finding a committed and faithful partner was a significant factor in the survival of a child (Washburn and Lancaster 1968; Lancaster and Lancaster 1987; Stewart-Williams and Thomas 2013; Schacht and Kramer 2019). Finally, having a high-status partner can be advantageous for both men and women because social status is often linked with access to resources, opportunities, and influence within a community (Mulder and Beheim 2011; Nelissen and Meijers 2011; von Rueden 2014). Cognitive skills allowed individuals to adapt their behavior to specific situations in a complex and changing word (Brosnan et al. 2010; Taborsky and Oliveira 2012). Nowadays, obtaining higher education may also be valued because it is seen as prestigious or is an indicator of resources (Fales et al. 2016). Social skills help us understand other people's emotions. Instead of reacting instinctively to what we see or hear, we process this information which allows us to respond appropriately and take advantage of opportunities in social environment ultimately improving chances of survival and success (Walters and Sroufe 1983; Paul et al. 2005; Taborsky and Oliveira 2010).

However, finding a partner who is close to perfection in all the aforementioned categories is rare. In most cases, people on the dating market usually agree to trade-offs, forsaking some desired traits or accepting undesirable ones if there is an opportunity to obtain another more favorable trait (Csajbók and Berkics 2017; Csajbók et al. 2022). Given that people with a RSO tend to engage in long-term relationships, while people with a USO choose short-term relationships more often, it is reasonable to assume that people are guided by different criteria when entering romantic relationships. Studies show that when looking for a long-term partner, traits associated with family (i.e., warmth, fidelity, honesty) and cognitive skills (i.e., intelligence, ambition, education) are more important (Regan et al. 2000; Fletcher at al. 2004; Castro and Lopes 2011). For women, resource-related traits also play a significant role in long-term relationships (Regan et al. 2000; Fletcher et al. 2004; Castro and Lopes 2011; Buss 2015; Fales et al. 2016; Jonanson et al. 2017; Thomas et al. 2019). In contrast, when looking for a short-term partner, external attributes like physical appearance are more desirable for both men and women (Simpson and Gangestad 1992; Gangestad 1993; Kenrick et al. 1993; Regan and Berscheid 1997; Wiederman and Dubois 1998; Stewart et al. 2000; Jonanson et al. 2017; Schwarz et al. 2020). Other traits favored in a short-term relationship are related to social skills (i.e., sense of humor, sociability) (Simpson and Gangestad 1992; Sprecher and Regan 2002).

Since sociosexual orientation relates to the type of relationships a person will be more likely to engage in (Simpson and Gangestad 1991), it should also relate to the preferred traits in a potential partner. In 1992, Simpson and Gangestad examined that relation by asking the participants to rate the importance of 15 partner attributes which related to either (1) personal/parenting qualities or (2) attractiveness/social visibility. Individuals with RSO rated attributes indicating the first one highly, while individuals with USO put more importance on the attributes related to the second one. Other studies have also attempted to link partner trait preferences and SO (Muggleton and Fincher 2017; Wilbur and Campbell 2017; Marcinkowska et al. 2021), however, in all of these studies respondents were asked to select their preferred traits with a distinction between a short-term and long-term partner, which immediately suggested to the respondents that the set of traits for the ideal partner would vary depending on the context of the relationship.

In addition, our main motivation for conducting the present study was not to examine correlations, which are already well documented, but rather to see if sociosexual orientation could be determined based on the traits selected by those surveyed. The Sociosexual Orientation Inventory (SOI) designed by Simpson and Gangestad in 1991 addressed the previous shortcomings of sociosexuality measures that tended to focus more on examining permissiveness to extramarital sex (Jurich and Jurich 1974; D'Augelli and D'Augelli 1977; Kelley 1978; Jessor et al. 1983), rather than willingness to engage in sex without love, commitment and emotional closeness. The SOI also addressed the problem of the often-weak relationship between attitudes and behavior (Wicker 1969) as expressing a willingness to engage in sex without emotional intimacy is quite different from actually doing so. Simpson and Gangestad (1991) developed and validated a short self-report measure, the Sociosexual Orientation Inventory (SOI) intended to assess sociosexuality which included e.g., attitudes towards non-committal sex and sexual behavior. Higher scores indicate a more non-restrictive orientation, while lower scores indicate a more restrictive orientation.

Despite the wide popularity of the SOI, the questionnaire has been repeatedly criticized. The main criticism has been the doubt that a single unidimensional aspect can accurately reflect differences in sociosexuality. Attention has also been drawn to the psychological heterogeneity of the questions and the distorted distribution of scores, the open-ended questions encouraging exaggerated responses with low reliability, and the phrasing of one of the questions in a way that makes the SOI inappropriate for singles (Townsend et al. 2005; Webster and Bryan 2007; Penke and Asendorpf 2008). For the above reasons, Penke and Asendorpf (2008) modified the SOI and the questionnaire became a multidimensional measure of three aspects of sociosexuality: (1) the behavioral dimension (e.g. the number of former sexual partners); (2) the attitude dimension concerning attitudes towards and acceptance of casual sexual contact devoid of emotional commitment; (3) the desire dimension concerning the intensity of fantasies about sexual contact with persons with whom the respondent is not in a relationship. All questions are closed, and answers are reported on a nine-point (optionally five-point) Likert scale. As with the SOI, responses from all nine questions are summed according to set rules (see Jankowski 2016), with higher scores indicating a non-restrictive orientation and lower scores indicating a more restrictive orientation.

A major problem with both SOI and SOI-R is the directness and intimacy of the questions, which means that many people, especially from countries with more closed and conservative backgrounds, may refuse to answer the questionnaire. Hence there is a need for a new measure of sociosexual orientation that does not include such questions and potentially can reach a larger group of respondents.

Here we use a new, unique method in that the respondents had to choose six of the 10 characteristics given. Each characteristic was assigned to one of the five categories. This arrangement required at least one category to be selected twice (both traits from a given category were selected by the subject). This is a simple and effective method, providing similar results to complex survey instruments, which can be used in many other studies. The purpose of the study is to observe the trade-off made when choosing a partner and see if it is possible to determine sociosexual orientation based on preferred characteristics in a potential partner.

Our hypotheses are as follows:

- people with an unrestricted sociosexual orientation will be more likely to look for attractiveness-related traits in a potential partner;
- those with a restricted sociosexual orientation will pay more attention to traits related to commitment;
- seeking resources, cognitive and social skills will be more important for those with a restricted sociosexual orientation and therefore will be more related to the good prospects for parental investments.

Materials and methods

Participants

A total of 416 Polish speaking people took part in the survey. Excluded were the people who did not finish the survey (51) and whose sexual orientation was other than heterosexual (66). Thus, 299 subjects (62.9% (N=188) women) between 18 and 62 years of age (average age of 25.7 and 26.3 respectively for women and men) were included in the analysis. 59.9% (N=179) were in a relationship, of which 22.9% (N=41) were married. 15.4% (N=46) of respondents had children. The majority (64.5%, N=193) completed high school and 32.5% (N=97) graduated from university. Participants under 18 years old were not allowed to continue completing the survey.

Measures and procedure

The data were collected through Qualtrics' online platform. The survey link was shared through groups and online forums. Surveys were collected from February to April 2022.

The study was completely anonymous, the respondents gave their consent at the beginning of the study and were informed they could withdraw from the survey at any time. The questionnaire consisted of 3 parts: (1) sociodemographic data (age, sex, sexual orientation, and education), (2) a personal ad questionnaire, and (3) the Revised Sociosexual Orientation Inventory.

In the personal ad part, regardless of their current marital status, respondents were asked to imagine that they have no partner(s) and had to create their own personal ad. This section, like most personal ads, consisted of an "I'm looking for" section, in which respondents selected six characteristics of their dream partner(s) from the list of 10 characteristics. Respondents were not allowed to add their own adjectives to the list. Respondents were also asked to rank the selected adjectives from the most important to the least important of the six selected traits.

The traits were pre-assigned to five categories (two in each category) – (1) attractiveness – attractive, sensual; (2) cognitive skills – intelligent, college-educated; (3) social skills – with a sense of humor, sociable; (4) resourcefulness – entrepreneurial, high-earning; and (5) commitment – affectionate, faithful.

The form of the questionnaire, which required the selection of exactly six traits (there was no possibility to select fewer or more than six) meant that at least one (and up to three) of the five categories had to be repeated. The repeated categories are understood to be when both proposed traits have been selected from a given category. They were then treated as those playing the most important characteristic sought in a potential partner. One person could repeat a minimum of one category of features, and a maximum of three categories. One category was repeated by 94 people, two categories by 197 people, and three categories by eight people.

In the last section, respondents were asked to complete a 9-item validated Polish version of the Revised Sociosexual Orientation Inventory (SOI-R, Penke and Asendorpf 2008) to measure their level of sociosexual orientation. The SOI-R consists of nine questions, three items for each dimension of sociosexual orientation - (1) behavioral, (2) attitudes, and (3) fantasies. Questions are answered using a 9-point scale. After averaging the answers to these three questions, an indicator for each dimension was calculated. The total score for sociosexuality was the average from these three scales, where higher values in the score indicate a less restrictive sociosexual orientation and lower values more restrictive sociosexual orientation.

Analysis

The non-parametric Mann-Whitney U test was used to test the relationship between the level of sociosexuality and gender because the data were not normally distributed. The Chi-square test of concordance was used to test the relationship between the frequency of repeating a given category of a potential partner's characteristics and gender. The relationship between the level of sociosexual orientation and the type of repeated category of traits sought was examined using the Mann-Whitney U test. Analyses were performed in Statistica 13 software.

Results

The SOI-R results on sex differences (Table 1) confirm that men have higher scores than women in all domains and the general SOI-R score.

Figure 1 shows how often each category of traits was repeated in the description of the potential partner, and therefore which category was most important to the respondents. In the case of attractiveness, men repeated this category more often than women ($X^2(1, N=299) = 16.09$; $p<0.001$). Women, on the other hand, were more likely to repeat (or seek) cognitive skills ($X^2(1, N=299) = 5.85$; $p<0.05$).

Table 1. Comparison of the median level of sociosexual orientation domains between women and men

	Median		p	Z
	Women (N=188)	Men (N=111)		
SOIR	2.67	3.67	<0.001	-6.01
SOIR behavioral domain	1.33	1.67	<0.001	-3.42
SOIR attitudes domain	3.67	5.67	<0.001	-4.7
SOIR fantasies domain	2.00	3.67	<0.001	-5.68

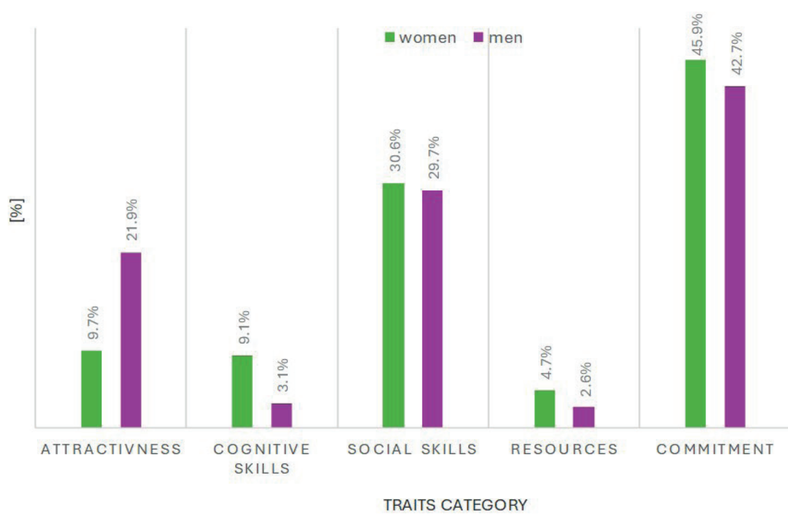

Fig. 1. Frequencies of repeated categories of preferred traits in a potential partner

The median levels of SOI-R and all its domains were then compared separately within each sex for all trait categories that were sought (repeated traits) in a potential partner characteristic (Table 2). The best assessment for SOI-R was found for repeating commitment for both sexes. Apart from behavioral dimension for men, both men and women who repeated commitment category have lower SOI-R and its other domains scores than those who did not repeat this category. This means that both men and women repeating this trait when seeking a sexual partner have more restricted sociosexuality. Seeking attractiveness in a partner can be also a relatively good SOI-R assessment for both sexes. Both men and women who repeated attractiveness category have higher general SOI-R scores than those who did not repeat this category. For women, what is interesting, this is mainly driven by attitude and fantasies domain. In both these domains women who repeated category attractiveness have higher scores. Out of the other three categories, only repeating category social skills is negatively associated with general SOI-R and attitude dimension for women and positively associated with attitude dimension for men.

Table 2. Comparisons of the median scores of the sociosexual orientation for repeated or not repeated traits sought in a partner in the sample of women

Traits category	SOIR domains	Median for repeated category	Median for not repeated category	Z	p
Attractiveness	SOI-R	3.44	2.33	4.41	<0.001
	SOI-R behavioral	1.33	1.33	1.00	0.32
	SOI-R attitudes	5.33	3.67	3.46	<0.001
	SOI-R fantasies	4.00	2.00	4.28	<0.001
Cognitive skills	SOI-R	2.78	2.56	-2.56	0.80
	SOI-R behavioral	1.33	1.33	-0.35	0.73
	SOI-R attitudes	3.67	3.67	0,51	0.61
	SOI-R fantasies	2.67	2.00	-1.29	0.20
Social skills	SOI-R	2.33	2.94	-2.52	0.01
	SOI-R behavioral	1.33	1.33	-0.59	0.56
	SOI-R attitudes	3.67	4.00	-2.42	0.02
	SOI-R fantasies	2.00	2.33	-1.37	0.17
Resources	SOI-R	2.78	2.67	-0.66	0.51
	SOI-R behavioral	1.33	1.33	-0.20	0.85
	SOI-R attitudes	4.00	3.67	-0.22	0.83
	SOI-R fantasies	2.67	2.00	-1.06	0.29
Commitment	SOI-R	2.44	3.44	3.42	<0.001
	SOI-R behavioral	1.33	1.67	2.65	0.008
	SOI-R attitudes	3.67	5.00	2.74	0.006
	SOI-R fantasies	2.00	3.00	3.10	0.002

Table 3. Comparisons of the median scores of the sociosexual orientation for repeated or not repeated traits sought in a partner in the sample of men

Traits category		Median for		Z	p
		repeated category	not repeated category		
Attractiveness	SOI-R	4.06	3.33	2.39	0.02
	SOI-R behavioral	1.83	1.67	1.28	0.20
	SOI-R attitudes	6.33	5.33	1.86	0.06
	SOI-R fantasies	4.00	3.33	1.56	0.12
Cognitive skills	SOI-R	4.06	3.56	-0.17	0.87
	SOI-R behavioral	2.17	1.67	-1.62	0.11
	SOI-R attitudes	6.33	5.67	-0.57	0.57
	SOI-R fantasies	2.67	3.67	1.22	0.22
Social skills	SOI-R	3.78	3.44	0.95	0.35
	SOI-R behavioral	1.67	1.67	-1.05	0.29
	SOI-R attitudes	6.00	5.50	1.38	0.17
	SOI-R fantasies	3.67	3.33	0.85	0.40
Resources	SOI-R	2.89	3.78	1.85	0.06
	SOI-R behavioral	1.67	1.67	0.50	0.62
	SOI-R attitudes	3.67	5.67	2.02	0.05
	SOI-R fantasies	3.33	3.67	0.19	0.84
Commitment	SOI-R	3.33	4.11	-2.66	<0.01
	SOI-R behavioral	1.67	1.67	-0.28	0.78
	SOI-R attitudes	5.33	6.67	-2.34	0.02
	SOI-R fantasies	3.17	4.67	-2.15	0.03

Discussion

The aim of our study was to test new method allowing to assess sociosexuality and therefore potential sexual strategy pursued by women and men. We analyzed if creating a personal ad with an emphasis (repeating some category) on a specific trait in a potential partner (e.g., attractiveness, commitment or social skills) allows to assess sociosexuality measured by SOI-R.

We confirmed that the level of sociosexual orientation differs between sexes. Women are characterized by lower levels of sociosexual orientation (SO) in each domain (Buss and Schmitt 1993; Barta and Kiene 2005; Lippa 2009; Brase et al. 2014). This result indicates that in relationship to sex differences in sociosexuality our sample was not biased and can be treated as a representative for the studied population.

As expected, we showed that people with an USO focus mainly on traits related to attractiveness in a potential partner, while those with a RSO pay more attention to traits indicative of commitment (our two first hypotheses). It is also consistent with the previous research (Simpson and Gangestad 1992; Hackathorn and Brantley 2014). Additionally, the trade-off between commitment and attractiveness appeared more exacerbated for women than for men. This result is also consistent with the previous studies indicating that physical attractiveness is important to men regardless of the type of relationship (Kenrick et al. 1993; Regan et al. 2000; Fletcher et al. 2004; Castro and Lopes 2011) so giving up the attractiveness of a potential partner will be more frequent for women than for men.

According to our study, commitment and attractiveness are the traits sought in a potential partner that allow the best assessment of a person's general SOI score. In the case of women, higher expectation of attractiveness in a potential partner reflects mainly two domains of their SOI i.e. attitude and fantasy. It is noteworthy that the method we used is a better predictor of SOI-R and its domains for women than men. The relationships between SOI-R, SOI-R A, or SOI-R F, and both attractiveness and commitment sought are higher for women than men.

