

# **ANTHROPOLOGICAL** ***REVIEW***



WYDAWNICTWO  
UNIWERSYTETU  
ŁÓDZKIEGO

# **ANTHROPOLOGICAL** ***REVIEW***

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# The ancient people of Balinese: a study of health status based on paleopathology remains

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**ABSTRACT:** Humans have been constantly exposed to the environment in their daily activities, which may result in some pathological changes. Paleopathology research is rare, particularly on ancient humans who lived in Bali, Indonesia. This research is intended to gain more insight into the history of diseases and the community in the past to assist future health researchers. This study was conducted to understand variation in disease among the ancient populations in Bali, Indonesia. The study examines skeletons from the Gilimanuk, Semawang, and Pacung Sembiran sites in Bali, Indonesia, collected from 1964 to 2008. A total of 336 individuals were identified, including 63 adult males, 75 adult females, and 56 adults whose sex could not be determined. In addition, 142 children were included in the analysis. The examination of the individuals revealed the presence of various dental pathological changes, such as caries and enamel hypoplasia, as well as periodontitis. Furthermore, several bone-related diseases and abnormalities were identified, such as osteophytes, fractures, porotic hyperostosis, and spina bifida occulta. Available evidence suggests a similarity in the types of pathological changes among the ancient populations of Gilimanuk, Semawang, and Pacung Sembiran. While a comprehensive explanation of the paleopathological variations observed in these populations necessitates further investigation, the present findings hold considerable value for medical experts and anthropologists seeking to better understand patterns of disease distribution among ancient Indonesian populations.

**KEY WORDS:** ancient, population, teeth, skeleton, paleopathology.



Original article

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## Introduction

Life in the past is a considerably compelling point of discussion. The period before the 4<sup>th</sup> century AD was the ancient period, as indicated by the discovery of inscriptions in Kutai of East Kalimantan (Wiradnyana 2010), which served as the demarcating line of the historical and non-historical period in Indonesia. Just as anatomy and forensic medicine seek to answer questions of the present, paleopathology also uses human remains as research material to reconstruct life in the past to obtain a better understanding of the present (Roberts and Manchester 2005). This is helped by a large number of ancient (before 4<sup>th</sup> century) human bone remains found in Indonesian regions, such as in Gilimanuk (Bali), Semawang (Bali), Plawangan (Java), Sumatra (Aceh), Sulawesi (Makasar), Kalimantan (Banjarmasin), West Nusa Tenggara and East Nusa Tenggara.

Finding out someone's health status in the past can be achieved by examining skeletons that can be used to reconstruct ancient human life (Jacob 1972, 1977; Bosch 2000; Suriyanto 2020). For instance, changes in diet can lead to various diseases because diet is recorded on masticatory organs, such as teeth (Steckel and Rose 2002; Steckel 2005; Suriyanto 2020). Teeth and mouth are organs that also served as social organs in the past. In the ancient period, human social status was indicated by teeth modification and the use of dental accessories, such as ablation, staining, and the addition of non-biological materials such as rocks are examples of such modifications (Suriyanto and Koesbardiati 2010; Koesbardiati et al. 2015, 2018; Koesbardiati and Murti 2019).

The disease can also be triggered by contact with other populations (Larsen

et al. 2007; Suriyanto 2020). These diseases spread from local to regional areas and can sometimes turn into epidemics, such as plague. The practice of colonization had facilitated outbreaks of many diseases and, arguably, gave origin to various epidemics, such as avian influenza, swine flu, plague, and smallpox. The easier and faster mobility coupled with an easy access to transportation, the faster and the wider the spread of diseases from one area to another. Ancient people recorded the experienced by them life events in various cultural artifacts (Buzon et al. 2005; Suriyanto 2020). Some notable events related to diseases, such as outbreaks, epidemics, or pandemics, also attracted their attention, which is recorded in rock drawings, pottery, reliefs, and inscriptions. This information can not only provide a better understanding of diseases in the past but also can help in dealing with similar diseases in the future (Bosch 2000; Suriyanto 2020).

The Gilimanuk site (2000 BP) is located by the side of the beach. Gilimanuk used to be once a coral reef ocean area. It was surrounded by water during the Pliocene era but now is a part of the mainland. The combination of land and calm waters calms the east side of Gilimanuk, accumulating fine sediment. This sediment then serves as a habitat for the development of mangrove forests (Soenarto 1993). Mangrove forests provide a safe habitat for fish and other animals. There is enough fresh water in the surrounding environment to support life. People at the time were most likely able to make a living as fishermen thanks to their easy access to the sea (Aziz 1995). This community consumed marine animals and land animals as evidenced by the discovery of the remains of bones

and teeth of pigs and chickens (Soejono 1977). The first study published in 1962 revealed human skeletons and pottery. Based on these findings, further research was carried out from 1963 to 1977 with a total of 40 sectors and the opening of 37 excavation boxes (Soejono 1977). The human skeletons in Gilimanuk which are stored at the Bioanthropology and Paleoanthropology Laboratory come from excavations from 1964 to 1977.

The Semawang site is located on Sanur Beach, in the South Denpasar District of Badung Regency, Bali Province, Indonesia. At the Semawang Site, the soil is composed of loose sand, sandstone, shells, and coral fragments (Harkantiningih 1990). It was first discovered by a resident upon whose report a research survey was conducted followed by three excavation phases in 1986, 1988, and 1990. Frames I–III were unearthed in 1986, frames IV–VIII in 1988, and frames IX–XV in 1990. The human skeletons were excavated in 1986–1988 at the Semawang site. Grave goods found at the site included animal skeletons, kereweng (a type of Balinese gamelan instrument), metal objects, shells, and beads (Yuliati 1990). The fauna of the area includes monkeys, starlings, pangolins, porcupines, pigs, monitor lizards, turtles, dogs, and snakes. Meanwhile, mangroves, coconuts, ketapang, and camplung are common coastal plants, and many rice fields are planted with rice, secondary crops, and lowland horticulture (Gautama 2011).

Nestled on the northern coast of Bali, Pacung Sembiran stands as twin sentinels of a rich maritime heritage. Situated within the Tejakula District of Buleleng Regency, this site occupies a strategic position, marked by their precise coordinates of 8°31'45" East Longi-

tude and 8°7'32" South Latitude. Poised at an elevation of 25 meters above sea level, Pacung Sembiran unfurls along a 700-meter stretch, 250 meters from the coastline. The strategic location has long captivated traders from distant lands, making it a bustling hub of maritime commerce for over two millennia. Archaeological investigations at the Pacung Sembiran sites commenced in 1987 and continued with multiple excavation seasons until 2008, with a resumption in 2012. These endeavours yielded a remarkable abundance of artifacts, including Indian pottery, pig's teeth, and bronze tools crafted in Pacung Sembiran. This rich archaeological record points to the existence of thriving communities in Pacung Sembiran dating back to at least the first century AD, corresponding to Bali's paleometallic or iron-bronze period (Ardika et al. 1997; Calo et al. 2015). Fresh water is only available in areas near the beach. The human skeletons were excavated in 2004–2005 at the Pacung Sembiran site. The drying of the land means that people must work as fishermen, traders, or gardeners. This location has been occupied by humans for an estimated 2200 years. Pacung Sembiran has a calm coastline and a deep bay, making it easier for ships to anchor (Ardika 1991; Suastika 2008).

Drawing upon the rich archaeological record of Bali's past, this study seeks to elucidate the types and prevalence of diseases that afflicted ancient populations in Gilimanuk, Semawang, and Pacung Sembiran. By delving into the paleopathological landscape of these communities, this study can provide a better understanding of diseases in the past so that it can be useful for dealing with these diseases in the future.

## Materials and Methods

### Sample

This study examines the morphology of the human skeletons derived from three locations in Bali, Indonesia: Gilimanuk (114°26′–114°56′30″ E and 80°5′20″ S), Semawang (111°35′04″ E, 6°3′33″ S), and Pacung Sembiran (8°27′12″ E, 8°42′19″ S), collected between 1964 and 2008 (Fig. 1). The human skeletons were obtained from the Gilimanuk (300 individuals) site, the Semawang (11 individuals) site, and the Pacung Sembiran (25 individuals) site. To reconstruct the health condition of the representatives of the examined past populations from these sites, a paleopathological approach (Rühli et al. 2016) was used through macroscopic observations of the undamaged remains of the bones and teeth. Data were collected in December

2023. This study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada (No. KE/FK/1913/EC/2023).

### Procedure

The first stage of the study included making macroscopic observations of the skeleton to determine the availability of the skeleton as a sample. In the next stage, the surface of the bones was cleaned from dust and sand to enable observations on the surface of the bones. The following stage involved sex identification by looking at the sex markers (nuchal crest, mastoid process, supraorbital margin, glabella, mental eminence) on the skull bone (Buikstra and Ubelaker 1994). If no sex markers were found, sex was categorized as unidentified.

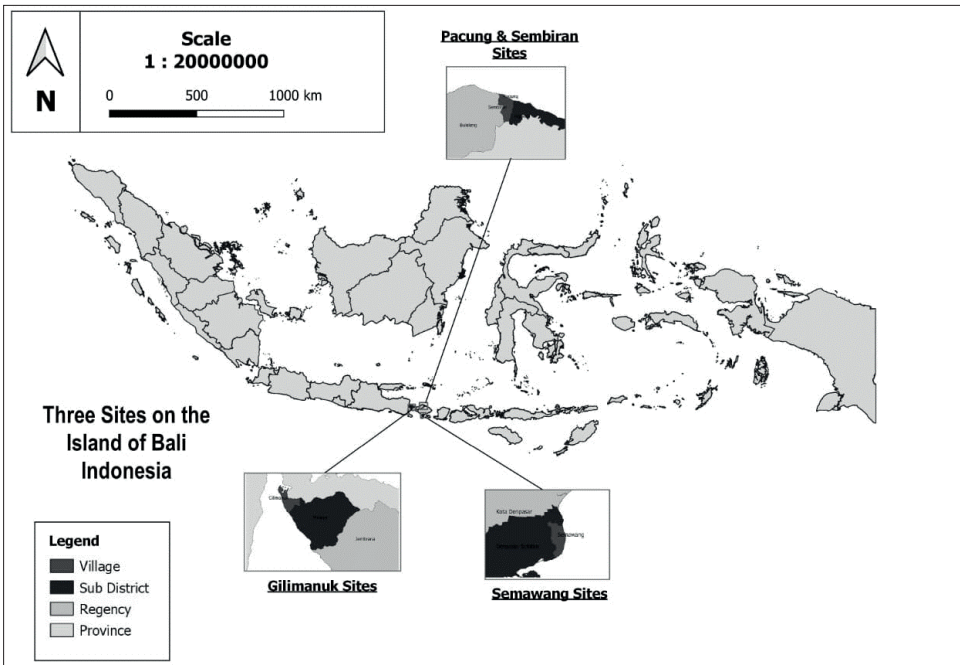


Fig. 1. Location map of Gilimanuk, Semawang, and Pacung Sembiran sites, Bali Indonesia

The method used in this study for detecting pathological changes in skeletal remains involved meticulously examining individual bones and teeth. Each specimen was scrutinized, and, to minimize the risk of misdiagnosis, the bones or teeth were compared to healthy specimens. The next stage consisted of determining the age at death by assessing the auricular surface, pubic symphysis, or the sutures of the skull and teeth. Age at death was determined by examining the closure of the cranial sutures (Buikstra and Ubelaker 1994), and severe attrition (Lovejoy 1985). Subsequently, disease on the surface of bones and teeth was identified (Aufderheide et al. 1998; Ortner 2003; Roberts and Manchester 2005). Assessment of the diseases was conducted as follows: caries (Lukacs 1989), Enamel hypoplasia (Hillson 1997), periodontitis (Lukacs 1989), severe attrition (Lovejoy 1985), calculus (Brothwell 1981), osteophytes (Jurmain 1990), porotic hyperostosis (Stuart-Macadam 1985), and spina bifida occulta (Aufderheide and Rodríguez-Martín 1998). During the final stage of the study an analysis of the results of observations to determine the type of disease and disorder was conducted.

## Results

Descriptive statistics on the subjects and types of pathological changes at the Gilimanuk, Semawang, and Pucang Sembiran sites are listed in Table 1. Figures 2–4 show dental caries and calculus, and osteophytes on lumbar vertebra of the individual derived from the Gilimanuk site. Figure 5 shows the pangur on the incisor teeth from the Pacung Sembiran site.

The Gilimanuk site (2000 BP) used to be a community group residing on the

beach. This study examined 300 Gilimanuk skeletons consisting of 50 adult males, 63 females, 135 children, and 52 sex-unidentified adults. The age range for adults was between 16 and 60 years, and for children between 0–15 years. Most of the skeletons were in a fragmentary state. The identified pathological changes in this population were trauma-related, such as fractures or myositis ossificans, degenerative diseases, such as osteoarthritis, infections, and congenital abnormalities such as closed ear canals. Dental diseases, such as caries, periodontal disease (periodontitis), enamel hypoplasia, or traces of consumption of areca were also common. In addition, severe attrition and calculus were found in the ancient Gilimanuk community (Tab. 1).

The Semawang population on Bali's Sanur Beach lived in a similar location to the community of Gilimanuk site, with relatively the same culture during the Paleometallic period around 2000 BP (Yuliati 1990). The skeleton population from Semawang consisted of 11 individuals (five males, five females, and one child aged around 10 years). The age range was 25–40 and 20–40 years for males and females respectively. There was no visible disease in the children. In contrast, the adult individuals showed caries, enamel hypoplasia, and changes caused by periodontitis (Tab. 1), similar to the ancient Gilimanuk people. The ancient Semawang people were also found to have severe attrition and calculus on their teeth. Some individuals demonstrated visible teeth modification locally known as pangur (teeth filling) and traces of betel nut consumption. One individual (female) showed an abscess on the mandibular first molar, possibly indicating that the periodontal disease has reached a severe level (Ortner 2003).

Table 1. Paleopathological types of skeletons of ancient populations in Gilimanuk, Semawang, and Pacung Sembiran, Bali Indonesia

Population	Skeleton	Paleopathological lesions	Sex				Number of individuals
			M	F	UN		
					A	NA	
Gilimanuk (300)	Teeth	Caries	–	4	–	1	5
		Enamel hypoplasia	5	6	–	–	11
		Periodontitis	1	1	–	–	2
		Abscess	–	1	–	–	1
		Severe attrition	6	10	1	5	22
		Calculus	13	17	–	–	30
	Bones	Osteophytes	10	10	2	–	22
		Fracture	5	3	–	1	9
		Porotic hyperostosis	4	1	–	–	5
		Spina Bifida Occulta	2	2	–	–	4
Semawang (11)	Teeth	Caries	2	1	–	–	3
		Enamel hypoplasia	1	–	–	–	1
		Periodontitis	2	2	–	–	4
		Abscess	–	1	–	–	1
		Severe attrition	3	2	–	–	5
		Calculus	3	2	–	–	5
	Bones	Osteophytes	–	–	–	–	–
		Fracture	–	–	–	–	–
		Porotic hyperostosis	1	–	–	–	1
Pacung Sembiran (25)	Teeth	Caries	2	–	3	1	6
		Enamel hypoplasia	1	1	–	1	3
		Periodontitis	–	–	–	–	–
		Severe attrition	2	1	3	–	6
		Calculus	2	4	4	1	11
	Bones	Osteophytes	–	–	–	–	–
		Fracture	1	–	1	–	2
		Porotic hyperostosis	2	–	–	–	2

M: male; F: female; UN: unknown; A: adult; NA: non-adult

Situated on the northern coast of Bali, the Pacung Sambiran sites lie 250 meters above sea level. The Pacung Sembiran people, who inhabited Bali during the early first century AD, lived during the Paleometallic or iron bronze period (Calo

et al. 2015). A total of 25 skeletons were examined, comprising 8 adult males, 7 adult females, and 6 children while sex of 4 adult individuals could not be determined. The age range spanned from 16 to 45 years for adults, and 8 to 10 years



for children. Various pathological changes were identified among these individuals, including enamel hypoplasia, caries, porotic hyperostosis, and fractures (Tab. 1). Additionally, a supratrochlear foramen was found on the humerus. There were also a lot of severe attrition and dental calculus observed in individuals from the three above-mentioned sites which could be considered as a degenerative or physiological change rather than a disease.

## Discussion

The majority of pathological changes identified in human skeletal remains obtained from Indonesian sites typically manifest in the teeth. This is primarily due to the teeth's exceptional durability, making them the most resilient part of the body, capable of preserving evidence of pathological changes long after other tissues have decomposed (Roberts and Manchester 2005). Furthermore, teeth serve as the initial point of entry for food, increasing their susceptibility to the accumulation of various pathogenic agents (Ungar 2014). Among dental diseases, caries, enamel hypoplasia, and periodontitis are the most prevalent findings.

Dental caries, a prevalent disease in modern society, serves as a mirror reflecting an individual's oral health and dietary habits. This infectious disease of the teeth, caused by the activity of bacteria like *Streptococcus mutans* and *Lactobacillus acidophilus* (Roberts and Manchester 2005), typically damages the tooth's structure, crown, or roots, potentially disrupting mastication processes and diminishing overall quality of life (Sheiham 2006). The presence of dental caries in all three populations examined at this research site, both in

the maxilla and mandible teeth, can be attributed to dietary shifts from hunting to agriculture, leading to increased consumption of carbohydrates and vegetable protein, consequently fostering the development of caries (Klaus 2017). The high frequency of caries alongside tooth loss may also indicate underlying health issues, particularly those related to dietary habits (Ungar 2014). This study found that females have a higher prevalence of caries compared to males, which is supported by Boonlop (2018), who studied 362 prehistoric human skeletons (5000 BP) from 13 archaeological sites in Thailand and discovered that females were more likely than males to have dental caries. Dental caries disease was found in 3 out of 11 individuals from the Semawang site, in 5 out of 25 individuals from the Pacung Sembiran site, and in 5 out of 300 people from the Gilimanuk site (Tab. 1), where many female individuals were affected by this disease (Fig. 2).



Fig. 2. Dental caries on maxillary premolar and molar at the Gilimanuk site (individual IA)

Enamel hypoplasia (EH) is a dental condition characterized by defects in the enamel, manifested as lines, small holes, or indentations (Roberts and Manchester 2005). It can be associated with various diseases, including syphilis, rickets, dental trauma, tuberculosis, and malnutrition (Ortner 2003). Although EH is not as prevalent as severe attrition, calculus, or caries, it was observed in specimens derived from these three sites. In the Pacung Sembiran populations, around 3 out of 25 individuals exhibited EH, while in the Semawang population, 1 out of 11 individuals were affected. In contrast, only around 11 out of 300 individuals of the Gilimanuk people experience this condition (Tab. 1). Several factors contribute to EH, including trauma, metabolic stress, genetics, and environmental pressures. Research by Koesbardiati et al. (2018) on ancient populations revealed the presence of EH in 14% (7 individuals) of individuals from the Liang Bua site and 16% (3 individuals) from the Melolo site in East Nusa Tenggara, Indonesia. Noerwidi (2020) study of the Javanese Binangun and Leran populations, residing on Java's north coast, indicated that 5 out of 10 individuals (50%) experienced EH. These findings suggest that life on Java's north coast may have been challenging in the past, exposing the population to many pressures. King et al. (2005) highlighted the potential of using EH as an indicator of assessing childhood growth and patterns of morbidity and mortality in past populations. However, only one child found in the Pacung Sembiran sites exhibited EH. Klaus (2020) proposed that the frequency of enamel hypoplasia in a population tends to increase with the intensification of agricultural practices, population growth, and rising social complexity.

Concurrent with enamel hypoplasia, periodontitis was also observed in the studied populations. Periodontitis, also known as gum infection, is a destructive that affects soft tissues, supporting bone structures, and can also affect teeth. It arises as a complication of untreated gingivitis, an inflammation of the gums. Periodontitis has been documented since 130 years ago in Neanderthal Kaprina, Croatia (Murti et al. 2023). The incidence of periodontitis in the sample of the individuals from the Gilimanuk site was lower compared to other dental diseases, found in only two individuals (one male and one female) from the Gilimanuk site, while there were four individuals (one man and three women) at the Semawang site were affected, whereas no cases were found at the Pacung Sembiran site (Tab. 1). Research conducted by Murti et al. (2023) on a skeletal population from Kotabaru, South Kalimantan, Indonesia, revealed the presence of periodontitis affecting the maxillary anterior teeth, attributed to a high-carbohydrate plant-based diet.

Betel nuts are commonly consumed in Asia and the Pacific. Betel nut leaves reddish-brown stains on the teeth (Koesbardiati and Murti 2019). Betel nut chewing has also been proposed as a potential contributing factor to periodontitis, with its ingredients potentially triggering inflammation in the periodontal tissues. In addition, oral health status and vitamin C deficiency may play a role in the development of this disease (Noerwidi 2020). According to archaeobotanical reports, areca pulp (*Areca catechu* L) can chew betel (Zumbroich 2007/2008). Meanwhile, Rusyad and Koesbardiati (2010) discovered tooth coloring in Polynesia and Micronesia, using lime, areca nut (*Areca catechu*), gambier (*Uncaria*

*gambir Roxburgh*), and betel (*Piper betel*). Nguyen (1990) described the widespread practice of applying black varnish to teeth in Vietnam and its impact on the incidence of dental caries, whereas Suddhasthira et al. (2006) reported that this habit is commonly practiced by Thai people and, according to archaeological remains, this tradition was carried out around 5000–4000 years ago.

Apart from the three diseases described above, many individuals from the above-mentioned sites were found with calculus and severe attrition. Tooth attrition involves a gradual loss of tooth structure or tissue due to contact between teeth. This erosion of the tooth layer occurs over time as a result of excessive friction between the upper and lower teeth during chewing (Sperber et al. 2017). In ancient societies, severe tooth attrition was often observed due to dietary habits and rudimentary food processing techniques. Heavy diet patterns, such as the consumption of abrasive materials mixed with food, coupled with limited use of fire to soften food, contributed to the high prevalence of tooth wear in these populations (Roberts and Manchester 2005). In the Semawang population, 5 out of 11 individuals showed signs of tooth wear, while in the Pacung Sembiran population, 6 out of 25 individuals exhibited signs of tooth wear. In contrast, the residents of Gilimanuk showed incidents of tooth wear in 22 out of 300 individuals. As noted by Sperber et al. (2017), wear patterns on teeth can provide valuable insight into the properties of food materials consumed by a population. It might be that severe attrition observed in this ancient human populations residing in coastal habitats was primarily caused by the ingestion of sand particles carried by

soft-bodied organisms, such as shellfish or snails, which were likely part of their diet. As suggested by Noerwidi (2020), ancient people in Binangun and Leran sites, North of Central Java consumed snails as a part of their diet.

Dental calculus, also known as tartar, is a hardened form of dental plaque, a sticky film of bacteria that adheres to the surface of teeth. This calcification occurs when minerals from saliva and gingival crevicular fluid interact with plaque, leading to the formation of a hard, crusty deposit. The presence of tartar on the teeth of individuals who lived millennia ago offers a valuable insight into their dietary habits, types of bacteria that inhabited their oral cavities, and their overall oral health (Roberts and Manchester 2005). Among the dental changes identified, calculus exhibited the highest prevalence in the Pacung Sembiran sites compared to the Gilimanuk and Semawang sites. For example, 5 out of 11 individuals from the Semawang site exhibited calculus, while in Pacung and Sembiran 11 out of 25 individuals showed calculus (Tab. 1). In the Gilimanuk population, the incidence of calculus accounted for 30 out of 300 individuals. The formation of calculus is linked to the accumulation of food debris between teeth that remain uncleaned (Fig. 3). A high prevalence of calculus is typically associated with high consumption of carbohydrates and proteins or an environment with acidic water (Roberts and Manchester 2005). Noerwidi (2020) posited a link between calculus formation and the consumption of sugary foods or beverages. Research by Prayudi and Suriyanto (2018) revealed the presence of calculus and periodontal disease, in individuals from the Paleometallic period.



Fig. 3. Dental calculus on the maxillary premolar and molars at the Gilimanuk site (individual IA)

In addition to the periodontal disease mentioned earlier, other prevalent bone pathological changes included osteophytes, fractures, porotic hyperostosis, and spina bifida occulta. Osteophyte disease manifests itself as a form of the body's reaction to disorders surrounding the joints. Variations in age, sex, population, and mechanical stress are known to influence bone osteophyte changes. The incidence of osteophytes in ancient populations was not observed at the Semawang and Pacung Sembiran sites, whereas at the Gilimanuk site approximately 22 out of 300 individuals exhibited signs of osteophytes (Fig. 4). Our data suggest that the incidence of osteophytes at the Gilimanuk site does not differ between males (10 individuals) and females (10 individuals), while 2 cases of osteophytes were detected in children. This is not in line with the Kim et al. (2012) study that stated that more severe osteophytes occur more often in men be-

cause, compared to women, they tend to perform heavier physical activity. In this study, osteophytes were frequently found on the vertebrae and metatarsals.



Fig. 4. Osteophytes on a lumbar vertebra at the Gilimanuk site (individual VIII)

Fractures, a common bone disease in humans that have been present throughout history, were also observed in the Gilimanuk, Pacung, and Sembiran populations. Fractures were found in nine out of 300 individuals at the Gilimanuk site and two out of 25 individuals at the Pacung Sembiran site, with a higher frequency in males. This trend is consistent with the findings of Domett and Tayles (2006), who studied the skeletons of the adult Thai population (2000–400 BC) and reported that the number of male sufferers (12 individuals) was higher than that of females (7 individuals). These individuals' fractures are most likely the result of a combination of everyday accidents, such as agriculture and animal husbandry, as evidenced by fractures in long bones. Moreover, fractures in the skull might indicate interpersonal

violence. Fractures in the Gilimanuk population were primarily found in the rib bones, while fractures in the Pacung Sembiran sites were more common in molar teeth, which are fractures of the tooth crown that occur while the individual is still alive. Further examination revealed that all fractures in the costal bones had healed, as evidenced by the presence of bones that had reconnected but were not evenly connected, which suggests a natural healing process.