Contrary to the attractiveness that is supposed to indicate biological condition or fertility (Wiederman and Dubois 1998; Stewart et al. 2000; Schwarz et al. 2020) and commitment securing long-term relationship (with higher chances to raise offspring successfully), resources and cognitive skills are of a lower priority. We should, however, remember that our subjects are relatively young (around 26 y.o.) and come from a Western, Educated, Industrialized, Rich and Democratic (WEIRD) society. In the case of resources, it is likely that our subjects had a relatively good financial situation and therefore did not pay a lot of attention to this trait in a potential partner. In addition, in the studied society, there is a negative stereotype of people focusing on resources when seeking a partner, and therefore it is likely that to avoid social criticism, advertisers may be reluctant to admit they seek this trait in a potential partner. This problem, however, can be more important for women subjects. It is because according to many studies, men are less likely to seek resources (Kenrick et al. 1993; Regan et al. 2000; Fletcher et al. 2004; Castro and Lopes 2011).

The intriguing part of our results is the relationship between sociosexuality and seeking social skills in a partner. Women who paid more attention to social skills in a potential partner had lower SOI-R which confirms one part of our third specific hypothesis, but contradicts previous research by Simpson and Gangestad (1992). It is worth mentioning that men and women repeated this trait category equally often (respectively 29.7% and 30.6%). To describe social skills, we used "with a sense of humor" and "sociable," and what is of interest, both men and women were more likely to choose "with a sense of humor" rather than "sociable." A possible explanation might be the positive relationship between this trait and chances to acquire a partner with higher social status and with more resources and these attributes are mainly sought by women in a long-term partner (Kenrick et al. 1993; Regan et al. 2000; Fletcher et al. 2004; Castro and Lopes 2011). Well-developed social skills were shown to be associated with holding leadership positions e.g., in high school (Kuhn and Weinberger 2005). Furthermore, social skills can facilitate social interactions, which in turn can lead to greater conscientiousness and efficiency at work (Witt and Ferris 2003; Beheshitfar and Norozy 2013). In the case of entrepreneurs, it has been shown that a high level of social skills often helps in gaining access to investors or potential customers, which strongly influences success at work (Baron and Markman 2000). However, for men we found opposite relationships, men with USO in attitude dimension paid more attention to social skills. Other SOIR domains although not statistically significant also showed a trend in this direction. To sum up, we confirmed our first and second hypotheses, but the third one was confirmed only for social skills and only for women.

To the best of our knowledge, this study is the first attempt to examine the relationship between the sought traits in a potential partner and sociosexual orientation using new simple (relatively non-invasive, at least in some more traditional societies) way. The form of the survey based on creating a personal ad puts the subjects in a situation as close as possible to the one they might encounter in real life, which makes the obtained results reliable. The survey also made it possible to select more than one category of characteristics repeated, which made it possible to better observe what the preferences of each category of traits are according to the SO level than if only one of the categories could be repeated. However, further research is needed that would allow still more accurate SOI or reproductive strategy assessment.

The study has a few potential limitations. Poland is a conservative country, and many people did not want to complete a survey in which they had to answer such intimate questions, which significantly limited the number of respondents. There also seems to be a problem with some adjectives used in the study. The fact that we found no significant results for cognitive skills may be either related to the lower (than attractiveness and commitment) meaning of these traits at the mating market or was due to an inappropriate selection of adjectives. The adjectives describing this category were – "intelligent" and "collage-educated." The adjective "intelligent" was chosen by 67% of the respondents, while the adjective "with a college education" was chosen by only 9% of the respondents. This is the largest difference observed in the choice of adjectives within a single category of traits, which may suggest that higher education was not the optimal trait for this category, making the category of cognitive skills a less frequently repeated category. To select adjectives that best describe the studied trait categories, it would be worth to conduct a survey in which subjects would determine to what extent a given adjective accurately reflects the category to which it belongs. Moreover, our subjects are relatively young (around 26 yrs) and come from a WEIRD society, and therefore our results (including those related to the resource category) cannot be generalized for the whole population, and more so for other populations, for instance living in a harsher environment or in developing countries.

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Conflict of interest

The authors declare no competing interests.

Ethics statement

The study was completely anonymous and non-invasive. The respondents gave their consent at the beginning of the study and were informed they could withdraw from the survey at any time without any consequences, so it did not require formal ethical approval to conduct it.

Statement of contributions by individual authors

Justyna Kajstura – data collection, data analysis, manuscript writing; Bogusław Pawłowski – study conceptualization, planning a procedure, correction of analysis and manuscript

We declare that the paper has not been previously published or concurrently submitted to an editorial office of another journal, and also it is approved by all authors.

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Chemical Characterization of Archaeological Pulp Stones from 18th–19th Century Radom, Poland: A Pilot Study

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ABSTRACT: The purpose of this study was to evaluate the calcium (Ca) and phosphorus (P) contents of pulp stones in a historical population (18^{th}–19^{th} centuries) from Radom, Poland. Ten molars from adults from the Radom cemetery (18^{th}–19^{th} centuries) were used in the study. The crowns of the teeth were mechanically opened, and visible pulp stones were examined with a scanning electron microscope equipped with a Si(Li) energy dispersive detector (energy dispersive X-ray spectrometry detector, which uses a lithium (Li)-doped silicon (Si) single-crystal semiconductor as a detector element). Enamel presented higher values of Ca/P ratio compared with the dentine and/or pulp stones. Moreover, in one case, the external layer of the pulp stone had significantly lower Ca and P contents compared with the internal layer (Ca: 13.97±0.31 vs. 34.2±0.37 [wt%]; P: 6.13±0.21 vs. 16.5±0.34 [wt%]). Evaluation of the Ca and P composition of the analyzed enamel and dentine in the Radom samples from the 18^{th}–19^{th} centuries produced findings that are consistent with studies conducted on teeth from contemporary populations. In the case of one individual, two chemically distinct layers were diagnosed in the pulp stone. It is difficult to interpret this finding based on a single case, but it may be due to the fact that the outer layer is characterized by higher organic structure.

KEYWORDS: pulp stones, calcium, phosphorus, mineralization

Original article

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Pulp stones are calcified structures that occur in the coronal and radicular pulp, where they may be free, attached, or embedded in the dentine. Free pulp stones are present within the pulp tissue proper and are the most commonly seen type on radiographs (Goga et al. 2008; Al-Ghurabi and Najm 2012). Pulp stones can be observed in deciduous and permanent dentition. Moreover, pulp stones can occur in both erupted and unerupted teeth, as supported by previous studies (Şener et al. 2009; Çolak et al. 2012; Kaabi et al. 2024).

The exact cause and mechanism of pulp calcification are still largely unknown and have been the subject of a great debate (Mitręga and Dreher 1968; Barnaś et al. 1972; Goga et al. 2008; Marshall et al. 2023). Among the main etiological factors mentioned in the literature are: the age of the individual, trauma, periodontal disease, dental caries, abrasion, and genetic predisposition (Ozkalayci et al. 2011; Bahetwar et al. 2012; Çolak et al. 2012). Diseases of the metabolic and/or cardiovascular systems may also co-occur with pulp stones (Huang and Chen 2016; Nicklisch et al. 2021). In our previous study (Tomczyk et al. 2017), we showed that pulp calcification is not connected with the age of the individual; rather, it is caused primarily by pulp irritation. This irritation may be the result of either mechanical factors (dental wear) or disease processes (dental caries and periodontal diseases). The type of diet is also discussed regarding the formation of pulp stones (Raj et al. 2012; Tomczyk et al. 2014). Nicklisch et al. (2021) showed that individuals with higher $\delta^{15}N$ isotope values in their bone collagen appear to be more frequently affected by pulp stones. This finding implies that diets containing higher amounts of animal protein may influence the formation and development of pulp stones.

In dental examinations, the most common pulp stones are diagnosed using intraoral radiography. However, this applies only to structures that exceed 200 μm in diameter. Smaller pulp stones are diagnosed using cone-beam computed tomography (Gulsahi et al. 2009; Sisman et al. 2012). In selected cases, the diagnosis of pulp stones can be based on histological or micro-CT analysis. However, the examination procedure in both methods is time-consuming and costly (Goga et al. 2008; Marshall et al. 2023). The evaluation of pulp stones increasingly uses advanced technology, such as inductively coupled plasma atomic emission spectroscopy (ICP-AES), which makes it possible to analyze their structure and chemical properties. This method is based on the excitation of atoms and ions with plasma. The result is the emission of electromagnetic radiation at wavelengths specific to the element (Milcent et al. 2019). The results from this evaluation have confirmed earlier reports that the density and chemical composition of pulp stones is very similar to dentin (Mitręga and Dreher 1968; Barnaś et al. 1972), mainly regarding the contents of the two main elements, calcium (Ca) and phosphorus (P) (Le May and Kaqueler 1993).

The presence of pulp stones as well as their structure and composition are a topic of clinical research (Turkal et al. 2013; Gaddalay et al. 2015; Palatyńska-Ulatowska et al. 2021; Mirah et al. 2023). This is because knowledge of pulp stones is important in endodontic treatment. Unfortunately, only a few studies have addressed pulp stones in historical populations. The authors of these papers have typically focused on the occurrence of pulp stones and their location (Elvery et al. 1998; Tomczyk et al. 2014; Tomczyk et al. 2017; Nicklisch et al. 2021). There are several reasons for the limited number of studies on pulp stones in historical populations. First, it is impossible to subject all specimens to radiography; usually, only random samples of specimens are examined. Second, highly specialized evaluations are invasive in the sense that they require mechanical opening of the tooth chamber, which leads to permanent damage to the tooth. The historical value of archaeological materials prevents conducting such analyses. Therefore, only certain materials from archaeological sites can be subjected to such examination (Nicklisch et al. 2021). Such an opportunity to study pulp stones has arisen for several specimens from a historical cemetery from Radom (Poland).

The present study evaluated the Ca and P contents in the pulp stones of individuals from a historical population from Radom (18^{th}–19^{th} centuries). This study aimed to answer the following research questions: (i) Do the Ca and P contents vary among the studied dental structures? (ii) Do the pulp stones exhibit differences in Ca and P content between their outer and inner layers?

Materials and methods

Dental material of adult individuals from the historical population of Radom (Poland), which came from the cemetery used in the years 1791–1811, was selected for the study. The dental material used for the research is stored at the Institute of Biological Sciences, Cardinal Stefan Wyszynski University in Warsaw. Material was stored in a dry place, stored from possible contamination that could affect the results of the study. The material used in the present study had been analyzed previously in terms of the frequency and location of pulp stones (Tomczyk et al. 2017). The presence of pulp stones was determined with a portable X-ray machine (EZX-60, Edlen Imaging, USA). Due to the invasive (destructive) nature of the study, 10 molars were selected from the described material for further analysis.

First, crowns of the teeth were opened with a dental drill. Next, the edges of the crowns were filed down so that there were leading-edge pulp stones. The transversely cut teeth with the pulp chamber filled with pulp stones were polished gently with sandpaper and rinsed in an ultrasonic bath in cold deionized water. After air drying, each tooth specimen was mounted in a microscopic holder with carbon conductive paint (EM-Tec C33, Rave Scientific, USA) and stored under vacuum overnight. The exposed surfaces of enamel, dentine, and pulp stones were positioned parallel to the holder surface. After coating with a thin layer of carbon (JEC-530, JEOL, Tokyo, Japan), the samples were observed in scanning electron microscope (SEM) (JSM-5410, JEOL) equipped with an Si(Li) energy-dispersive detector (EDS; Noran Instruments Inc., the Netherlands). To determine qualitatively the elemental composition of all dental structures, X-ray microanalysis was performed with the following conditions: an accelerating voltage of 15 keV and an absorption current of 100 pA as measured for the aluminum stub, and a 25° take-off angle for the EDS detector, which was positioned 30 mm from the specimen to achieve a 0.033 sr solid angle. Each analysis was performed in the raster mode at 750× magnification to obtain the total X-ray intensities emitted from the enamel, dentine, and pulp stones not exceeding 30% of the EDS detector deadtime. The same analytical conditions were used for quantitative estimation of the Ca and P contents. For this endeavor, the apatite standard was measured (SPI Supplies, West Chester, PA, USA).

Three measurements were taken at random locations (the test locations were estimated visually) on each tested dental structure (enamel and dentin). In addition, three random measurements (averaged before the statistical analysis) were taken from the pulp stones from the central part (internal layer) and the periphery (external layer). All spectra were processed to obtain the peak-to-background (P/B) Ca and P values (Roomans 1988). Next, the P/B values derived from the apatite standard were compared with the P/B values for unknown enamel, dentine, and pulp stones and the Ca and P contents [wt%] were calculated. Differences in the Ca and P contents as well as the Ca/P ratio were estimated using the non-parametric Kruskal–Wallis test (Statistica, TIBCO Software Inc., Palo Alto, CA, USA). The Shapiro–Wilk test was used to test for normality. A p-value <0.05 was considered to indicate a statistically significant difference.

Results

X-ray microanalysis of three parts of teeth – enamel, dentine, and pulp stones – revealed no significant differences in the Ca and P contents (Table 1).

The enamel part was the most mineralized, with a notably higher continuum radiation (background) intensity compared with the dentine and pulp stones (Fig. 1). This effect is due to the strong contribution of elements with a higher atomic number, that is, Ca (Z=20), in the generation of bremsstrahlung rather than elements of organic matter, that is, H (Z=1), C (Z=6), N (Z=7), or O (Z=8) (Goldstein et al., 2018). Indeed, enamel presented a significantly higher Ca/P ratio compared with the dentine or pulp stones ($p=0.048$) (Fig. 2).

Although the Kruskal–Wallis test revealed no statistically significant differences in calcium (Ca) and phosphorus (P) contents individually among enamel, dentine, and pulp stones (Table 1), the Ca/P ratio differed significantly. This apparent discrepancy can be explained by the cumulative effect of even minor, statistically insignificant variations in Ca and P contents, which collectively affect their proportional relationship, resulting in a significant difference in the Ca/P ratio. In one case, the pulp stone showed two layers that had a distinct elemental composition. The external layer had markedly lower Ca and P contents compared with the internal layer (Ca: 13.97±0.31 vs. 34.2±0.37 [wt%]; P: 6.13±0.21 vs. 16.5±0.34 [wt%]) (Fig. 3). The other pulp stones did not show such variation.

Table 1. The results of X-ray microanalysis of phosphorus (P) and calcium (Ca) content [%wt] in teeth enamel, dentine and pulp stones parts. The values are means ± standard deviations

	Amount [%wt]			
Elements	Enamel	Dentine	Pulp stones	p-value*
P	16.41±0.78	16.55±0.64	16.71±0.73	0.0796
Ca	35.43±1.72	33.51±0.84	33.36±1.20	0.0685

*Kruskal–Wallis statistical analysis

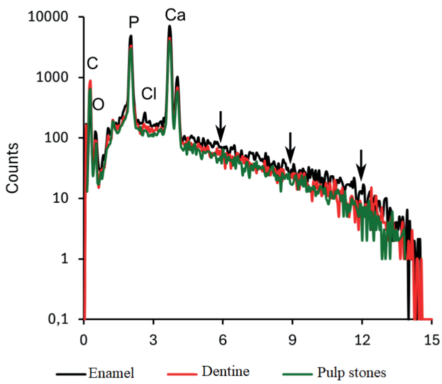

Fig. 1. The representative spectra obtained during X-ray microanalysis of the tooth enamel, dentine, and pulp stones. The dentine and pulp stone spectra are similar in their background intensity; however, the enamel spectrum shows a higher background (arrows). This indicates a greater contribution of the mineralized part of the enamel structure compared with the dentine and denticle

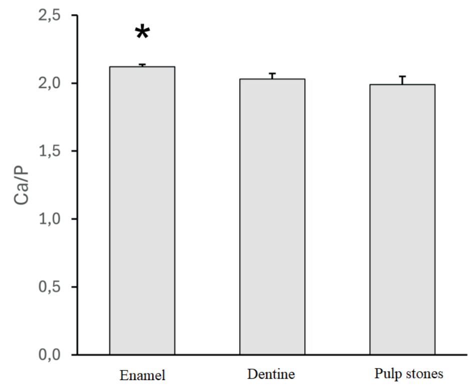

Fig. 2. The calcium-to-phosphorus (Ca/P) ratio for the tooth enamel, dentine, and pulp stones. The asterisk (*) indicates a significantly higher Ca/P ratio for enamel compared with the dentine and pulp stones (Kruskal–Wallis test, $p<0.05$).

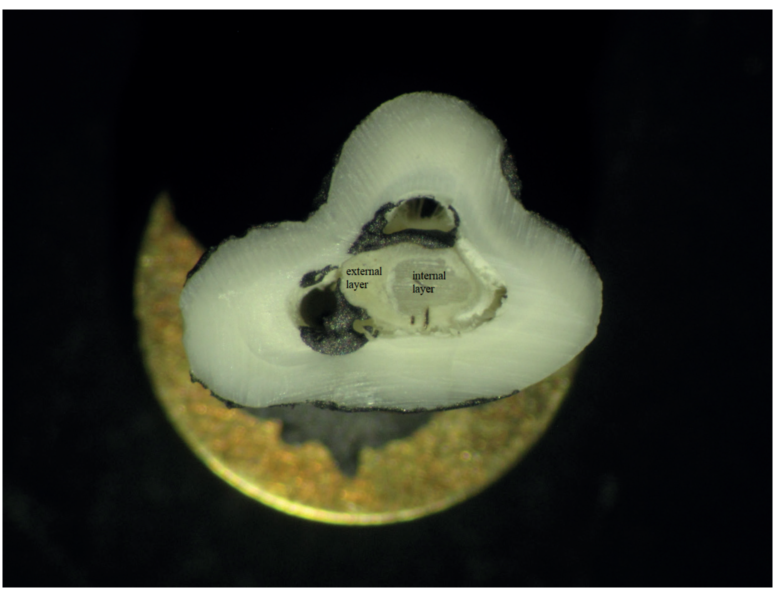

Fig. 3. A picture of a pulp stone with external and internal layers

Discussion

Ca and P are the main elements that make up the structure of teeth, so these elements have been widely evaluated (Arnold and Gaengler 2007; He et al. 2011; Liu et al. 2013; Palatyńska-Ulatowska et al. 2021; Sarna-Boś et al. 2022); however, the authors of these studies focused on contemporary populations. Murray (1936) reported a Ca/P ratio variation for human teeth that varied from 1.92 (dentin) to 2.15 (enamel). Hillson (1996) found that the biogenic Ca/P ratio varied from 1.91 to 2.17 for enamel and from 2.1 to 2.2 for dentin. Different Ca/P ratios indicate the different nature of enamel and dentin (Arnold and Gaengler 2007). This ratio decreases in the case of demineralization of enamel due to dental caries (Robinson et al. 1995; Sakoolnamarka et al. 2005). The Ca and P contents of the analyzed teeth from the 18th–19th centuries in Radom are consistent with studies conducted on teeth from modern populations (Arnold and Gaengler 2007; He et al. 2011; Sarna-Boś et al. 2022). Moreover, the Ca/P ratios calculated for the enamel and dentin are within the range of variation of this indicator for contemporary populations (Hillson 1996; Sarna-Boś et al. 2022). The content of elements in teeth can fluctuate due to, among other things, a diverse diet, which is the source of these elements. This means that in different historical periods, when there were different dietary trends, the elemental content of teeth can vary (e.g. Nedoklan et al. 2021). The elemental composition, including Ca and P, is also influenced by the environmental conditions in which the skeletal materials were deposited. For example, alkaline soil better protects bones and teeth compared with acidic soil, which easily destroys hydroxyapatite (Stipisic et al. 2014). The lack of differences in the abovementioned studies may be due to a similar diet for the current population as well as the population in the 18th–19th centuries. This may be confirmed by other anthropological studies (Pach et al. 2023; Perkowski et al. 2024).

The Ca/P ratio did not differ significantly between the dentin and pulp stones. However, there was a significant difference between the enamel and the dentin and pulp stones (Fig. 2). These results indicate that pulp stones have chemical properties that are similar to dentin, a finding that is consistent with the results of other studies conducted on teeth extracted from modern patients (Milcent et al. 2019). Consistently, Le May and Kaqueler (1993) pointed out that pulp stones chemically resemble dentin, with average Ca and P contents of 32% and 15%, respectively. Milicent et al. (2019) found differences between pulp stones and dentin. They noted that pulp stones are characterized by the presence of water and more organic material. These features observed on the teeth from contemporary patients were also captured in the historical population.

Of note, one pulp stone showed two distinctly different areas (the external and internal layers) based on the Ca and P contents. Pulp stones of the other individuals did not show chemical differentiation of their layers. Milcent et al. (2019) made similar observations with teeth that were extracted from patients. According to this study the inner surface of pulp stones shows increased levels of oxygen, calcium, and phosphorus compared to the outer surface which might be attributed to higher organic structure.

It is well known that pulp stones show very high heterogeneity in their structure and composition, so it is difficult to make far-reaching interpretations on the basis of a single case (Berès et al. 2016).

Limitations

This study has its limitations. The main limitation of most historical population studies is their small size. As mentioned, the examinations performed are destructive in nature, as they require mechanical opening of the tooth chamber. Such a procedure cannot be standardly performed on historical materials.

Contribution to the field

There is still controversy over both the causes and mechanisms of the formation of pulp stones. This issue is important for several reasons, including for endodontic treatment. While research on the teeth of modern patients is common, examination of odontological material from histological specimens is extremely rare. The results from the present study confirm the observation that pulp stones are chemically similar to dentin. This means that dentin irritation is an important cause of pulp stone formation. In addition, the formation of pulp stones occurs via a certain process that involves the mineralization and calcification of these structures. This can be useful in the treatment process when assessing the degree of development of pulp stones in the tooth.

Conclusion

Studies of pulp stones from historical populations are not often carried out due to the uniqueness and specificity of the dental material. In the present study, we analyzed the chemical composition of pulp stones; it corresponded to the chemical composition of dentin. Enamel showed the most mineralized structure, as the intensity of continuous (background) radiation was significantly higher compared with the dentin and pulp stones. In one pulp stone, we noted two chemically distinct areas (the internal and external layers). Further analysis of larger samples is needed to assess whether such layering is a common feature in historical pulp stones.

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Conflict of interest

Lead author Jacek Tomczyk is the President of the Polish Anthropological Society (Polskie Towarzystwo Antropologiczne) of which Anthropological Review is a flagship journal. He was not involved in the editorial handling of this article.

Author contributions

JT – research concept and design, critical revision of the article; **PR** – writing the article, final approval of the article; **KG** – collection and/or assemble of data, final approval of the article; **GT** – data analysis and interpretation, writing the article, final approval of the article.

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Ethics statement

The human remains are curated at the Institute of Biological Sciences, Cardinal Stefan Wyszynski University. Research on the presented material did not require ethics committee approval.

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Prehistory and Protohistory in Sicily. A Geometric Morphometrics Approach to Study the Biological History of Early Human Peopling of the Island

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ABSTRACT: In recent years, the debate about the early human peopling of Sicily has almost exclusively focused on the archaeological evidence. The dispersal patterns and the possibility for, and degree of, admixture caused by ancient migratory flows have been only investigated in limited anthropological studies conducted on a short time spans. Recent craniofacial morphometric analyses that considered migratory flows and population influx have provided a more comprehensive approach. These analyses go beyond archaeologically based settlement hypotheses by merging previous archaeological evaluations and paleoclimatic studies with an anthropological approach. This study expands upon earlier morphometric work and provides an overview covering the period from the Upper Palaeolithic to the Iron Age. For this study, human skulls from Sicilian Prehistory and Protohistory were considered. These skulls were divided into six periods based on the dating of associated archaeological artifacts. Sample selection was based on a detailed bibliographic review of previously published archaeological and historical works. With the aim of associating the diachronic changes in cranial morphology with population migrations and admixture we performed a 3D geometric morphometrics (GM) comparative analysis. The data reported demonstrate that the first colonization of Sicily started during the Upper–Paleolithic when stable climatic conditions allowed hunter-gatherers to move from the Italian Peninsula to the island. Moreover, the results show a cyclical occupation of the land counterclockwise direction completed only with the hinterland colonization in the first historical periods with Greek and Punic colonization.

KEYWORDS: geometric morphometrics, skulls, biological history, human peopling, Sicily

Original article

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Introduction

The biological history of the human peopling of Sicily presents many interesting aspects. Its proximity to both the Italian Peninsula and North Africa and its unique physical geography have made the island a crossing point for several living species (e.g., *Leithia melitensis, Aquila fasciata, Chamaerops humilis, Pinus halepensis etc.*) (Bosch 2010; Massa 2011; Surdi 2011, Pignatti 2011). Sicily is the largest island in the Mediterranean Sea and is itself divided into a mosaic of habitats (due to coastlines, sea valleys, small cliffs, hills, and mountain ranges), each with its own microclimate (Ruggieri 1973; Massa et al. 2011). At the same time, it is isolated biogeographically and is characterized by numerous endemic species of flora and fauna (Pignatti 2011). Environmental adaptation and/or natural selection (climatic factors, bottleneck effect, genetic drift, gene flow, or random evolutionary processes caused by neutrality) have produced profound coevolutionary changes in all species in the region (Sineo et al. 2015). *Homo sapiens* was no exception and even before the intense cultural and biological contributions left by the Greek, Carthaginian, Roman, Byzantine, Islamic, and Norman/Swabian colonizers (Lauria et al. 2023), early Sicilians were subjected to and strongly influenced by the environmental conditions. These conditions quickly and significantly influenced the island's gene pool and the human phenotype as early as Prehistory (D'Amore et al. 2009, 2010; Galland et al. 2019).

In recent years, the debate about the early human peopling of Sicily (and the related dispersal patterns) has almost exclusively focused on the archaeological evidence. The possibility for and degree of admixture caused by ancient migratory flows have been only investigated in limited anthropological studies conducted on a short time spans (D'Amore et al. 2009, 2010; Galland et al. 2019). The archaeological based settlement hypotheses assume an early colonization of Sicily's northern coast during the Palaeolithic, initially driven by economic needs and the related subsistence strategies (Beretta et al. 1986; Beloch 1989; Di Salvo et al. 2008; Barucco et al. 2021). This was followed by a new colonization and partial substitution during the Mesolithic (Galland et al., 2019). The island was then significantly impacted by Neolithic and post-Neolithic demic movements (Fernandes et al. 2020) that determined the colonization of the hinterland and of the southeast coast during protohistoric periods. These movements were driven by cultural and social factors (Serratti 2000; Di Salvo et al. 2008) such as the foreign invasion of the island which was carried out by several populations (Siculi, Ausoni, Morgeti and Elimi – Holloway 2002).

Recent craniofacial morphometrics analyses (Lauria and Sineo 2023) that considered migratory flows and population influx have provided a more comprehensive approach to understanding settlement patterns. These analyses go beyond archaeologically based settlement hypotheses by merging previous archaeological evaluations and paleoclimatic studies with an anthropological approach.

This study expands the sample upon earlier morphometric work (D'Amore et al. 2009, 2010; Galland et al. 2019; Lauria and Sineo 2023) and provides an overview focusing the period from the Upper Palaeolithic to the Iron Age (14.500 B.C.E. – 900-800 B.C.E.). To reconstruct the biological history of the early human settlement in Sicily we analysed the shape variation (polarity and magnitude) of facial features in order to associate the changes in cranial morphology with population influx. Hypotheses were formulated by performing a Procrustes coordinates PCA to understand the separation between the specimens and the groups reasonably. Instead, PCA is an exploratory analysis we use its similarity with the discriminant analyses to draw our conclusions.

Materials and methods

For this study, human skulls from Sicilian Prehistory and Protohistory were considered. Approval to examine the skulls was issued by Department of Sicilian Cultural Heritage and the Gemmellaro Archaeological Museum. These skulls were divided into six periods (Table 1) based on the 14C cal B.P for SanTeodoro 1 (Sineo et al. 2002), Molara (Leighton 1999) and Oriente (Modi et al. 2022) and based on archaeological artifacts (industry and pottery) for SanTeodoro 2, Uzzo, Marcita and Polizzello (Leighton 1999) (Table 2). Sample selection was based on a detailed bibliographic review of previously published archaeological and historical works as well as previous anthropological and palaeoecological studies (Table 3). Additionally, the A. Salinas, Baglio Anselmi, and L. Bernabo' Brea' museum catalogues were consulted. A preliminary visual examination of the skeletal remains was carried out to assess their morphological completeness and to determine if they were suitable for inclusion in this study. To avoid any error related to approximation, broken, incomplete (skulls not in anatomical connection and/or lacking landmarks necessary for the GM study discussed below) and restored skulls were a priori excluded and not considered for the study.

Table 1. Main Sicilian Prehistoric and Protohistoric Periods – B.C.E. Before Common Era

Main Sicilian Prehistoric a nd Protohistoric Periods:
B.C.E. Before Common Era
Prehistory
• Upper Paleolithic: 38.000–8.000 B.C.E.
• Mesolithc: 8.000–6.000 B.C.E.
• Neolithic: 6.000–4.000 B.C.E.
• Eneolithic/Copper Age: 4.000–2.500 B.C.E.
Protohistory
• Bronze Age: 2.500–1.100 B.C.E. Early Bronze Age: 2.500–2.000 B.C.E. Middle Bronze Age: 2.000–1.500 B.C.E. Late Bronze Age: 1.500–1.100 B.C.E. • Iron Age: 1.100–700 B.C.E.

Table 2. Sample Site; Key; Number of Specimens; 14C Dating cal B.P. and Periods

Site	Key	Specimens	14C Dating cal B.P.	Periods
Cave of San Teodoro	ST	2	14.500	Upper-Paleolithic
Cave of Uzzo	Uz	2	–	Mesolithic
Cave of Molara	Mo	1	8.600	Mesolithic
Cave of Oriente	Or	1	10.544	Mesolithic
Ragusa	Ra	3	–	Eneolithic(Copper Age)
Marcita	Ma	4	–	Bronze Age
Polizzello	Po	2	–	Iron Age

Table 3. Previously published archaeological and historical works as well as previous anthropological and palaeoecological studies

Archaeological and historical works	Anthropological and palaeoecological studies
Belvedere et al. 2017	Becker 1995–2000
Bonfiglio et al. 2001	Castellana & Mallegni 1986
Borgognini et al. 1985–1993	Di Salvo 1991
Borgognini & Repetto 1986	Di Salvo et al. 1998–2007–2012
Chilardi & Galdi 2012	Galland et al. 2019
Conte et al. 2017	Garilli et al. 2020
Costantini 2014	Incarbona et al. 2010a–2010b
De Miro 1988	Mannino et al. 2017
Hodos 2018	Messina et al. 2008
La Rocca 2011	Miccichè et al. 2018
Panvini et al. 2020	Schimmenti & Di Salvo 1997
Tusa 1994	Sineo et al. 2002–2005

In total, 16 adult (Ubeleker 1989; Scheuer and Black 2000; Buikstra and Ubelaker 1994) human skulls (Table 4) from eight different settlements were selected (Figure 1, Table 2). The spatial bias in the map (Figure 1) is mainly due to a lack of specimens from the south-eastern part of the island, which saw scarce colonization (lack of settlements) of *Homo* before the historical periods. Despite the small number of specimens and settlements, the sample effectively represents the inhabited regions of Sicily before the historical conization.

Table 4. Table reporting details of the Sample Size/Composition: Site, Key, Catalogue's Label, Sex

Site	Key	Label	Sex
Cave of San Teodoro	ST1	ST1	Female
	ST2	ST2	Male
Cave of Uzzo	Uz1	Uz4a	Female
	Uz2	Uz5	Male
Cave of Molara	Mo	Mo2	Male
Cave of Oriente	Or	OrB	Male
Ragusa	Ra1	RaT2S2	Male
	Ra2	RAT2S1	Male
	Ra3	RaT2S3	Female
Marcita	Ma1	MaTC8	Male
	Ma2	MaTC18	Male
	Ma3	MaTC20	Female
	Ma4	MaTC16	Female
Polizzello	Po1	No label	Female
	Po2	No label	Male

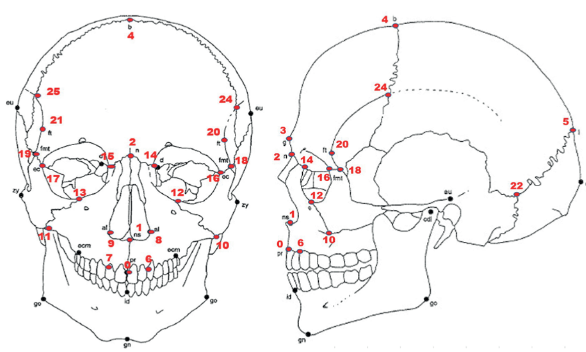
Fig. 1. Map of Sicily displaying the sample sites locations. Figure was edited and downloaded from Google Earth content for purposes of research and education. The software is either freely available for download or the licenses have been bought with institutional funding

With the aim of associating the diachronic changes in cranial morphology (Relethford 2002, 2004a, b; Roseman 2004; Taubadel and Lycett 2008; von Cramon-Taubadel 2008; 2009a; 2009b; 2009c; 2014; Betti et al. 2009; Smith 2009; 2011; von Cramon-Taubadel and Weaver 2009; Matsumura et al. 2018; Manthey and Ousley 2020; Klingenberg 2022) with population migrations, we performed a 3D geometric morphometrics (GM) comparative analysis. This study followed the approaches proposed by several authors (Bruner and Manzi 2004; Bruner 2007; D'Amore et al. 2009, 2010; Baab et al. 2010; Smith 2011; Fredline et al. 2012; Matusmura et al. 2018, 2022; Galland et al. 2019; Hubbe et al. 2020; Lauria and Sineo 2023; von Cramon-Taubadel and Lycett, von Cramon-Taubadel 2008, 2009a, 2011, 2014, 2017; Grine 2023a, 2023b; Gunz and Freidline 2023, Ribot et al 2023). Sex was estimated by evaluating the skulls' morphological characters according to Acsàdi-Nemeskèri (1970) as reported in Walrath (2004) and Minozzi and Canci (2015). The 3D models from Grotta di SanTeodoro, Grotta della Molara, and Grotta D'Oriente were acquired using computed tomography (CT) while the specimens from Grotta dell'Uzzo, Ragusa, Partanna, Marcita, and Polizzello were obtained through photogrammetric reconstruction following the protocol proposed by Lauria et al. (2022). The method consists of a Structure-from-Motion (SfM) photogrammetry that uses a single camera to capture chromatic details and reconstruct shape. A series of photos were taken forming circles around the target that will completely be covered and reconstructed by allying the photos and building a cloud of point before the polygonization of the model in the end.