Porotic hyperostosis is exhibited by small, pore-like holes typically found on the frontal or parietal bones of the skull, with a few occasionally present on the occipital bone. These holes are usually symmetrical and, in advanced stages, may give the impression of skull thickening. According to Aufderheide et al. (1998), these holes are indicative of anemia but can also arise from other diseases such as leukemia, thalassemia, aplastic anemia, or potential malnutrition. Blom et al. (2005) observed a higher prevalence of porotic hyperostosis in coastal populations compared to those residing in highlands. The incidence of porotic hyperostosis at the Semawang site was 1 out of 11 individuals, at Pacung Sembiran 2 out of 25 individuals, while at the Gilimanuk site, 5 out of 300 individuals exhibited porotic hyperostosis (Tab. 1). Notably, the parietal bones were the most commonly affected by porotic hyperostosis. Associated with porotic hyperostosis is cribra orbitalia, which was also found in one juvenile at the Gilimanuk site. Cribra orbitalia was not observed in individuals from (Semawang and Pacung Sembiran). Klaus (2020) posits that porotic hyperostosis and cribra orbitalia have long been associated with metabolic disorders linked to anemia. Cribra orbitalia and porotic hyperostosis

are morphological syndromes that can be manifested as downstream consequences of various secondary conditions associated with chronic anemia. Walker et al. (2009) also highlight that porotic hyperostosis and cribra orbitalia are among the most frequently observed pathological lesions in ancient human skeletal assemblages. Since the 1950s, chronic iron deficiency anemia has been widely accepted as a plausible cause of both conditions.

Spina bifida occulta (SBO) is a spinal disorder that often associated with chronic back pain. According to the Spina Bifida Association (Aufderheide et al. 1998), approximately 10–20 percent of individuals are affected by SBO. What makes SBO unique is that many individuals who have this condition are unaware of its presence, as symptoms may be absent. SBO can occur along any part of the spine but is most prevalent in the sacral region (Aufderheide et al. 1998; Merbs 2004), and, in this instance, it is referred to as sacral spina bifida occulta (SSBO). In this study, SBO was only observed at the Gilimanuk site, affecting four adult individuals (two males and two females). Research by Molto et al. (2019) on a skeletal population inhabiting the Dakhleh Oasis, Egypt, reported that around 15.6% of the sample exhibited SSBO, with a temporal increase in males. In addition, in both sexes SSBO tended to increase with advancing age. All of the four individuals with SBO at the Gilimanuk site were found in the sacrum bone. The age range for the male individuals was between 20 and 40 years, while the female individuals were between 25 and 30 years old.

In addition to the above-mentioned bone and dentition diseases, evidence of dental modifications, such as *pangur* is (tooth-filing) and betel chewing, has



been unearthed at these archaeological sites. As noted by Noerwidi (2020), the examination of cultural practices on human skeletal remains provides valuable insights into living habits and cultural identity in the past, often manifested in the form of morphological alterations to specific body parts, such as teeth. Dental modifications in the forms of *pangur* and betel chewing have been observed at the Gilimanuk, Semawang, and Pacung Sembiran sites. At the Pacung Sembiran site, there were around 12 out of 25 individuals exhibiting signs of betel chewing, as evidenced by tooth discoloration. At the Gilimanuk site, 17 individuals engaged in betel chewing practices, while approximately 22 individuals displayed *pangur* tooth modifications. Among the population at the Semawang site, six individuals exhibited tooth filling, but no evidence of betel chewing was found. *Pangur* typically involves filing the visible portions of the teeth from the outside of the mouth. This practice persists in several regions of Indonesia, including Bali, and may serve as a marker of gender distinction post-puberty (Prayudi et al. 2020). The majority of tooth modifications observed at the three aforementioned sites are located on the maxillary and mandibular incisors. Noerwidi (2020) study on ancient populations at the Binangun and Leran sites on the North Coast of Java reported evidence of betel chewing practices, with remnants of areca nut and chalk observed on the buccal and lingual surfaces of several molar teeth.

This study offers valuable insight into the types of pathological changes and health conditions that prevailed among ancient populations of Gilimanuk, Semawang, and Pacung Sembiran, situated in coastal regions on the island of Bali. Several dental diseases predom-

inated in these populations. This study, however, has its limitations and requires further refinement. More research, incorporating microscopic observations and molecular approaches, is needed to gain a more comprehensive understanding of the specific diseases and infections that afflicted the ancient inhabitants of these sites. The outcomes of this research will contribute to addressing contemporary health challenges and inform preventive measures.



Fig. 5. *Pangur* on maxilla teeth at the Semawang site (individual XII)

The results of the identification of 336 skeletons of ancient populations from the Gilimanuk, Semawang, and Pacung Sembiran sites on the Indonesian island of Bali show the dominance of caries and enamel hypoplasia. Bone diseases and abnormalities that can be observed include osteophytes, porotic hyperostosis, fractures, and spina bifida occulta. Moreover, there are traces of betel nut consumption and tooth modification in the form of *pangur* (teeth filing) in some individuals.

The presence of disease in ancient humans at these sites indicates similar types of disease with unequal prevalence. However, the absence of disease markers in certain individuals doesn't necessar-

ily imply they were healthy during their lifetime. Alternative possibilities include diseases that didn't leave skeletal traces, such as skin diseases, or the individual's death before the disease manifested in skeletal lesions.

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### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication.

### Authors' contributions

NTR – was the lead researcher, conceived the concept and design, identified samples, data collection, article writing, and critical revision of the article for important intellectual content; AP – identified samples, performed data collection, and critical revision of the article for important intellectual content; RAS – identified samples, performed data collection and compilation; JH – performed data collection and compilation; FA – performed data collection and the photographer; ARH – performed data collection. All authors discussed the results and contributed to the final manuscript for publication.

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# Hominin musical sound production: palaeoecological contexts and self domestication

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**ABSTRACT:** In this article we seek to integrate theories of music origins and dance with hominin fossil anatomy and the paleoecological contexts of hominin evolution. Based on the association between rhythm in music, dance and locomotion, we propose that early bipedal hominins may have evolved neurobiological substrates different from other great apes due to the rhythmic aspects of bipedal walking and running. Combined with the emancipation of the hands resulting from erect posture, we propose that the neurobiological changes necessary for technological innovation, cultural practices and human musical abilities may have evolved, at least in incipient form, much earlier than previously thought. The consequent ability to synchronize movement and sound production may have also proved beneficial as early bipedal hominins ventured out of late Miocene and early Pliocene woodland and forested habitats and into more open habitats with increased predation risk. We also postulate that, along with bipedalism, paedomorphic morphogenesis of the skull at the base of the hominin clade was a necessary prerequisite for the evolution of vocal modulation and singing in later varieties of hominin. To date research into the evolution of music and dance has yet to be integrated with the fossil and paleoecological evidence of early hominin evolution. This paper seeks to fill this lacuna in the extant literature on human evolution. We also suggest that auto-catalytic feedback loops evolving synergistically with hominin erect posture, skull and hand morphology, neurochemical processes and the self-domestication syndrome, have been operative from early hominins some 6 Ma to the present. We document this process by reference to primatological, ethnographic, neurochemical and archaeological data.

**KEY WORDS:** evolution of music, evolution of dance, early hominins, bipedalism, paleoecology, locomotion, archaeology, *Ar. ramidus*, self-domestication.



Original article

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## Introduction

In recent decades there has been a resurgence of interest in the evolutionary foundations of music and dance (Falk 2004; Brown et al. 2006; Mithen 2009; Brown 2017; Clark and Henneberg 2017; Dissanayake 2021; Benítez-Burraco and Nikolsky 2023). Various approaches have been adopted to elucidate the evolutionary origins and adaptive significance of such human behaviours. For example, based on evidence from chimpanzee rhythmic perception, it has been argued that the prerequisites for music and dance probably existed in the common ancestor shared by humans and chimpanzees (Hattori and Tomonaga 2020). In a similar vein, based on vocal tract anatomy and skull architecture, it has been proposed that early hominins evolved an anatomical configuration more conducive to musical vocalization than that evident in chimpanzees – and that consequently research into the evolutionary building blocks of music and language should focus on late Miocene and early Pliocene fossil anatomy (Clark and Henneberg 2017).

There has also been important research looking into the relationship between locomotion and rhythmic aspects of music and dance. This work has illuminated the degree to which the rhythmic aspects of walking may share neurobiological substrates with the rhythmic structuring of music and dance (Friberg and Sunberg 1999; Shove and Repp 1995; Thaut 2009; Bengtsson et al. 2009; Grahn and Rowe 2009; Thaut 2013). Additionally, it has been proposed that the evolution of the human bipedal locomotor adaptation gave rise to forms of metrically synchronised bodily entrainment using the upper and lower limbs, a form

of entertainment that was to later form the basis of rhythmic vocal chorusing and music (Brown 2022).

While links have been made between the emergence of erect trunk bipedalism and the evolution of music and dance (Mithen 2009), very little research has explored this association in the context of emerging fossil and palaeoecological data regarding the emergence of erect bipedalism in early hominins. Given facultative bipedalism is believed to have emerged in early forest and woodland dwelling hominins such as *Ar. ramidus*, which then developed into obligate bipedalism among Australopiths (Lovejoy and Latimer et al. 2009; Kimbel et al. 2014), the association between music, dance and locomotory adaptations warrants further exploration in the context of emerging evidence from the fossil record.

The concept of self-domestication has been used to explore the origins of both language and music, highlighting associations with cultural transmission, pro-sociality and neurochemical regulation (Clark and Henneberg 2017; Thomas and Kirby 2018; Benítez-Burraco and Nikolsky 2023). Importantly, oxytocin is thought to be associated with in-group cohesion in chimpanzees and bonobos (Brooks et al. 2022) as well as the prosocial and affiliative aspects of music and dance in humans (Dissanayake 2021; Harvey 2020). While the oxytocin system is thought to be an important component of hominization and the evolution of cooperative and alloparental breeding systems (Hrdy 2009; Lovejoy 2009), it has yet to be fully explored in the context of the evolution of music, dance and relevant fossil and palaeoecological data. For example, while Clark and Henneberg (2017) analyse the possible relevance of the oxytocin system

in explicating the evolutionary building blocks of music and language in early hominins such as *Ar. ramidus*, to date such an approach has not been explored in the context of early hominin paleoecology and the demographic expansion of the Australopithecines into non-arboreal habitats.

Archaeological approaches to the evolution of music have been discussed by numerous researchers (Blake and Cross 2008; Morley 2013; Fazenda et al. 2017; Turk et al. 2018). However, taphonomic issues may problematize such research as the earliest evidence for such behaviors may not have been preserved in the archeological record (Bednarik 1994). Despite such problems, putative musical instruments have been attributed to not only *Homo sapiens* but also Neanderthals (Turk et al. 2018). Additionally, caves possess acoustic properties that have long been exploited by humans (Fazenda et al. 2017). This behavioral phenomenon is very suggestive in light of emerging evidence of cave occupation by other hominin species in addition to *Homo sapiens* (Fuentes et al. 2023; Jaubert et al. 2016). For example, is it possible that occupation of caves by hominins may have contributed to an enhancement of auditory perception and sound production capabilities?

There has also been research exploring the previously overlooked sound producing properties of stone tools (Blake and Cross 2008). Increasing evidence pushing back the antiquity of tool manufacture to the Australopithecines (Harmand et al. 2015) suggests the possibility that early hominins were using physical objects to produce sound. Given chimpanzees use objects such as trees in such a manner (Eleuteri et al. 2022) it is possible that Australopithecines may have used

stone tools not only in procuring food but also to produce sound.

Based on their tool manufacturing abilities, it has been suggested Australopithecines had elevated levels of hand motor control resulting from reorganization or expansion of the brain's various motor regions (Harmand et al. 2015). Combined with enhanced manipulative abilities resulting from bipedalism, which enables emancipation of the forelimbs from locomotion (Lemelin and Schmitt 2016), it seems possible that Australopithecines may have possessed sound production abilities beyond that evident in other great apes. While it has been previously suggested that stone tools may have been the first musical instruments (Montagu 2004), this theory has not been investigated in the context of early hominin anatomy, archaeology and paleoecology. Given the previously overlooked sound producing properties of stone tools it has been argued that existing lithic collections could be reanalysed in order to discover possible evidence of early human sound production (Blake and Cross 2008: 17).

It has also been argued that the collaborative synchronization underpinning music and dance may have evolved as a form of coalitionary signaling (Hagen and Bryant 2003: 24); additionally it has been proposed that such forms of coordinated sound production may have been utilized by hominins to deter predators, particularly as they moved out of arboreal habitats into more open habitats with higher predation risk (Jordania 2014, 2020). Importantly, one of the major shifts in hominin phylogeny was the demographic expansion of Australopithecines out of forested and woodland areas, and into more open and diverse habitats as obligate terrestrial bipeds (Meindl, Chaney, and Lovejoy 2018).

In this article we explore possible evidence for early forms of hominin sound production. We propose that as early hominins became obligate bipeds and moved out of arboreal forest and woodland habitats, the anatomical configuration associated with the skull, hands and body would have become part of an adaptive suite facilitating enhanced sound production using both the voice and objects. We propose that these abilities may have evolved as part of a broader adaptive complex involving enhanced levels of in-group cooperation. It was these early forms of sound production, and the associated changes in motor systems and neurobiological substrates, that formed the phylogenetic building blocks of later forms of communication, music, dance and ritual behaviors. We also suggest ways in which this hypothesis can be tested by increased awareness of other forms of sound production that may have left traces in the archeological record and which to date have been overlooked.

### **Canines, predators and synchronized sound production**

In this section we outline a model of human music, language, dance and sound production grounded in evolutionary percussors that constitute the putative phylogenetic building blocks of these forms of cultural expression. This will involve synthesizing data on paleoecology, predation pressure and hominin fossil anatomy. Importantly, we will develop a broader conception of sound production than that normally conceived of in modern cultures. For example, an evolutionary and cross species conception of sound production includes song, nonverbal vocalization, sound made with the

body, physical substrates and manufactured objects – which in the case of past hominin cultures may include the sound producing properties of stone tools or the acoustically resonant spaces inside cave structures (Blake and Cross 2008, 2015; Fazenda et al. 2017). Many of these forms of sound production or acoustic signaling are embedded in ecological and cultural contexts in which auditory forms of sociality would have had survival benefits for those individuals or groups that possessed them (Blake and Cross 2015).

The unique features of hominin sound production, whether it be vocal, bodily or produced with objects, seem to be related to the unique hominin anatomical configuration. These include erect bipedalism, non-locomotory hand morphology, a flat face and loss of the canine honing complex characteristic of other primates. We will argue these traits, while not being sufficient, are nevertheless necessary preconditions for the evolution of the motor systems and neurobiological substrates that subserve vocal modulation, tool manufacture and the creation and use of musical instruments. We also explore evidence that obligate bipedalism led to the evolution of neurobiological systems that subserve not only the pace and timing of locomotion, but also rhythmic perception necessary for the temporal structuring of music and dance (Thaut 2013). Given these anatomical features are very ancient we explore their original emergence and their important role in hominization.

Researchers have argued that synapomorphic traits that differentiate hominins from other great apes include a more anteriorly positioned and horizontally oriented foramen magnum associated with vertical neck posture in orthograde bipedal locomotion, as well as dental



traits such as reduced canines (Mongle et al. 2019). Importantly, a recent analysis of 300 fossils spanning 6 million years has, according to the authors, demonstrated that ‘male canine size reduction occurred early in human evolution, broadly coincident with the adoption of bipedality’ (Suwa et al. 2021: 1). Importantly, a centrally positioned foramen magnum characteristic of hominin bipedalism, combined with reduced facial projection and lack of aggressive canine armory, gives rise to the unique hominin skull and vocal tract configuration that is a necessary prerequisite for the evolution of spoken language and singing (Clark and Henneberg 2017).

The majority of Miocene hominoids are believed to have been arboreal or semi-arboreal (Rose 1993). Additionally, there is evidence that early hominins were arboreal facultative bipeds (Lovejoy and Simpson et al. 2009). It therefore seems likely that early hominins sought refuge from predators in trees – as is the case in other primates (Baldwin et al. 1981: 482; Hamilton 1982: Boesch 1991: 228; Iwamoto et al. 1996: 393). In addition to such ecological factors, there is also a vocal component to primate predator defence involving loud vocalisations and alarm calls (Tutin et al. 1981; Tsukahara 1993; Boesch 2009: 22–23 and 52–53). There may also be predation induced selection on social structure associated with non-arboreal habitats; for example, savanna-living chimpanzees travel in large numbers when moving between patches of trees, which may be an anti-predator response (Tsukahara 1993) while baboons who evolved in open savanna or semidesert habitats jointly drive predators from the group (Kummer 1967: 154–155). Additionally, geladas who also inhabit more open habitats, utter alarm

calls and form multilevel fission-fusion social structures in response to predation – which has been proposed as a possible model for early hominin occupation of non-arboreal habitats (Lin et al. 2020).

What are the implications of the above observations for the evolution of hominin sociality and sound production? We suggest that when erect bipedal hominins moved out of woodland and forested ecologies into more open habitats, they may have also used vocalisation, alarm calls and various forms of non-vocal sound production to deter predators. Importantly, it has been argued that hominins may have been subject to predation, from *Ar. dipithecus ramidus* to modern humans, and that consequently social cooperation and associated neuroendocrine systems may have evolved as a response to early hominins being initially a prey species – a theory that finds support from evidence that cooperation can reduce lethal outcomes resulting from predator attacks (Hart and Sussman 2011, 2019). We would add to this observation the possible role synchronised sound production and movement may have played in this putative adaptive suite.

Many of the features we analyze in early arboreal facultative bipeds such as *Ar. ramidus* seem to be associated with forested and woodland ecologies. Evidence for this view is based on paleohabitat and dentition (White et al. 2009). While this position has been questioned based on the existence of contemporaneous ‘tree or bush savanna’ in the region (Cerling et al. 2010), White and colleagues assert that *Ar. ramidus* did not occupy such an ecological niche, with the species’ primary habitat consisting of closed forest and woodland (White et al. 2010). It has also been argued that *Ar. ramidus* may not be a hominin but

a fossil relative of chimpanzees – and that additionally there is little evidence of bipedalism in the species (Senut 2015: 2053–2054). This perspective, however, seems at odds with a more recent analysis of character sampling that suggests *Ar. ramidus* was in fact a basal hominin (Mongle et al. 2019). This position is further supported by evidence of facultative bipedality in the *Ar. ramidus* cranial base, which is believed to show extensive affinities with *Australopithecus* and *Homo* (Kimbel et al. 2014). Further, the cranial base angle, as well as facial, jaw and canine morphology in *Ar. ramidus* are distinctly different from chimpanzees, showing pronounced affinities with hominins (Clark and Henneberg 2015, 2017). Importantly, erect bipedalism also means hominins do not have hands specialized for locomotion as in quadrupedal apes. It is this lack of specialization in the forelimbs that ultimately underpins human technological and cultural evolution as well as coevolutionary processes between the hands and the brain (Lundborg 2013).

The absence of large aggressive canines and canine sexual dimorphism in hominins has long puzzled evolutionary theorists and numerous explanations have been put forward to account for this unusual phenomenon. For example, in *The Descent of Man* Darwin argued that with the invention of clubs and other weapons, canines were no longer necessary in male on male conflict (Darwin 2004:73). However, this does not seem to be a complete solution for it does not postulate a selective mechanism for canine reduction; it merely suggests weapons could have taken the place of canines without explaining what potential selective pressures may have been involved in canine reduction.

It has been suggested that selection for the diminution of aggression and changes in hormonal regulation may have been a possible evolutionary mechanism resulting in canine reduction (Holloway 1967). Others have proposed similar solutions to the dilemma of canine reduction with a shift in social and mating behavior and associated neurochemical regulation being considered a possible explanation (Lovejoy 2009; Clark and Henneberg 2017, 2015). Some researchers have suggested that this may have involved a socio-behavioural shift that minimized male–male aggression possibly mediated by female mate choice (Suwa et al. 2021). What is significant is that early hominins such as *Ar. ramidus* seem to have lost the aggressive canine armory characteristic of many other primate species prior to becoming terrestrial obligate bipeds. It is important to note that *Ar. ramidus* still possessed a grasping big toe and that the species most likely climbed and walked bipedally among the large trees of late Miocene and early Pliocene Africa (Lovejoy and Simpson et al. 2009; Lovejoy and Latimer et al. 2009).

Importantly, some species such as baboons evolved extreme forms of canine sexual dimorphism, which are in part related to the need for predator defense in what are essentially hostile savanna habitats with high predation risk (Plavcan and van Schaik 1992). Having lost aggressive canine armory in forested and woodland habitats, the question arises as to how early hominins would have survived when they ventured into habitats lacking trees that could serve as places of refuge from predators? Is it possible that coordinated sound production and body movement were part of an antipredator adaptive suite?



Based on the above observations, we hypothesize that early hominins such as *Ar. ramidus* may have already possessed social adaptations that enabled significant levels of prosocial coordination and cooperation. While such adaptations no doubt would have evolved for many reasons, they would have been useful when encountering predators, and such encounters may have been one component in a complex selective regime favoring their enhancement. Consequently such cooperation, combined with synchronized vocalization and sound production may have manifested itself in a form of “territorial chorus” that provided some of the building blocks for the evolution of music and language (Brown 2017). Our argument is premised on the notion that pro-sociality and cooperative breeding correlate across primate taxa with reduced canine size and reduced canine sexual dimorphism – social adaptations that may explain such reduction in early hominins (Lovejoy 2009; Clark and Henneberg 2015). Importantly, co-operatively breeding primates show elevated levels of generalized pro-social behavior (Burkart et al. 2007) as well as coordinated and synchronized vocalization abilities akin to human conversation (Choi et al. 2015). This has led to speculation that co-operative breeding in *Ar. ramidus* may have been accompanied by increased vocal synchrony and ability to modulate vocalizations – an assertion that finds additional support from the species skull architecture and vocal tract anatomy which is more human-like when compared with chimpanzees (Clark and Henneberg 2017).

Significantly, as noted by Schruth and colleagues among anthropoids only the monogamous and swinging lesser apes seem to share the human aptitude for spectral musicality (Schruth et al. 2023).

Consequently, given the association between such aptitudes and forest dwelling arboreal primates, they propose musical ability as a primitive trait characteristic of Miocene apes that was consequently lost, or which became atrophied as primate species ventured into more terrestrial habitats. Humans are unusual among terrestrial primates in that we retain such musical abilities, which are much more common in arboreal species. As the authors suggest in ‘strictly terrestrial primates, previously evolved associations between musical calling and [arboreal] locomotion appear to have atrophied’ (Schruth et al. 2023: 9). This may be due to the fact that singing in open terrestrial habitats many attract predators, whereas this may not be an issue in forest canopies which serve as refugia (Jordania 2020). From such a perspective, as opposed to losing such abilities when they ventured into more terrestrial habitats, hominins retained them, which then served as the phylogenetic building blocks for later forms of musicality. The question that needs addressing is why did terrestrial hominins retain musical calling?

The association between arboreal forest ecologies, monogamy and protomusical calling noted by Schruth and colleagues, is also very suggestive in the context of early hominin social and mating behaviour. It suggests that early hominins such as *Ar. ramidus* may have not only been anatomically similar to arboreal lesser apes (Lovejoy 2009) but may also have shared with such species aspects of mating, social and musical behavior (Schruth et al. 2023: 9; Clark and Henneberg 2017). This may have involved pair-bonding and forms of cooperative or alloparental care of offspring (Lovejoy 2009) possibly mediated by forms of prosocial vocal synchrony (Clark and Henneberg 2017).

While there is extensive evidence of cooperative breeding and allomaternal care in *Homo sapiens* (Hrdy 2009) it is unclear when this breeding strategy evolved. Consequently, it is very difficult to infer social and mating systems from fossil anatomy with any certainty. However, reduced canine size and dimorphism does correlate across primate taxa with reduced male on male aggression and *Ar. ramidus* canine size and dimorphism is nearly human-like; consequently, it has been argued this evolutionary pattern in *Ar. ramidus* indicates a 'profound behavioural shift associated with comparatively weak levels of male aggression early in human evolution, a pattern that was subsequently shared by *Australopithecus* and *Homo* (Suwa et al. 2021: 1). Such an adaptive complex in *Ar. ramidus* may have involved cooperative breeding and male investment in the maternal metabolic budget as an alternative reproductive strategy to male on male tournament behaviour (Clark and Henneberg 2015). This may mean that the system of cooperative breeding that is believed to have facilitated the increase in brain size in the *Homo* lineage (Isler and Schaik 2012a) may have evolved much earlier at the base of the hominin clade (Lovejoy 2009; Clark and Henneberg 2015). This position finds support in the fact that cooperatively breeding primates such as marmosets, who engage in forms of vocal communication similar to humans, have a low encephalisation quotient (Ghazanfar and Takahashi 2014) and that human co-operation is not dependent on advanced cognitive abilities, nor on large brain size, and that therefore human pro-sociality may have evolved before the emergence of large brained hominins (Isler and Van Schaik, 2012a).

Additionally, it has been suggested that increases in body and brain size in excess of earlier Australopithecines, would have required contribution to the infant metabolic budget by individuals other than the mother – and that consequently cooperative or alloparental care provides a plausible explanation for the increase in brain size in the *Homo* lineage (Hrdy 2009; Isler and Van Schaik 2012b). From this perspective, "emotional modernity" including cooperative breeding, uniquely human forms of mind reading and intersubjectivity, may have evolved long before increases in brain and body size – in fact such adaptations may have been necessary for such evolutionary trends, which require increased metabolic allocation to infant and childhood growth from other individuals in addition to the mother (Hrdy 2009).

One of the important issues related to the adoption of obligate erect bipedalism in the *Homo* genus, is that it may have facilitated increased metabolic allocation to infant growth and a slower pace of development for infants resulting from the need to learn more complex tool use and foraging technologies (Potts 2011). Significantly, based on correlations between brain size and life history trajectories (Smith and Tompkins 1995) it has been argued that *Ar. ramidus* life history was similar to chimpanzees (Clark and Henneberg 2015). As brain and body size increased in *Homo erectus*, it has been proposed that life history was slowed down to include extended childhood and adolescent phases of development during which social learning could occur, thus enabling the acquisition of language skills (Locke and Bogin 2006) as well as cultural competencies such as increasingly complex tool manufacture (Bogin 2003; Sterelny 2012). Such changes in life his-

tory may have underpinned the socio-behavioural adaptations contributing to the demographic expansion of *Homo erectus* (Hawkes and Coxworth 2013; Hrdy 2009; O'Connell et al. 1999). We would add to this adaptive complex the important role of music and dance in the social transmission of cultural knowledge relating to hunting and kinship systems; for example, in extant gathering and hunting cultures music and dance are the primary means of transmitting knowledge regarding waterholes and hunting grounds from one generation to the next – transmission that is particularly important during adolescent initiation rites (Strehlow 1971; Stanner 2014).

We suggest that the building blocks of this adaptive suite, at least in incipient form, may have been in place at the base of the hominin clade – an inference based on the above mentioned reduction in both canine size and dimorphism (Lovejoy 2009). Significantly, it has been argued that reduced canine and body size sexual dimorphism in hominins suggests that co-operative breeding and allo-parental care may have provided the social contexts for language evolution to develop – and particularly the acquisition of language abilities by infants and juveniles due to intensified interaction with numerous carers (Fitch 2007). We seek to extend this observation in our analysis of the evolution of coordinated sound production and bodily movement. For example, if cooperative breeding did evolve in early small brained hominins, is it possible that vocal cooperation of the kind we find in other cooperatively breeding primates was part of this adaptive complex? And could such cooperation and synchronised sound production have formed the early phylogenetic building blocks upon which later forms of music and dance were built?

The thesis outlined above is obviously a tentative hypothesis and other scenarios are possible. For example, others have argued, based on measures of bodily size sexual dimorphism, that early hominins evolved a gorilla-like polygynous mating system, and that this consequently evolved into human social and mating systems (Geary, Bailey, and Oxford 2011). This approach, while convincing on some grounds, does not account for why early hominins lost the large aggressive canine complex – additionally it has been argued that sexual dimorphism in early hominins falls within the range of modern humans (Reno and Lovejoy 2015). This debate is still ongoing and inferences from fossil anatomy to social and mating systems remain highly contested. In this paper we adopt, as a working hypothesis, the theory that reduced male on male aggression as evidenced by loss of large aggressive canines signals an important behavioural shift involving elevated levels of pro-sociality and possibly forms of alloparental care of offspring – including male investment in the maternal metabolic budget (Clark and Henneberg 2015). We also suggest that adaptations such as increased levels of pro-sociality in *Ar. ramidus* would have benefited later hominin species such as the Australopithecines when they ventured out into more open nonarboreal habitats with increased predation risk.

More specifically, we suggest that coordinated group behavior involving synchronized vocalization, synchronized sound production using physical objects or substrates, in addition to synchronized body movements, could have deterred predators (Jordania 2009, 2020). Because hominins had already developed cooperative social structures in forested and wooded ecologies they could

consequently cope with high predation risk in more open nonarboreal habitats. These adaptations, we suggest, were exapted as the climate changed and the shrinkage of the great forest ecosystems of the Miocene meant that great apes and hominins needed to rely more and more on forms of terrestrial locomotion – with great apes adopting quadrupedal knuckle walking and hominins obligate erect bipedalism. In this sense both erect bipedality and pro-sociality in *Ar. ramidus* were necessary pre-requisites of the Australopith demographic expansion as obligate terrestrial bipeds.

### The phylogenetic depth of the hominin adaptive suite: bipedality, vocal tracts and hand morphology

In this section we propose that erect trunk orientation and the associated skull and hand morphology have much greater phylogenetic depth than traditionally supposed. There are two important consequences of erect trunk orientation that relate directly to the origins of vocal

abilities such as language and singing, as well the motor control necessary for using objects to create sequentially structured rhythm. Firstly bipedalism, due to where the spinal cord enters the skull and connects with the brain, contributes to the shortening of the horizontal vocal tract, with such shortening being a necessary precondition for the evolution of human language and singing (Figs 1 and 2). Secondly, obligate bipedal locomotion emancipates the forelimbs from locomotion (Lemelin and Schmitt 2016). This is the crucial factor underpinning the evolution of hominin hand morphology that enables the development of precision grasping and the consequent co-evolution between the brain and the hands that underpins human technological and cultural accomplishments – from the earliest stone tool industries to classical piano playing (Lundborg 2013; Wilson 1999). We also speculate that the creation of such physical objects out of stones or other materials would have provided hominins with additional means of creating sound that could potentially deter predators – that is an evolutionary precursor of “drumming” (Jordania 2009).

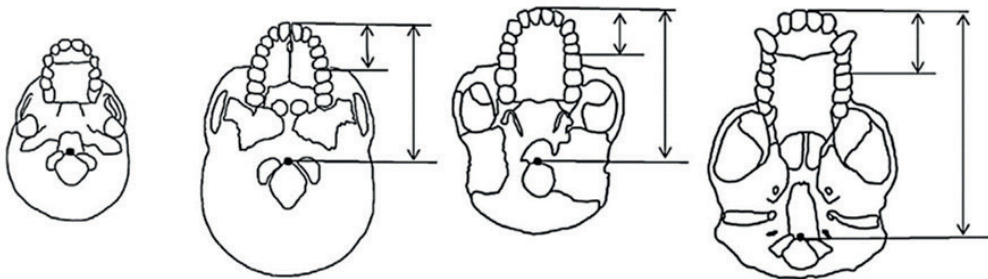


Fig. 1. Length of the face, palate and horizontal vocal tract. This figure graphically illustrates the differences between chimpanzee and *Ar. ramidus* skull architecture. Note the more posterior position of the foramen magnum and basion (black dot) in the adult chimpanzee and the greater resemblance of both humans and *Ar. ramidus* to the infant chimpanzees. From left: infant chimpanzee, adult human, *Ar. ramidus* and adult chimpanzee. From Clark and Henneberg (2017)



Fig. 2. Skull shape and facial projection. Note the spinal cord (black bar) enters from the bottom of the skull in *Ar. ramidus* and the infant chimpanzee and from the rear in the adult chimpanzee. This feature, in addition to reduced facial projection, suggests the evolution of pedomorphic skull morphogenesis in *Ar. ramidus*. From left: adult chimpanzee, *Ar. ramidus* and infant chimpanzee. From Clark and Henneberg (2017)

A crucial perspective we explore in this article is that the skull anatomy of non-human great apes, and particularly that associated with quadrupedal knuckle walking, represents an obstacle that prevents the evolution of human-like vocal ability – and that the absence of this obstacle in early hominins opened the way for language and vocal ability to evolve (Clark and Henneberg 2017). As already noted, many researchers have suggested that bipedalism evolved much earlier than traditionally supposed, with some arguing unique features of the hominin lineage may have their origin in Miocene and Pliocene ecological contexts. For example, there seems to be evidence of bipedal locomotor morphology in the European Miocene, which has been postulated as the ancestral form from which both humans and great apes evolved (Böhme et al. 2019). Other researchers have argued that hominin bipedalism and forelimb morphology may be primitive, with chimpanzee anatomy associated with quadrupedal knuckle walking being derived (Lovejoy et al. 2009; White et al. 2015). This view is consistent with evidence that humans did not evolve from a knuckle walking ancestor as previously assumed and that human biped-

alism originally evolved as an adaptation to arboreal habitats (Kivell and Schmitt 2009). Others, on the other hand, have questioned the above interpretation, arguing that the last common ancestor humans share with great apes was in fact similar to chimpanzees in terms of possessing knuckle walking quadrupedal locomotor morphology (Chaney et al. 2022; Prang 2019; Prang 2021; Williams et al. 2023).

Whatever the case regarding the last common ancestor hominins share with chimpanzees and bonobos, there seems to be a consensus regarding the common ancestor of hominins. For example, mounting evidence from the Miocene and early Pliocene, suggests that the common ancestor of early hominins such as *Ardipithecus*, *Orrorin*, and *Sahelanthropus* was likely adapted to vertical climbing and perhaps suspension – and that the common ancestor of hominins was orthograde and not a knuckle walking terrestrial quadruped (Ward 2013).

Importantly, the grasping toe in *Ar. ramidus* was ‘retained for several million years on a foot otherwise adapted for terrestrial bipedalism’ (Williams et al. 2022: 71). This suggests, that in hominins at least, an early form of bipedal

posture existed in conjunction with arboreal, climbing adaptations in the foot. The occurrence of erect facultative bipedality in forest and woodland dwelling hominins capable of tree climbing, is further supported by the cranial base and position of the foramen magnum in *Ar. ramidus*, which is similar to other bipedal hominins and distinct from other great apes (Kimbel et al. 2014).

It has been argued that bodily based metric entrainment amongst members of a social group may have evolved in the hominin lineage before the metrical elements of vocal musicality (Brown 2022). This theory is based on the observation that bodily and haptic based coupling in chimpanzees occurs more frequently when locomoting in an upright bipedal posture (Lameira, Eerola and Ravignani 2019). The implication of these findings is that early erect bipedal hominins such as *Ar. ramidus* may have had increased capacity for bodily based coupling and entrainment than other great apes. If so this would mean the building blocks of synchronized bodily movement (that is the phylogenetic precursors of dance) may have existed at least in an incipient form in early bipedal hominins. Consequent to this stage, such ability to metrically synchronize bodily movements would have formed the basis of metrical entrainment of vocalizations and the consequent evolution of synchronized and rhythmically structured group singing (Brown 2022).

If the above scenario is correct, it would mean the ability to synchronise body movements may have conferred evolutionary benefits on erect bipedal hominins as they ventured out of forested and woodland ecologies and into more open habitats. Consequently, when thinking about the emergence of obligate bipedal-

ism the question that perhaps should be asked is “why did early hominins remain orthograde when they began exploiting terrestrial niches?” (Ward 2013: 1379). We could also add to this observation and ask what were the socio-behavioural and neurochemical factors associated with remaining orthograde and exploring terrestrial niches as erect trunk obligate bipeds? And what is the relevance of this anatomical configuration in subserving the neurobiological substrates of communicative capacities such as music and dance?

As already mentioned, the important point to emphasize when considering hominin brain evolution is that bipedalism emancipates the forelimbs and hands from locomotion. The consequences of this are that early bipedal hominins lacked the specialized wrist and hand morphology evident in chimpanzees, which prevents these apes from evolving complex manipulative abilities. It has been argued that retaining a more generalized configuration more conducive to precision grasping is what facilitated the coevolution between the brain and the hands characteristic of the hominin lineage (Lemelin and Schmitt 2016; Lundborg 2013; Napier 1993, 1962). Additionally, it has been suggested that neural reorganization and expansion of motor regions associated with hand motor control may have emerged with the Australopithecines (Harmand et al. 2015). It is these developments associated with bipedal locomotion and hand morphology that may have signaled the beginning of a unique form of neurochemical regulation involved in motor control – that is upregulation of the dopaminergic system. We explore this issue further later in this article.

The relationship between vocal abilities, motor control and movement have



been extensively explored (Fitch 2011; Feenders et al. 2008; Lieberman 2009). For example, it has been argued erect bipedal locomotion provided the initial selective force for the enhancement of the subcortical sequencing ability involved in both motor control and cognition (Lieberman, 2009). Consequently, it has been asserted that the selective pressures that resulted in the evolution of the sequential processing necessary for tool manufacture, as well as human speech 'may ultimately derive from upright bipedal locomotion, the initial hominid adaptation' (Lieberman, 2009: 143 and 151). There is also significant evidence of analogies between motion and music, with researchers finding links between music and the force patterns associated with walking as well as relationships between physical motion and musical tempo (Friberg and Sunberg 1999; Shove and Repp 1995). Additionally, the 'felt pulse' patterns involved in locomotion and rhythmic perception are both believed to be based on the entrainment of oscillator circuits in the brain (Thaut 2013: 7 and 9).

Importantly, areas involved in rhythmic perception are related to those that regulate movement; for example cortico-cerebellar circuits that subserve conscious and subconscious responses to temporal structure are involved in rhythmic synchronization and rhythmic motor control (Thaut 2009), while music has been found to activate motor and premotor cortices that are not part of the classical auditory system of the temporal lobe (Bengtsson et al. 2009). Additionally, the basal ganglia shows a specific response to beats during rhythm perception, with a cortico-subcortical network and coupling of motor and auditory areas being associated with musicality (Grahn and Rowe 2009). This association is also

supported by evidence that listeners often experience music as a type of virtual movement analogous to physical motion, and that during ontogeny mastering melodic leaps and steps accompanies learning to walk, and that during childhood play, musical patterns are often associated with the affective characteristics of the accompanying locomotion (Nikolsky 2023).

Significantly, in animals that have high levels of vocal learning, spontaneous rhythmic movement to auditory rhythms seems to be more common than in species that lack such learning. Additionally in high vocal learners, motor planning regions are in tight reciprocal communication with forebrain auditory regions throughout life, suggesting that vocal learning may have been a preadaptation for the evolution of human beat perception and synchronisation (Patel 2021). This perspective seems to differ from Brown's thesis discussed above that bipedal bodily based synchronisation and metric entrainment were evolutionary precursors to the metrical and melodic aspects of music (Brown 2022). While postulating vocal learning as a preadaptation for spontaneous rhythmic movement to auditory rhythm is different in emphasis from seeing bodily synchronization as an evolutionary precursor to synchronised metrical singing, both theories do link the evolution of rhythmic perception with vocalisation and melodic vocal synchrony. The difference between the two approaches is which has evolutionary priority – vocal learning or bodily based synchronized entrainment?

In terms of the model outlined in this paper the question that arises from the above discussion is: did vocal learning in early hominins such *Ar. ramidus* and the Australopithecines evolve prior to the

evolution of beat perception? Related to this question is the evolutionary priority of the hominin vocal tract facilitating vocalization and language relative to obligate erect bipedalism. In terms of the issues discussed above it is important to emphasize that we see a shift to a human-like vocal tract in *Ar. ramidus* long before we see the emergence of obligate terrestrial bipedalism in the Australopithecines. Does this imply that vocal learning, may have preceded the kind of bodily based rhythmic entrainment associated with bipedalism? Or did, as intimated by Brown's model, the emergence of obligate bipedalism in Australopithecines form the evolutionary basis of later forms of vocally based metrical synchronization – forms of synchronization that were absent in *Ar. ramidus*?

Resolving these issues with any certainty is far beyond the purview of this essay. However, what our model of sound production informed by ecological and fossil data provides is a framework for thinking about which aspects of the modern adaptive suite may have had evolutionary priority. What is clear is rhythmic perception associated both with locomotion and music seem to be linked. The question is which components of this aspect of the modern human adaptive suite have the greatest phylogenetic depth? That is, did vocal learning give rise to beat perception or are the metrical aspects of musical rhythm products of locomotor adaptations? As we speculate below, given early hominins may have been forest and woodland dwelling singers before becoming obligate bipeds, could this mean that vocal learning had priority in the evolution of beat perception and synchronization?

The link between musical perception and locomotion is also suggested by re-

search findings that patients with Parkinson's disease, or who have suffered strokes or traumatic brain injury, benefit from rhythmic auditory stimulation (Thaut et al. 1997; Hurt et al. 1998; Thaut et al. 2001; Thaut 2013). Significantly, Parkinson's disease is a neurological disorder involving the progressive degeneration of the dopaminergic system (Raglio 2015), a system which is believed to be central to the emergence of obligate terrestrial bipedalism in the genus *Homo* (Previc 2009). Based on such findings, it has been suggested that the ability to maintain an internal rhythm associated with bipedal locomotion and fluid walking, would have spread over into a capability for maintaining rhythmic sound – and the associated freeing of the arms, the hands, and the upper torso, in addition to enhanced muscular control may have underpinned the evolution of dance within the *Homo* genus (Mithen 2009).

What the above discussion suggests is that hominins have unique neurobiological adaptations associated with entrainment to an external pulse, and that this ability to rhythmically structure sound and movement is related to bipedal locomotion. Given obligate bipedalism seems to be very ancient it is reasonable to suppose that early hominins such as the Australopithecines possessed a form of rhythmic sound production and perception that may have been more advanced than in other great apes. We suggest such capacity for rhythmic sound and movement associated with bipedal locomotion may have been crucial to the collaborative synchronization underpinning music and dance based coalitionary signaling (Hagen and Bryant 2003: 24). Further, such an adaptation may have been important not only in signaling be-



tween groups of hominins, but also during interactions with predators. In what follows we flesh out these ideas in more detail and how they relate to the demographic expansion of Australopithecines.

### **Canine reduction and synchronized sound production: reconsidering the *Australopithecus* demographic expansion**

In the following we assume *Ar. ramidus* to be ancestral to *Australopithecus*. (White et al. 2015; White et al. 2009; Kimbel et al. 2014). More specifically, we suggest as a working hypothesis that elevated levels of pro-sociality, and possibly cooperative breeding or alloparental care of offspring, were adaptations already in place in *Ar. ramidus* and that these adaptations were necessary for the successful demographic expansion of *Australopithecus*. As already noted *Australopithecus* shares with earlier hominins such as *Ar. ramidus* a centrally positioned foramen magnum as well as loss of the aggressive canine armory characteristic of other primates (Suwa et al. 2009; Kimbel et al. 2014; White et al. 2015). However, they possess adaptations suggestive of a greater degree of terrestrial bipedalism than earlier forest and woodland dwelling hominins. It is this adaptation that is believed to be associated with their demographic expansion into more diverse habitats than those occupied by earlier hominins (Meindl, Chaney, and Lovejoy 2018).

Significantly, early hominins such as *O. tugenensis* and *Ar. ramidus* may have avoided predators through cryptic habitation in forests (Treves and Palmqvist 2007). However, having expanded into

nonarboreal habitats, it is likely that *Australopithecus* became subject to increased predation pressure – an observation supported by extensive analysis of the fossil remains of this genus (Brain 1983). Significantly, as a result of predation pressure, it has been argued that hominins inhabiting Pliocene African savanna-woodlands might have engaged in more visual, and possibly auditory vigilance, than those living in closed forested habitats (Treves and Palmqvist 2007: 367).

The evolution of African carnivores coincided with a decrease in woodland relative to grassland with the consequence that the type and number of carnivores changed throughout the course of hominin evolution. For example, between 6 and 3.6 Ma there were five genera of large carnivores without extant analogues, and from the mid-Pliocene (3.6 Ma) these groups were joined by an additional eight new genera of carnivores (Treves and Palmqvist 2007: 357). Additionally, from 1.8 Ma onward archaic carnivores went extinct in Africa, partly as a result of a global carnivore guild turnover and species replacement. The important issue here for the adoption of obligate erect trunk bipedality are the significant number of predators that existed between 3.6 and 1.8 Ma – a period which coincided with the *Australopithecus* demographic expansion into nonarboreal habitats. As Terves and Plamqvist write:

*Given the existence of numerous ambush predators between 3.6–1.8 Ma, hominins would have experienced strong selection for efficient vigilance. Large parties of apes organized like those of chimpanzees are conspicuous and costly in terms of individual vigilance, competition for food and*

*agonistic social interactions, hence we propose early hominin foraging parties would have adopted more cohesive and calmer social organization to maintain efficient vigilance and reduce conspicuousness to carnivores during diurnal foraging. Groups formed of trusted and familiar individuals often forage and travel with high levels of interindividual proximity, experience minimal conflict, and coordinate vigilance more easily.*

(Treves and Palmqvist 2007: 370)

One of the reasons postulated for the absence of singing in the majority of terrestrial primates is that singing will attract the attention of ground dwelling predators (Jordania 2020). Humans are an exception to this trend being one of the few singing terrestrial primates (Schruth et al. 2024). Is it possible that early hominins retained singing from their arboreal ancestors with one of its uses being an anti-predator adaptation? In this sense, far from singing attracting predators it may have been, along with high levels of interindividual proximity and coordinated vigilance, an effective means of deterring them and consequently reducing mortality risk.

It has been argued that the demographic shift of Australopiths into non-arboreal habitats necessitated a change in social structure to one unlike that evident in any other extant non-human great apes (Meindl, Chaney, and Lovejoy 2018). For example, such expansion into nonarboreal habitats would have exposed young and adult females to high levels of extrinsic mortality due to predation. However, if the early hominin social structure was one based on allo-parental care, reduced intragroup conflict, group level cooperation, and male forms

of group protection and investment, then female survivorship could be enhanced both before and after sexual maturity, leading to population increase and demographic expansion (Meindl, Chaney, and Lovejoy 2018).

Importantly, large groups of animals are more likely to encounter a predator, but less likely to be attacked by it, which may result from inherent benefits of group living and cooperative breeding systems (Sorato et al. 2012). Further, given cooperative defence and shelter construction are some of the primary benefits of sociality, it has been argued that predation risk may be fundamental for the transition toward complex social organization (Groenewoud et al. 2016). Additionally, numerous animals engage in coalitionary vocal signaling; for example female lions roaring in chorus deters alien and potentially infanticidal males, gibbons use communal screaming to defend group boundaries and repel predators (Hagen and Bryant 2003: 26–27) while chimpanzees employ group level co-operation mediated by vocal calls as a means of predator defense (Boesch 2009: 22–23 and 52–53). Additionally, baboons jointly drive predators from the group (Kummer 1967: 154–155) while geladas utter alarm calls and seem to form multilevel fission-fusion social structures in response to predation (Lin et al. 2020).

Support for possible predation on early hominins comes from the Swartkrans deposits, which suggest Australopiths may have been attacked while in the caves, a distinct possibility given that carnivores also use such caves as dens (Brain 1994; Treves and Palmqvist 2007: 363). Given the likelihood of predation pressure on Australopiths, then what role would have synchronized vocalization and sound production played as they

expanded out of forest and woodland ecologies and out into diverse non-arbo-real habitats? In the absence of aggressive canine armory, is it conceivable that early hominins used synchronized sound production and body movement to deter predators?

When dealing with this issue it is important to acknowledge that early forms of sound production may not have only been vocal but may also have been produced by the body, such as stomping on the ground or clapping, or by hitting physical objects together. Similar to humans, many other animals produce both vocal and somatic sounds. For example many mammals communicate non-verbally by drumming on their body or a substrate in order to attract mates, signal to predators or to establish territorial ownership – signals which may be a ritualization of phylogenetically older behaviors associated with running or digging (Randall 2015).

In gorilla's such non-vocal sound production is evident in chest beating which is believed to convey information about size and competitive ability (Wright et al. 2021). Chimpanzees produce resonant sound using their body by moving one external object against another such as throwing rocks at tree trunks (Kalan et al. 2019). They also drum on trees which enables them to communicate long distances (Eleuteri et al. 2022) and at times such drumming may be integrated with vocalization such as the pan hoot (Arcadi, Robert, and Boesch 1998). Chimpanzees have also been observed performing so-called "rain dances" (Whiten et al. 1999), they show evidence of rhythmic swaying induced by sound (Hattori and Tomonaga 2020) they are able to synchronise their movements to an auditory rhythm (Hattori, Tomonaga, and Matsuzawa 2013) in addition to exhibiting spontaneous whole-

body entrainment between two peers, suggesting possible empirical evidence for the phylogeny of human dance (Lameira, Eerola, and Ravignani 2019). Based on these findings it has been suggested the prerequisites for music and dance are deeply rooted in hominoid phylogeny and probably existed in the common ancestor shared by humans and chimpanzees approximately 6 million years ago (Hattori and Tomonaga 2020).

Elaborating on these observations it has been argued that such bodily and 'haptic coupling may have been the earliest means for producing rhythmic entrainment between two agents in non-human primates.' Additionally, given such behaviour is restricted to the bipedal manner of locomotion in chimpanzees, such studies 'might have implications for the evolution of both bipedalism and dance' (Brown 2022: 9). Given that *Ar. ramidus* was a facultative biped we can speculate that such haptic based locomotor coupling may have been more common than in chimpanzees who are infrequent bipedalists, with a locomotor anatomy designed for quadrupedal knuckle walking. This is even more so with the emergence of obligate bipedalism in the Australopithecines.

Given the ubiquity of sound production using the body and objects in mammals and primates, we can assume with some justification that a variation on such forms of communication existed in *Ar. ramidus* and *Australopithecus*. However, the unique anatomy of these early hominins would suggest that such abilities may have been much more complex than in other primates. For example, *Australopithecus* hand proportions are human-like primarily because of the unique locomotor adaptation of hominins (Almécija, Moyà-Solà, and Alba 2010). Importantly, greater

manipulative capacity of *Australopithecus* relative to other great apes is suggested by Lomekwi stone technologies, which include hammers, anvils and sharp-edged flakes, which have been dated to 3.3 Ma (Harmand et al. 2015). Additionally there is evidence of stone-tool-assisted consumption of ungulates by *Australopithecus afarensis* (McPherron et al. 2010). Lomekwi technologies suggest that their makers had elevated levels of hand motor control resulting from reorganization or expansion of the brain's various motor regions – and that such reorganization could have occurred before 3.3 Ma (Harmand et al. 2015). It is also possible that these adaptations provided the social niche in which selection for upregulation of neurochemicals associated with complex sequential processing and motor control may have occurred – as we explore in more detail below.

If the co-evolution of the brain, hands and motor systems had developed in *Australopithecus* to the point where these small-brained hominins were able to create hammers and anvils and use stone tools to butcher ungulates, then the question arises as to what other uses such objects and abilities were put? It has been proposed that the rhythmic percussive pattern produced by hitting two stones together to make tools may have been the world's first musical instrument; for example 'two flint knappers, chipping in ear-shot of each other, could have been the first musicians to produce rhythmic counter point as they interlocked their rhythms' (Montagu 2004: 171). It has also been argued that hominins would have been aware of the acoustic properties of flint knapping and it is possible that they would have exploited such properties for communicative and social purposes (Blake and Cross 2008).

Concurring with the above authors we propose that the long sequence of strikes required to make such tools, the hitting of stones together, and the use of anvils, have acoustic properties that would most likely have appealed to early hominins. Would they have taken pleasure in the rhythmic structure of repeated blows to an object? Would they have used such objects to create sound in a similar manner to chimpanzees throwing rocks at or drumming on trees? Importantly, hard rocks, such as basalt, flint or chert, suitable, and actually used in the Lower Paleolithic for production of weapons and tools with sharp cutting edges and fine points, due to their crystalline nature, produce distinct clear sounds when struck with a hard object (a "hammer"). These sounds are unlike those occurring in nature. Consequently, they may have attracted the attention of tool-makers leading them to experiment with their production and acoustic properties.

When moving out of forested ecologies caves may have provided hominins with shelter and protection from predators – as they do for baboons where degree of inaccessibility to predators seems to be one of the factors involved in the choice of sleeping sites such as caves (Hamilton 1982). However, this may have been a double-edged sword as caves may have been dangerous for hominins as they may also serve as dens for predators (Treves and Palmqvist 2007: 359). Importantly, deposits in the Swartkrans caves contain fossilised remains of Australopithecus, baboons as well as the extinct carnivorous cat *Dinofelis*. While there is some uncertainty in interpreting the remains, it has been suggested that early hominins may have been attacked while in the caves, a distinct possibility given that carnivores also use such caves as dens (Brain

1994; Treves and Palmqvist 2007: 363). Given increasing hominin occupation of caves as they ventured out of arboreal habitats, it is worth considering the degree to which sound production within such spaces may have effectively deterred predator attacks. Importantly for the evolution of sound production and auditory perception, there are resonant spaces inside cave structures that produce echoes (Fazenda et al. 2017). Additionally, reverberations in caves, particularly when occupied by a concentrated group of people, are thought to convert melodic intervals into harmonic intervals by prolonging the “tails” of preceding melodic tones (Nikolsky and Benítez-Burraco 2022). It is also worth speculating if early hominins may have explored the acoustic properties of caves, not only using their voices to produce sound, but also objects such as stone tools and perishable items that may not have survived in the archaeological record.