Photogrammetric and CT models were scaled with the software used to build the models (Metashape 1.5.1 for photogrammetry and Slicer 5.2.2 for CT) and after exported in PLY format. The GM analyses were based on a configuration of 26 Landmarks (Buikstra and Ubelaker 1994) (Figure 2) positioned on the suture boundaries (Landmarks Type 1) and on the anthropometric points (Landmarks Type 2) (Bookstein 1991). Using the software "Landmark3.6" (Wiley et al. 2007), 78 Raw Coordinates (RCs) were acquired for each specimen and were then analysed using the software programs "MorphoJ 2.0" (Klingenberg 2011) and "PAST 2.0" (Hammer and Harper 2001).

MorphoJ was employed to subject the RCs (exported from "Landmark") to a Generalized Procrustes Analysis (GPA) (Dryden and Mardia 2016) to remove the effects of translation and rotation and to standardize each specimen to unit centroid size (Gower and Payne 1975, Rohlf and Slice 1990, Goodall 1991). The resulting Procrustes Fitted Coordinates (PFCs) were visualized using shape change graphs, including Lollipop and Wireframe Graphs, which allow three-dimensional forms to be visualized in two dimensions (Hammer and Harper 2008; Klingenberg 2013). These graphs illustrated the shape changes from a starting shape (the mean shape in the sample) to the target shape (the most extreme of the specimens) (Klingenberg 2013). These graphs were used to capture shape variation (direction and magnitude) across time (Harvati et. 2007, 2010, Bruner and Ripani 2008, Baab et al. 2010, Galland et al. 2016, 2019, Lauria and Sineo 2023). To highlight the positions of the specimens and groups within the sample (Hammer and Harper 2008), the RCs (exported from MorphoJ) were procrustized again in PAST to perform a Principal Component Analysis (PCA) based on a Covariance Matrix (represents the change of each variable relative to the others, including itself). The PCA separately analysed the PFCs of the single specimens, the average PFCs of each site, and the average PFCs of period.

Always considering that PCA is an exploratory analysis that allows for the formulation of a hypothesis based on the visualization (Le Maître and Mitteroecker 2019) and that discriminant analyses were not applicable (MANOVA and CVA are possible only when the variables are



Key	Landmarks Configuration	Type
0	Prosthion	1
1	Nasospinale	1
2	Nasion	1
3	Glabella	2
4	Bregma	1
5	Lambda	1
6–7	Point between the dental alveoli I2/C	1
8–9	Alare	2
10–11	Zygomatic-Maxillary suture – lower margin	1
12–13	Zygomatic-Maxillary suture – upper margin	1
14–15	Maxillary-Frontal suture	1
16–17	Ectoconchion	1
18–19	Fronto-Temporal-Malar	1
20–21	Frontotemporal	1
22–23	Occipital-Temporal-Parietal intersection	1
24–25	Stephanion	1

Fig. 2. Anatomical Landmarks Configuration. Drawn from Buikstra and Ubelaker (1994). The numeration starts from zero according to the "Landmark3.6" software

less than the specimens, Bronstain et al. 2006, Hammer and Harper 2008), we used the similarity between PCA and other discriminant analyses (Lauria and Sineo 2023) to reasonably understand the separation between the specimens and the groups. PAST was also employed to create a cluster analysis (CA) (UPGMA cluster procedure and Euclidean distance matrix) (Saitou and Nei 1987), leaving the software to recognize the outgroup.

Finally, on the PFCs, were conducted two "one-way ANOVA tests", the Levene's test for homogeneity of variance (Levene 1960; Derrick et al. 2018) and the more robust Shapiro-Wilk test (Shapiro and Wilk 1965) to evaluate the null hypothesis (H0) of equal multivariate means between the groups.

Results

Eigenvalue and percentage of variance

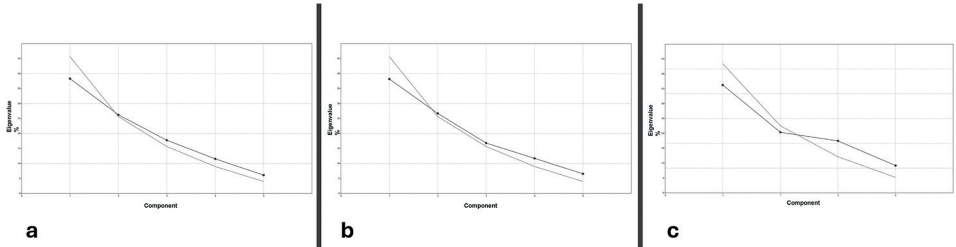
The plots of the PFCs (Single Specimens: Figure 3a; Averages of Each Site: Figure 3b; Averages of Each Period: Figure 3c) show that only the first two Principal Components (PCs) are significant. In detail, for the single specimens, the % of variance is 38,317% and 26,252% and the eigenvalue stands at 37,4197 and 26,252%, respectively. For the average of each site the % of variance is 38,188% and 26,717% while the eigenvalue are 33,4632 and 23,4111. Finally considering the averages of each period the values are 43,531% and 24,462% for the % of variance and 28,2558 and 15,8780 for the eigenvalue. In general, both the eigenvalues and the percent variance decrease gradually, converging toward similar values (Figure 3d-f), with a slightly more marked trend between the first two PCs (Figure 3). This suggests greater variations between the Paleolithic and the Mesolithic components (hunter-gatherers of the Stone Age) compared to the farmers-shepherds of the Metal Ages.

Shape variation

Considering that sample size and sample composition (number of specimens and sexual dimorphism) can influence the analysis, the shape variations show that the most notable changes are located on the neurocranium and the lower face, while minor changes affected the upper face (landmarks 4 and 19–22), the nose, and the orbits (Figure 3g). The frontal bone generally increased in size, becoming more elongated and lower (Figure 3h-i), while the width remained almost unchanged (Figure 3l). The parietal bones slightly reduced in size, showing decreases in length, the height of the posterior portion (Figure 3i), and in the width of the superior part (Figure 3l). The nose became slightly elongated (Figure 3g), however its size and position remained almost unchanged (Figure 3i-l). The orbits showed minimal changes in size and position (Figure 3l), only moving slightly downward.

Principal component analyses and cluster analyses

The PCA performed on PFCs of the single specimens (Figure 4a-b) shows the two Upper Paleolithic specimens from San Teodoro (Incarbona et al. 2010a) lying exactly on the negative axes of PC1, separated from the other specimens. In contrast, all the Mesolithic hunter-gatherer specimens from Molara, Uzzo, and Oriente are located on the positive axes of PC1. The Uzzo specimens are positioned on the negative side of the PC2 axes and those from Molara and Oriente are on the positive side of the PC2 axes. Notably, the Oriente specimens appear separated from both the Molara ones and all other individuals. In general, the hunter-gatherer specimens from the Sicilian Stone Age occupy an inhomogeneous morphospace surrounding the specimens representing the farmers-shepherds of the Sicilian Metal Ages. The farmers-shepherds of Ragusa (Copper Age/Eneolithic), Marcita (Bronze Age), and Polizzello (Iron Age) are all grouped in a homogenous morphospace near the centre of PCs axes (Figure 4a-b).



d

PC	Eigenvalue	% variance
1	37,4197	38,317
2	25,6376	26,252
3	17,3455	17,762
4	11,2833	11,554
5	5,97175	6,1150

e

PC	Eigenvalue	% variance
1	33,4632	38,188
2	23,4111	26,717
3	14,7418	16,823
4	10,2621	11,711
5	5,74861	6,5603

f

PC	Eigenvalue	% variance
1	28,2558	43,531
2	15,8780	24,462
3	13,6134	20,973
4	7,16247	11,035

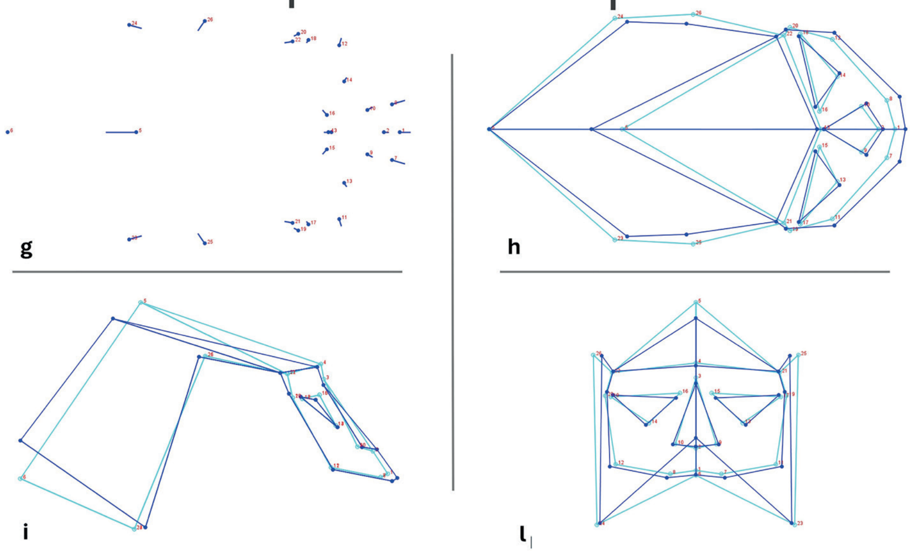


Fig. 3. Scree Plot (with Broken Stick in red), Eigenvalue and % of Variance of the PC covered by: Specimens (a-d); Average of each site (b-e); Average of each period (c-f). Cranial Shape Variation (light blue-dark blue) – Lollipop Graph Superior View (g); Wireframe Superior View (h); Wireframe Lateral View showing the changes of the height (i); Wireframe Anterior View (l)

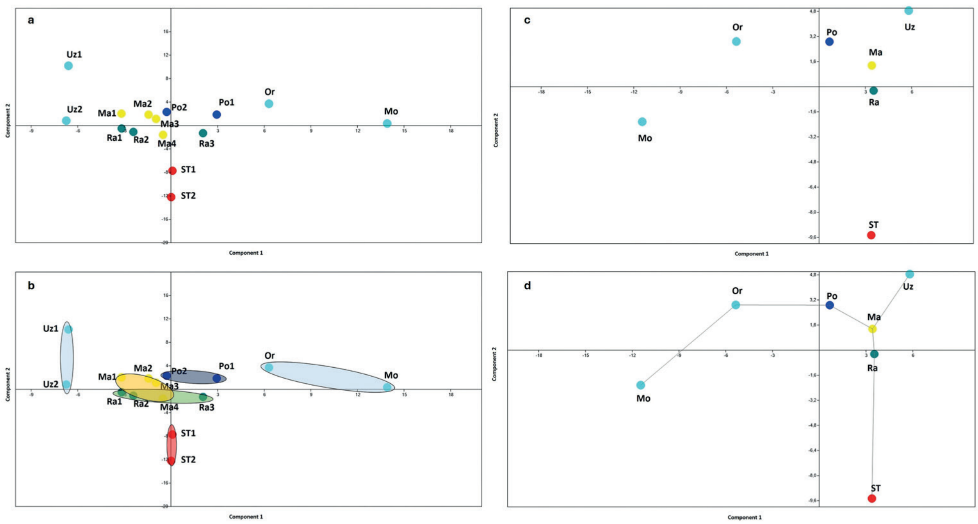


Fig. 4. PC1vsPC2 Specimens Procrustes Coordinates (a); PC1vsPC2 Box Color Specimen Procrustes Coordinates (b). ST: Cave of San Teodoro; Mo: Cave of Molara; Uz: Cave of Uzzo; Or: Cave of Oriente; Ra: Ragusa; Ma: Marcita; Po: Polizzello. PC1vsPC2 Sites Averages Procrustes Coordinates (c); PC1vsPC2 Spanning Tree Averages Sites Procrustes Coordinates (d). ST: Cave of San Teodoro; Mo: Cave of Molara; Uz: Cave of Uzzo; Or: Cave of Oriente; Ra: Ragusa; Ma: Marcita; Po: Polizzello

Further PCA analyses, based on the averages of the PFCs (average of each site: Figure 4c-d; average of each period: Figure 5a), reinforce the previous findings; the Paleo-Mesolithic sites of San Teodoro, Molara, Uzzo, and Oriente each occupy different quadrants in the PCA, surrounding the Copper, Bronze, and Iron Age sites of Ragusa, Marcita, and Polizzello, respectively (Figure 4c-d). Among these last three sites (all positioned on the positive PC1 axis), it should be noted that the Iron Age site of Polizzello appears slightly distant from the sites of Ragusa and Marcita, which are close to each other but separated by the PC2 axis (Figure 4c-d). These results also show a significant distance between the Paleolithic and Mesolithic periods (both hunter-gatherers, separated by the PC1 axis; Figure 5a), which in turn are far from the Copper, Bronze, and Iron Ages (all farmers-shepherds), grouped together in the upper right quadrant (positive PC1 and PC2 axes) close to each other but each in their own position (Figure 5a). The CA results (Figure 5b), based on the averages of the PFCs for each period, confirm the PCA findings. San Teodoro is automatically recognized as the outgroup, while the Paleolithic group retains some affinities with the Mesolithic specimens, which cluster separately from both the Paleolithic and the more recent farmers-shepherds from the Metal Ages. Additionally, the Prehistoric Eneolithic/Copper Age and the Protohistoric Bronze Age are still clustered together and, in turn, separated from the Iron Age group, which is chronologically closest to the most recent historical periods.

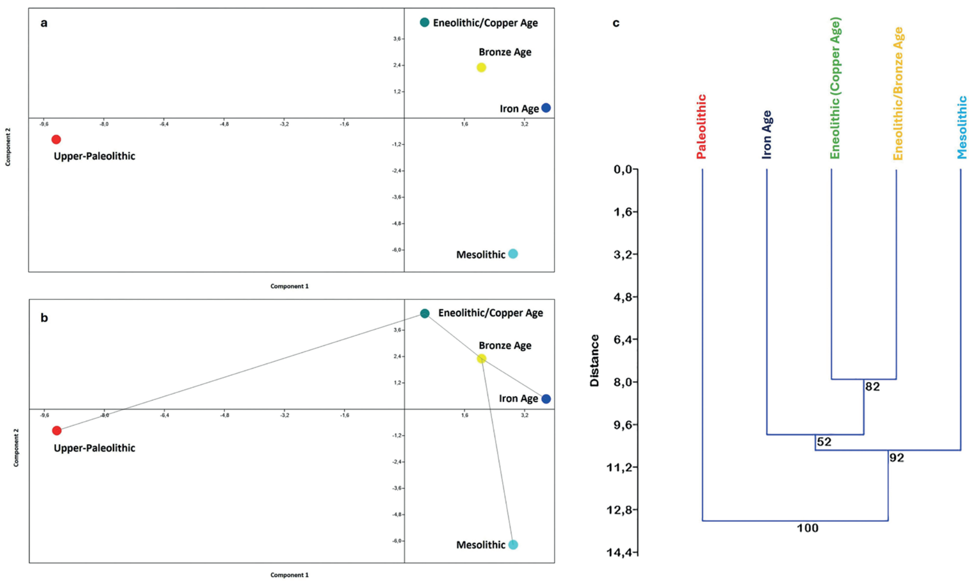


Fig. 5. PC1 vs PC2 Period Averages Procrustes Coordinates (a); PC1 vs PC2 Spanning Tree Averages Period Procrustes Coordinates (b). Cluster Analyses representing the divergences in Sicily from the Paleolithic to the Iron Age (c)

One-way ANOVA

The Levene's test for homogeneity of variance to evaluate the H0 returned in all the case p (same) value < 0.001 (in a significance level of $\alpha=0.05$), in detail PFCs specimens p (same)=$3{,}569E^{-31}$, PFCs sites p (same)=$1{,}915E^{-10}$ and PFCs periods p (same)=$9{,}223E^{-12}$ that rejects the H0. In the same way the Shapiro-Wilk test returns PFCs specimens p (same)=$6{,}067E^{-19}$, PFCs sites p (same)=$1{,}6E^{-12}$ and PFCs periods p (same)=$1{,}016E^{-7}$ that also in this case rejects the H0.

Discussion

Overall, the gradual decrease in eigenvalues and the percent variance suggests a slow progressive increase in variability (Figure 3) caused by primitive low-density migrations. These migratory events of the Paleolithic and Mesolithic (Stone Age hunter-gatherers) exhibit spatial clustering distinct from the later Metal Age farmer-shepherds.

The shape variations across prehistory and protohistory (Figure 3g) show a general trend of dolicefalization of the neurocranium (becomes more elongated and narrower). In contrast, the lower face became more elongated but wider, while the nose and orbits largely maintained their size and position. This pattern suggests phenotypic and genotypic changes due to neutral forces such as environmental factors and the stochastic forces that generally evolve in a neutral manner (Smith 2011). These acted in parallel with the population influx caused by migratory flows from the content (Betti et al. 2009). The scatterplots generated by the PCAs (Figures 4–5) and by the CA (Figure 5) show a decrease in the biological distance from the Paleolithic to the Iron Age, with short distances between the Sicilian Metal Age groups. Specifically, the hunter-gatherer migratory flows of the Stone Ages (Paleolithic and Mesolithic) were always characterized by sporadic, low-density migrations that produced cyclical and discontinuous occupations of the island. From the Eneolithic/Copper Age onward, there was a slow increase in both frequency and density of migratory flows, continuing through the Bronze and Iron Ages. Although these demic migrations produced some population discontinuity, the concentric arrangement indicates a limited but constant degree of admixture among the mentioned groups. This implies no significant morphometric variation between Prehistory and Protohistory populations. Finally, these dynamics produced a not negligible allometry supported by the one-way ANOVA tests, that both returned p (same) values < 0.001 that rejects the H0.