Given the above-mentioned evidence of chimpanzees swaying rhythmically to sound, synchronizing their movements to an auditory rhythm, as well as spontaneous whole-body entrainment between two peers, it does seem plausible that the hand morphology and tool making ability of *Australopithecus* would suggest increased capacity to produce sound and entrain to a felt pulse in ways more complex than that evident in chimpanzees. Significantly, entrainment seems to be associated with rhythmic knapping and collective manufacturing of stone tools (Zubrow and Blake 2006). Given Australopithecines were bipedal, which may have increased general rhythmic capabilities, as well as improving manipulative capacities due to the associated hand morphology, it seems possible that such forms of entrainment between numerous

individuals may have been enhanced relative to other great apes. This perspective is consistent with the view that neural reorganization and expansion of motor regions associated with hand motor control may have emerged with the Australopithecines (Harmand et al. 2015).

It is important to reiterate the point that chimpanzees are limited in the extent to which they can develop the abilities outlined above due to the hand morphology associated with their locomotor adaptations. Additionally they would also lack the neurobiological substrates associated with both bipedal walking and associated forms of rhythmic perception – although the above examples of entrainment to a beat suggest chimpanzees may possess such abilities in an incipient form. However, we suggest that Australopithecines may have evolved a more refined sense of rhythm due to being obligate bipeds – and this process of refinement would have continued in later species of hominin through autocatalytic feedback loops (Henneberg and Eckhardt 2022). Significantly, there is evidence in *Australopithecus africanus* of an external auditory meatus more conducive to the perception of high frequencies than that evident in chimpanzees, a derived hominin trait that is thought to facilitate short-range intragroup communication in open habitats (Quam et al. 2015). Combined with the increased ability to manufacture and use hammers and anvils, which may have produced loud rhythmically sequenced sound, there seems to be a plausible case for *Australopithecus* using synchronized sound production and body movement as an adaptation to non-arboreal ecologies with elevated predation risk.

It has been argued that our ancestors were vulnerable hominins living in open

habitats with limited weaponry, and that they may have survived by increasing the range and diversity of their vocal calls. For example, ‘...lions prowling in the dark may have been more wary of approaching a noisy bunch of females and infants if unexpected pitch variations made it difficult to estimate group size and risk’ (Knight and Lewis 2017:437). Group rhythmic singing and dancing has also been called an effective intimidation tool that may have been a survival strategy of ancestral hominins when they moved out of forested habitats (Jordania 2020). Such synchronized vocalization and body movement may have been combined with hitting stones (hammers on anvils) resulting in vigorous ‘drumming sessions’ during scavenging confrontations with large African predators such as lions (Jordania 2014: 94).

As already noted many primate species use vocalisation in their attempt to deter predators. Some primate species may use alarm calls to deter predators that depend on surprise attacks – that is such calls communicate to the predator that they have been seen and that consequently it is unprofitable to continue the attack (Zuberbühler et al. 1999). They may also combine vocalisations with physical attack in mobbing behaviour, which involves two or more prey animals distracting or repelling a predator by making repeated advances usually while vocalizing and displaying in a conspicuous fashion (Treves and Palmqvist 2007: 368).

Chimpanzees have been observed uttering despaired calls while climbing up into a big tree (Boesch 1991: 228); effectively chasing leopards away using loud synchronized barking and branches to fight them off, with group level co-operation mediated by vocal calls being one

of the most effective means of predator defense (Boesch 2009:22–23 and 52–53); responding to lions by climbing in to trees and eliciting alarm calls and whimpers (Tsukahara: 1993); as well as climbing high into trees where they remained uttering frequent loud vocalizations until potential predators left the area (Tutin et al. 1981: 139). In geladas, vocalisations may serve to alert other members of the group that a predator is nearby or to alert a leopard that it had been seen; additionally male geladas have been observed emitting loud barks and bluff-charging to within three metres of a leopard while females and smaller juveniles sheltered in nearby trees and bushes (Lin et al. 2020: 11). Significantly, hunter-gatherer peoples use rhythmic clapping, drumming, chanting, and choral singing explicitly to keep wild animals away (Lewis 2009; Thin 1991).

In addition to vocalisation there seems to be a relationship between predation and social structure, with increased group cohesion reducing predation risk. For example, chimpanzee group level co-operation mediated by vocal calls may be one of the most effective means of predator defense in this species (Boesch 2009: 22–23 and 52–53). As Boesch notes, predation pressure in chimpanzees results in individuals of both sexes spending more time together, which means they are less likely to ‘be singled out in a leopard attack’ (Boesch 2009: 2). It is important to note that there also seems to be an ecological component in the relationship between social structure and predation. For example, savanna-living chimpanzees travel in large numbers when moving between patches of trees, which may be an anti-predator response (Tsukahara 1993), suggesting that as the number of trees decrease in a habitat group cohesion may become more important.



Concurring with this observation, in baboons who adapted to open savanna or semidesert habitats where trees are rare as a source of protection from predators, resulted in the evolution of large aggressive males who can jointly drive predators away from the group (Kummer 1967: 154–155). Significantly, baboons have evolved extreme degrees of canine sexual dimorphism, which are in part related to the need for predator defense in what are essentially hostile savanna habitats with high predation risk (Plavcan and van Schaik 1992).

It has been argued that several million years ago, both hominins and the theropithec ancestors of modern geladas transitioned from living in woodland-dominated habitats to more open-country environments. The consequent reduction in the availability of refugia may have resulted in similar adaptations in both groups such as a fission-fusion way of life and formation of multilevel societies (Lin et al. 2020). Significantly, geladas are thought to engage in vocal synchrony akin to human choral singing, both species using rhythm and melody to resolve emotional conflicts (Richman 1987). Therefore it seems possible that such socioecological adaptations may have been similar in geladas and Australopithec. In our model we hypothesize that elements of a prosocial adaptive suite, and possible cooperative breeding, were already in place in *Ar. ramidus* and that these adaptations were enhanced as Australopithec evolved social adaptations to cope with expansion into more diverse and challenging habitats.

Our analysis provides both fossil and palaeoecological support for the notion that elements of both music and language may have evolved from an evo-

lutionary precursor form of “territorial chorus” (Brown 2017) – although, as we suggest below, such an adaptive complex may have been built upon preexisting mammalian neurochemistry. The “territorial chorus” thesis is based on the assumption that music and language evolved in the context of egalitarian social dynamics that promoted group-level communication, cohesion and co-operation (Brown 2007:16). In later periods of evolution, this primitive form of sound production may have bifurcated into sequentially structured language and harmonic musical forms, with such musical forms using isometric rhythms and pitch blends and language using words and propositional syntax (Brown 2001). In our model we propose that such a precursor may have already been evident in early hominins such as *Ar. ramidus* and that it was amplified through autocatalytic feedback loops (Henneberg and Eckhardt 2022) as Australopithecines moved into nonaraboreal habitats and relied more and more upon group level sound production. This socio-behavioral shift may represent the very ancient building blocks of affect based sound communication in the hominin lineage – that is primordial hedonistic stimulation, which is believed to be a universal feature of music associated with affective bonding (Benítez-Burraco and Nikolsky 2023).

### **Self-domestication and the evolution of music and language**

The concept of self-domestication has been proposed as a central component in human evolution (Hare 2017; Clarke and Henneberg 2015 and 2017). Based on analogies with domesticated animal breeds, this thesis seeks to explain certain observable trends in the hominin



fossil record and features of modern human behavior and psychology; these include reduced cranial robusticity, shortened facial region, reduced levels of aggression, social tolerance, elevated levels of pro-sociality, reduced sexual dimorphism, increased disease risk and the retention of juvenile or paedomorphic features into adulthood (Hare 2017; Clark and Henneberg 2015; Leach 2003; Bednarik 2020). While the process has been postulated to explain the transition from more robust to more gracile and “feminized” morphology within the *Homo* genus (Cieri et al. 2014; Bednarik, Saniotis, and Henneberg 2022), it is also believed to have produced the more paedomorphic morphology and psychology of bonobos relative to chimpanzees (Hare, Wobber, and Wrangham 2012) as well as the paedomorphic skull architecture of *Ar. ramidus*, suggesting that the process of self-domestication began at the base of the hominin clade (Clark and Henneberg 2017, 2015).

Self-domestication is also believed to have been crucial to the evolution of music (Clark and Henneberg 2017; Benítez-Burraco and Nikolsky 2023) as well as being a precondition for the emergence of language (Thomas and Kirby 2018). This thesis is based on the assumption that language presupposes a system of social transmission and learning, and that such a system could have evolved through self-domestication. As the authors write, rather than ‘accounting for language structure itself, the key task for biological evolution lies in accounting for the foundational traits that make a process of structure-creating cultural evolution possible.’ Consequently, they argue that ‘the cultural evolution of language structure is rooted in an earlier process of self-domestication’ (Thomas and Kirby 2018:23).

Importantly, the skull morphology of *Ar. ramidus* shows greater similarity to infant chimpanzees than it does to adult chimpanzees (Fig. 1 and 2). These similarities include position of the foramen magnum, short relative length of the face and horizontal vocal tract in relation to the length of the skull, as well as the degree of cranial base flexion. Consequently, it has been argued that these paedomorphic features provide evidence for self-domestication at the base of the hominin clade (Clark and Henneberg 2017). It has also been argued that elevated levels of prosocial neurochemicals such as oxytocin may have evolved in *Ar. ramidus* (Lovejoy 2009; Clark and Henneberg 2017). It is important to note that elevated levels of oxytocin and serotonin are characteristic of species that have been domesticated by humans – further these neurochemicals seem to be part of the self-domestication complex of hominins (Hare 2017).

The important point to note here is that the changes in the *Ar. ramidus* skull that correlate with elevated levels of pro-sociality and evidence for self-domestication, are the same that are required to evolve skull morphology and a vocal tract necessary for vocal modulation – that is a centrally positioned foramen magnum (itself a product of erect bipedalism), loss of canine armory and reduced facial prognathism (Clark and Henneberg 2017). This observation provides detailed anatomical evidence for the contention that ‘the cultural evolution of language structure is rooted in an earlier process of self-domestication’ (Thomas and Kirby 2018: 23). As noted by Clark and Henneberg (2017), these deep interconnections between the anatomical basis of both vocal tract anatomy and social evolution evident in early hominins, have

yet to be adequately explored by paleo-anthropologists and scholars researching the origins of music and language. We hope this paper goes some way to rectifying this lacuna in the extant literature. In what follows we elaborate on this perspective in more detail, highlighting how neurochemical regulation and the process of self-domestication may have facilitated the growth of technology and musical ability throughout the course of hominin evolution.

### **Neurochemical regulation in the hominin lineage**

It has been argued that the evolution of human mental capacities was not particularly dependent on changes in brain size and structure, but rather on alteration of its neurotransmitter and neurophysiological regulation involved in information processing and emotional states (Previc 2009; Saniotis et al. 2019; Henneberg and Saniotis 2016; Previc 1999; Saniotis and Henneberg 2012). However, current knowledge of neurobiological processes underlying cognitive abilities in ancestral hominins is still scant since neuro-biochemical effects do not fossilise (Saniotis and Henneberg 2011). That said, in the above discussion we have made some tentative suggestions in which we have sought to infer changes in neurochemical profiles that may have accompanied changes in skeletal anatomy – which do fossilize. This was based on comparison with other species and evidence of neurochemical regulation associated with the self-domestication syndrome.

In what follows we discuss the role of oxytocin, serotonin, and dopamine and how these neurochemicals may be related to the fossil and paleo-ecological evidence discussed above. Importantly,

it has been argued that the process of enculturation was largely influenced by neuro-hormonal regulation, especially from the Paleolithic period onwards where sophisticated social behaviors, technology and art developed (Bednarik et al. 2022) and that the social transmission of linguistic structures was dependent on self-domestication (Thomas and Kirby 2018) which involves altered neurochemical regulation (Hare 2017).

Oxytocin is a deeply conserved neurochemical primarily associated with female mammalian nurturant behavior (Panksepp 2004). Importantly, in both bonobos and chimpanzees the oxytocin system is involved in group cohesion – but it functions in species specific ways. For example, in chimpanzees the oxytocin system seems to be associated with in-group bonding particularly in the context of intergroup aggression, while in bonobos it seems to facilitate both in-group cohesion as well as affiliative behaviour between groups (Brooks et al. 2022). Given chimpanzee in-group cohesion is believed to be related to predation pressure and intergroup conflict (Boesch 2009), it seems the oxytocin system can facilitate sociality in the face of external threat or competition. However, it is unlikely that these are the reasons for the oxytocin system evolving in chimpanzees – that is it is more likely the oxytocin system, originally evolving in the context of female mammalian nurturant behavior, was exapted in adulthood group bonding. That the oxytocin system can be exapted in species specific ways, is evidenced by the fact that in bonobos it is not associated with intergroup conflict but forms the physiological basis for increased motivation to cooperate as well as intergroup affiliation (Moscovice et al. 2019).

In humans oxytocin is involved in sexual bonding (Light, Grewen, and Amico 2005; Panksepp and Biven 2012: 241) parental psychology (Gordon et al. 2010) as well as generalised co-operation (Rilling et al. 2012). It is also involved in the social dimensions of music, such as trust and cooperation within groups of culturally compatible but not necessarily genetically related individuals – aspects of sociality that are believed to stimulate reward and motivation due to music's impact on the limbic system (Harvey 2020). Importantly, dance has been shown to induce pleasurable arousal and positive prosocial mood via the release of endorphins and neurohormones such as oxytocin (Laland, Wilkins, and Clayton 2016). The oxytocin system may also be associated with a form of primordial hedonistic stimulation, a putatively universal feature of music associated with affective bonding that underpins diverse cultural forms of music expression (Benítez-Burraco and Nikolsky 2023).

While the oxytocin system, and its role in the evolution of early hominin musical behavior, may have been important for group cohesion in the face of external threat or intergroup conflict – as it is in chimpanzees – it may not have originally evolved for such purposes. For example, it has been argued that the oxytocin system and the coevolved ritualized facial, bodily, and vocal signals of affiliative intent by ancestral mother–infant pairs, formed the original adaptation that was then exapted in music, dance, group ritual and other social bonding behaviours (Dissanayake 2021).

This perspective has been developed in the context of the Australopithecine demographic expansion, suggesting that “motherese” or infant-mother vocalisations were able to establish a form

of nontactile contact comfort, which then provided the neurobiological basis of adulthood music and social bonding (Falk 2004). This theory is related to the vertical vector of bipedalism, which makes it difficult for hominin infants to ride on their mother's back in the manner of quadrupedal apes such as chimpanzees. Additionally, a putative decrease in infant grasping abilities, and specifically the lack of a grasping big toe associated with bipedalism, would have required mothers to place their infants on the ground while they foraged, with reciprocal vocalisation maintaining nontactile contact (Falk 2004).

While the notion of such infant-directed speech has been found to exist in various forms cross-culturally suggesting an evolved universal communicative system (Hilton et al. 2022), the motherese thesis has been called into question due to a lack of anthropological support for the theory (Rosenberg, Golinkoff and Zosh 2004). While accepting aspects of the motherese thesis, and the possibility that mother infant vocalisations may have formed the neurobiological substrates of prosocial melodic vocalisation, we see this form of social bonding in a broader social context, and that infant vocalisations may also serve to elicit responses from not just the mother but also alloparents – a position that does seem to find support from cross cultural data (Hrdy 2009: 123). In this sense vocally mediated bonding between infants, the mother and other members of a cooperatively breeding social unit, may have been exapted in adult social bonding, providing the neurobiological foundation and ontogenetic precursors for such socioemotional traits – as has been claimed for cooperation, empathy and altruism

more generally (Preston 2013). Given oxytocin functions in species specific ways in bonobos and chimpanzees, it likely would have also done so in early hominins – and as is the case with modern humans it may have subserved early forms of coordinated movement and sound production.

Various authors have suggested that serotonergic regulation became increasingly employed in the hominin clade where it functioned in impulse control and delayed gratification (Raghanti et al. 2008; Azmitia 1999; Saniotis et al. 2021; Soubrié 1986). Furthermore, it has also been found that increasing serotonin levels in the striatal areas of the brain further reinforced limbic inhibition which was crucial in the development of tool production, language and affiliative behaviors (Raghanti et al. 2018). It has also been suggested that the serotonin receptor 5-HT<sub>2A</sub>R may have had a significant role in human evolution by improving neuroplasticity and adaptive behaviors in adverse environments (Ettrup et al. 2014). This has obvious implications for hominin expansion into nonarboreal habitats with increased predation risk.

Significantly, serotonin and BDNF (brain derived neurotrophic factor) work to reinforce each other. For instance, BDNF enhances serotonergic expression of raphe neurons, as well as upregulating serotonergic uptake and modifying serotonergic neuron firing rates (Goggi et al. 2002; Martinowich and Lu 2008; Zhou, Sari, and Zhang 2000). It has also been argued that BDNF underwent positive selection due to increasing physical activity levels (i.e. persistent hunting) from *H. ergaster* onwards, which produced more BDNF which in turn enhanced its synergistic neurotrophic and cognitive roles with the serotonergic system (Saniotis and

Henneberg 2013). Further, alteration in BDNF expression may have been exapted in early hominin social activities which entrained affective states via rhythmic motor sequences – for example those involved in dance (Brown, Martinez, and Parsons 2006). Importantly, dance has been shown to increase neurotrophins such as BDNF which assist in neuroplasticity and cognitive function (Brown, Martinez, and Parsons 2006) while music, dance and ritual are believed to result in forms of prosocial synchronization and merging between self and other facilitated by endorphin release (Tarr, Launay and Dunbar 2014). While it is unclear when these neurochemicals were upregulated in the hominin lineage, we suggest that such a process may have begun with early hominins such as *Ar. ramidus*, and particularly the Australopithecines when coordinated movement, sound production and vocalisation increased social cohesion and predator defence in nonarboreal habitats.

It is important to note the lifestyle and dietary changes that occurred as hominins ventured into nonarboreal habitats and developed a broader diet. It has been suggested that the climatic changes occurring over the last several million years in sub-Saharan Africa contributed to increased meat consumption and elevated thyroid and dopamine production as hominins expanded their locomotor range, engaged in chase hunting, and adapted to ecologies inducing increased thermoregulatory stress (Previc 2009, 1999). Additionally, the consequent increasing dietary levels of the omega-3 DHA in ancestral hominins probably increased thyroxine (T<sub>4</sub>) which is involved in creativity, language fluency and memory (Previc 2002). Thyroxine is implicated in converting tyrosine to the

dopamine precursor L-Dopa. Significantly, it has been shown that T4 levels in humans are approximately 30% higher than in chimpanzees (Previc 2002).

Dopamine is a significant neurotransmitter which is involved in planned movement, neuromodulation, spatial memory, motivational behaviour and cognitive function (Klein et al. 2019; Berridge and Kringelbach 2008; Salamone and Correa 2012). The medial caudate nucleus which forms the striatum with the putamen in basal nuclei has increased dopaminergic activity compared to non-human primates, supporting more flexible cognitive abilities and behaviours (Raghanti et al. 2016). Importantly, dopamine is believed to be an important factor in sequential learning and planning of motor tasks (Badgaiyan, Fischman, and Alpert 2007). Additionally, the motor system is thought to play a central role in musical and rhythmic perception, suggesting that motor planning is not only involved in movement but is also recruited for music perception even in the absence of actual physical movement (Gordon, Cobb, and Balasubramanian 2018). We suggest that the regulation of oxytocin, serotonin and dopamine would have been under significant selective pressure as hominins adapted to changed ecological conditions, resulting in enhanced prosocial behaviors, motor control and synchronized sound production and bodily movements.

The model outlined in this article focusing on anatomy, social structure and neurochemical regulation may prove useful given the discovery of small brained hominins such as *Homo naledi*. For example, *Homo naledi* possesses many human-like anatomical traits associated with the hand, foot, lower limb, dentition and cranium – yet significantly it has

a brain size equal to that of australopiths (Berger et al. 2017). What this suggests is that posture, hand morphology, altered neural architecture and associated neurochemical regulation may give rise to what were once considered uniquely human social behaviors – behaviors that, based on the *Homo naledi* evidence, do not seem to require large brain size. For example, recent excavations have led to claims that *Homo naledi* seems to possess cultural traits characteristic of modern humans, including engraving, fire and using forms of collaborative planning and coordination to bury their dead in caves (Fuentes et al. 2023). Given the occupation of caves by this species, is it possible that *Homo naledi* took pleasure in, and experimented with, the resonant spaces and reverberatory potential of caves? To our knowledge there is no evidence as yet that they did so – however absence of evidence is not evidence of absence. While the findings of the *Homo naledi* excavations are promising, and potentially revolutionary for our understating of hominin evolution, more research is required to establish the veracity of the claims mentioned above; for a critical analysis of these claims and possible avenues for further research see reviewers' comments in Berger et al. (2023). One possible avenue of future research may be to ascertain any previously unnoticed evidence of musical behaviors among *Homo naledi* artefacts.

Our model may also help illuminate the neurobiological substrates of social cognition among the Dmanisi hominins, which have cranial capacity ranging from 545 to 760 ml (Lordkipanidze 2017, p. 49). Importantly, the lower limit of the Dmanisi fossils of 545ml is only slightly larger than the upper limit of chimpanzees, which is 500ml (Tobias 1971). It is

unlikely that such a small difference in brain size can account for the differences in ecological niche, subsistence patterns and socio-behavioral adaptations between these two species.

Significantly, the Dmanisi hominins are believed to have used an Oldowan Mode 1 tool kit, to have been cooperative hunters who had access to large game, who had a diet broader than *H. habilis* (Lordkipanidze et al. 2013; Pontzer, Antón, and Lordkipanidze 2014) and who were highly social in terms of care for conspecifics (Lordkipanidze et al. 2005). Given that geographic dispersal is believed to be dependent on high levels of sociality, combined with the small body and brain size of the Dmanisi fossils, it has been suggested that the earliest hominin presence in Eurasia predated increases in body size and brain size (Lordkipanidze et al. 2013). Given the small brain size of these hominins, combined with evidence of many aspects of the human adaptive suite, it seems reasonable to propose that their psychosocial adaptations result from altered brain architecture and neurochemical regulation associated with hominin bipedalism. Significantly, there is evidence of antemortem damage to the fossils that has been attributed to predation (Margvelashvili 2022; Lordkipanidze et al. 2023). It is interesting to speculate the possible forms of sound production employed by these small brained hominins – given they could make stone tools and given evidence of possible predation would they have used coordinated sound production to deter predators? That is given their ability to engage in complex forms of sequential motor control in the making of weapons and tools, we also suggest that these hominins may have employed these abilities in forms of synchronized

sound production and bodily movement – to either enhance group cohesion or as a form of predator defense.

### **Archeological evidence of sound production and sequential processing**

In this article we have argued that the sequential processing and neurochemical regulation resulting from erect bipedalism may have evolved through autocatalytic feedback loops that can be traced back to early hominins such as *Ar. ramidus* and the Australopithecines (Henneberg and Eckhardt 2022). We also suggested that increased capacity for such processing and enhanced motor control, in the context of a prosocial egalitarian social system, is essential for human technological evolution and the structural properties of human communicative capacities. As opposed to seeing modern linguistic and behavioral capacities evolving with the emergence of larger brained and more gracile members of the *Homo* genus (Diniz-Filho et al. 2019; Benítez-Burraco and Kempe 2018; Neubauer, Hublin, and Gunz 2018) we suggest that many of the adaptations associated with behavioral modernity were already in place among *Homo erectus* (Bednarik 2013; Webb 2006; Sterelny 2012; Bednarik 2015) and that they originally evolved through a social niche involving self-domestication processes, cooperative breeding and intergenerational knowledge transfer that may reach back to *Ar. ramidus* and the Australopithecines (Clark and Henneberg 2021a; Clark and Henneberg 2021b). In the following we explore this perspective in greater detail as a means of interpreting the archeological evidence for music – from ancient lithophones to purported flutes found by archaeologists



from the Aurignacian, a technocomplex of hominins transitional between robust and gracile *Homo sapiens*, i.e., Neanderthaloid humans (Bednarik 2020).

It has been argued that self-domestication led to increased skull gracility and globularity within the *Homo* genus, which was accompanied by changes in neural architecture that gave rise to modern forms of cognition and language use within anatomically modern humans (Benítez-Burraco and Kempe 2018). However, this position has been questioned based on evidence that other factors such as mastication may contribute to differences between robust and gracile members of the *Homo* genus – and that consequently robust varieties going back as far as 600k may have been behaviourally modern (Clark and Henneberg 2021). If this position turns out to have any merit then the question arises: to what degree did music form part of behavioural modernity in earlier varieties of the *Homo* genus?

One of the problems in establishing the evolutionary origins of music is most types of musical instrument would have been made of perishable materials usually lost from the archaeological record due to taphonomic processes (Bednarik 1994). This also means that the absence of such artifacts does not necessarily mean that earlier hominins lacked the socio-cognitive ability to produce and use them. As opposed to inferring the emergence of musical abilities by reference to archaeological finds of musical instruments, we interpret such evidence as some of the most recent examples of hominin sequential processing, rhythmic perception and musical abilities that occurred as result of architectural and neurochemical alterations of the hominin brain.

Significantly, in palaeoart, we have a number of finds that are attributable

to robust rather than gracile hominins (Bednarik 2017), and there is a tantalisingly small number of apparent musical instruments from Middle Palaeolithic contexts. About thirty presumed flutes or pipes have been reported from Upper Palaeolithic settings, but as mentioned, they need not necessarily be attributed to gracile *H. sapiens*. The best-known examples are those from the Aurignacian of Hohle Fels, Vogelherd and Geißenklösterle in Germany (Hahn and Münzel 1995; Conard, Malina, and Münzel 2009), Spy in Belgium (Otte 1979), and Isturitz, Abri Blanchard and Mas d’Azil in France (Passemar 1944; Harrold 1988).

As already noted, caves are an important competent in hominin evolution going back to at least *Homo naledi* if not the Australopithecines. Caves may have provided shelter and protection from predators in the absence of arboreal forms of refuge as well as being places of symbolic or ritual behaviour (Jaubert et al. 2016). Importantly, many of the paintings of the Upper Paleolithic are situated in the most resonant areas of the caves, which includes stalactites which are reported to have been used as natural tone producing ‘lithophones’ (Morley 2013: 115–117). Significantly, it has been argued that the acoustic properties of such spaces amplify pitch value directing the attention of singers to fundamental frequencies and harmonicity (Benítez-Burraco and Nikolsky 2023). Additionally, the acoustic properties of caves have led to the suggestion that early forms of music and visual art may have developed as part of cave based ritual practices (Morley 2013).

Some researchers have argued that Neanderthals exhibit evidence of cave based behaviours traditionally associated with *Homo sapiens*. For example, the Middle Palaeolithic Bruniquel Cave de-



posits in France contain evidence of anthropogenic geometric structures made of stalagmites, suggesting forms of social organization among Neanderthals more complex than previously thought (Jaubert et al. 2016). However, it is unclear how ancient forms of ritual and associated musical behaviors are. We suggest it is likely they emerged prior to the evolution of anatomically modern humans, having developed among robust hominins such as Neanderthals. It may even be the case that very early versions of ritual behavior, at least in incipient form, were present among small brained hominins as suggested by the *Homo naledi* finds. It will be interesting to see how future research illuminates this issue further.

Importantly, predation on hominins appears to have been common during the Pleistocene among early anatomically modern humans as well as Neanderthals (Camarós et al. 2016). For example Neanderthal fossils from The Cova Negra in Spain have cranial punctures similar to those evident in the Australopithecus cranial fragment SK-54 from Swartkrans in South Africa, which are believed to have resulted from a leopard attack (Camarós et al. 2016). It is unclear if cave occupation during this period was a response to predation pressure or other factors. What seems to be clear is caves were a place conducive to the production and amplification of sound, that early members of the *Homo* genus occupied them, and that they most likely did so for shelter and possible cultural reasons. Predation may have been one factor engendering this practice although it seems difficult at present to ascertain its relative role with any certainty.

There have been occasional reports of flute-like objects from Middle Palaeolithic contexts, of which one example

deserves closer attention (Fig. 3). It is the Mousterian bone flute from Divje babe I, Slovenia, from layer 8, the lowest of five Mousterian strata (Turk 1997; Turk, Dirjec, and Kavur 1995). Since it was first reported, it has led to intensive debates (d'Errico et al. 2003; Chase and Nowell 1998; d'Errico and Stringer 2011; d'Errico and Villa 1997). The tubular fragment of a juvenile cave bear femur bearing a series of holes is dated to about 50 ka. Advocates of the replacement of replacement of robust members of the *Homo* genus by more gracile forms, argue that the four regularly spaced holes result from carnivore activity, yet they lack indications of compression or crushing and any counter-traces on the underside. Moreover, experimentation has demonstrated that the object has a two-and-a-half-octave compass that extends to over three octaves by over-blowing, and perfectly melodious tunes can be played on it (Turk et al. 2018). It would be readily accepted as a flute if it were from an Aurignacian context because replacement scholars attribute that musical tradition to gracile *H. sapiens*.

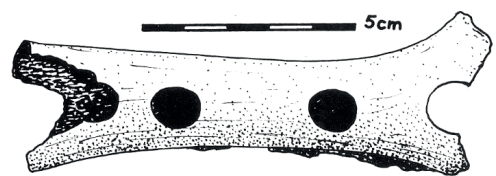


Fig. 3. Presumed flute of the Mousterian of Divje babe I, Slovenia, made of a cave bear bone

Flutes or pipes are not the only musical instruments described from Palaeolithic contexts. Skiffles, rasps or scrapers have been reported from several Upper Palaeolithic occupation layers (Geiringer 1982: 13–14; Kuhn and Stiner 1998;

Huyge 1991; Maringer 1982; Vincent 1988; Dauvois 1989, 1999). One such possible scraped idiophone is attributable to the Middle Palaeolithic. It is the fragment of a mammoth long bone (probably a femur or tibia) found with a cold fauna and Mousterian flint implements at Schulen, Belgium (Huyge 1990). The oblique fracture to create a pointed end was achieved by a cut scored with a stone tool, along which the bone was then snapped off. The deeply cut subparallel grooves extending from the point downwards (Fig. 4) have been so intensively worn by transverse rubbing, especially near the point, that their original number is difficult to establish. There seem to have been twelve grooves initially, and their separating ribs, as well as the edge's underside, are covered by an intense tribological gloss featuring micro-striations parallel to the tool's edge. The gloss is absent in the groove floors, which have retained the longitudinal striae of the lithic tools used to create them.

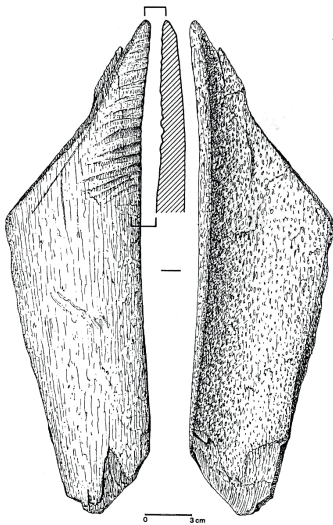


Fig. 4. Presumed skiffle on a mammoth bone fragment from Schulen, Belgium

Free aerophones, usually known as bullroarers, have also been attributed to the Palaeolithic, as have osseophones (struck bones), albeit with less persuasive examples. The evidence is much clearer for another form of idiophone, the lithophones. These are rocks struck to vibrate, producing clear sounds audible over great distances (Boivin et al. 2007; Boivin 2004). Lithophones can be identified and may provide the earliest evidence available of music-making – although we suggest this ability may in fact be an extension of the hominin sound producing capability that can be traced back to Lomekwi artefacts. The quality and tone of the sound they yield depend on the shape and material properties of the stone and the amount of contact it has with other rocks: minimal contact facilitates more effective vibration. Therefore, stalactites in limestone caves are acoustically suitable candidates. Several cave sites have been proposed to have furnished such evidence in the form of marked or struck speleothem formations (Glory 1964, 1965; Vaultier, Santos, and Glory 1965), such as the 'organ sanctuary' in Nerja Cave, Spain (Dams 1984, 1985). However, these are Upper Palaeolithic, although the Escoural Cave examples could conceivably be Middle Palaeolithic as Upper Palaeolithic occupation evidence is lacking at that site.

Most known lithophones (Querejazu Lewis and Bednarik 2010) or 'rock gongs' are large slabs of rock featuring cupules, a type of cup-shaped indentation that is the most common petroglyph on the planet (Bednarik 2008). These may occur singly on the lithophone's spot most conducive to emitting good sound, but different parts of a lithophone may yield different notes

(Bednarik 2010a). Therefore, most specimens bear several cupules, even hundreds (Fig. 5). Cupules were made by percussion and can occur on any rock type, and most of them are not found on rocks suitable as lithophones. They have been made from the Lower Palaeolithic to the 20<sup>th</sup> century (Fig. 6). Replication studies have determined that those made on the hardest rocks, such as granite, quartzite and in rare cases, even massive quartz, can require up to hundreds of thousands of blows with stone hammers (Kumar and Krishna 2014). Most of the earliest known cupules occur on rocks that may not be good lithophones, such as those at Daraki-Chattan (India), Nchwaneng and Potholes Hoek (both South Africa) or Sai Island (Sudan) (Bednarik 2017: 43–44, 115–118). A notable exception is the upright quartzite slab in Auditorium Cave (India; *op. cit.* Fig. 38), located in a space of distinctive acoustic properties. The cupules of these five sites have been attributed to the Lower Palaeolithic, although dating these phenomena remains notoriously difficult (Bednarik 2010b).



Fig. 5. Granite lithophone covered by cupules; Serra do Papagaio III, Santana do Matos, Rio Grande do Norte, Brazil

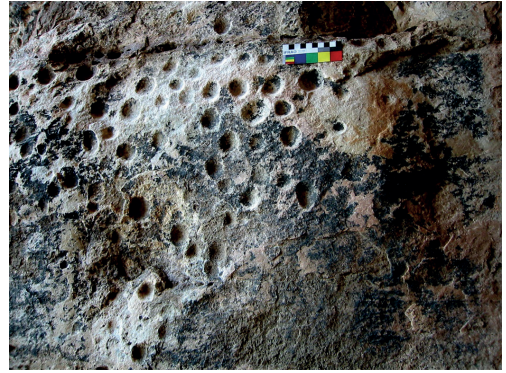


Fig. 6. Some of the 550 cupules on the walls of the quartzite cave Daraki-Chattan, Bhanpura, central India, 28 of which have been excavated in Lower Palaeolithic strata

Of particular interest here are the sensory effects of the rhythmic sound of cupule production sessions, combined with the tremendous physical exertion demonstrated by the replication experiments that revealed the involvement of immense ‘commitment, stamina and patience’ (Kumar and Krishna 2014). Cupules on tough rock, especially those on lithophones, could have been either produced by countless short sittings over many generations; or they may be the result of many monotonous sessions lasting hours at a time, introducing a trance-like state in the operative. The timing of striking the rock favours intervals reflecting the rebound characteristics of the stone hammers, i.e., strikes are very precisely spaced in time. The kinetic mechanics of the process manifest the tribological properties of the elements involved, establishing a specific rhythm, and it can seem to the operator that the rock determines the rate of striking. This establishment and governing of rhythm over a lengthy period, combined with distinctive pitches of sound produced, is a prime candidate for embedding the

rudiments of music production. Although it does not fix the timing of the advent of music production any more than the archaeological data listed above, any form of natural patterning would have helped hone hominin cognition. Further, such evidence for cognitive honing may be an extension of processes that can be traced back to the earliest stone tool technologies.

In conclusion, our analysis offers a model of human music origins and dance grounded in the palaeoecological contexts of early hominin evolution. We propose that changes in skull architecture, locomotion, hand morphology, neurobiology and ecology, may have favored the uses of coordinated sound production and movement as part of a more generalized adaptive suite. We further argue that this very primitive form of movement and sound production provided the phylogenetic building blocks that were consequently amplified through autocatalytic feedback loops during subsequent periods of hominin evolution.

It is also a model that opens up potentially new avenues of research. For example, some stone tools have been shown to have traces of use as sound producing objects. The veracity of our model may be able to be tested by re-analyzing Pleistocene artefact assemblages for any hints of their use as “sound tools”. As Blake and Cross argue, the realisation of the potential – and often overlooked – sound producing properties of stone tools may necessitate the ‘seemingly monumental task of re-analysing or re-considering excavated lithic collections’ a research project that ‘should target sites where other forms of musical or quasi-musical behaviours have survived’ (Blake and Cross 2008: 17). The presence of such evidence

among early hominin archaeological deposits could be used to test our model. If such evidence was forthcoming it would push back music origins into much earlier periods of paleohistory than previously thought. We predict such an archaic origin for musical abilities based on the unique anatomical configuration, and associated neurobiology, of hominin bipedalism, canine anatomy and hand morphology. The challenge for future researchers is to discover clues for such an ancient origin despite the taphonomic processes that make finding such evidence in older archaeological layers less likely.

### **Conflict of interests**

Authors declare no conflict of interests.

### **Authors’ contributions**

GC was the primary author of the paper. All authors were involved in the discussion and conception of the hypothesis. AS and ML contributed material on neurohormonal regulation and RB on archaeology. MH contributed material on palaeoanthropology and developed the overarching conceptual orientation.

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# Morphology and morphometry of frontal sinuses as a tool in sex determination based on 17<sup>th</sup>–18<sup>th</sup> century skulls from Poland

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**ABSTRACT:** Morphological analysis of the frontal sinuses (FS) is one of the methods used to assess the sex of human remains. Depending on the methods, the results indicate the effectiveness of using the FS in the assessment of sex at the level of 60–85.9%. Our goal was to determine whether the morphological and morphometric methods of sex assessment based on the FS can be used for examining historical populations in anthropological studies. We assessed FS both morphologically and morphometrically on a sample of 76 dry skulls (41 females and 35 males) from 17<sup>th</sup>–18<sup>th</sup> century from Poland to evaluate the potential of applying this method for sex estimation in human remains. A total of 76 X-rays were taken in both frontal and lateral views. The morphology and morphometry of the FS were assessed with ImageJ software. There were no significant differences between the sexes in assessing the outline of the upper border of the FS, as well as the number of partial septa. There was a statistically significant difference between the sexes in the width of the right FS, the height of the right and left FS, and the right and left area of the FS. The lateral view indicated a statistically significant difference between the sexes regarding the depth and area of the FS. The accuracy rate in classifying males and females using FS morphometry ranged from 59.09% to 69.57%. The FS in the lateral view (69.57%) and the height of the left FS in the frontal view (68.18%) are the most appropriate regressors for sex determination. Statistically significant differences in some FS measurements between the sexes do not appear to be a sufficient indicator of sex. Morphological and morphometrical characteristics of the FS should not be used as a guideline for sex assessment in the historical Polish population.

**KEY WORDS:** frontal sinuses, sex dimorphism, morphology, morphometry.

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## Introduction

Frontal sinuses (FS) are paired structures situated within the anterior cranial vault, posteriorly to the superciliary arches (Yoshino et al. 1987; Moideen et al. 2017). These sinuses are pneumatic cavities lined with mucosa, located between the internal and external laminae of the frontal bone. FSs are almost always asymmetric and separated by a septum. Each sinus extends superiorly to the medial end of the eyebrow ridge and back into the orbital portion of the frontal bone (Falguera et al. 2012). Their inferior margin represents the superior margin of the orbital rim and their medial margin is shared with the contralateral sinuses (Yoshino et al. 1987).

At birth, FS are small, closed pouches (Patil et al. 2012). FS pneumatization begins about two years of age, becomes significant in early adolescence, and is completed in the late teens (Hopkins 2016). The two FSs develop independently from the anterosuperior pneumatization of the frontal recess into the frontal bone and show considerable anatomical variation in size and shape. There are four basic morphological types of FS in terms of size: aplasia, hypoplasia, medium size, and hyperplasia (Guerram et al. 2014). One or both FS may be hypoplastic or absent, although bilateral aplasia is rare (McLaughlin et al. 2001). FS can also be unilateral or bilateral. The prevalent asymmetry of FS can be attributable to the fact that they develop from two independent structures (Hopkins 2016).

Research on the uniqueness of FS structures began in the early twentieth century. Schuller (1921), who examined the frontal sinuses for the first time in 1921, provided information about their unique shape, complexity, and individ-

uality, with relevance for human identification in post-mortem cases. The special characteristics of frontal sinuses in terms of their size, shape, and position made them unique personal identification tools, especially due to their irregular shape and inter-individual uniqueness (Camargo et al. 2007; Cox et al. 2009; Tang et al. 2009; Uthman et al. 2010; Xavier et al. 2015). Most studies indicate that FS can potentially be used in sex differentiation during anatomical and anthropological studies, as differences in size and configuration have been observed in males and females (Patil and Mody 2005; Uthman et al. 2010; Kim et al. 2013; Hamed et al. 2014; Sai Kiran et al. 2014; Michel et al. 2015; Thottungal et al. 2024).

However, the biological significance of the frontal sinuses is not yet clear although it has been hypothesized to have olfactory, respiratory, phonetic, static, thermal, and/or mechanical functions (Patil et al. 2012; Nikolova et al. 2017; Godinho and O'Higgins 2018). According to the prevalent theory, its presence decreases skull weight and mechanically fixes the bones of the splanchnocranium (McLaughlin et al. 2001; Teul et al. 2005; Guerram et al. 2014; Nikolova et al. 2017).

Estimation of the sex is a fundamental step in performing anthropological examinations of skeletal material (Phenice 1969; Lovell 1989; Bruce and MacLaughlin 1990; Buikstra and Ubelaker 1994; Hsiao et al. 1996; Ubelaker and Volk 2002; Spradley and Jantz 2011; Inskip et al. 2018). As the skull is one of the best-preserved body parts after death, it is considered a good material for assessing sex (Rogers 2005). The frontal bone is one of the most sexually dimorphic elements of the human



skull, due to features such as glabellar prominence, frontal eminences, and the massiveness of the supraorbital ridges (Williams and Rogers 2006). However, these features may not be applicable for sex identification when skulls are fractured and deformed. Material from archaeological sites or ossuaries is often fragmentary and thus sex assessment is often performed based on single bone fragments, which requires extracting as much information as possible from such material (Ubelaker and Wu 2020). Fragmentation of the skull prevents the use of most conventional markers for sex estimation. When bone fragmentation does not allow the use of standard methods of sex assessment, anthropologists may consider alternative methods, such as X-ray, CBCT, or histological analysis.

The present study aimed to evaluate the morphological structure of FS using posteroanterior and lateral radiographs of crania of the 17–18<sup>th</sup> century crania from Poland and to compare the findings for males and females. Metric analysis was performed and the morphological diversity was estimated in terms of FS type, the number of scallops, and septa. We estimated the height and width range for medium-sized (normal) and hyperplastic FS types for the entire sample and both sexes separately.

This study also aimed to evaluate the accuracy rate of sex identification based on frontal sinus index and area measurements and determine the dimorphic potential of the frontal sinuses. In addition, we aimed to assess the accuracy of sex estimates based on a logistic regression model, and to test a hypothesis regarding the usefulness of frontal sinus morphology as a tool for anthropologists when determining the sex of skeletal material in historical samples.

## Materials and methods

The research material consisted of a series of skulls from Krakow cemeteries used by people of different socio-economic status, dating back to the 17<sup>th</sup> and 18<sup>th</sup> centuries: 1. The cemetery next to St. Mary's Basilica used by wealthy burghers (Bieniarzówna and Małecki 1984; Żukow-Karczewski 1989); 2. The cemetery next to St. Mark's Church, where a group of Krakow craftsmen of relatively low social status were buried (Zaremska 1974; Myszka 1996); 3. The cemetery next to St. Peter the Little in the hamlet of Garbary, which was located outside the city walls, on the outskirts of what was then Krakow. This cemetery used to be a burial place of the poorest Krakow residents and the inhabitants of nearby villages (Pieradzka 1931; Kracik 1989; Wyzga 2014). Figure 1 illustrates the location of all of these sites in Poland.

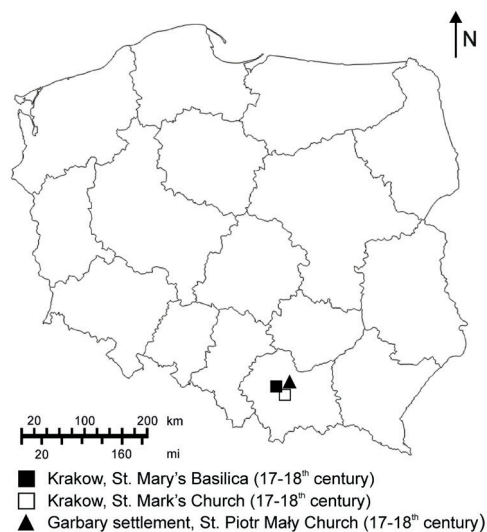


Figure 1. Map of Poland showing the geographic locations of the sites included in this research

A total of 76 skeletons that exhibited a complete epiphyseal fusion were analyzed (35 males and 41 females). The skeletons included in this study met two primary criteria. The first criterion was the completeness of the material allowing the estimation of sex and age at death, which were estimated using anthropological methods. All individuals were analyzed using the long bones, cranium, and pelvis (Phenice 1969; Lovell 1989; Bruce and MacLaughlin 1990; Buikstra and Ubelaker 1994; Ubelaker and Volk 2002; Spradley and Jantz 2011; Inskip et al. 2018). An overall sex estimate was made for each skeleton based on all the traits assessed. When general skull and pelvic estimates provided conflicting results, more weight was placed on pelvic traits which have been shown to be more reliable in sex identification compared to solely using skull features (Inskip et al. 2018). Skeletons that were ambiguous in estimating sex or those whose preservation state made it impossible to determine sex were excluded from the analysis. The estimation of age at death included evaluations of pubic symphysis (Meindl et al. 1985; Brooks and Suchey 1990), which has been shown to provide reasonable age estimates for individuals <40 years of age (Hens et al. 2008), and the eight-phase Lovejoy auricular surface method (Lovejoy et al. 1985), which provides reasonable estimates for individuals >40 years of age because the auricular surface more frequently survives taphonomic insult and its morphological changes continue well into the sixth decade of life (Hens et al. 2008). The lower limit of the age range was set at 25, as it exhibits a complete epiphyseal closure of the sternal end of the clavicle (Scheuer and Black, 2000). The second inclusion criterion was good condition of the skele-

ton, and especially a well-preserved facial part of the skull with the frontal bone.

All X-ray images were acquired by a radiologist at the Department of Descriptive Anatomy, Pomeranian Medical University to eliminate technical bias. The skulls were placed on a special platform that allowed repeatable radiographic positioning. To visualize the frontal view of the sinuses, we used the PA Axial Caldwell Method, which is a caudally angled radiograph, with its postero-anterior projection. In the Caldwell position, the FS outlines are the clearest, minimizing the risk of interpretation errors. According to Caldwell's method, the skull forehead was placed against the image detector so that the orbitomeatal line was running perpendicular to the detector. The petrous ridge was below orbits. The skulls angled caudally at 15°, the orbitomeatal line was parallel to the floor so that the central ray exited the nasion. The mid-sagittal plane was perpendicular to the midline of the image receptor. For the lateral view, we used a common routine method. The skull lay flat, raised on a thin radiolucent foam rubber pad. The infraorbitomeatal line from the inferior orbital margin to the upper border of the external auditory meatus was set perpendicularly to the table.

Only images with visible FS margins were selected for analysis. X-ray images were acquired according to the commonly accepted methodology at a focus distance of 1 m, an exposure time of 1 s, a voltage of 90–95 kV, and a current of 125 mA. The applied linear image enlargement was estimated at 1.13. In the calibration measurements, photographic film was scanned, calibrated, and saved in .jpg format. The morphological types of FS were estimated according to Gueram et al. (2014) (see Fig. 2), where aplasia indicates the absence of frontal pneu-

matization, hypoplasia indicates that FS is limited to the area under the supraorbital line, medium size indicates that FS is limited to the area medial to the mi-

dorbital line, while hyperplasia indicates that FS extends in the area lateral to the midorbital line. Some variations of FS pneumatization are shown in Figure 3.

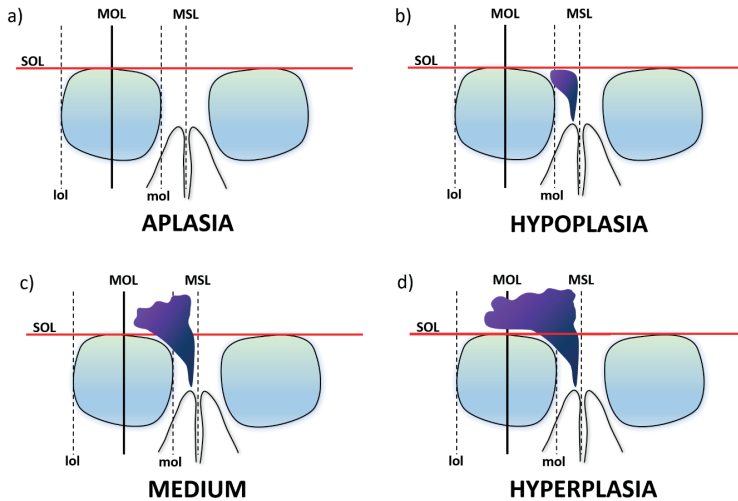


Figure 2. Morphological types of the frontal sinuses after Guerram et al. (2014): a) aplasia, b) hypoplasia, c) medium, d) hyperplasia. Where: MOL – midorbital line; SOL – supraorbital line; MSL – midsagittal line; mol – medial orbital line (vertical line passing through the most medial orbital point); lol – lateral orbital line (vertical line passing through the most lateral orbital point)

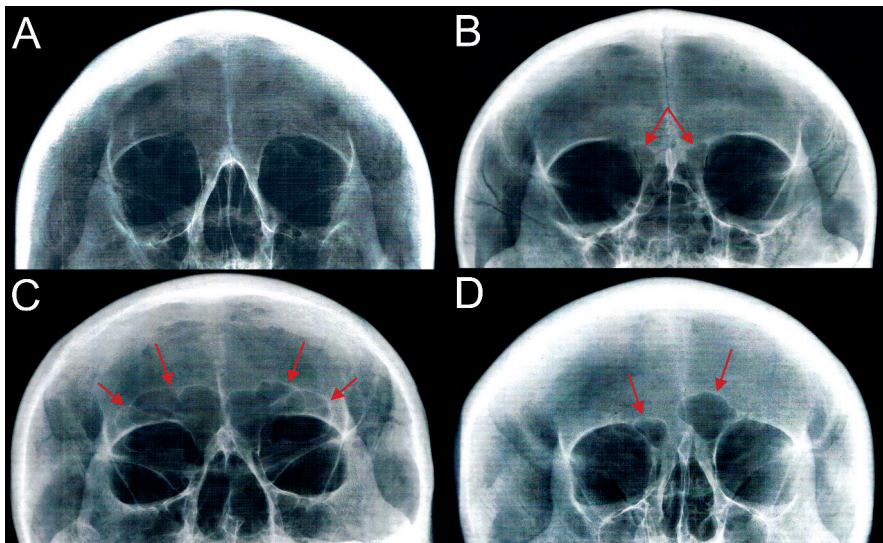


Figure 3. Variations of the frontal sinus pneumatization: A – bilateral aplasia; B – bilateral hypoplasia; C – bilateral hyperplasia; d – visible asymmetry in FS size

Based on Caldwell's diagrams (Fig. 4), the following measurements of the frontal sinuses were taken: right height, left height, right width, left width, left area, right area, and total area obtained only for the portion of the FS projected above the baseline (A). The separation between the left and right sides of the sinus was based on the frontal sinus septum to ensure that only one width was measured on each side. The highest height of each side (measurements B and C) was estimated from the maximum distance between the base and upper lines of the FS, while the largest width (measurements F and G) was estimated from the maximum distance between the medial and lateral lines of the right and left sides of the FS. The linear measurements obtained from each

radiograph were expressed in millimeters (mm), while the areas were expressed in square millimeters (mm<sup>2</sup>). Figure 5 shows a diagram of the FS measurement on lateral cephalograms. The highest (H) and lowest (L) points were observed. The maximum height (A) of the FS was obtained by connecting these points. The maximum depth (B) was measured by drawing a perpendicular line to A connecting the anterior wall of the frontal sinus in its deepest part. All measurements were obtained using the program ImageJ for Windows, and the obtained values are the arithmetic mean of three subsequent measurements. Next, the range of measurement variability was estimated separately for the normal and hyperplastic types of the FS.