Although some of our landmarks (such as glabella or inion) involves sexually dimorphic cranial areas males and females have not influenced the scatterplots. Indeed, no differences were observed between groups from all sites. Male and female individuals from the same site are often very close to each other (Galland et al. 2019). When evaluating the variation between human groups arriving to a localized geographical region (like an island), it is important to consider that the genetic pool is often stressed by genetic drift phenomena such as the bottleneck and founder effect (Manica et a. 2007). In addition to these stochastic forces, adaptive changes (such as the masticatory-inducted phenotype) are in parallel impacted by cultural variations with the same plasticity but with a slow degree of diversification (Harvati and Weaver 2006). In particular, patterns of the cranial vault and the upper face are evolving largely neutrally (Smith 2011). Nevertheless, the large differentiation of facial shapes during the centuries could not only be explained by adaptive changes but also by the arrival of new genetic components (Betti et al. 2009).

Conclusion

According to the paleoclimatic data (during the last glacial peak, Sicily was characterized by a steppe or semi-steppe environment and extremely low rainfall values) (Incarbona et al. 2010a, b; Sadori et al. 2008) a stable occupation by *Homo sapiens* of the island was possible not before the Upper–Paleolithic. Right in that period early migratory flows arrived from the continent on the northwest coast and continued exclusively along the northern coastline, moving east to west in a counterclockwise direction. PCA analyses show that, during the Sicilian Stone Age, the hunter-gatherer's colonizers cyclically occupied the land establishing settlements in the proximity of caves, close to the coastlines. Moreover, the scatterplot displays that only during the transition to a mobile-forager/semi-sedentary ecology during protohistoric period allowed a gradual increase in the frequency and density of migratory flows. Although the degree of admixture was limited, semi-migratory farmers-shepherds began abandoning caves for small villages. This shift marked the start of the colonization of the southeast cost and hinterlands, a process that was only completed in the first historical periods with Greek and Punic colonization (700 B.C.E.).

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Conflicts of interest

The authors declare that they have no conflict of interest and no competing interests.

Ethics statement

The interpretations reported in the present study are based on the analysis of skeletal findings obtained through excavations and authorized by institutional permits.

Statement of contributions from authors

Conceptualization and Investigation: G.L. and L.S.; Methodology and Software: G.L.; Formal Analysis and Data Curation: G.L.; Data Interpretation, Writing and Editing: G.L. and L.S.; Supervision, Funding acquisition and Project administration: L.S.

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Eruption of Permanent Teeth in Bulgarian Children Aged 5–12 Years

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Abstract: The time of eruption and the number of permanent teeth together with the time of sexual maturation and ossification of the skeleton are important indicators of the biological maturity and health of children. The aim is to evaluate the eruption of permanent teeth in Bulgarian children aged 5–12 years and to assess its relationship with age and sex. The present cross-sectional study included 709 individuals from 5 to 12 years. The oral and dental status was checked and included the number of erupted teeth (NET). Statistical analyses were performed to compare the sexes and ages. Statistically significant differences between sexes are observed only in teeth 17, 27, 33 and 43. The sequence of tooth eruption was examined, and it is almost identical in male and female subadults. Mandibular teeth erupt earlier than maxillary teeth, excluding first premolars for both sexes and canines in males. Females have earlier tooth eruption and more permanent teeth compared to males. A significant association between age and NET is established. Sex does not have a significant effect on tooth eruption. The time of eruption of permanent teeth is influenced significantly by age, but not by sex in our sample. Differences between males and females are found in the sequence of eruption only of canines and premolars. Females tend to have earlier tooth eruption and more erupted teeth. Lower teeth erupt earlier than upper teeth, excluding first premolars in both sexes and canines in males.

Keywords: children, tooth eruption, sequence, sex differences, age differences

Original article

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Introduction

The time of eruption and the number of permanent teeth (NET) together with the time of sexual maturation and ossification of the skeleton are important indicators of the biological maturity and health of children (Nikolova et al. 2023). Tooth eruption is a long physiological process during which the forming tooth penetrates the oral mucosa, and the incisal edge or tip of a cusp is seen in the oral cavity (Peneva et al. 2009:141–2). As an indicator of the development of the maxillofacial complex, tooth eruption is used in dentistry to evaluate disturbances in the process of eruption and to determine measures and prepare plans for treatment and optimize children's oral health (Leroy et al. 2003; Wright 2010; Kutesa et al. 2013).

In forensic odontology and paleoanthropology, the formation and eruption of teeth are used to identify human remains and to estimate age and diet of individuals. Factors which are used for determination of dental age are degrees of formation of unerupted teeth, clinical eruption, degree of completion of roots of erupted teeth, degree of resorption of deciduous teeth, which are all part of the process of tooth eruption (Jayakrishnan et al. 2021). Several anthropological studies have shown that the time of eruption of permanent teeth varies between different populations. A lot of factors influence this process, including genetics, hormones, age, sex, ethnicity, socioeconomic status, nutrition, craniofacial morphology, pathology (e.g., Almonaitiene et al. 2010; Kutesa et al. 2013; Kurosaka et al. 2022:159–164).

Eruption of permanent teeth follows a specific sequence, and it is related to the age of the individual. It is clinically observed at the age of 6 and ends around 12 years of age for permanent teeth (Peneva et al. 2009; Paz-Cortés et al. 2022). In Bulgaria, there are few studies investigating the time and sequence of dental eruption and its relationships with different factors (Peneva 1978; Mladenova 2003; Tineshev 2009). The importance of the present study is to provide new data on the mean age and sequence of eruption of permanent teeth in Bulgarian children. The aim is to evaluate the eruption of permanent teeth in Bulgarian children aged 5–12 years and to assess its relationship with age and sex.

Materials and methods

A total of 709 children from 4 kindergartens and 11 schools, were investigated during the period 2020–2023 by the first author. This study received ethics approval by the Ethical Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences (Protocol 10/08.07.2020). Before starting the examination, information about the purpose of the study was given and written informed consent was obtained. The study was conducted in accordance with the principles of the Declaration of Helsinki for human studies (World Medical Association 2008) (see details in the Ethics Statement below). The individuals/participants were randomly selected according to the following criteria:

- Inclusion criteria: Children aged 5–12 years in good physical and psychological health; informed written consent given by parents or guardians.
- Exclusion criteria: children with systemic and chronic diseases and lack of age data.

The examination was performed in the medical rooms in the schools and kindergartens. The oral and dental status was checked directly by intraoral examination with a dental mirror and a probe under artificial light. Permanent teeth were categorized into two grades: 1 – erupted and 0 – unerupted, and all erupted teeth were assessed for each child. The tooth was registered as erupted when any part of the clinical crown was present inside the oral cavity. The number of erupted permanent teeth was evaluated for the studied age groups.

Data were analyzed using Statistical Package for Social Sciences (SPSS Inc, version 16.0 for Windows, IBM Corp Chicago, IL, USA). Pearson Chi-square test, Cramer's V and Odds ratio were used to study the association between age, sex and eruption of permanent teeth. Binary Logistic Regression also was performed. The Student's t-test and One-Way ANOVA (with post hoc Scheffe multiple comparison procedures) were applied to estimate differences between sexes and age groups. The differences were considered statistically significant at $p<0.05$.

Results

The present cross-sectional study included 361 (50.9 %) male and 348 (49.1 %) female subadults. The examined participants did not differ significantly in mean age (males – 8.79 (± 2.20) and females – 8.68 (± 2.20) years, $p>0.05$). The average age of tooth eruption is presented on Table 1. Statistically significant differences between sexes are observed only in teeth 17, 27, 33 and 43 /upper second molars and lower canines/, which erupted earlier in females ($p < 0.05$).

Table 1. Mean age of tooth eruption by sex and jaw

Males			Females		**T**-test (p value) ♂/♀
		Maxilla			
Right (I)	Left (II)	Tooth	Right (I)	Left (II)	
6.53	6.67	**1/I^1**	6.64	6.64	
7.38	7.49	**2/ I^2**	7.34	7.35	
10.18	10.26	**3/ C**	10.36	10.45	
9.22	9.37	**4/ P^1**	9.37	9.37	**17** – .039*; **27** – .023*
10.29	10.38	**5/ P^2**	10.32	10.27	
6.45	6.47	**6/ M^1**	6.50	6.48	
12.09	12.20	**7/ M^2**	11.89	11.84	
		Mandibula			
Right (IV)	Left (III)	Tooth	Right (IV)	Left (III)	
6.37	6.36	**1/I_1**	6.27	6.28	
7.38	7.36	**2/ I_2**	7.30	7.29	
10.24	10.39	**3/ C**	10.15	10.11	
9.31	9.44	**4/ P_1**	9.38	9.38	**33** – .027*; **43** – .004*
10.14	10.22	**5/ P_2**	10.30	10.32	
6.40	6.38	**6/ M_1**	6.42	6.37	
11.87	11.82	**7/ M_2**	11.76	11.73	

Level of significance: * $p<0.05$

The sequence of tooth eruption is almost identical in male and female individuals and is as follows:

In males: I_1, M_1, M^1, I^1, I_2, I^2, P^1, P_1, P_2, C^1, C_1, P^2, M_2, M^2

In females: I_1, M_1, M^1, I^1, I_2, I^2, P^1, P_1, C_1, P_2, P^2, C^1, M_2, M^2

Mandibular teeth erupt earlier than the maxillary teeth, excluding first premolars for both sexes and canines in males. Differences are observed only in the eruption of the premolars and canines. In males, teeth 35 and 45 erupt before the canines followed by upper premolars, while in females, teeth 33 and 43 erupt before the premolars followed by upper canines.

During the studied age period females have earlier tooth eruption and more permanent teeth compared to males. The mean number of erupted teeth varied between 1.36 (± 1.27) at the age of 5 and 26.03 (± 2.41) at the age of 12. In males the mean number of erupted teeth is lower and varied between 1.84 (± 2.84) and 25.60 (± 3.4). A significantly higher NET in females is observed in the age groups 7, 10 and 11 years (Fig. 1).

A significant association between age and NET is established ($\chi^2(7, N = 709) = 352.08$, $p < 0.0001$, Cramer's V = 0.705,). This is the reason to consider that age is an important factor in tooth eruption. The results from logistic regression model show that older age has a significant role in tooth eruption (OR=14.420, $p < 0.0001$, 95 % CI (7.583–27.422)). For every year increase in age the odds are 14.4 times larger.

Unlike age, sex does not significantly affect tooth eruption ($\chi^2(1, N = 709) = 0.039$, $p = 0.843$, Cramer's V = 0.007). The null hypothesis that the two variables are independent of each other cannot be rejected. The females and males seem to have nearly equal odds (OR = 1.05) of erupted teeth (Table 2).

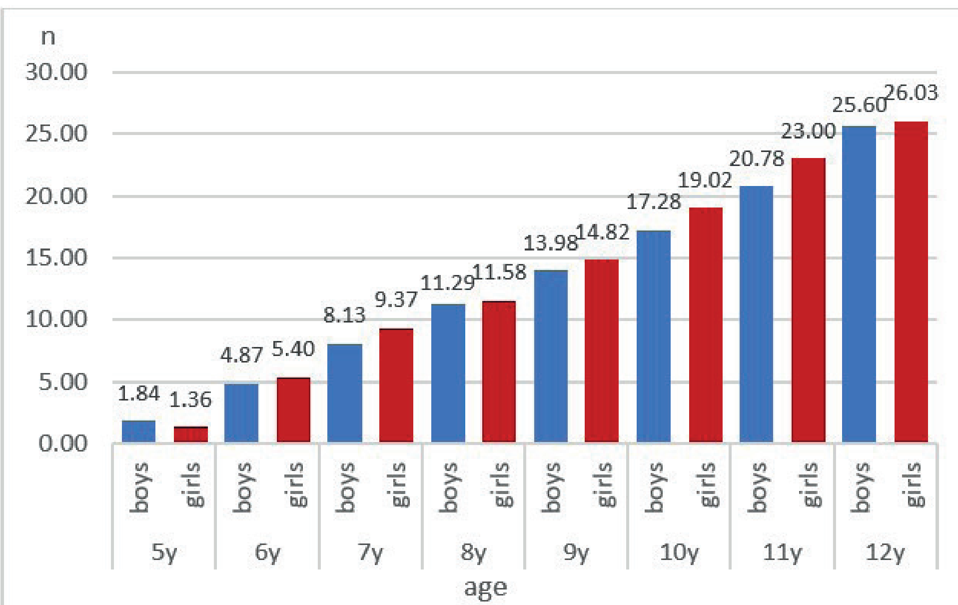

Fig. 1. Number of erupted teeth according to age and sex

Table 2. Percentage of erupted and unerupted teeth in 5–12-year-old children

			Permanent teeth		Total
			Un-erupted	Erupted	
Sex	Males	n	39	322	361
		%	10.8%	89.2%	100.0%
	Females	n	36	312	348
		%	10.3%	89.7%	100.0%
Total		n	75	634	709
		%	10.6%	89.4%	100.0%

Discussion

Tooth eruption is an essential part of children's growth and development. It is genetically determined and X chromosome linked inheritance. Reduplication in the X chromosome in females can be associate with higher intra individual correlations between factors of tooth eruption (Garn et al. 1965).

Some authors have established earlier tooth eruption and more erupted teeth in females at ages 6 to 14 in comparison to males (Amidu et al. 2013; Sheetal et al. 2013:178–80; Öznurhan et al. 2016; Hassan and Shahid 2018). In our study the average age of tooth eruption is almost identical for both sexes, but eruption in females started earlier than in males. Our data show that the first teeth to erupt in both sexes are the lower central incisors (31, 41). The biggest difference, between the two sexes is found in canines – 4 to 6 months earlier eruption in females. Bayrak et al. (2012) present results that show difference in the eruption times between males and females up to 18 months. In the study conducted by Wedl (2004) the eruption times of the incisors in both sexes are lower compared to our results, but all other examined teeth have higher mean age of eruption. Kapusi-Papp et al. (2023) also found statistically significant earlier mean eruption times for all teeth in females than in males, except for the mandibular central incisors. The difference varies from 1.9 up to 8.9 months. According to Eskeli et al. (2016) and Šindelářová et al. (2018) there were significant differences in the eruption status between both sexes in Finland and Czech populations, respectively. Rehmawati et al. (2022) established differences between males and females only in the eruption of teeth 16, 26, 36 and 46. Earlier physical development in females is a factor that determines their earlier tooth eruption (Paz-Cortés et al. 2022). Expression of genes coded in the Y-chromosome is believed to be connected with delayed skeletal maturity and tooth eruption in males (Nassif and Sfeir 2020).

The first clinically visible stage of the process of eruption is the penetration through the oral mucosa of the tip of the dental crown and is associated with the formation of enamel and dentin layers (Kutesa et al. 2013).

Aris et al. (2020) examined daily secretion rates (DSRs) of enamel in anterior teeth in males and females from ancient and modern British populations. They did not find differences in enamel DSRs between male and female groups, but comparing the different British populations the authors observed significantly delayed enamel DSRs in the modern-day populations compared to ancient ones. Those results are leading to the conclusion that the process of forming the enamel is not determined by sex differences in tooth eruption and sequence of eruption. Aris (2022) also compared enamel DSRs in anterior and posterior teeth and did not find any trend in which teeth tend to grow faster.

The timing of tooth eruption varies among different populations. Baka pygmy individuals are found to have the earliest tooth eruption compared to other human populations (Ramirez Rozzi 2016). Olze et al. (2007) in their study compared eruption of wisdom teeth in individuals from different ethnicities. They found that black South African population are reaching eruption stages faster than the other examined populations and Japanese are the slowest. Examining the mean eruption times in males and females aged 4–15 years from Uganda Kutesa et al. (2013) established that the values were comparable to Ghanaian and Nigerian children but lower than these of subadults from Belgium, USA, Australia, Iran and Pakistan.

Differences between sexes are also observed in the sequence of dental eruption. Females, more frequently than males, are found to have the most common sequence of eruption (Suri et al. 2004; Peneva et al. 2009; Arid et al. 2017; Reis et al. 2021; Kurosaka et al. 2022), in which lower teeth erupt earlier than the upper ones (Mladenova 2003; Amidu et al. 2013; Nikolova et al. 2023). Our results show that the eruption in both sexes starts with the lower central incisors, after them lower first molars erupt, upper first molars, upper central incisors, lower lateral incisors, upper lateral incisors, upper first premolar, lower fist premolar. Difference is observed in the sequence of eruption of second premolars and canines – for males the order of eruption is: lower second premolars, upper canines, lower canines, upper second premolars and for females – lower canines, lower second premolars, upper second premolars and upper canines. After that, tooth eruption is similar for both sexes and continues with lower second molars and upper second premolars. For both females and males is valid eruption firstly of the lower teeth and after them the upper, with the exclusion of first premolars for both sexes and canines in males which erupt firstly in upper jaw.