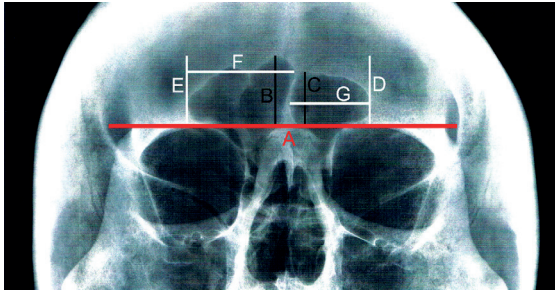


Figure 4. Diagram of Caldwell with the demarcation of the borders of the frontal sinus and identification of the measurements collected with the aid of a reference baseline: A – supraorbital line corresponding to the superior margin of the orbits; B – the height of the right FS; C – the height of the left FS; D and E – lateral lines of the FS right and left side; F – width of the right FS; G – width of the left FS

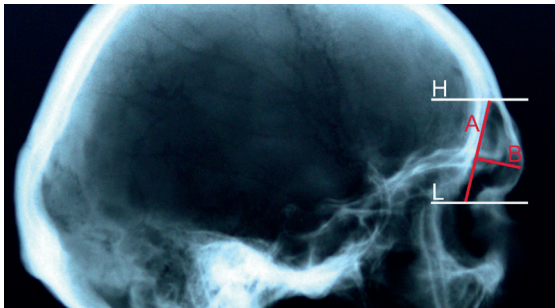


Figure 5. Frontal sinus measurements on the lateral cephalogram. H – the highest point of the FS; L – the lowest point of the FS; A – maximum height, B – maximum depth of the FS

The frontal cephalograms were divided into two main parts. The first step of the analysis involved the evaluation of the FS morphological type of FS, the variability of the upper border of FS and the classification of the number of partial septa. The second step consisted of estimating the range of variability, mean value, and standard deviation for the sinus measurements analyzed, as well as estimating the significance of sex differences. That step also included analysis of the estimated values of asymmetry indices (AI), which were calculated according to Szilvassy (1974, 1982) and Tang et al. (2009):

$$AI = \frac{\text{The value of the smaller frontal sinus measurement}}{\text{The value of a larger frontal sinus measurement}}$$

If both frontal sinuses are of the same size, the index in this case is 100 (no asymmetry). The closer the index is to 100, the smaller the asymmetry. Then, based on the features that differentiated male and female skulls, a simple logistic regression model was built.

The analysis of the lateral cephalograms included estimation of the descriptive statistics and assessment of the significance of the differences between male and female skulls. Similar to the frontal cephalograms, a logistic regression model was constructed for features that show differentiation.

Sex differences in the sinuses were also evaluated in terms of the number of scallops on the superior border (right, left and total), the number of partial septa (right, left and total), and the unilateral/bilateral presence or absence of partial septa. If the outline of the anterior margin was scalloped or lobulated, these structures were counted. A similar procedure was followed for number of partial septa. Figure 6 shows the applied method for evaluat-

ing the number of scallops and septa (after Yoshino et al. 1987), while Figure 7 shows an example of FS with scallops and septa. The outline of the upper border of the FS on each side was divided into the following categories: absent; smooth/scalloped with 1 arcade; scalloped with 2 arcades; scalloped with 3 arcades; scalloped with 4 arcades; scalloped with 5 arcades; and scalloped with 6 arcades.

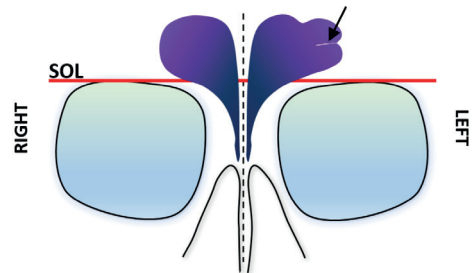


Figure 6. The upper FS border is scalloped with 3 arcades on the left and smooth on the right side. One partial septum (marked by black arrow) is present in the left sinus

To assess the inter-rater reliability study, a subset of 30 cephalometric radiographs (15 males and 15 females) were randomly chosen to be reviewed by the different observers. Observers independently drew measuring lines and made the same measurements. The intraclass correlation coefficient (ICC) was then determined in SPSS statistical package version 23 (SPSS Inc, Chicago, IL). We used ICC2 (according to McGraw and Wong's (1996) terminology) or ICC (2,k) (in Shrout and Fleiss's (1979) convention), which is a Multiple-Rating, Absolute-Agreement, 2-Way Random-Effects Model. ICC estimates indicate good reliability: ICC = 0.793 for male samples, with 95% confidence interval = 0.750–0.836, and ICC = 0.821 for female samples, with 95% confidence interval = 0.759 and 0.854.



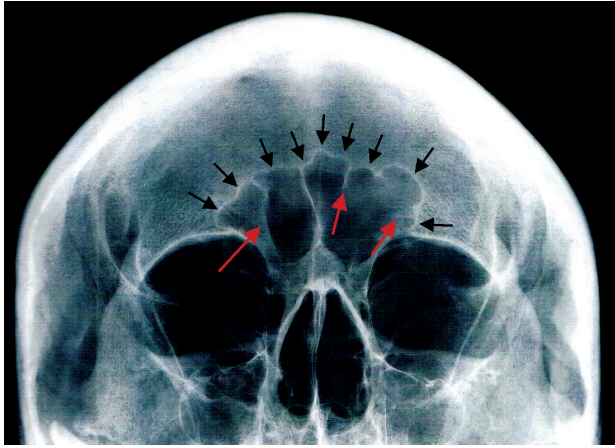


Figure 7. The upper FS border is scalloped with a few arcades (black arrows), both on the left and right sides. Red arrows indicate the septa, respectively a partial septa on the left and a complete septum on the right

## Results

First, the frequency of the various FS morphological types was calculated (Tab. 1). A comparison of their estimated frequency in males and females using the Chi-squared test showed no significant differences ( $p = 0.3954$  for the right side and  $p = 0.3122$  for the left). In two cases, bilateral aplasia of the FS was observed. Both of those cases were male skulls with a metopic suture.

Second, the morphology of the superior border of the FS was observed and the number of scallops was noted (Tab. 2 and Tab. 3). Skulls with bilateral sinus aplasia were not included in the analysis. However, skulls with unilateral sinus aplasia were included, denoted by the column named "Absent" (Tab. 2). Data were analyzed using the Chi-squared test for comparison of the frequency of morphological type for the two sexes. No statistically significant differences between skull sides and sexes were observed ( $p > 0.05$ ).

Table 1. Distribution of frontal sinus types in the analyzed skulls

Sex	Side	Frontal sinus type			
		Aplasia n (%)	Hypoplasia n (%)	Medium size n (%)	Hyperplasia n (%)
Female N = 41	Right	0 (0.00)	6 (14.63)	33 (80.49)	2 (4.88)
	Left	0 (0.00)	5 (12.20)	31 (75.61)	5 (12.20)
Male N = 35	Right	2 (5.71)	4 (11.43)	26 (74.29)	3 (8.57)
	Left	2 (5.71)	3 (8.57)	23 (65.71)	7 (20.00)
Total N = 76	Right	2 (2.63)	10 (13.16)	59 (77.63)	5 (6.58)
	Left	2 (2.63)	8 (10.53)	54 (71.05)	12 (15.79)



Table 2. The classification of the outline of the upper border of the frontal sinus

Sex	Side	Outline of upper border						
		Absent	Smooth / Scalloped with 1 arcade	Scalloped with 2 arcades	Scalloped with 3 arcades	Scalloped with 4 arcades	Scalloped with 5 arcades	Scalloped with 6 arcades
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Female N = 38	Right	3 (7.90)	12 (31.58)	13 (34.21)	7 (18.42)	2 (5.26)	0 (0.00)	1 (2.63)
	Left	2 (5.26)	12 (31.58)	11 (28.95)	7 (18.42)	5 (13.16)	1 (2.63)	0 (0.00)
	Chi-squared test R vs L					$p = 0.6326$		
Male N = 31	Right	2 (6.46)	6 (19.35)	9 (29.04)	6 (19.35)	5 (16.13)	2 (6.45)	1 (3.22)
	Left	1 (3.22)	5 (16.13)	11 (35.49)	4 (12.91)	6 (19.35)	3 (9.68)	1 (3.22)
	Chi-squared test R vs L					$p = 0.9664$		
Total N = 69	Right	5 (7.25)	18 (26.09)	22 (31.88)	13 (18.84)	7 (10.14)	2 (2.90)	2 (2.90)
	Left	3 (4.35)	17 (24.64)	22 (31.88)	11 (15.94)	11 (15.94)	4 (5.80)	1 (1.45)
	Chi-squared test R vs L					$p = 0.7499$		
Chi-squared test – sex differences					$p = 0.1877$			

Table 3. The classification of the number of the partial septa

Sex	Side	N	Number of partial septa			
			Absent	1 partial septum	2 partial septa	3 partial septa
			n (%)	n (%)	n (%)	n (%)
Female N = 36	Right	35	28 (80.00)	4 (11.43)	3 (8.57)	0 (0.00)
	Left	36	29 (80.55)	6 (16.67)	1 (2.78)	0 (0.00)
	Total	71	57 (80.28)	10 (14.09)	4 (5.63)	0 (0.00)
Chi-squared test R vs L					$p = 0.4957$	
Male N = 31	Right	30	23 (76.67)	5 (16.67)	1 (3.33)	1 (3.33)
	Left	31	20 (64.52)	7 (22.58)	2 (6.45)	2 (6.45)
	Total	61	43 (70.49)	12 (19.67)	3 (4.92)	3 (4.92)
Chi-squared test R vs L					$p = 0.7567$	
Total N = 67	Right	65	51 (78.46)	9 (13.85)	4 (6.15)	1 (1.54)
	Left	67	49 (73.13)	13 (19.40)	3 (4.48)	2 (2.99)
	Total	132	100 (75.76)	22 (16.67)	7 (5.30)	3 (2.27)
Chi-squared test R vs L					$p = 0.8408$	
Chi-squared test – sex differences					$p = 0.2333$	

The lack of significant sex differences suggests that the morphological features of the frontal sinuses are not a good predictor of sex assessment, at least in the Polish historical population.

Table 4 summarizes the descriptive statistics for sinus measurements and *p*-values for Student's *t*-tests, which was used to compare the significance of differ-

ences between the sides and sexes. Prior to the test, the normality of distribution was established. The results indicate the absence of any statistically significant differences between the body sides in terms of height, width, and area. In turn, statistically significant differences were observed between sexes, among all measurements except for the width of the left FS.

Table 4. Range of variability and mean values of the FS frontal view for females, males, and both sexes combined

Measurements	Sex									Student's t-test – sex differences
	Female			Male			Total			
	n	Range	Mean (SD)	n	Range	Mean (SD)	n	Range	Mean (SD)	
WFS_R [mm]	35	6.64– 33.85	21.13 (7.63)	29	5.67– 41.31	26.94 (7.52)	64	5.67– 41.31	23.86 (8.03)	<i>p</i> = 0.004
WFS_L [mm]	36	5.84– 42.78	23.15 (8.45)	30	8.87– 51.18	26.57 (10.36)	66	5.84– 51.18	24.71 (9.45)	<i>p</i> = 0.145
Student's t-test – WFS R vs L	<i>p</i> = 0.3385			<i>p</i> = 0.8751			<i>p</i> = 0.5849			
HFS_R [mm]	35	0.87– 25.58	11.45 (5.81)	29	1.43– 30.15	14.87 (5.49)	64	0.87– 30.15	13.00 (5.88)	<i>p</i> = 0.019
HFS_L [mm]	36	2.12– 21.55	12.41 (4.92)	30	2.94– 35.26	16.15 (7.73)	66	2.12– 35.26	14.11 (6.57)	<i>p</i> = 0.020
Student's t-test – HFS R vs L	<i>p</i> = 0.3133			<i>p</i> = 0.4688			<i>p</i> = 0.3138			
AFS_R [mm <sup>2</sup> ]	35	0.70– 49.72	19.95 (13.76)	29	0.59– 66.84	30.28 (14.90)	64	0.59– 66.84	24.72 (15.10)	<i>p</i> = 0.005
AFS_L [mm <sup>2</sup> ]	36	0.86– 56.88	23.29 (14.01)	30	1.95– 94.10	34.76 (23.44)	66	0.86– 94.10	28.41 (19.51)	<i>p</i> = 0.017
Student's t-test – AFS R vs L	<i>p</i> = 0.4559			<i>p</i> = 0.3833			<i>p</i> = 0.2298			

WFS\_R – width of the right frontal sinus; WFS\_L – width of the left frontal sinus; HFS\_R – height of the right frontal sinus; HFS\_L – height of the left frontal sinus; AFS\_R – area of the right frontal sinus; AFS\_L – the area of the left frontal sinus; WFS – width of the frontal sinus; HFS – the height of the frontal sinus; AFS – the area of the left frontal sinus

The asymmetry index (AI) was calculated according to Szilvassy (1974, 1982) and Tang et al. (2009). Descriptive statistics and the evaluation of differences in the mean AI value between the sexes are summarized in Table 5. The average values of height-width indices are very similar for both sexes and indicate the lack of asymmetry in both the female sample (72.19 WFS AI and 37.01 HFS AI) and the male sample (77.33 WFS AI and 69.33 HFS AI). Slightly lower average values were obtained for the area index (60.13 AFS AI in women and 58.68 AFS AI in men). No statistically significant differences between males and females were found in mean AI values for all the measurements analyzed.

Table 6 summarizes the descriptive statistics for sinus measurements along with *p*-values for Student's *t*-tests, which was used to determine whether there were significant sex differences. Prior to the test, the normality of distribution and the ratio of FS height and depth (Frontal Sinus Index – FSI) was calculat-

ed. The results indicated no significant sex differences in FS height and frontal sinus index (FSI). In contrast, significant differences were found for FS depth (DFS) and FS area (AFS) measurements.

In the next part of the study, a simple logistic regression model was built based on different variables presented in Table 7. In the case of osteological materials, damage to the squamous part of a frontal bone is often observed, which may result in the inability to perform all required measurements. When considering the potential fragmentation of the frontal bone in the supraorbital region, we decided to present the results for single variables, which may be more useful in the daily work of anthropologists.

The accuracy rate for classifying females and males ranged from 59.09% to 69.57%. The model shows that the frontal sinus area in the lateral view (69.57%) and the height of the left frontal sinus in the frontal view (68.18%) are the most suitable regressors for sex determination.

Table 5. Asymmetry index (AI)

AI	Sex								
	Female			Male			Total		
	n	Range	Mean (SD)	n	Range	Mean (SD)	n	Range	Mean (SD)
WFS AI	33	24.06–98.95	72.19 (18.63)	28	25.15–99.03	77.33 (18.06)	61	24.06–99.03	74.55 (18.40)
Student's <i>t</i> -test – WFS AI	<i>p</i> = 0.534								
HFS AI	33	7.84–99.24	73.01 (25.54)	28	18.03–99.31	69.33 (19.25)	61	7.84–99.31	71.32 (22.76)
Student's <i>t</i> -test – HFS AI	<i>p</i> = 0.280								
AFS AI	33	3.10–99.67	60.13 (27.81)	28	4.68–92.97	58.68 (23.44)	61	3.10–99.67	59.47 (25.70)
Student's <i>t</i> -test – AFS AI	<i>p</i> = 0.829								

WFS AI – asymmetry index of the frontal sinus width; HFS AI – asymmetry index of the frontal sinus height; AFS AI – asymmetry index of the frontal sinus area

Table 6. Range of variability and mean values of the FS lateral view for females, males, and both sexes combined

Measurements	Sex								
	Female			Male			Total		
	n	Range	Mean (SD)	n	Range	Mean (SD)	n	Range	Mean (SD)
DFS	37	3.26–12.72	7.39 (2.03)	32	3.35–16.83	9.04 (3.35)	69	3.26–16.83	8.15 (2.83)
Student's t-test WFS – sex differences	$p = 0.015$								
HFS	37	9.86–35.79	23.21 (6.45)	32	4.09–48.25	26.00 (8.65)	69	4.09–48.25	24.51 (7.62)
Student's t-test HFS – sex differences	$p = 0.131$								
AFS	37	2.33–30.65	14.65 (6.43)	32	1.92–48.28	19.61 (11.75)	69	1.92–48.28	16.95 (9.54)
Student's t-test AFS – sex differences	$p = 0.030$								
FSI	37	1.29–6.58	3.29 (1.09)	32	0.73–5.81	3.05 (1.06)	69	0.73–6.58	3.18 (1.08)
Student's t-test FSI – sex differences	$p = 0.367$								

DFS – depth of the frontal sinus; HFS – height of the frontal sinus; AFS – area of the frontal sinus; FSI – Frontal Sinus Index – the ratio of height and depth of the frontal sinus

Table 7. Simple logistic regression analysis of sex by different variables

Variables	Coefficient	SE	Wald	$p$ value	-2 Log likelihood	$\chi^2$ for model fit	$p$ value	% of overall correct classification
Frontal view								
HFS_R	1.1202	0.5028	4.9052	0.0267	41.2021	5.7552	0.0164	67.19
Constant	-1.6601	0.7197	5.3202	0.0211				
HFS_L	0.9658	0.4376	4.8701	0.0273	41.6604	5.6283	0.0177	68.18
Constant	-1.5524	0.6723	5.3322	0.0209				
WFS_R	1.0242	0.3879	6.9703	0.0082	39.8270	8.5055	0.0035	65.63
Constant	-2.6713	0.9929	7.2387	0.0071				
WFS_L	0.3984	0.2750	2.0987	0.1474	44.3781	2.1930	0.1386	59.09
Constant	-1.1717	0.7301	2.5752	0.1085				
AFS_R	0.5124	0.1954	6.8715	0.0088	40.8203	8.0836	0.0045	63.08
Constant	-1.4294	0.5537	6.6638	0.0098				

Variables	Coefficient	SE	Wald	<i>p</i> value	-2 Log likelihood	$\chi^2$ for model fit	<i>p</i> value	% of overall corrected classification
AFS_L	0.3319	0.1470	5.0991	0.0239	41.7589	5.8361	0.0157	67.69
Constant	-1.1634	0.4909	5.6176	0.0178				
Total area	0.4091	0.1386	8.7157	0.0032	41.3680	10.5980	0.0011	67.65
Constant	-2.2191	0.7300	9.2397	0.0024				
Sinus Index	-0.2117	0.2333	0.8233	0.3642	47.2218	0.8481	0.3571	59.42
Constant	0.5258	0.7750	0.4603	0.4975				
Lateral view								
HFS	0.5069	0.3391	2.2341	0.1350	46.4587	2.3742	0.1234	63.77
Constant	-1.3920	0.8721	2.5477	0.1105				
DFS	2.2836	0.9939	5.2790	0.0216	44.5877	6.1163	0.0134	65.22
Constant	-2.0051	0.8416	5.6760	0.0172				
AFS	0.5986	0.2889	4.3010	0.0381	45.2047	4.8823	0.0214	69.57
Constant	-1.1572	0.5428	4.5456	0.0330				

HFS\_R – height of the right frontal sinus; HFS\_L – height of the left frontal sinus; WFS\_R – width of the right frontal sinus; WFS\_L – width of the left frontal sinus; AFS\_R – area of the right frontal sinus; AFS\_L – the area of the left frontal sinus; HFS – the height of the frontal sinus; DFS – depth of the frontal sinus; AFS – the area of the left frontal sinus; SE – standard error of the mean

After applying a multivariate logistic regression model for all measurements of the frontal projection (including measurements from both sides), the mean correctness of the sex evaluation was 73.77% ( $\chi^2$  for model fit was 21.0510 at  $p = 0.00703$ ). Regarding measurements performed in the lateral view, the mean correctness of the sex evaluation was 68.11% ( $\chi^2$  for model fit was 6.1494 at  $p = 0.1046$ ).

Therefore, including all measurements in the frontal sinus morphometry-based sex assessment regression model generally increases its reliability by slightly more than 5.5% to less than 15%. However, the use of all possible measurements in the model does not allow us to conclude whether the reliability of the method itself increases significantly.

Contrary to expectations and despite obtaining statistically significant results, the morphometry of the frontal sinuses does not constitute a reliable sex discriminant in the skeletal series we examined.

## Discussion

Numerous attempts have been made to assess the usefulness of the upper border morphology of the FS. In order to determine the dimorphic potential of the FS, many researchers have evaluated the accuracy rate of the frontal sinus index (FSI) and area measurements in sex estimation (Yoshino et al. 1987; Riepert et al. 2001; Christensem 2005; Camargo et al. 2007; Goyal et al. 2013; Sai Kiran et al. 2014; Luo et al. 2018; Almeida Prado et al. 2021; Thottungal et al. 2024).

In this study, we aimed to compare the morphology of the sinuses in males and females and to determine whether morphometric differences can be used as an additional anthropological tool in determining the skeletal sex of historical populations.

The frontal sinus may be absent or even extend beyond the frontal region. In the skeletal series analyzed, we observed two male skulls with bilateral FS aplasia. The typical configuration of FS features two sinus cavities separated by a bony septum, but variations in the number of sinus cavities have been reported.

The typical FS configuration features two sinus cavities separated by a bony septum, but variations have been reported regarding the number of sinus cavities present. The FS may be absent or even extend beyond the frontal region. The presence of three or more sinus lobes is considered to be quite rare (Phrabhakaran et al. 1999), while some studies suggest that double and triple (Schaeffer 1916; Açar et al. 2020), or even quadruple and quintuple cavities (Cryer 1907) are quite common.

In the present study, FSs were classified into four types of pneumatization. The medium-sized FS type was the most prevalent on both sides, both in males and females (77.63% and 71.05% on the right and left sides, respectively), which is consistent with the results reported by Guerram et al. (2014). In turn, Yazuci (2019) reported that hyperplasia was the most widespread type of FS in males, while aplasia and hypoplasia were predominant in females. The predominance of hyperplastic FS in males may be due to their overall relative size.

In the analyzed skeletal series, we observed two male skulls with bilateral aplasia of the FS (2.63%). Studies indicate that complete absence of FS oc-

curs in approximately 0–15% of adults (Yoshino et al. 1987; Çakur et al. 2011; Guerram et al. 2014; Belaldavar et al. 2014; Yüksel Aslier et al. 2016; Duzer et al. 2017; Pajic et al. 2017; Luo et al. 2018; Butaric et al. 2020) and the rate may vary between different geographic groups, climate, extent of supraorbital ridges, ancestry, cranial indices, or presence of a metopic suture (see, e.g., Ikeda 1980; Gulisano et al. 1987; Harris et al. 1987; Donald et al. 1994; Christensen and Hatch 2018). An interesting case of high percentages of aplasia was observed in the Alaskan Inuit population: 36% of females and 25% of males (Duzer et al. 2017) and the Canadian Inuit population: 40% of females and 43% of males (Hanson and Owsley 1980).

In the present study, the upper border of the frontal sinuses scalloped with two arcades was the most frequent type of morphology, both in females and males (31.88% total), while Yoshino et al. (1987) reported FS smooth or scalloped FS with one arcade as the most frequent in the Japanese population. In turn, in Tang et al. (2009) the most frequently recorded FS type was scalloped with two arcades on the left side (26.7%) and scalloped with three arcades on the right side (27.3%). These discrepancies are probably due to population or geographical differences.

The study by Yoshino et al. (1987) and Luo et al. (2018) and our findings suggest the absence of a partial septum as the most common morphological feature in the populations studied or skeletal series. The authors also obtained similar values for the FSI for women and men in the lateral view.

Some researchers have attempted to use morphometric diversity of the frontal sinuses and frontal sinus indices to assess sex (Goyal et al. 2013; Belaldavar



et al. 2014; Sai Kiran et al. 2014; Michel et al. 2015; Luo et al. 2018). Some studies showed a strong indication of a difference between groups of men and women for most of the variables studied (Belaldavar et al. 2014, our data). Some studies indicated that single indicators, such as width of frontal sine (Sai Kiran et al. 2014), height and depth of frontal sine (Hamed et al. 2014; Shamlou and Tallman 2022), or total FS volume/area, are the most discriminant (Michel et al. 2015). However, in most cases, the frontal sinus index revealed statistically significant sexual dimorphism ( $p > 0.05$ ) and was thought to be a comparatively better indicator of sex determination. According to Grummons et al. (1987), ratios are more reliable compared to individual absolute measurements because of the inherent shortcomings of cephalometry, such as magnification.

According to a study by Tang et al. (2009), the height and width asymmetry index of bilateral frontal sinuses in the Chinese Han population was slightly higher (both height and width were in the range of 80–100) compared to those reported in this study (average 71.32 for height and 74.55 for width). In our study the height and width ratio was not statistically significant compared to individual measurements. This might be due to variation between skeletal series, and differences in the literature data in this respect could be linked to the variability of sexual dimorphism in different populations. However, some researchers argued that although the FSI method was superior to others, it had little potential because it consistently produced a low percentage of correct classifications, only slightly better than individual measurements (Buckland-Wright 1970; Cameriere et al. 2005; Goyal et al. 2013; Belaldavar et al. 2014).

All skulls in the present study had asymmetric FS, which is attributable to independent pneumatization processes of the right and left FS. According to the literature, 85.7% of individuals exhibit asymmetry of FS height and width: 50.1% have left-sided dominance and 35.6% have right-sided dominance while the remaining 14.3% reveal symmetric sinuses (Basic et al. 2004; Kanat et al. 2015). Larger left FSs in both sexes were also recorded by Spaeth et al. (1997) and Pondé et al. (2003) and was observed in the present study for all three types of pneumatization separately and for all types combined.

Previous studies reported variation in FS size depending on the individual's sex (Ruiz et al. 2004). Our results indicate that all absolute means measurements were higher/wider for male skulls, including the FS area (both in frontal and lateral view), but not all reached statistical significance. Buckland-Wright (1970) was one of the first authors to report sex differences, stating that the FSs in males were approximately twice as large as in females. The morphological differences in the cranium between the two sexes are considered to be mainly caused by genetic factors, more so than by nutritional, hormonal, or muscular factors (Quatrehomme et al. 1996; Patil and Mody 2005), which can explain why the FSs of men are on average larger compared to those observed in women (Szilvassy 1981).

In our study, statistically significant differences were obtained for most measurements in the frontal view (except for the width of the left side) and for the depth and the area of the frontal sine in the lateral view. Similarly significant differences between men and women in a variety of metric (e.g., total volume, width, height, depth, angle) and non-metric traits

(bilateral asymmetry, sinus shape) were obtained in several other studies (Kim et al. 2013; Hamed et al. 2014; Michel et al. 2015; Wanzeler et al. (2019).

In contrast, Yoshino et al. (1987) and Cox et al. (2009), who also evaluated anteroposterior radiographs, found no significant sexual dimorphism. Both studies indicated a sceptical view regarding the value of FS in sex assessment, suggesting too high interindividual variability of the sinuses.

Our results of the correct discrimination rate are within the range reported by other researchers, i.e., 60.0–85.9% (Camargo et al. 2007; Uthman et al. 2010; Goyal et al. 2013; Belaldavar et al. 2014; Hamed et al. 2014; Sai Kiran et al. 2014; Michel et al. 2015; Luo et al. 2018; Almeida Prado et al. 2021).

Discriminant analysis reported by Uthman et al. (2010) indicated the highest empirical values. The authors correctly predicted sex from FS in 76.9% of the cases (in conjunction with skull measurements, the overall accuracy rate was 85.9%). In the model reported by Uthman et al. (2010), the height of the left sinus was the best discriminating variable, followed by the width of the right sinus.

It should be emphasized that the lower average correct discrimination rate was obtained based on morphological characteristics such as the number of scallops on the superior border of the sinuses, the number of partial septa, and the unilateral/bilateral presence or absence of partial septa or supraorbital cells. Higher values were obtained based on metric measurements of the height, width, and area of the FS.

In summary, FS provides an average accuracy for estimating sex across different populations. This may be due to their substantial morphological variability. Some researchers have suggested

that different populations express sexual dimorphism by different patterns and to different degrees, depending on variation among proximate and ultimate mechanisms, such as sexual selection, mating patterns, variation in body size, economic patterns, and non-economic role patterns (Bastir et al. 2011). Populations may show greater or lesser sexual dimorphism, translating into average male and female morphology (Walker 2008). Thus, some groups may reveal significant differences, while in others those differences may not reach statistical significance.

A significant limitation of the present study is the small sample size that might be not enough to capture the complete range of variation, as well as the fact that the exact anatomy of the frontal sinus may not be captured on radiographs due to shot angle and distance, thus reducing the reliability of research results using cephalograms (Cameriere et al. 2005). In general, the use of FSs for anthropological purposes requires a certain degree of caution. Certain environmental factors, such as hyperpneumatization caused by sport activities, disease, trauma, post-mortem changes, and differences in techniques and radiographs such as distance, angle, and orientation of the skull, can modify the image of the frontal sinus, distorting its anatomical features and morphometric analysis (Quatrehomme et al. 1996; Cameriere et al. 2005).

Biological anthropology distinguishes between estimating sex by visually examining skeletal features (nonmetric methods) and using equations based on skeletal dimensions (metric methods). Growing concerns about the subjectivity of nonmetric sex assessments have led to the implementation of ordinal scoring systems, statistical methods, and the quantification of discrete trait mor-

phology with geometric morphometric techniques that examine shape differences (Ajanović et al. 2023). Metric sex estimation is preferred due to the objectivity associated with osteometric data, but it is only possible if equations from geographically and temporally appropriate reference samples are available and if the material is not fragmentary (Chovalopoulou et al. 2018).

Despite statistically significant differences in linear measurements and areas of the FS between the sexes, our results do not support the hypothesis of the usefulness of the frontal sinuses in assessing the sex of historical skeletal materials. The accuracy rate obtained (from 59.09% to 69.57%) is insufficient. The results of this paper suggest that frontal sinuses may have limited application as predictors of sex. The obtained values of the correctness of the assessment are not high, which allows us to conclude that, when we have mixed bones (e.g., ossuaries), the assessment is unreliable, in contrast to the promising results of work in the field of forensic medicine.

In summary, the method of sex assessment based on frontal sinus morphology, which has garnered great interest in forensic medicine and forensic anthropology, is not necessarily applicable to the study of prehistoric populations.

### Conflict of interests

The authors declares that there is no conflict of interests.

### Authors' contributions

KM – writing-original draft preparation, writing-reviewing and editing, investigation, data curation, methodology; JM – conceptualization, formal analysis,

methodology; BB – investigation, resources, supervision; AB – investigation, validation, supervision; BL – methodology, data curation; AW – methodology, data curation; IW – investigation, resources, supervision.

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# The anatomical bases of the 3D digital facial approximation of the Zlatý kůň 1 woman (ca. 43,000 BP)

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**ABSTRACT:** In 1950 on Mount Zlatý kůň ('Golden Horse') in modern-day Czech Republic a system of caves was discovered. During many years of research in this area, human and animal osteological remains have been excavated, among which the most interesting ones were nine fragments of a female skull, now dated to ca. 43,000 yrs BP which are one of the earliest known anatomically modern humans in Eurasia. The aim of this research was to use purely digital techniques to: (1) to reconstruct the skull based on the 3D data of preserved fragments, (2) to approximate the probable appearance of the female it belonged to, and (3) to analyze the calculated shape of the reconstructed mandible and volume of the neurocranium in the context of similarities and differences with other representatives of the genus *Homo*. Computer techniques used in this research constitute a new, original approach to the problem of 3D analyses and may be useful primarily in bioarchaeological sciences, where metric analyses of the most valuable bone artifacts are often severely limited due to the incompleteness of the material available for research. The digital techniques presented here may also contribute significantly to the field of surgery, with the possibility of being adapted for applications in cranial prosthetics and post-traumatic reconstructive surgery.

**KEY WORDS:** Zlatý kůň 1; facial approximation; digital; anatomy; prehistory; anthropology.

Original article

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## Introduction

In 1950, during the works on the explosion of a large limestone rock on Mount Zlatý kůň ('Golden Horse'), the Koněprusy cave system (in modern-day Czech Republic) was discovered by construction workers. Its exploration over the following years revealed the presence of human and animal remains, as well as stone- and bone-made artifacts attributed to the early Upper Palaeolithic period. Attention was paid to the presence of what initially appeared to be two separate skulls, but later, when the pieces of bone were assembled, were argued to belong to a single individual. Contradictory studies continued to emerge, as, given the structural robustness, the remains were firstly attributed to a male although subsequent analyses indicated an adult female. A similar problem arose in the case of assessing the antiquity of the remains: initially, estimated to be ca. 30,000 years old by the archaeological stratigraphic method and then, using radiocarbon dating, reconsidered to be much younger, ca. 15,000 before present (BP). However, a craniometric analysis found the remains compatible with a chronology anterior to the Last Glacial Maximum (LGM, ca. 20,000 years ago). Later  $^{14}\text{C}$  tests dated the remains back to ca. 34,000 years BP, but it was believed that the skeleton was too contaminated by an animal glue found on the skeletal elements to yield a reliable absolute dating. Genomic analyses carried out on the remains and Bayesian tip dating suggested a much earlier chronology of ca. 43,000 years ago, making the Zlatý kůň 1 woman one of the earliest known anatomically modern humans preserved from the stock of the first Eurasian inhabitants (Posth 2021; Prüfer et al.

2021; Rmoutilová et al. 2018; Svoboda 2000). Palaeogenetically, this female individual's genome was shown to have a limited amount of Neanderthal admixture and it is worth underlining how the lineage she belonged to did not contribute genetically to later generations of people in Europe or Asia (Churchill et al. 2022; Prüfer et al. 2021). In this article, a 3D facial approximation based on an anatomical analysis is offered using novel digital techniques.

## Materials and methods

### A. Concepts, software and hardware

Forensic facial reconstruction (FFR) or forensic facial approximation (FFA) (Stephan 2015) is an auxiliary recognition technique that predicts facial appearance and is used when little information is available to identify an individual based on their remains (Pereira et al. 2017). It should be stressed that this technique is not about making an exact identification, such as those provided by DNA tests or comparative analysis of teeth, but, rather, it relies on the recognition of the facial aspects that may indirectly lead to individual's identification. As previously noted, for the FFA process to be feasible, it is necessary to first obtain the appearance of the skull itself.

This skull image was acquired in a purely digital process, based on previously published methodology, an approach which will be described in further detail later. Facial approximation followed the same approach described in Abdullah et al. (2022) and Moraes (2023), although with small modifications. The modeling process was carried out in the Blender 3D software, running the add-on OrtogOn-

Blender ([http://www.ciceromoraes.com.br/doc/pt\\_br/OrtogOnBlender/index.html](http://www.ciceromoraes.com.br/doc/pt_br/OrtogOnBlender/index.html)) and its submodule ForensicOnBlender. The program and add-on are free, open source and multiplatform. They can run on Windows ( $\geq 10$ ), MacOS ( $\geq$  BigSur) and Linux (=Ubuntu 20.04).

In the present work, a desktop computer with the following characteristics was used:

- Intel Core I9 9900K 3.6 GHZ/16M processor;
- 64 GB of RAM memory;
- GeForce 8 GB GDDR6 256-bit RTX 2070 GPU;
- Gigabyte 1151 Z390 motherboard;
- SSD SATA III 960 GB 2.5";
- SSD SATA III 480 GB 2.5";
- Water Cooler Masterliquid 240V;

like in the list above Linux 3DCS (<https://github.com/cogitas3d/Linux-3DCS>), based on Ubuntu 20.04.

### **B. Three-dimensional skull reconstruction**

The skull known as Zlatý kůň 1 consists of nine fragments (codes: AP2, AP3, AP9, AP10, AP12, AP15, AP18, AP18 and AP21), which are stored at the Anthropology Department of the National Museum, located in Prague, Czech Republic. Despite covering a considerable part of the surface of a composite skull (cranium and mandible), the structure has some missing regions, such as the nasal bone, part of the maxilla, the left orbit, and the left part of the frontal bone. In 2018, a multinational team of researchers carried out the work of three-dimensional reconstruction of the missing regions, using statistical data extracted from a group made up of 31 skulls: 30 modern ones (15 males and 15 females, scanned using computed tomography at the Center

Hospitalier Universitaire, CHU, in Bordeaux, France) and one of the Moča skull, found in Slovakia, dated to 13,100 years BP. The researchers initially mirrored the 3D mesh to reconstruct the missing regions, using the original anatomy of the skull. Since the image obtained using this method still had some empty areas, it was then complemented basing on statistical data extracted from the aforementioned tomography and fossils, which led to obtaining an image of a complete skull (Rmoutilová et al. 2018). Unfortunately, the authors of this study did not have direct access to the Zlatý kůň 1 fossils, therefore they decided to reconstruct the skull based on data available in scientific publications (Posth 2021; Prüfer et al. 2021; Rmoutilová et al. 2018; Svoboda 2000).

This study uses the approach of Moraes et al. (2023), previously used, among others, in the reconstruction of the skull of Pharaoh Tutankhamun. The facial approximation discussed in this research used as a reference the skull reconstructed by Rmoutilová et al. (2018), based on the images available in the publication (open access under the *Creative Commons* license) in order to deform the skull of a virtual donor over the spatial references, thus correcting the structure with the measurement data present in the same material (Rmoutilová et al. 2018), and reinforcing the precision of the scale with data collected from Prüfer et al. (2021). The anatomical deformation resulted in a skull closely approximating the fossil Zlatý kůň 1 (Fig. 1A). At first, the structure of the zygomatic arch, close to the *porium*, seemed to differ from the expected anatomical pattern. Aiming to compare with modern individuals, a series of 30 skulls of different population affinities

and sexes received a two-dimensional tracing with orthographic observation along the X axis in order to establish a lateral pattern of the region (Fig. 1 B). Two other fossils received the same graphic treatment and, at the end, the set of 30 modern samples (in gray) were compared to the fossils Zlatý kůň 1 (in green), Mladeč 1 (in red, Moraes et al. 2022) and Nazlet Khater 2 (in blue, Moraes and Santos 2023). This comparison indicates a similar initial elevation of the zygomatic arch in the three fossils, which clearly differs from the group of modern skulls (Fig. 1C). With the issue related to the zygomatic arch overcome, attention turned to other regions, with the projection of lines and limits expected for the skull and soft tissue (Moraes et al. 2022a; Moraes and Suharschi 2022; two classes on this approach are available online, 1 of 2: <https://youtu.be/U6oYkEmfyWo>, 2 of 2: <https://youtu.be/Vcz2e5uSFX8>). The projection of the lines was compatible with the skull reconstructed by Rmoutilová et al. (2018), except the orbital frontomalar distance (fmo-fmo) and the projection of other measurements based on the fmo-fmo ratio. According to the fmo-fmo proportion, the limit of the incisors would be below the reconstructed skull, as well as below the chin (Fig. 1D, in blue). However, when taking into account the expected mean for such regions, the lines are close and within one standard deviation (Fig. 1E). The difference between the fmo-fmo ratio and the average can be explained by the distance between the gonions, generally compatible with the fmo-fmo distance, but which, in this case, was significantly smaller. While the fmo-fmo distance was ca. 102 mm, compared to the general average of ca. 97 mm (<https://bit.ly/3NRw2KW>), the

go-go distance was  $\sim 94$  mm, compared to an average of ca. 97 mm. Therefore, the mandible proportion is more appropriate to the average than the proportion expected by the fmo-fmo distance. The data reinforces the statistical coherence of the reconstruction carried out by the multinational team in 2018 (Rmoutilová et al. 2018).

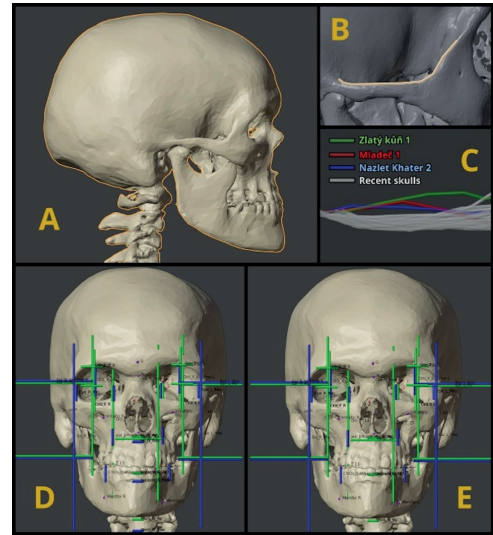


Fig. 1. Three-dimensional reconstruction of the skull

### C. Forensic facial approximation

Since the human skull discussed in this work has poorly understood context of population affinities (apparently the population to which this individual belonged did not genetically contribute to either Europeans or modern Asians, Prüfer et al. 2021), this work disregarded the use of thickness markers of soft tissues and the authors chose to use only the anatomical deformation on the fossil. This approach proved to be very compatible with the parameters coming from the soft tissue thickness tables, keep-



ing the limits within the SD in other approaches that used the technique (vd. *OrtogOnLineMag* #5: [https://ortogonline.com/doc/pt\\_br/OrtogOnLineMag/5/](https://ortogonline.com/doc/pt_br/OrtogOnLineMag/5/) and *OrtogOn-LineMag* #6: [https://ortogonline.com/doc/pt\\_br/OrtogOnLineMag/6/](https://ortogonline.com/doc/pt_br/OrtogOnLineMag/6/).. To reinforce volumetric precision, two 3D meshes from virtual donors were imported, including the skull, endocranium and soft tissue of a man and a woman, both adults (class available on anatomical deformation: [https://youtube.com/watch?v=xig5\\_EcIFWA](https://youtube.com/watch?v=xig5_EcIFWA)). The deformed meshes received a line indicating the profile of the face, interpolating the two limits of the skin resulting from the anatomical deformation (Fig. 2 A, B), which were compatible with the nasal projection based on statistical data collected in computed tomography scans of living individuals belonging to different population affinities (Moraes et al. 2021; Moraes and Suharschi 2022). The limits projected from the skull were compared with the deformed mesh and adapted to the expected parameters, including the size of the nose on the X axis, the size of the eyes on the X axis, the position of the eyeball on the X, Y and Z axes, the size of the ears on the Z axis and the size of the lips on the X axis (Fig. 2C). Thanks to the pre-segmented structure, it was possible to adjust the mesh so that it resulted in the volume of the endocranium, whose data will be detailed later (Fig. 2D). Following the approach exposed in Abdullah et al. (2022) and Moraes (2023), a bust from another facial approximation was imported and adjusted to provide a mesh composed of four-sided faces and with a previously configured texture (Fig. 2E). Unlike the approaches mentioned above where a version with eyes open was available, the present work only has a version with eyes closed, in order to reduce the subjective elements of the structure, as

will be explained in the *Results*. The mesh underwent detailing via digital sculpting, texture adjustment and scene lighting configuration so that the final images could be generated (Fig. 2. F, G).

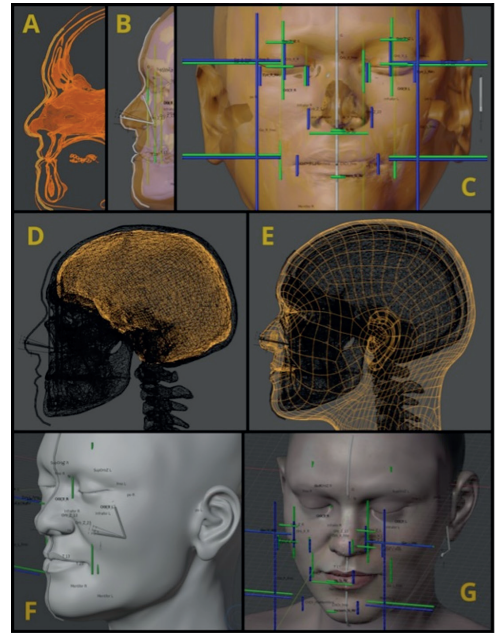


Fig. 2. Steps of the digital facial approximation

## Results

The final images were generated using Blender 3D's Cycles renderer (<https://www.blender.org/>) and consisted of views of the bust composed to show the most objective elements of the face, focusing on the general structure. For the images with objective elements, the eyes were closed, the image was converted to gray scale, and the head did not have hair (Figs. 3–6). For images with speculative elements, the eyes were opened, the hair was configured, and the colors were maintained (Figs. 7–9).



Fig. 3. 3/4 image of the face approximated with objective elements

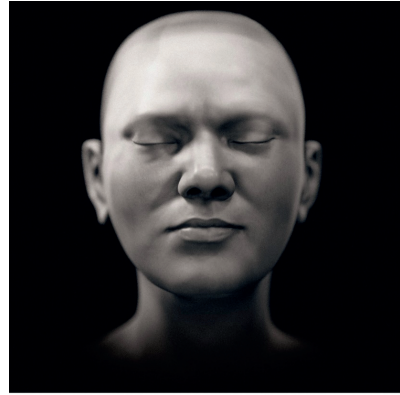


Fig. 6. Frontal image of the face approximated with objective elements



Fig. 4. Side image of the face approximated with objective elements



Fig. 7. 3/4 image of the face approximated with speculative elements

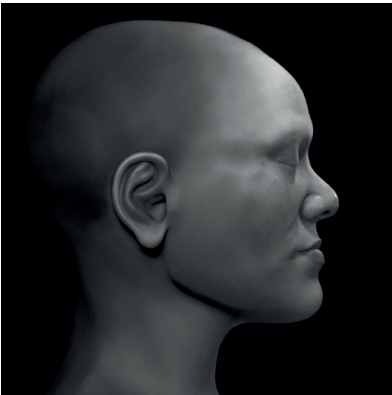


Fig. 5. Profile image of the approximate face with objective elements



Fig. 8. Side image of the face approximated with speculative elements



Fig. 9. Frontal image of the face approximated with speculative elements

## Discussion

The 3D approximation of the examined specimen, obtained according to the adopted methodologies, makes it possible to make inferences on the morphology of the mandible and the endocranium volume between different species of *Homo*. An attempt to understand the differences between the mandibles of fossils dating from 32,000 to 45,000 BP, the bones of 30 modern individuals and the fossils Zlatý kůň 1, Oase 1 (Crevecoeur 2012) and Nazlet Khater 2 (Moraes and Santos 2023) were measured at two distances, sch-scp and pch-acp (Fig. 10), both only on the Y axis, with sch being point on superior margin of the condyle head, scp – point on superior margin of the coronoid process, pch – point on the posterior margin of the condyle head and acp – most anterior point on the anterior aspect of the ramus border (Lestrel et al. 2013). A graph (Fig. 11) created on the base of pch-acp measurements compares three fossils: Oase 1 (approx. 40,000 years ago), Nazlet Khat-

er 2 (32,000–44,000 years ago) and Zlatý kůň 1 (approx. 43,000 years ago), as well as broad group of modern human mandibles. As can be seen, Zlatý kůň 1 is characterised by the significantly most robust mandible structure. By adding data for four mandibles belonging, according to Rosas et al. 2019, to *H. neanderthalensis* (Atapuerca-605, Atapuerca-905, Mauer and Arago 2), three distinct groups can be created (Fig. 12). First, a broader one, consists of the *H. neanderthalensis* individuals, which slightly intersects with second group – modern humans. This group is characterised by the smallest mandibles. The third group is formed by *H. sapiens*, which comprises fossils aged from 32,000 to 45,000 yrs BP. It is clearly visible that this group is distant from modern humans but intersects with *H. neanderthalensis*. Something similar happens with the data related to the endocranium volume (X axis) and head circumference (Y axis, see Fig. 13). Points representing fossils of individuals living between 31,000 and 45,000 yrs BP, for the most part, touch the ellipse composed of *H. neanderthalensis*, *H. rhodesiensis* and

*H. heidelbergensis*. The volume of Zlatý kuň 1's endocranium resulted in ca.1590 cm<sup>3</sup>, a value above one SD from the av-

erage of current female. In relation to the head circumference, with 59.08 cm, also above one SD from the average.

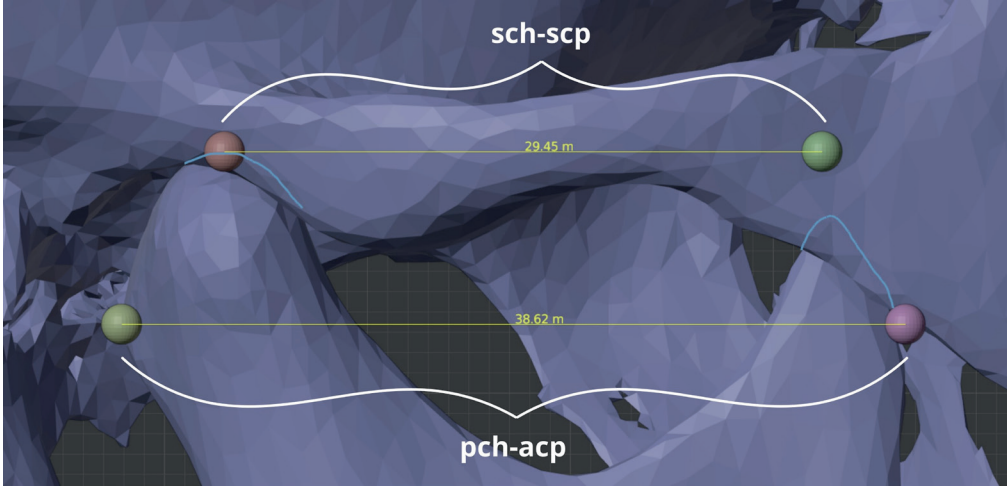


Fig. 10. Measurements taken on the mandible (Y axis)

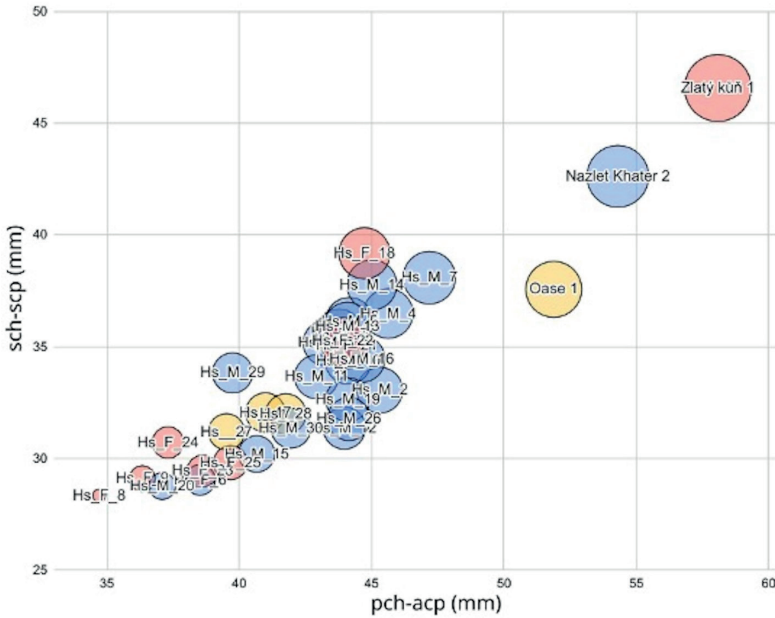


Fig. 11. Distribution of pch-acp measurements on the X axis and sch-scp on the Y axis, the diameter of the spheres is proportional to the sum of the two measurements (pch-acp)+(sch-scp). The colors represent sex, with male blue, female red and undefined yellow

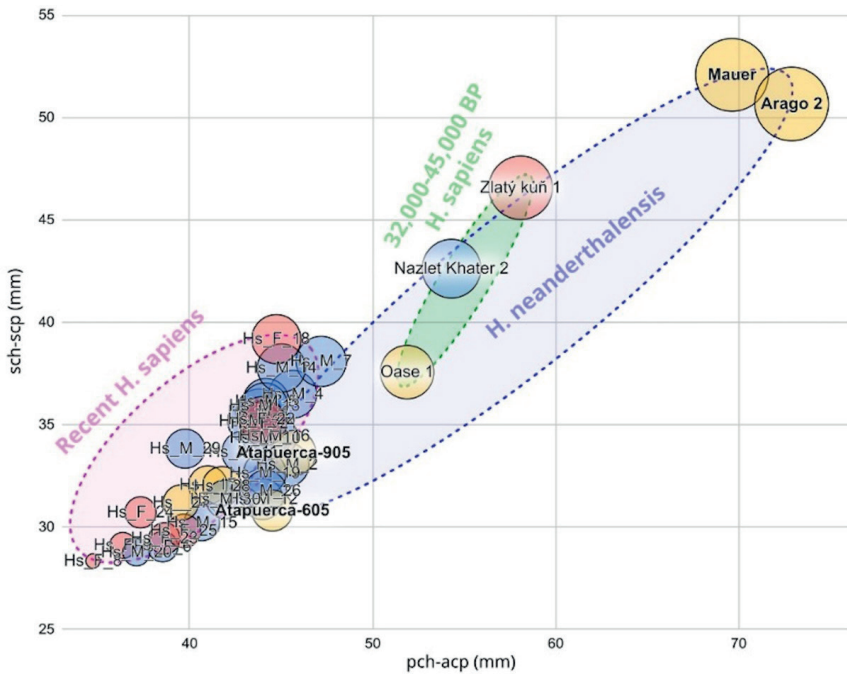


Fig. 12. Distribution of pch-acp measurements on the X axis and sch-scp on the Y axis, with the addition of the *H. neanderthalensis* group

The endocranium volume and circumference graph was generated based on data for a group of 50 *H. sapiens* endocrania, 31 male and 19 female (Fig. 13). When comparing this data to outcomes based on noticeably larger numbers (up to 2750 for each sex, da Costa et al. 2022; Neubauer et al. 2018; Ritchie et al. 2018), similar sample's distribution is still visible (Fig. 14). When applying the factor of -9.81% to convert the volume of the Zlatý kůň 1 endocranium into brain volume (Moraes et al. 2023), 1434 cm<sup>3</sup> is reached, that is, 318 cm<sup>3</sup> above the average, which is 1116 cm<sup>3</sup>. Therefore, when using data from Ritchie et al. (2018), Zlatý kůň 1's brain is 3.53 SD above the average for females. Even if compared to males, the brain would be 2.0 SD above the average, which is 1234 cm<sup>3</sup>. The head circumfer-

ence, which in Zlatý kůň 1 individual resulted in 59.08 cm, is 2.08 SD above the female average according to Costa et al. (2022). When taking into account the group of both sexes of *H. sapiens*, the endocranium (not the brain) of Zlatý kůň 1 is also 1.6 SD above the general average, according to Neubauer et al. (2018). The volumetric and linear data measured on the fossil Zlatý kůň 1 are based on the information provided by Prüfer et al. (2021) and Rmoutilová et al. (2018). Zlatý kůň 1's encephalic size is compatible with evolutionary trends affecting anatomically modern *H. sapiens* in that during the Holocene period (last 10,000 years, hence much later than the time in which this individual lived) brain size shrunk by approximately 10% (Ruff et al. 1997; Henneberg 1988; Neubauer et al. 2018).