Our results contrast with the studies by Peneva (1978), Mladenova (2003) and Nikolova et al. (2023) on Bulgarian children. According to these authors the first teeth to erupt are the lower first molars followed by the incisors. Other differences are established in the eruption of the premolars and canines. The sequence described by Peneva (1978) for males is: lower first premolars, lower canines, upper canines, upper second premolars, lower second premolars and for females it is: lower canines, lower first premolars, upper second premolars, upper canines, lower second premolars, which quite differs from our results. Earlier tooth eruption in the lower jaw, excluding premolars, is found by Peneva (1978). This conclusion is based on the earlier development of lower tooth germs and the more active mandible in the process of mastication. Our results for the sequence of eruption of the permanent teeth are similar to results presented by Oznurhan et al (2016) – the difference is in upper canines, which erupt later than second premolar in males and lower canines which erupt earlier than the first and second premolar. Almost identical difference is found in the study done by Nassif and Sfeir (2020). In their study difference between the sexes is not presented and again upper canines erupt later than premolars and lower canines. A similar sequence is found in the study made by Bayrak et al. (2012) and Kapusi-Papp et al. (2023) who reported that the lower canine erupts earlier than the lower premolar. In their study, no difference is established between the sexes in eruption of permanent teeth of lower jaw.

Conclusion

The time of eruption of permanent teeth is influenced significantly by age, but not by sex. Differences between males and females are found in the sequence of eruption only of canines and premolars. Females tend to have earlier tooth eruption and more erupted teeth during the age period 5–12 years. Lower teeth are erupting earlier than the upper, excluding first premolars for both sexes and canines in males.

The results present contemporary view of tooth eruption in Bulgarian children, which can be used from a variety of clinicians such as orthodontists, pediatric dentists, pediatricians and forensic pathologists.

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Conflict of interests

No conflict of interest is declared by the authors.

Ethics approval

The study was approved by the Ethical Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences (Protocol 10/08.07.2020). Before starting the examination, information about the purpose of the study was given and written informed consent was obtained. The study was conducted in accordance with the principles of the Declaration of Helsinki for human studies (World Medical Association 2008).

Author contributions statement

BK and IYP design the study; BK and IYP collected the data; BK, IYP, YZ oversaw the statistical analysis and interpretation; BK, IYP and YZ were the authors of the written content; All authors agree to be accountable for all aspects of the work.

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Cardiovascular Risk Factors at Different Stages of Menopause: A Study among Bengali-Speaking Hindu Ethnic Group, India

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Abstract: A reduction in the estrogen and progesterone levels during midlife leads to adverse changes in body fat distribution, insulin and lipid metabolism, and endothelial dysfunction; all of these increase the risk of cardiovascular disease (CVD). However, scholars are not unanimous on whether menopause enhances the CVD risk, independent of the normal process of aging and other confounding factors. Despite the cardio-protective effect of endogenous estrogen during their premenopausal years, the increased life expectancy of women exposes them to a greater lifetime risk of CVD compared to men. The aim of the present study was to understand the cardiovascular risk factors associated with different stages of menopause. This study was cross-sectional in nature and was carried out in the Howrah district of West Bengal, India. Two hundred and one participants were recruited for the study (Premenopausal 71, Perimenopausal 61, and Postmenopausal 69). Data on body fat distribution, blood glucose levels, and total cholesterol, blood pressure, and socio-demographic, menstrual and reproductive history, and lifestyle characteristics were obtained following standard protocols. Multivariate analysis of covariance was performed to understand how menopausal status impacted CVD risk factors after controlling the effects of the confounders. Menopausal status significantly predicted the CVD risk factors and body fat measures after removing the effects of the confounding variables, reinforcing the role of estrogen in the development of CVD. An overwhelming majority of the participants in our study have central obesity; so, this group is more prone to developing CVD in the near future. Future cross-cultural studies are required to understand how the link between menopause and CVD varies across different cultural groups and throughout the menopausal transition.

Keywords: menopause, midlife health, CVD, blood glucose level, body fat distribution

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Introduction

Menopause signifies the end of the reproductive phase and the initiation of the post-reproductive phase of a woman's life. An estimated 1.5 million women go through the menopausal transition (MT) each year (Santoro et al. 2005). The phases of MT and postmenopause have a significant impact on women's midlife health. During this menopausal transition, most women passed through some long-term and short-term adverse physiological changes owing to the decline in the sex hormone levels (Carr 2003). The short-term changes may involve the experience of menopausal symptoms, and the long-term changes include the occurrence of cardiovascular risk factors (Matthews et al. 2009). Worldwide, cardiovascular diseases (CVD) are emerging as the primary cause of death, especially among those women living in middle and low-income countries (Matthews et al. 2009). Studies show that women have a reduced risk for CVD than men before the fifth decade of life. After reaching menopause, this gender gap declines, making women more susceptible to CVD risk factors (Satyavani et al. 2015). For instance, menopause results in some adverse biological changes like, redistribution of body fat from periphery to the center, impaired glucose and insulin metabolism, dyslipidemia, fibrinolysis, coagulation, vascular endothelial dysfunction, and low bone mineral density (Carr 2003).The receptors of estrogen are present in the myocardium, tissues of vascular smooth muscle, endothelium, and in coronary arteries and function as a cardio-protective hormone with both long and short-term benefits on the CVD system (Matthews et al. 2009). Furthermore, estrogen helps to maintain a favorable lipid level, changes the vascular tone, protects the vascular endothelium from the adverse impacts of low-density lipoprotein cholesterol (LDL) by inhibiting the oxidation of LDL, and stabilizes the endothelial cells (Inaraja et al. 2020). All these are the cardio-protective mechanisms of estrogen which is reduced during and after menopause. Thus, the postmenopausal years are linked to cardiovascular health concerns like diabetes, hypertension, dyslipidemia, heart disease, and osteoporosis (Carr 2003; Inaraja et al. 2020). According to the Framingham Heart Study, women with natural menopause (aged 50–59 years) are four times more likely to develop CVD than the age-matched premenopausal women but the result was unadjusted for age and smoking (Lisabeth et al. 2009). Previous cohort studies, including SWAN (El Khoudar et al. 2017), the Melbourne Women's Midlife Health Project (Burger et al. 2007), the Healthy Women Study (Davis et al. 2012), the Penn Ovarian Aging Study (Freeman et al. 2016), and the Seattle Women's Health Study (Thomas et al. 2018) indicated a significant relationship between menopause and CVD risk factors independent of aging; but the trend is not uniform across other studies (Casiglia et al. 2008; Lisabeth et al. 2009; Trikudanathan 2013). Scholars are not unanimous on whether menopause independently enhances the risk of CVD, separate from the normal process of aging (Pardhe et al. 2017). Thus, the question remains unresolved whether natural menopause serves as an independent predictor of CVD across all populations.

Studies in various middle-income countries of the world, including India show that women reach menopause at an advanced age than the developed countries (Syamala and Sivakami 2005). Despite the cardio-protective effect of endogenous estrogen during their premenopausal years, the increased life expectancy of women exposes them to a greater lifetime risk of CVD compared to men (Carr 2003). Thus, the contribution of estrogen deficiency in the occurrence of CVD in women is emerging as a conceivable therapeutic challenge of the present century. Most of the Indian studies related to menopausal women were aimed at understanding the attitude and perception of women regarding menopause, menopausal symptoms, and age at menopause (Dasgupta and Ray 2015; Mozumdar and Aggarwal 2015). Despite few studies (Nag and Ghosh 2013) there is a dearth of data on the relationship between CVD risk factors and menopausal status among Indian populations.

We aimed to compare CVD risk factors and body fat patterning of women of differential menopausal stages and to understand the link between menopausal status and CVD risk factors, after controlling the effects for confounding variables.

Materials and methods

Study area

The study was carried out among the Hindu Bengali-speaking populations of the state of West Bengal, India. Data for this cross-sectional study were collected from the city of Howrah, which is an urban agglomerate of Howrah district of West Bengal, India. The participants were recruited from four municipal wards (namely, ward numbers 42, 44, 47, and 48) of the Howrah Municipal Corporation of Howrah *Sadar* Subdivision. These municipal wards have been selected for operational convenience.

Study participants

Initially, a total number of 300 women were approached for this study based on certain inclusion criteria. The criteria were as follows: have attained natural menopause (in the case of postmenopausal participants), married with at least one surviving child, and have no documented history of any kind of metabolic diseases. A total number of 99 women were eliminated from the study, either because they did not fit into the inclusion criteria (n=50) or they denied participating (n=49). Women who reached menopause surgically (n=15), or who were on medications (n=35) for any metabolic disorders were excluded. Most of the participants who denied participating in the study was reluctant to participate when they learned that the study involves the collection of blood samples. Unmarried and nulliparous women were removed to confirm that all the women had experienced some specific reproductive processes (contraception use, pregnancy, parity, and lactation). The hormonal modifications associated with these reproductive events are found to have an impact on menopause as well as CVD (Patchen et al. 2017). Finally, a total of 201 participants were incorporated in this study (71 premenopausal, 61 perimenopausal, and 69 postmenopausal). The criteria of Stages of Reproductive Aging Workshop (STRAW) were followed to categorize the participants into differential menopausal stages (Soules et al. 2001). Participants who had experienced regular menstrual bleeding during the past three months and were not experiencing any irregularities were considered premenopausal. Participants whose menstruation had stopped for more than three months but less than twelve months and were experiencing irregular period during the last twelve months were considered perimenopausal. Participants who reported that their bleeding has ceased spontaneously for the last twelve months were considered postmenopausal. The purpose of the research was conveyed to the participants, and they provided written informed consent. The study was approved by the Institutional Human Ethical committee, University of Calcutta (protocol 06/WT/19-20/1763).

Data collection

A structured schedule that was previously applied in the same population was used to obtain data on socio-demographic, menstrual, reproductive, and lifestyle characteristics (Dasgupta and Ray 2015). Data related to socio-demographic characteristics involve participants age at the time of data collection (years), occupational types and educational attainment of participants and their spouses, and monthly per capita household expenditure (in Indian rupees). Reproductive and menstrual history of the participants include age at menarche (years) and age at marriage (years), history of pregnancy and breastfeeding (for the last child), and contraceptive use. The participants were asked to report the actual date when they experienced menarche, or the closest month of the event. Some of the participants could not recall their age at menarche. The first author was required to provide some hint on specific personal events (like her own birthday or academic standards) that occurred around the time of her menarche. This approach helped the participants to report the date of their menarche accurately. Age at marriage was cross-checked by participant's husbands and/or mothers. For postmenopausal participants, they were asked to report how many years prior to the date of the interview their menstrual bleeding had ceased. The first author provided some hints in relation to some personal occasions, like the birthday of her grandchildren or any other specific event that happened around the time of her last menstruation. This helped the participants to recall the time of their final menstrual bleeding (Kar and Roy 2023). Finally, the participant's age at the time of the interview was subtracted from the number of years following menopause to determine the age at menopause. Pregnancy records include age at first and last pregnancy and total number of pregnancies (live births, stillbirths, and miscarriages). The above-mentioned methodology has been used in our previously published study investigating the same population but addressing different research questions (Kar and Roy 2023).

A pretested food frequency schedule was used to obtain data on the food consumption patterns of the participants (Harmouche-Karaki et al. 2020). The schedule consists of a total of eighteen items that are generally consumed by the Bengali Hindu populations and are available in the study area. The participants were asked to indicate how frequently they consumed these food items in the past week before the date of the interview. Every food item was divided into eight response groups, with zero denoting never and seven denoting every day of the week. Finally, three categories of food consumption pattern were created- regularly (consumption of 5–7 days), occasionally (consumption of 2–4 days) and rarely or never (never or consumption of less than two days).

A pretested physical activity schedule was used to obtain data on the physical activity levels of the participants (Rääsk et al. 2017). The schedule consists of the physical activities of the participants about their daily activities, like cooking, mopping, dusting, ironing, washing clothing, washing dishes, watching television, listening to music, bicycling, exercising, walking, and creating handicrafts. The participants were asked to indicate how many days they got involved in these activities in a typical week and also how much time (in minutes) on a typical day they spent on these activities. Later, we used the International Physical Activity Questionnaire (IPAQ) to determine the Metabolic Equivalent (MET) score (Rääsk et al. 2017). Metabolic Equivalent minutes per week served as a measure of the degree of physical activity. Metabolic Equivalent minutes exhibited how much energy was required to perform a particular physical activity. The following scores were given for particular physical activities: moderate activities were given 4 METS, walking was given 3.3 METS, vigorous activities were given 8 METS, and sedentary activities (watching television, listening to music, etc.) were given 1 MET. We estimated MET minutes per week by multiplying the provided MET score by the number of minutes the activity was performed in a typical day and by the number of days the activity was carried out in a particular week. For instance, if a participant performed dusting for 20 minutes (in a typical day) for 4 days in a typical week, then the MET score would be 4 (that is the assigned MET score for household chores) × 20×4= 320 MET. Finally, to obtain a total MET score of a typical week, we added the MET scores of each physical activity category (moderate, sedentary, and vigorous activities).

Total cholesterol (TC) (mg/dl) and random blood glucose levels (mg/dl) were assessed for each participant. The use of random blood glucose testing to estimate diabetes has certain drawbacks. The guidelines of International Diabetes Federation have suggested testing for participants with random glucose values ≥ 200 mg/dl (Engelgau et al. 2010). Accu-check active blood glucose monitoring kit (Model no. GB10803608) and multi-care-in meter (Model no. IN2140129) were used for estimating blood glucose and TC levels, respectively. We collected blood samples from the tip of the left hand's second finger. An automatic blood pressure monitor (Omron's blood pressure monitor, Model no. HEM-7121) was used to measure the blood pressure level (mmHg) of the participants. After a 10-minute interval, two independent blood pressure readings were obtained, and the average was calculated (Kar and Roy 2023). Each participant's mean arterial pressure (MAP) was determined using the formula MAP= SBP+2(DBP)/3.

Omron's Body Composition Monitor (Model No. HBF-362) was used to measure body weight (to the nearest 0.1 kg), skeletal and subcutaneous fat for the whole body and trunk, visceral fat, and percent body fat (PBF) for each participant who were dressed in minimal clothing and without shoes. The Rossmax Body Fat Monitor was employed to assess the muscle mass content of the body (Model no. WF260). To confirm that the instruments were reliable, each measurement was obtained twice.

A portable GPM anthropometer was used to assess height (to the nearest 0.1 cm) for each participant standing on a horizontal plane without shoes (Lohman et al. 1988). Waist circumference (WC) was determined at the minimum circumference of the torso between the rib cage and the iliac crest with a fiberglass insertion tape over minimal clothing. Hip circumference (HC) was assessed horizontally at the widest extension of the hips around the buttocks. Some of the anthropometric indices such as fat mass (FM), fat-free mass (FFM), and waist-hip ratio (WHR) were assessed following standard formulae:

WHR=WC (cm)/HC (cm)

FM=PBF/100 × Weight (kg)

FFM=weight-FM

The study was undertaken during the time period of February 2021 to June 2022.

Statistical analysis

Descriptive statistics were performed to examine the distribution of socio-demographic, reproductive, and menstrual characteristics, food consumption patterns, physical activities, TC level, blood glucose and blood pressure levels, and body fat pattern (skeletal and subcutaneous fat of the whole body and trunk, PBF, WC, HC, visceral fat, WHR, FM, FFM, and muscle mass) of the participants. The Kolmogorov-Smirnov test was applied to check the normality of each variable. We applied the analysis of variance (ANOVA) test to compare the variables of CVD risk factors (total cholesterol, blood glucose, and blood pressure levels) and body fat content across the menopausal groups. A post-hoc test (Scheffe's test) was employed to find out the differences between each menopausal group. The Kruskal-Wallis test was used as an alternative to ANOVA for variables that did not adhere to the normal distribution. Multiple linear regression analysis (stepwise) was employed to identify the predictors of CVD risk factors and body fat patterning. The variables that showed significant differences among the three menopausal groups were incorporated as dependent variables, while socio-demographic, menstrual, and reproductive characteristics, food habits, and physical activities were incorporated as independent variables. The independent variables collinearity was also determined. Multivariate Analysis of Covariance (MANCOVA) was applied to understand how menopausal status impacted CVD risk factors after eliminating the impacts of the confounding variables (socio-demographic, reproductive, and lifestyle variables). Menopausal status was incorporated as the grouping variable in MANCOVA. CVD risk factors and body fat measures that differed significantly across the groups in ANOVA (FFM, FM, HC, WHR, PBF, whole body, trunk and leg skeletal fat, and muscle mass, blood pressure, and blood glucose levels) were included as dependent variables. Homogeneity of variances and covariances was checked by performing Box's M test. Mahalanobis distance was calculated to find out the multivariate outliers in the groups of independent variables in the context of each dependent variable. The variables that appeared as significant predictors of body fat measures and CVD risk factors in multiple linear regression were included as confounding variables. Chi-square test was applied to examine the distribution of CVD risk factors across the menopausal groups. The Asian-specific (WHO 2008) cut-off for women has been employed to evaluate the prevalence of CVD risk factors. A WC of >88cm was considered to be in the risk category, whereas a WHR of ≥0.85 was assessed to be in the risk category. Those with blood glucose level of <200 mg/dl were classified in the non-diabetic category, and those with a level of ≥200mg/dl were classified as diabetic; A TC level of ≥240 mg/dl was determined as a high cholesterol level, whereas a TC level of <240 mg/dl was assessed as a normal level; SBP value of ≥140 mmHg and DBP value of ≥90 mmHg and MAP value of ≥100 were marked as the hypertensive category (WHO 2008). A minimum 'p' value of 0.05 was determined to be a statistically significant level for all inferential statistics. The whole data was assessed with the help of statistical package for social science version 20.0 (IBM Corporation 2011).

Results

Table 1 shows that the participant's age reported at the time of the interview differed significantly across the menopausal groups. The majority the postmenopausal participants were homemakers (68.2%); while a majority of the participants from the premenopausal group were working (46.4%), followed by the perimenopausal participants (44.2%). Table 1 further shows that the majority of the participants and their spouses have completed secondary level education irrespective of their menopausal status (Table 1).

Table 1. Socio-demographic characteristics of the participants (n=201)

Socio-demographic variables	Pre (n=71)	Peri (n=61)	Post (n= 69)	F value/Kruskal-Wallis test/ Chi square test/ Fisher's exact test	p value
Participant's age (years) at the time of interview(mean±SD)	32.28±4.57	42.34±4.34	53.12±7.48	235.01	0.01
Participant's working status				3.52	0.17
Working	33(46.4)	27 (44.2)	22 (31.8)		
Homemaker	38 (53.6)	34(55.8)	47 (68.2)		
Occupational categories of the spouses					
Service	56 (78.9)	49 (80.3)	49 (71.0)	3.57	0.46
Business	13 (18.3)	8 (13.1)	14 (20.3)		
Others*	2 (2.8)	4 (6.6)	6 (8.7)		
Educational categories of the participants					
Non-literate	–	4 (6.6)	7 (10.1)		
Primary	7 (9.9)	9 (14.8)	7 (10.1)	–	–
Secondary	34 (47.9)	31 (50.8)	35 (50.7)		
Higher-secondary	12 (16.9)	7 (11.5)	10 (14.5)		
Graduate and above	18 (25.4)	10 (16.4)	10 (14.5)		
Educational categories of the spouses					
Non-literate	–	4 (6.6)	–		
Primary	8 (11.3)	14 (23.0)	12 (17.4)	–	–
Secondary	31 (43.7)	24 (39.3)	41 (59.4)		
Higher-secondary	12 (16.9)	7 (11.5)	4 (5.8)		
Graduate and above	20 (28.2)	12 (19.7)	12 (17.4)		
Monthly household expenditure (per capita) (INR)	96.39**	97.51	108.83	1.93	0.38

*Others: e.g., non-working, retired, labor; **mean rank; Figures in the parenthesis represent percentage values

Table 2 shows that the age at first and last pregnancies, age at menarche and marriage, and the duration of breastfeeding did not differ significantly across the menopausal groups, while the number of total pregnancies and duration of oral-contraceptive use differed significantly across the menopausal groups. Parity appeared to be significantly higher among the postmenopausal participants (53.6%), followed by the perimenopausal participants (44.3%). The table further shows that the majority of the participants did not experience any fetal loss irrespective of their menopausal status. The majority of the participants from the postmenopausal group (69.6%) did not use any kind of contraceptive (Table 2).

Table 2. Reproductive and menstrual characteristics of the participants (n=201)

Reproductive and menstrual variables	Pre (n=71)	Peri (n=61)	Post (n= 69)	F value/Kruskal-Wallis test/ Chi square test/ Fisher's exact test	p value
Age at menarche (years) (mean±SD)	12.00±1.39	13.00±1.44	12.00±1.85	1.87	0.15
Age at marriage (years) (mean±SD)	18.00±3.44	19.00±4.17	18.00±4.77	0.12	0.89
Age at first pregnancy (years) (mean±SD)	20.68±3.55	20.00±4.43	20.00±4.99	0.28	0.75
Age at last pregnancy (years) (mean±SD)	27.17±4.31	26.67±4.76	26.45±5.04	0.28	0.76
Number of pregnancies					
One	31 (43.6)	16 (26.2)	11(15.9)	19.09	0.01
Two	25 (35.2)	18 (29.5)	21(30.4)		
More than two	15 (21.2)	27(44.3)	37 (53.6)		
Ever experienced fetal loss				0.18	0.91
Yes	12 (16.9)	9 (14.8)	10 (14.5)		
No	59 (83.1)	52 (85.2)	59 (85.5)		
Duration of breastfeeding(month)	99.15*	103.50	100.70	0.18	0.91
Ever use of contraceptive					
Yes	43 (60.6)	30 (49.2)	21 (30.4)	12.96	0.001
No	28 (39.4)	31 (50.8)	48 (69.6)		
Duration of OCP use (month)	111.40*	103.77	87.85	7.30	0.02

*Mean rank

Table 3 shows that barring regular consumption of soya products, meat, and aerated drinks, the majority of the participants reported having consumed cereals, pulses, green leafy vegetables, roots and tubers, fish, snacks, and tea on a regular basis irrespective of their menopausal status. Further, it appears that the participants differed significantly in roots and tubers, sweets, and egg consumption patterns across the menopausal groups. The participants differed significantly in moderate (household chores) and sedentary activities. Sedentary activities were found to be higher among the premenopausal participants followed by the postmenopausal participants. The participants differed significantly in the frequency of outside meal consumption. The majority of the participants from the premenopausal group (64.8%) reported consuming outside meals followed by the postmenopausal participants (49.3%). It also appears that 15.9% of the participants from the postmenopausal group reported having chewed tobacco on a daily basis followed by the premenopausal participants (9.9%). None of the participants reported consuming alcohol (Table 3).

Table 3. Food consumption pattern and physical activity level of the participants (n=201) (recall period based on the last seven days)

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of cereals					
Everyday/Regularly	71 (100)	60 (98.4)	69 (100)	–	–
Occasionally	–	1 (1.6)	–		
Never/Rarely	–	–	–		
Consumption of pulses					
Everyday/Regularly	43 (60.6)	41 (67.2)	39 (56.5)	3.75	0.43
Occasionally	7 (9.9)	2 (3.3)	8 (11.6)		
Never/Rarely	21 (29.6)	18 (29.5)	22 (31.9)		
Consumption of green vegetables					
Everyday/Regularly	68 (95.8)	56 (91.8)	63 (91.3)	–	–
Occasionally	–	3 (4.9)	2 (2.9)		
Never/Rarely	3 (4.2)	2 (3.3)	4 (5.8)		
Consumption of roots and tubers					
Everyday	65 (91.5)	57 (93.4)	54 (78.3)	7.66	0.02
Never/Rarely	6 (8.5)	4 (6.6)	15 (21.7)		
Consumption of soya products					
Everyday/Regularly	8 (11.3)	6 (9.8)	4 (5.8)	3.55	0.47
Occasionally	4 (5.6)	8 (13.1)	7 (10.1)		
Never/Rarely	59 (83.1)	47 (77.0)	58 (84.1)		
Consumption of meat					
Everyday/Regularly	8 (11.3)	3 (4.9)	–	–	–
Occasionally	6 (8.5)	5 (8.2)	5 (7.2)		
Never/Rarely	57 (80.3)	53 (86.9)	64 (92.8)		

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of fish					
Everyday/Regularly	45 (63.4)	44 (72.1)	50 (72.5)	7.07	0.13
Occasionally	16 (22.5)	5 (8.2)	7 (10.1)		
Never/Rarely	10 (14.1)	12 (19.7)	12 (17.4)		
Consumption of egg					
Everyday/Regularly	30 (42.3)	25 (41.0)	17 (24.6)	2.13	0.01
Occasionally	8 (11.3)	12 (19.7)	6 (8.7)		
Never/Rarely	33 (46.5)	24 (39.3)	46 (66.7)		
Consumption of fruits					
Everyday/Regularly	25 (35.2)	20 (32.8)	22 (31.9)	3.56	0.47
Occasionally	4 (5.6)	2 (3.3)	8 (11.6)		
Never/Rarely	42 (59.2)	39 (63.9)	39 (56.5)		
Consumption of milk					
Everyday/Regularly	26 (36.6)	19 (31.1)	20 (29.0)	–	–
Occasionally	–	1 (1.6)	1(1.4)		
Never/Rarely	45 (63.4)	41 (67.2)	48 (69.6)		
Consumption of snacks					
Everyday/Regularly	52 (73.2)	53 (86.9)	60 (87.0)	–	–
Occasionally	–	–	1 (1.4)		
Never/Rarely	19 (26.8)	8 (13.1)	8 (11.6)		
Consumption of sweets					
Everyday/Regularly	21 (29.6)	24 (39.3)	38 (55.1)	11.75	0.01
Occasionally	2 (2.8)	2 (3.3)	4 (5.8)		
Never/Rarely	48 (67.6)	35 (57.4)	27 (39.1)		
Consumption of noodles					
Everyday/Regularly	3 (4.2)	1 (1.6)	4 (5.8)	5.39	0.23
Occasionally	3 (4.2)	7 (11.5)	2 (2.9)		
Never/Rarely	65 (91.5)	53 (86.9)	63 (91.3)		
Consumption of ghee/butter					
Everyday/Regularly	20 (28.2)	12 (19.7)	12 (17.4)	–	–
Occasionally	–	2 (3.3)	1 (1.4)		
Never/Rarely	51 (71.8)	47 (77.0)	56 (81.2)		
Consumption of tea					
Everyday/Regularly	47 (66.2)	52 (85.2)	61 (88.4)	–	–
Occasionally	1 (1.4)	–	–		
Never/Rarely	23 (32.4)	9 (14.8)	8 (11.6)		

Items of food	Pre (n=71)	Peri (n=61)	Post (n=69)	Chi-square test/ Fisher's exact test	p value
Consumption of aerated drinks					
Everyday/Regularly	11 (15.5)	7 (11.5)	5 (7.2)	–	–
Occasionally	1 (1.4)	1 (1.6)	–		
Never/Rarely	59 (83.1)	53 (86.9)	64 (92.8)		
Vigorous physical activity	98.35*	99.20	105.33	1.92	0.38
Moderate physical activity	117.00	95.12	89.73	8.64	0.01
Sedentary activity	113.37	90.34	97.70	5.78	0.05
Habit of chewing tobacco					
Yes	7 (9.9)	3 (4.9)	11 (15.9)	4.11	0.12
No	64 (90.1)	58 (95.1)	58 (84.1)	–	–

*Mean rank; Figures in parenthesis represent percentage values

Table 4 shows that PBF, HC, WHR, skeletal fat of the whole body, leg, and trunk, blood glucose level, SBP, DBP, MAP, muscle mass, and FFM differed significantly across the menopausal groups. It further appeared that PBF, WHR, blood glucose, and blood pressure levels were significantly higher among the postmenopausal participants than the pre and perimenopausal participants. It also appears that for body fat measures like HC, skeletal fat related to the whole body, leg, and trunk, FFM, and muscle mass, the premenopausal participants showed the highest values, followed by the peri and postmenopausal participants. The post-hoc tests showed significant differences between pre- and post-menopausal participants for the variables of body fat measures (barring a few) and CVD risk factors. Peri and post-menopausal participants differed significantly in the case of the whole body, leg, and trunk skeletal fat, muscle mass, and FFM (Table 4).

Table 4. Distribution of CVD risk factors and fat patterning of the participants (n=201)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Kruskal-Wallis test	p value	Post-hoc test
	mean±SD					
BMI	26.21±4.41	26.06±3.83	25.22±4.24	1.13	0.33	Pre and Peri (0.98) Pre and Post (0.38) Peri and Post (0.52)
PBF	34.37±4.39	35.35±4.01	37.13±4.25	7.59	0.01	Pre and Peri (0.42) Pre and Post (0.001) Peri and Post (0.05)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Kruskal-Wallis test	p value	Post-hoc test
		mean±SD				
WC (cm)	88.48±12.13	88.89±9.86	89.10±11.68	0.05	0.95	Pre and Peri (0.98) Pre and Post (0.95) Peri and Post (0.99)
HC (cm)	96.54±7.45	95.23±6.75	93.29±7.83	3.42	0.04	Pre and Peri (0.59) Pre and Post (0.04) Peri and Post (0.33)
WHR	0.91±0.08	0.93±0.08	0.95±0.09	4.29	0.01	Pre and Peri (0.39) Pre and Post (0.01) Peri and Post (0.34)
Visceral fat	7.93±4.13	8.78±4.11	8.86±4.94	0.93	0.39	Pre and Peri (0.55) Pre and Post (0.47) Peri and Post (0.99)
Whole body subcutaneous fat percentage	30.24±5.60	30.49±4.96	31.01±4.71	0.41	0.66	Pre and Peri (0.96) Pre and Post (0.67) Peri and Post (0.84)
Whole body skeletal fat percentage	23.75±2.02	22.92±1.84	21.701±1.79	20.71	0.01	Pre and Peri (0.04) Pre and Post (0.01) Peri and Post (0.01)
Trunk subcutaneous fat percentage	26.40±5.31	26.92±4.60	27.90±4.57	1.72	0.18	Pre and Peri (0.83) Pre and Post (0.19) Peri and Post (0.52)
Trunk skeletal fat percentage	18.19±2.13	17.29±1.88	16.04±1.92	20.64	0.01	Pre and Peri (0.03) Pre and Post (0.01) Peri and Post (0.01)
Arm subcutaneous fat percentage	46.73±6.78	47.27±6.15	48.41±5.29	1.35	0.26	Pre and Peri (0.88) Pre and Post (0.27) Peri and Post (0.57)
Arm skeletal fat percentage	24.99±4.64	24.51±3.98	23.61±3.87	1.96	0.14	Pre and Peri (0.80) Pre and Post (0.15) Peri and Post (0.47)
Leg subcutaneous fat percentage	43.19±7.32	42.59±6.51	42.11±5.43	0.49	0.61	Pre and Peri (0.87) Pre and Post (0.62) Peri and Post (0.91)
Leg skeletal fat percentage	36.69±2.52	35.79±2.41	34.29±2.47	16.88	0.01	Pre and Peri (0.11) Pre and Post (0.01) Peri and Post (0.03)

Variables	Pre (n=71)	Peri (n=61)	Post (n=69)	F value/ Kruskal-Wallis test	p value	Post-hoc test
	mean±SD					
Blood glucose level(mg/dl)	99.00*	105.00	104.00	9.63	0.03	Pre and Peri (0.03) Pre and Post (0.01) Peri and Post (0.94)
Blood total cholesterol level (mg/dl)	190.68±31.34	201.41±40.66	201.90±40.50	1.97	0.14	Pre and Peri (0.27) Pre and Post (0.21) Peri and Post (0.99)
SBP (mmHg)	116.00*	127.00	134.00	31.18	0.01	Pre and Peri (0.01) Pre and Post (0.01) Peri and Post (0.23)
DBP (mmHg)	81.73±8.59	86.72±11.58	87.14±10.73	5.90	0.01	Pre and Peri (0.01) Pre and Post (0.02) Peri and Post (0.97)
MAP (mmHg)	96.67±9.64	101.29±14.43	103.59±13.03	12.19	0.01	Pre and Peri (0.003) Pre and Post (0.01) Peri and Post (0.57)
Muscle mass percentage	32.27±2.67	30.26±2.15	28.85±3.28	27.16	0.01	Pre and Peri (0.01) Pre and Post (0.01) Peri and Post (0.02)
FM (kg)	21.43±6.29	21.43±5.08	21.61±5.79	0.02	0.98	Pre and Peri (1.00) Pre and Post (0.98) Peri and Post (0.99)
FFM (kg)	39.72±5.56	38.53±4.71	35.88±5.46	9.59	0.01	Pre and Peri (0.43) Pre and Post (0.01) Peri and Post (0.02)

*Mean rank

Table 5 shows that PBF, WHR, blood glucose, and blood pressure levels (SBP, DBP and MAP) showed a positive relationship with participants' age, while skeletal fat of whole body and trunk, and muscle mass showed an inverse relationship. Percent body fat showed a positive association with the number of pregnancies, while skeletal fat of the whole body and trunk, and muscle mass showed an inverse association with the number of pregnancies. It also appears that blood glucose levels were likely to lower with an increase in green-leafy vegetable consumption. Percent body fat, WHR, and blood glucose level shows an inverse association with age at menarche, while skeletal fat of the whole body and trunk showed a positive association with participant's age at menarche. The values of R square represent that the models can explain 6–50 percent of the variability in the dependent variables (Table 5).

Table 5. Predictors of CVD risk factors and fat patterning (n=201)

Dependent variable	Independent variables	Unstandardized coefficients	t value	p value	CI at 95% lower	CI at 95% upper	R square
PBF	Participant's age	0.12	3.63	0.0001	0.05	0.19	0.27
	Educational years of the participants	0.33	4.32	0.0001	0.18	0.48	
	Age at menarche	-0.57	-2.95	0.004	-0.96	-0.19	
	Number of pregnancies	1.66	3.67	0.0001	0.76	2.56	
	Total number of wastage	-1.45	-3.02	0.003	-2.41	-0.51	
HC (cm)	Completed years of education	0.82	8.61	0.001	0.48	0.87	0.26
	Weekly duration of walking	-0.009	-4.68	0.001	-0.01	-0.005	
WHR	Exclusively homemaker	0.03	2.41	0.01	0.007	0.06	0.20
	Age at menarche	-0.01	-2.74	0.007	-0.01	-0.003	
	Age at marriage	-0.006	-2.85	0.005	-0.01	-0.002	
	Participant's age	0.002	2.32	0.02	0.0001	0.003	
	Educational years of the participant's husbands	0.004	2.12	0.03	0.0001	0.007	
Whole body skeletal fat percentage	Participant's age	-0.09	-5.78	0.0001	-0.12	-0.06	0.36
	Number of pregnancies	-0.88	-4.30	00001	-1.29	-0.47	
	Age at menarche	0.23	2.65	0.009	0.06	0.41	
	Total number of wastage	0.73	3.35	0.001	0.30	1.16	
	Educational years of the participants	-0.08	-2.40	0.01	-0.15	-0.01	
Trunk skeletal fat percentage	Participant's age	-0.10	-6.88	0.0001	-0.13	-0.07	0.38
	Age at menarche	0.29	3.37	0.001	0.12	0.47	
	Exclusively homemaker	-0.93	-3.12	0.002	-1.53	-0.34	
	Number of pregnancies	-0.33	-2.78	0.006	-0.56	-0.09	
Leg skeletal fat percentage	Participant's age	-0.11	-5.28	0.0001	-0.15	-0.07	0.16
Blood glucose level (mg/dl)	Green-leafy vegetables consumption	-7.97	-2.63	0.009	-13.94	-1.99	0.13
	Age at menarche	-7.46	-2.72	0.007	-12.88	-2.04	
	Participant's age	0.89	1.98	0.05	0.002	1.79	
SBP (mmHg)	Participant's age	0.92	5.68	0.0001	0.60	1.24	0.18
DBP (mmHg)	Per capita monthly household expenditure	0.001	2.15	0.03	0.0001	0.001	0.06
	Participant's age	0.18	2.09	0.04	0.01	0.35	
MAP (mmHg)	Participant's age	0.45	4.24	0.0001	0.24	0.66	0.11

(Only significant values are presented)

Table 6 shows that menopausal status significantly predicted CVD risk factors and measures of body fat patterning after omitting the effects of the confounding variables (socio-demographic, reproductive, and lifestyle factors) (Table 6).

Table 7 exhibited that the prevalence of central obesity appeared to be higher among the majority of the participants irrespective of their menopausal status. It also appears that 60.9% of the postmenopausal participants were hypertensive followed by the perimenopausal participants (54.1%). For DBP, 39.3% of the perimenopausal participants appeared as hypertensive, followed by the postmenopausal participants (34.8%). The prevalence of high total cholesterol levels and diabetes appeared to be lower among all the participants irrespective of their menopausal status (Table 7).

Table 6. Results of Multivariate analysis of covariance of the participants (MANCOVA)

Fixed factors	Dependent variables	Type III sum of squares	F value	p value
Menopausal status	PBF	288.08	7.98	0.0001
	HC	384.22	3.49	0.03
	WHR	0.04	3.46	0.03
	Whole body skeletal fat	161.03	22.73	0.0001
	Trunk skeletal fat	171.79	21.85	0.0001
	Leg skeletal fat	224.75	18.52	0.0001
	Blood glucose level	29550.21	5.62	0.004
	SBP	10765.29	15.36	0.0001
	DBP	1322.56	6.24	0.002
	MAP	3962.95	12.88	0.0001
	Muscle mass percentage	414.69	27.23	0.0001

Table 7. Prevalence of CVD risk factors among the participants (n=201)

CVD risk factors	Pre (n=71)	Peri (n=61)	Post (n= 69)	Chi square test/ Fisher's exact test	p value
Blood glucose level(mg/dl)					
Diabetic	–	5 (8.2)	7 (10.1)	8.61	0.009
Non-diabetic	71 (100)	56 (91.8)	62 (89.9)		
Waist circumference (cm)					
Normal	34 (47.9)	27 (44.3)	37 (39.1)	1.09	0.57
Risk	37 (52.1)	34 (55.7)	42 (60.9)		
WHR					
Normal	12 (16.9)	8 (13.1)	9 (13.0)	0.54	0.76
Risk	59 (83.1)	53 (86.9)	60 (87.0)		

CVD risk factors	Pre (n=71)	Peri (n=61)	Post (n= 69)	Chi square test/ Fisher's exact test	p value
SBP (mmHg)					
Normal	64 (90.1)	43 (70.5)	43 (62.3)	15.09	0.001
Hypertensive	7 (9.9)	18 (29.5)	26 (37.7)		
DBP (mmHg)					
Normal	59 (83.1)	37 (60.7)	45 (65.2)	9.11	0.01
Hypertensive	12 (16.9)	24 (39.3)	24 (34.8)		
MAP (mmHg)					
Normal	54 (76.1)	28 (45.9)	27 (39.1)	21.67	0.0001
Hypertensive	17 (23.9)	33 (54.1)	42 (60.9)		
Total cholesterol level (mg/dl)					
Normal	63 (88.7)	55 (90.2)	58 (84.1)	1.24	0.53
High	8 (11.3)	6 (9.8)	11 (15.9)		

Discussion

We aimed to compare the body fat patterning and CVD risk factors among women of differential menopausal statuses (pre, peri, and post) belonging to the Bengali Hindu ethnic group. Our study indicates that postmenopausal participants have higher PBF and central obesity and reduced lean mass (skeletal fat of whole body and torso) and muscle mass compared to their pre and perimenopausal counterparts as found in studies carried out in India and elsewhere (Ferrara et al. 2002; Ghosh and Bhagat 2010). A decrease in sex hormones after menopause results in fat accumulation, particularly in the abdomen region because sex hormones are required for adipocyte metabolism (Ferrara et al. 2002). The higher prevalence of central obesity among the postmenopausal participants in our study perhaps justifies the sex hormonal depletion as a reason behind the higher prevalence of central obesity. Some longitudinal studies on postmenopausal participants from Europe (Poehlman et al. 1995) and the USA (Sowers et al. 2009) reinforce the role of estrogen in central obesity during midlife. Countering these studies, several studies reported that weight gain in middle-aged women was associated with an increase in age, rather than menopause. For example, the Healthy Women's Study exhibited that women accumulate approximately 0.7 kg every year during their fifth and sixth decades of life, independent of menopause (Wing et al. 1991). Despite these inconsistent results, the majority of the cross-sectional (Ghosh and Bhagat 2010) and longitudinal studies (Sowers et al. 2009) have reported an increase in abdominal obesity during menopause, independent of aging which is in agreement with the present study.

Our study reveals that peri- and post-menopausal participants have higher blood glucose levels compared to their premenopausal counterparts. This finding shows consistency with some earlier studies carried out in various parts of the world, including India (Revis and Keene 2006) though inconsistent results have also been reported (Pandey et al. 2010). Scholars found that a decrease in the sex hormone levels during MT and beyond reduces the sex hormone binding globulin (SHBG) level in the blood, increasing the risk of type II diabetes mellitus (Carr 2003); but the association between menopause and blood glucose level independent of aging remains inconsistent (Kim 2012). Although measuring SHBG concentration is beyond the purview of our research, the findings of our study indicate the role of SHBG in determining blood glucose level.

It appears from our study that postmenopausal participants have significantly higher SBP, DBP, and MAP levels compared to the pre and perimenopausal participants, corroborating with some earlier research findings (Lima et al. 2012). The link between menopausal status and blood pressure levels persisted even after controlling the confounders (socio-demographic, reproductive, and lifestyle variables), which is in agreement with some of the previous studies (Gupta et al. 2014; Son et al. 2015). For instance, a study involving Korean postmenopausal women reported a significant association between menopause and hypertension after controlling for the confounders like age, BMI, WC, vasomotor symptoms, triglycerides, and uric acid which is in partial agreement with the present study (Son et al. 2015). On the contrary, studies reported that the high blood pressure level among midlife women could be explained by factors like age and BMI (Matthews et al. 2009). For example, the SWAN longitudinal study reported that after adjusting for age and other confounders, MT had no effect on blood pressure levels (Matthews et al. 2021). The mechanism through which menopause and hypertension are related is unclear. This could be attributed to the direct protective mechanism of estrogen on the renin-angiotensin-aldosterone system, which reduces after menopause (Carr 2003). An increase in vasoconstrictors (angiotensin II and endothelin) and a significant drop in nitric oxide took place after menopause; as a result, renal vasoconstriction and endothelial dysfunction occurred, resulting in the development of hypertension (Yanes et al. 2010).

The participants of our study, irrespective of the menopausal status show high level of total cholesterol as found in some other studies (Inaraja et al. 2020; Matthews et al. 2021);but intergroup difference in cholesterol level is not statistically significant, which is partly in agreement with a study conducted in Rajasthan (Kanwar et al. 2014). The SWAN longitudinal study (20 year of follow-up) conducted among 1554 women reported that total cholesterol level increased dramatically throughout the MT until the first year of menopause, then significantly decreased during the postmenopausal years (Matthews et al. 2021); thus, no significant difference persisted between the peri and postmenopausal women which is consistent with the present study. Despite the substantial studies on the effects of estrogen on lipid metabolism, it is not clear whether there is any association between the change in sex steroid concentrations and alterations in the lipid profile. Some large population-based studies reported no association between menopause and HDL levels (Pasquali et al. 1997), but alterations in the proatherogenic lipid profile driven by menopause may have an impact on LDL, triglyceride, and total cholesterol levels (Inaraja et al. 2020). Scholars are of the opinion that estrogen absorbs free radicals and naturally occurring LDL in the blood, which are capable of damaging the arteries and other tissues. In the absence of estrogen circulation in the blood, these particles build up in the arteries and restrict the blood flow (Carr 2003).

Apart from the hormonal factors, socio-demographic, reproductive, and lifestyle factors significantly contribute to the risk factors related to CVD. In our study, educational attainment of the participants reported a positive link with body fat patterning, corroborating (De Silva et al. 2015) and contradicting (Sabanayagam et al. 2007) with studies from India and elsewhere. For instance, evidence from Asian and Western countries showed a positive link between educational status, income and body fat patterning (De Silva et al. 2015). Additionally, educational attainment of the spouses showed a positive relationship with central body fat of the participants; this is in disagreement with some previous studies (Torssander et al. 2009; Murakami et al. 2017). A study involving Japanese women reported that women whose spouses had only attained high school or less were more likely to be obese than women whose spouses had completed a higher level of education (Murakami et al. 2017); the present study contradicts this finding. Perhaps the completion of higher levels of education by the participants and their husbands is linked to higher income and higher social position, which may result in excess nutrition and the adoption of a sedentary lifestyle. Some Indian studies conducted in urban areas of West Bengal showed 56.1% incidences of central obesity among women, irrespective of their menopausal status, owing to the sedentary lifestyle and the south Asian genetic predisposition to central obesity (Acharyya et al. 2014); this is consistent with the present study.

Postmenopausal women's risk of midlife obesity and CVD risk factors might also be impacted by pregnancy and childbirth (Patchen et al. 2017). In our study, ages at menarche and marriage showed inverse associations with central obesity, while parity shows an inverse association with lean body mass, corroborating with previous studies (Lakshman et al. 2009). A recent study conducted among 6,103 Iranian midlife women reported that women with an early age at menarche had a higher risk of central obesity compared to those with late age at menarche (after 14 years of age). This finding is aligning with the present study since all the participants had attained menarche before 14 years of age (Kheradmand et al. 2023). On the contrary, some other population-based studies indicated that age at menarche was linked to generalized obesity, but not to central obesity (Trikudanathan et al. 2013). However, the physiological mechanism of this link is not well understood. There may be a shared etiology rather than a direct link between early menarche and midlife obesity. For example, Elks et al. (2011) found that a genetic locus, LIN28B, is linked with both age at menarche and central obesity. Menarche is also associated with a number of neuroendocrinological changes, such as increased levels of adrenal androgen and hyperactivity of the hypothalamic-pituitary-gonadal axis (Trikudanathan et al. 2013). This higher level of androgen promotes the development of central obesity later in life. Additionally, it appears that early age at marriage is associated with early age at first pregnancy, which may lead to disruption in educational and career achievements.

Younger age at childbirth and increased parity cause increased stress and bring changes in lifestyle factors causing hypothalamic-pituitary-adrenal hyperactivity. Both factors independently enhance the development of central obesity in women later in life (Patchen et al. 2017). Mean ages at marriage and first pregnancy were found to be earlier among the participants of the present study. This could partially explain the reason behind the higher incidences of central obesity among the participants, irrespective of their menopausal status. Future prospective studies are needed to establish this association.

Studies revealed that chronological aging is a significant predisposing factor in the development of CVD (Zierer et al. 2016). In the present study, participant's age showed a significant positive relationship with blood pressure levels and body fat patterning. This could be due to the age-related adverse changes at the cellular level, including chronic inflammation, oxidative stress, myocardial deterioration, and changes in calcium plumping capacity (Zierer et al. 2016). All these adverse changes enhance the buildup of diacylglycerol fatty acids and saturated ceramide, leading to an increase in visceral adipose tissue and total body fat content. Aging is also associated with an increase in reactive oxygen and nitrogen species (RONS), which leads to damage in lipids, DNA, and protein, causing dysfunction of vascular tissues and promoting the development of chronic diseases like CVD. However, it is difficult to distinguish between the impacts of aging from menopause because, by definition, post and perimenopausal women are older than the premenopausal women. Future studies are needed to establish the independent association of both these events with CVD risk factors.

The present study exhibited that blood glucose level was likely to increase with a decrease in vegetable consumption, corroborating with previous studies (Kartiko et al. 2020). This could be attributed to the presence of phytochemicals (Vitamin C, beta carotene, etc.) and anti-oxidative nutrients in fruits and vegetables which may improve cardiovascular health. The present study reported that participant's working status is a significant predictor of central obesity (WHR) showing consistency with previous studies (George and Chandan 2016). For example, an Indian study (Mumbai) showed that women who are working have a lower body fat content than non-working women (George and Chandan 2016). A longitudinal cohort study (SWAN study) reported that physical activity is inversely linked to the changes in PBF and WC, independent of menopausal status and aging (Sternfeld et al. 2004). The participants of the present study are mostly non-working; this might be an explanation for the increased level of body fat content among the participants. The present study further reported that walking on a regular basis helps to reduce body fat, conforming to previous studies (Sternfeld et al. 2004; George and Chandan 2016).

Finally, our study concluded that menopausal status is a significant predictor of body fat distribution, blood pressure, and blood glucose levels after controlling the effects of the confounding variables (socio-demographic, reproductive, and lifestyle variables), corroborating with previous studies (Carr 2003; Matthews et al. 2021). Additionally, an overwhelming majority of the postmenopausal Bengali Hindu participants of the present study have central obesity; thus, this group is more prone to developing CVD in the near future. The likelihood of Indian postmenopausal women suffering from CVD risk factors will increase if menopausal status appears as a strong independent predictor of cardiovascular risk factors, as found in the present study. Policymakers can use baseline information from this study to formulate appropriate health policies that will help postmenopausal women to lead a healthy life beyond menopause. This research underscores the importance of addressing cardiovascular health as a critical component of women's health initiatives. By prioritizing education and access to preventive care, health planners can help postmenopausal women to make informed lifestyle choices that mitigate their risk of CVD and enhance their quality of life. More studies targeting midlife women are crucial to expand our understanding of this seldom-explored field to improve the well-being of postmenopausal women in developing countries.

There are certain limitations in this study. Owing to the COVID-19 pandemic and subsequent nationwide lockdown, the fieldwork was impacted in part. As a result of which the estimated sample size for the present study was not covered. The estimation of the total lipid profile and fasting blood glucose level, along with a larger sample size could have enhanced the findings of this study. The findings might have improved by a more thorough observation of the lifestyle choices (maybe on a subsample) of the participants. Postmenopausal health problems of women could be attributed to genetics, disparities in perception and attitude, and finally, their differential access to health care services. Therefore, incorporating data on these aspects might enhance the understanding of the link between menopause and risk factors of CVD in the present study.

Conclusions

Prediction of the CVD risk factors among middle-aged women continues to lag behind that of men because women exhibit CVD events a few years later than men. Additionally, the occurrence of CVD risk factors among midlife women might be a complex interaction of menopause, social-cultural factors, differential attitudes and perceptions of women regarding midlife, and access to health care services. Further cross-cultural investigations are needed to better understand how the association between menopause and CVD varies across different cultural groups and throughout the menopausal transition by taking into account both biological and social-cultural aspects of postmenopausal health. An awareness of this complex scenario will help policymakers to develop appropriate strategies for postmenopausal women. These strategies could include targeted health education, accessible healthcare services, and community support programs that can address the unique needs of diverse populations. By fostering a deeper understanding of these cultural differences, the overall health and well-being of postmenopausal women can be enhanced. Additionally, providing awareness and specialized interventions can help mitigate the health risks faced by postmenopausal women. This approach not only encourages improved health outcomes but also empowers women to take charge of their health during this critical phase of life and ensure a successful transition from reproductive to the post-reproductive phase of life.

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Conflict of interests

The authors declared that they have no conflict of interest.

Ethics statement

The study was approved by the Institutional Human Ethical committee, University of Calcutta (protocol 06/WT/19-20/1763). The purpose of the research was explained to, and written informed consent was taken from the participants.

Author contribution

The first author (DK) contributed 50% by collecting data and analyzing the data and partially drafting the manuscript. The second author (SR) contributed 50% by designing the study and reviewing the manuscript.

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