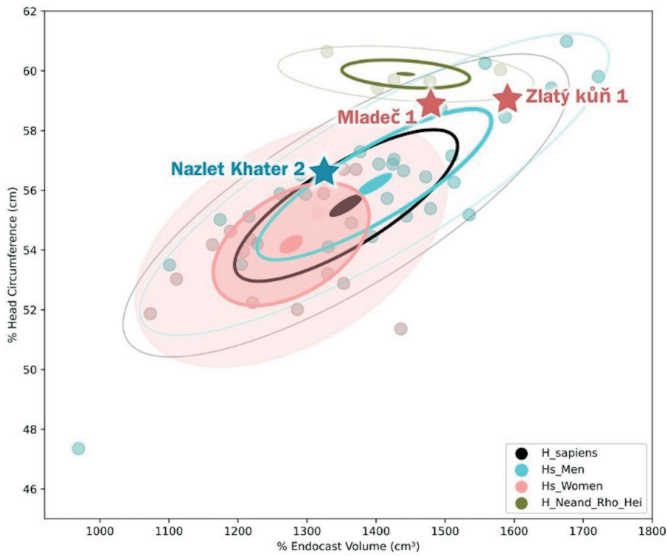


Fig. 13. Endocranium volume vs head circumference

MALE	Brain		Head Circumference	
Source	Moraes et al. (2023)	Ritchie et al. (2018)	Moraes et al. (2023)	Costa et al. (2022)
n	31	2466	31	1045
mean (ml)	1263	1234	56.2	56.2
SD	153	98	2.5	2.4

FEMALE	Brain		Head Circumference	
Source	Moraes et al. (2023)	Ritchie et al. (2018)	Moraes et al. (2023)	Costa et al. (2022)
n	19	2750	19	955
Mean (ml)	1162	1116	54.5	54.3
SD	98	94	2.0	2.3

GENERAL	Endocast		
Source	Moraes et al. (2023)	Neubauer et al. (2018)	Ritchie et al. (2018)
n	50	89	5216
Mean (ml)	1357	1328	1286
SD	158	164	105

Fig. 14. Comparison between different studies



## Conclusion

The rapidly developing visualisation and measurement techniques using 3D models are increasingly applied not only in engineering sciences, but also in medical ones, especially surgery, and scientific research, including physical anthropology. This study aimed to present a new, original method used in morphometric analyzes of bone remains and approximation of the real appearance of a face of the Zlatý kůň 1 woman, one of the earliest known Eurasian individuals. Due to the lack of access to the original remains, this study was based purely on digital data published in the scientific press. Based on digital data, the spatial arrangement of the preserved bone fragments was determined, and then the missing elements were reconstructed, ultimately obtaining an image of a complete skull and an approximation of the probable life appearance of the woman it belonged to. The obtained results were compared to those published in previous works that dealt with the reconstruction of the appearance of the Zlatý kůň 1 woman. Finally, the shape of the mandible reconstructed using digital methods and the volumes of the endocranium (1590 cm<sup>3</sup>) and brain (1434 cm<sup>3</sup>) were discussed, and then a comparison was made with currently known representatives of other species of the genus *Homo*. The calculated dimensions place the examined individual in the group of other examples of *H. sapiens* living between 45,000–32,000 years BP, which is consistent with the C14 dating of the material performed by independent researchers (43,000 years BP) and compatible with the described evolutionary trends of decreasing brain dimensions and decline massiveness of the mandible of our species. These studies show that

the use of modern research methods can significantly increase knowledge in the field of morphometric research, which is particularly valuable in the case of often fragmentarily preserved bone remains representing the ancestors of modern humans.

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## Conflict of interests

None to disclose.

### Authors' contribution

CM: conceptualisation, analysis, 3D modelling, writing of the first draft; FMG: conceptualisation, analysis, writing of the first draft, literature search; LS: analysis, literature search, critical revision of the first draft; JŠ: analysis, literature search, critical revision of the first draft; EV: conceptualisation, analysis, writing of the first draft, literature search; JM-S: analysis, literature search, critical revision of the first draft; NAF: analysis, literature search, critical revision of the first draft; MEH: analysis, literature search, critical revision of the first draft; TB: analysis, literature search, critical revision of the first draft, supervision.

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# Association between normal weight obesity and lipids profile in Slovak women aged 38 to 59 years

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**ABSTRACT:** Obesity and its associated health problems are an issue, especially when weight gain is not outwardly visible. Individuals with the same Body Mass Index (BMI) may differ in body fat percentage and may unknowingly suffer from normal weight obesity (NWO). Middle-aged women are at high risk if their body composition changes due to factors such as age, health status or reproductive history. This study investigated the relationship between NWO and lipid profile in middle-aged women. A sample of 122 women aged 38 to 59 years ( $47.17 \pm 5.24$ ) from Slovakia participated in this study. Using a questionnaire, participants answered questions about lifestyle, health background, sociodemographic classification, reproductive and menstrual history. The anthropometric parameters were determined using standard methods. Body composition was measured using a bioelectrical impedance analyzer. Biochemical parameters were determined from morning blood samples. Blood pressure was assessed in a sitting position using a digital sphygmomanometer. The primary aim of this study was to assess the differences in lipid profiles between NOW women and normal weight-lean (NWL) women. Our results showed statistically significantly higher values of uric acid in the women with NWO compared to NWL women ( $237.39 \pm 54.11 \mu\text{mol/l}$  vs.  $213.02 \pm 52.64 \mu\text{mol/l}$ ;  $p = 0.009$ ). Moreover, significant differences were noted between NWO women and NWL women in body height, body weight, waist and hip circumference, WHR, BMI and fat mass (%;  $p < 0.05$ ). Other biochemical variables showed no statistically significant differences between the study groups of women. Elevated uric acid levels in women diagnosed with NWO may serve as an indication of metabolic imbalance associated with undetected obesity. These results underscore the importance of implementing early detection and intervention methods for NWO to prevent related health issues. Further research is necessary to investigate the underlying factors contributing to these connections and evaluate the efficacy of customized interventions.

**KEY WORDS:** excessive fat mass, lipids, BMI, middle age.



Original article

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## Introduction

Weight gain is one of the biggest health problems in midlife. The changes in the body in midlife are due to many factors, such as age, health status, gender, genetic predisposition, and reproductive history. These changes can be assessed using anthropometric measures such as Body Mass Index (BMI), bioelectrical impedance analysis (BIA), or biochemical assessments (Luptáková et al. 2013a; Drozdová et al. 2016; Danková et al. 2017; Falbová et al. 2019, 2020, 2022a; Vorobeľová et al. 2021, 2023; Hurtado et al. 2024).

In recent decades, the global increase in obesity rates has been closely linked to a rising risk of chronic diseases such as diabetes, cardiovascular disease, and dyslipidemia (Fruh et al. 2017; Welsh et al. 2024). The World Health Organization (WHO) defines obesity as an excessive accumulation of fat that poses a health risk; this emphasizes the importance of considering not only Body Mass Index (BMI), a common measure used to classify overweight and obesity, but also fat mass (FM) to understand the complexity of the disease. Studies show that lean and obese individuals have different metabolic profiles, emphasizing the need to measure fat mass to gain a comprehensive understanding of obesity-related disease trajectories (Hirsch et al. 2016; Bosy-Westphal et al. 2021). In addition, participants with identical BMI may differ in terms of body fat percentage (BFP) and FM distribution, further highlighting the nuanced nature of obesity (Cota et al. 2021). The concept of normal weight obesity (NWO), first described by DeLorenzo in 2006 has emerged to address this phenomenon. This phenotype characterizes individuals with a BMI that indi-

cates a normal weight ( $\text{BMI} < 25 \text{ kg/m}^2$ ) but who have excess FM ( $\text{FM} > 30\%$ ). Despite being of normal weight, individuals classified as NWO have a similar risk of cardiovascular disease, type 2 diabetes, and metabolic syndrome as overweight and obese individuals (Cota et al. 2021; Ashtary-Larky et al. 2023). The discrepancy between BMI and the assessment of health risks arises due to the fact that BMI does not consider the distribution and percentage of body fat, which are crucial elements in determining an individual's overall health. As a result, individuals with NWO may not be identified as at-risk based on BMI alone, leading to a lack of appropriate interventions and an increased risk of health complications.

In addition, estradiol induces vasoprotective effects via multiple mechanisms, including alterations in plasma concentrations of lipoproteins (decrease in low-density lipoprotein cholesterol (LDL-C) levels, reducing oxidized LDL formation, increasing high-density lipoprotein cholesterol levels (HDL-C), hemostatic factors, glucose, and insulin (Dubey and Jackson 2001). Estrogen deficiency in midlife after menopause is the main reason for the deterioration of serum lipid profiles (Rexrode et al. 2003; Luptáková et al. 2012; Fonseca et al. 2017; Vorobeľová et al. 2019; Falbová et al. 2022b).

In this cross-sectional study, we attempted to clarify the association between NWO and lipids profile in middle-aged Slovakian women, due to the limited number of studies available in the literature on the topic and since the effect of increased of fat mass on lipid, inflammation or glycaemic status remains to be verified (Marques-Vidal et al. 2010).



## Material and methods

### Participants

This study was based on data collected from a cross-sectional survey in Slovakia. The women were recruited from various locations in Slovakia's west, south, and center (51.9% were born in towns), through an invitation letter, which was distributed prior to data collection with the aid of local physicians. The women were approached and recruited using a non-random procedure based on voluntariness and convenience. The studied sample consisted of 122 middle-aged women, aged between 38 and 59 years, with an average age of  $47.17 \pm 5.24$  years. All women were interviewed at a medical examination facility in the morning and assessed for medical, anthropometric, and lifestyle factors at local health centers. Each woman provided written informed consent for this study following the principles of the Declaration of Helsinki. Those who were unable to give a response due to severe physical or mental illness and on whom anthropometry and blood measurements could not be performed were excluded from the study. The women were divided into two groups according to their BMI and FM (%) – NWO women (BMI  $< 25 \text{ kg/m}^2$ , FM  $> 30\%$ ) and normal weight lean (NWL) women (BMI  $< 25 \text{ kg/m}^2$ , FM  $< 30\%$ ).

### Questionnaire

The women were interviewed using pre-tested questionnaires on their reproductive and menstrual history, socio-demographic background, lifestyle, and health status designed by Kaczmarek (Kaczmarek 2007) and validated in Polish studies (The Menopause-Specific Questionnaire, A. Mickiewicz University Poznań, Poland, Maria Kaczmarek). All

socio-demographic and lifestyle variables were self-reported. Smoking status was divided into current 'smokers' (smoking once a week to daily) and non-smokers (never smoked). Physical activity was categorized into two groups: regular and never, including occasionally. Education was divided into three groups: basic, secondary, and university education.

### Anthropometric analysis and body composition

Anthropometric measurements were taken after participants had removed their shoes and heavy clothing. Data were collected by trained anthropologists using standard Martin and Saller techniques. (1) height was measured to an accuracy of 0.5 cm using a Sieber and Hegner anthropometer at the head with the participant standing barefoot with the feet together; (2) body weight was measured on a personal weight scale to an accuracy of 0.1 kg; and (3) BMI was calculated as body weight divided by height squared. Waist and hip circumferences were measured according to the NHLBI Obesity Education Initiative (Audrain-McGovern and Benowitz 2011) and the WHO (2011). The WHR was calculated as the waist circumference divided by the hip circumference.

Body composition measurements were performed in the morning utilizing a bioelectrical impedance analyzer (BIA 101, Akern S.r.l.) at a signal frequency of 50 kHz, with a constant excitation current of  $800 \mu\text{A}$  and a four-electrode arrangement. Bioimpedance is a complex measurement composed of resistance (R, ohm) related to the amount of fluid and reactance (XC, ohm) associated with the cell membrane's capacitance. This study determined the FM (%) using Bodygram (version 1.21, Akern S.r.l) program.

### Biochemical analysis

Blood samples were collected in the morning after at least 12 hours of fasting. Biochemical values of bilirubin, glucose, gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), creatinine, uric acid (UA), total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) were analyzed from fasting plasma samples using routine laboratory procedures at the Clinical Laboratories Department of Bratislava Alpha Medical. Low-density lipoprotein cholesterol (LDL-C) was calculated from the total cholesterol, HDL-C, and triglycerides values using the Friedewald equation when triglycerides were 4.5 mmol/L. LDL-C was considered as absent if the serum triglyceride concentration exceeded this threshold. The atherogenic indices were calculated as follows: AI1 = TC (mmol/L) / HDL-C (mmol/L), AI2 (non-HDL-C) = TC (mmol/L) - HDL-C (mmol/L), and AI3 = LDL-C (mmol/L) / HDL-C (mmol/L). The atherogenic index of plasma (AIP) was calculated as the logarithmically transformed ratio of the TG to HDL-C molar concentrations (mmol/l).

### Blood pressure (BP) assessment

BP and pulse rate were measured in a sitting position using a digital sphygmomanometer (Omron M3). Each measurement was performed three times, and the average values for systolic blood pressure (mmHg; SBP), diastolic blood pressure (mmHg; DBP), and pulse rate (pulse/min) were then determined.

### Statistical analysis

All statistical analyses were performed using IBM SPSS for Windows (Statistical Package for the Social Science, version 24.0, Chicago, IL), with statistical

significance at  $p \leq 0.05$ . The obtained frequencies and percentages determined participants' responses, and the normality assumption hypothesis for continuous variables was tested by a one-sample Kolmogorov-Smirnov test. The differences between NWO and NWL women in the category variables were tested with Pearson Chi-square in contingency tables. The parametric Independent Samples T-Test and the non-parametric Mann-Whitney U test were used based on the normality distribution of the quantitative variables.

## Results

Table 1 depicts the baseline characteristics of the women under study, divided into the NWL group, comprised 57 participants (age  $46.56 \pm 4.78$  SD), and the NWO group (age  $48.11 \pm 5.46$  SD), comprised 65 individuals. Statistically significant differences between the two groups were observed in the following variables: body height (cm;  $p = 0.013$ ), body weight (kg;  $p < 0.001$ ), waist circumference (cm;  $p < 0.001$ ), hip circumference (cm;  $p < 0.001$ ), BMI ( $\text{kg}/\text{m}^2$ ;  $p < 0.001$ ), WHR ( $p = 0.015$ ) and FM (%;  $p < 0.001$ ).

On the contrary, no differences were observed in the variables age (y;  $p = 0.119$ ), SBP (mmHg;  $p = 0.438$ ), and DBP (mmHg;  $p = 0.577$ ). Overall, the NWO group presented higher values across all the mentioned variables except for body height (cm) and DBP (mmHg). Moreover, other factors such as smoking, sports activity, and education were reported across these two groups. Only 19 (33.34) women smoked in the NWL group, whereas 12 (18.46%) smoked in the NWO group ( $p = 0.060$ ). In the NWL group, 18 (31.58%) performed regular

sports activity, and 13 (20%) performed regular sports activity in the NWO group ( $p = 0.143$ ). In regards to education, in the NWL group, 7 (12.28%) had a basic level, 41 (71.93%) had a secondary level,

and 9 (15.79%) had a university degree; similarly, in the NWO group, 10 (15.38%) had a basic level of education, 46 (70.78%) secondary and 9 (13.84%) a university degree ( $p = 0.864$ ).

Table 1. Baseline characteristics of the study women

	NWL	NWO	
<b>Number of participants</b>	N = 57 (46.72%)	N = 65 (53.28%)	
	<b>Mean <math>\pm</math> SD</b>	<b>Mean <math>\pm</math> SD</b>	<b>p</b>
Age, y	46.56 $\pm$ 4.78	48.11 $\pm$ 5.46	0.119
Body Height (cm)	165.35 $\pm$ 5.56	162.89 $\pm$ 5.18	0.013
Body Weight (kg)	57.83 $\pm$ 6.30	62.55 $\pm$ 4.65	<0.001
Waist circumference (cm)	70.35 $\pm$ 7.08	76.67 $\pm$ 6.43	<0.001
Hip circumference (cm)	93.18 $\pm$ 4.84	97.60 $\pm$ 4.32	<0.001
BMI (kg/m <sup>2</sup> )	21.11 $\pm$ 1.65	23.55 $\pm$ 0.91	<0.001
WHR	0.75 $\pm$ 0.06	0.79 $\pm$ 0.07	0.015
Fat mass (%)	25.07 $\pm$ 4.37	32.88 $\pm$ 2.11	<0.001
SBP (mmHg)	116.05 $\pm$ 15.37	118.08 $\pm$ 15.28	0.438
DBP (mmHg)	75.79 $\pm$ 10.17	75.02 $\pm$ 9.10	0.577
	<b>N (%)</b>	<b>N (%)</b>	
<b>Smoking status</b>			<b>p</b>
Smokers	19 (33.34)	12 (18.46)	0.060
Non-smokers	38 (66.67)	53 (81.54)	
<b>Regular sport activity</b>			
Yes	18 (31.58)	13 (20.00)	0.143
No	39 (68.42)	52 (80.00)	
<b>Education</b>			
Basic	7 (12.28)	10 (15.38)	0.864
Secondary	41 (71.93)	46 (70.78)	
University	9 (15.79)	9 (13.84)	

Note: N, number of participants; p, value of statistical significance; SD, standard deviations; NWL, normal weight lean; NWO, normal weight obesity, SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body Mass Index; WHR, waist to hip ratio

Table 2 compares the mean values of selected biochemical variables and atherogenic indices between women with NWL and NWO. The primary objective of this study was to assess the variations in lipid profiles, which encompass Total Cho-

lesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG). The NWO group exhibited lower mean TC levels when contrasted with the NWL group. The mean TC for

the NWO group amounted to  $5.29 \pm 0.95$  mmol/L, whereas the NWL group displayed a mean TC of  $5.42 \pm 1.09$  mmol/L ( $p = 0.592$ ). Conversely, the NWL group had elevated LDL-C levels, with a mean of  $3.19 \pm 1.01$  mmol/L, in contrast to the NWO group, which had a mean of  $2.99 \pm 0.86$  mmol/L ( $p = 0.243$ ). Additional-

ly, the NWO group showed lower HDL-C levels, with a mean of  $1.70 \pm 0.50$  mmol/L, compared to the NWL group, which had a mean of  $1.79 \pm 0.43$  mmol/L ( $p = 0.299$ ). The NWO group had higher TG levels, averaging  $1.25 \pm 1.07$  mmol/L, whereas the NWL group averaged  $0.98 \pm 0.38$  mmol/L ( $p = 0.065$ ).

Table 2. Selected biochemical variables between NWL and NWO women

	NWL			NWO			<i>p</i>
	N	Mean	SD	N	Mean	SD	
Total cholesterol, (mmol/L)	57	5.42	± 1.09	65	5.29	± 0.95	0.592
Triglycerides, (mmol/L)	57	0.98	± 0.38	65	1.25	± 1.07	0.065
HDL-C, (mmol/L)	56	1.79	± 0.43	59	1.70	± 0.50	0.299
LDL-C, (mmol/L)	56	3.19	± 1.01	59	2.99	± 0.86	0.243
AI1 (TC/HDL-C)	56	3.16	± 0.90	59	3.28	± 1.18	0.946
AI2 (TC-HDL-C)	56	3.63	± 1.06	59	3.54	± 0.99	0.637
AI3 (LDL-HDL-C)	56	1.90	± 0.80	59	1.91	± 0.95	0.663
AIP log (TG/HDL-C)	56	-0.29	± 0.21	59	-0.21	± 0.28	0.115
Creatinine ( $\mu\text{mol/L}$ )	57	69.28	± 7.31	65	69.47	± 8.33	0.892
Bilirubin ( $\mu\text{mol/L}$ )	57	9.80	± 4.71	65	9.55	± 4.71	0.795
Glucose (mmol/L)	57	4.59	± 0.68	63	4.67	± 0.61	0.323
Gamma-glutamyltransferase ( $\mu\text{kat/L}$ )	57	0.29	± 0.18	65	0.29	± 0.17	0.898
Alanine aminotransferase ( $\mu\text{kat/L}$ )	57	0.25	± 0.08	65	0.29	± 0.20	0.736
Uric acid ( $\mu\text{mol/L}$ )	57	213.02	± 52.64	65	237.39	± 54.11	0.009
APO A1	56	1.77	± 0.27	59	1.76	± 0.29	0.972
ApoB/ApoA1	56	0.50	± 0.17	59	0.51	± 0.18	0.894

Abbreviations: N, number of participants; *p*, value of statistical significance; SD, standard deviations; NWL, normal weight lean; NWO, normal weight obesity; HDL-C, high-density lipoprotein – cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides; AI, Atherogenic index; APO, apolipoprotein

The women with NWO achieved statistically significantly higher mean values of UA compared to women with NWL ( $237.39 \pm 54.11 \mu\text{mol/l}$  vs.  $213.02 \pm 52.64 \mu\text{mol/l}$ ). No statistically significant differences were found in other biochemical variables between the women's study groups.

## Discussion

Our results show significant differences between the NWL and NWO groups in various anthropometric measures, such as body weight, waist circumference, hip circumference, BMI, WHR and FM (%). These findings suggest that despite both

groups being classified as normal weight by BMI, there are significant differences in body composition and fat distribution, which emphasizes the importance of considering waist circumference as a marker of central obesity. The fact that no significant differences in age and blood pressure were found between the two groups suggests that these factors are unlikely to influence the differences in anthropometric measures. The insignificant variations in blood pressure levels suggest that both groups exhibit comparable cardiovascular profiles in terms of hypertension risk. This observation is significant because it implies that the metabolic disturbances observed in the NWO group, such as increased uric acid levels, may not necessarily be reflected in conventional cardiovascular risk markers, such as blood pressure. This emphasizes the importance of examining beyond traditional metrics to gain a comprehensive understanding of metabolic health, especially in individuals with normal body weight. We can see insights into lifestyle factors such as smoking habits, sports activity and education level between the two groups. Although not statistically significant, the differences in smoking, physical activity and education levels are worth noting as they might have had an impact on overall health and risk factors associated with body composition (Falbová et al. 2023) and obesity (Vorobeľová et al. 2022). Smoking is widely recognized as a significant contributor to a variety of health issues, such as cardiovascular disease and metabolic syndrome. It can increase insulin resistance and inflammation, thereby exacerbating metabolic health in individuals with NOW (Chioloro et al. 2008). The potential impact of smoking on metabolic health in NWO individuals warrants further in-

vestigation, as addressing smoking cessation could be a critical intervention for improving health outcomes in this population. Moreover, it is crucial to engage in regular physical activity in order to preserve metabolic health and prevent obesity-related diseases. Although the difference in reported physical activity levels was not considered statistically significant, even minor variations in physical activity can have significant implications for one's health (Petridou et al. 2019). Future studies should consider more precise measures of physical activity and their direct effects on metabolic parameters in NWO individuals. Education level is often correlated with health literacy, access to healthcare, and lifestyle choices. Although our study found no significant differences in education levels, higher education may be associated with better health outcomes due to increased awareness and adoption of healthier behaviors (Zajacova et al. 2018). The role of education in influencing health behaviors and metabolic health outcomes in NWO individuals is an area that deserves more detailed examination. Similarly, studies by Opoku et al. (2023) and Lupťáková et al. (2013b) found significant associations between obesity, metabolic syndrome and various anthropometric measures such as body weight, waist circumference and BMI. Thus, the studies indicate that central obesity, as reflected by increased waist circumference, is associated with unfavorable metabolic outcomes regardless of BMI. The difference between the present study and the study by Opoku et al. (2023) is that the latter focuses more generally on obesity at different stages of menopause without considering NWO. In the present study, a significant difference in UA levels was found between the NWL and NWO

groups, with significantly higher levels observed in the NWO group. Several mechanisms may explain the association between elevated UA levels and obesity. Excess body fat may be associated with increased UA production and impaired secretion due to insulin resistance, leading to impaired UA metabolism and even hyperuricemia (Kızılay et al. 2019; Li et al. 2021). Elevated UA levels can also contribute to obesity by stimulating hepatic and peripheral fat synthesis (Johnson et al. 2011). This increase in UA levels is associated with metabolic and cardiovascular disease, suggesting possible effects of central obesity on metabolic health in women. In a study by Taheri et al (2020), a significant negative association was found between adiponectin levels and lipid profiles in women with NWO. Lower adiponectin levels correlated with higher levels of total cholesterol, LDL cholesterol and triglycerides, suggesting a possible protective function of adiponectin against dyslipidemia in this population. In a long-term surveillance study of 2873 women conducted over 30 years, 574 women exhibited initial clinical symptoms of coronary heart disease (CHD). Metabolic risk factors, such as cholesterol, glucose tolerance, uric acid, and menopause, were found to be correlated with the development of CHD. The study revealed that serum total cholesterol was a significant predictor of CHD in both men and women. However, impaired glucose tolerance eliminated the female advantage in CHD risk. Central obesity poses a higher CHD risk for women, and the risk is two to three times higher for postmenopausal women than for premenopausal women, as per Kannel's research in 1987. Impaired glucose tolerance is a prevalent issue among obese individuals and often leads to in-

ulin resistance and type 2 diabetes, as per Wondmkun's study in 2020. In obese patients, insulin resistance may also explain the connection between decreased bilirubin levels and increased body fat percentage. The activity of heme oxygenase-1, the enzyme responsible for bilirubin generation, appears to decrease in insulin-resistant conditions (Abraham et al. 2008; Belo et al. 2014). Kim et al. (2014) focused on Korean adults, women and men. Of the Korean adults with normal weight, approximately 20% were identified as having normal weight obesity based on body composition analysis using methods such as dual-energy X-ray absorptiometry (DEXA) or BIA. In addition, it was found that individuals with NWO were more likely to have cardiometabolic risk factors than normal weight lean individuals without excess adiposity, including elevated blood pressure, dyslipidemia, insulin resistance and inflammatory markers. In addition, another study by Bellissimo et al. (2019) showed that individuals with NWO had different patterns of metabolites compared to lean individuals. Metabolites associated with lipid metabolism, such as free fatty acids and phospholipids, were elevated in the plasma of individuals with NWO, indicating altered lipid homeostasis and possible dysregulation of lipid metabolic pathways. In addition, metabolites involved in glucose metabolism, such as amino acids and glycolytic intermediates, showed a different abundance in the two groups, suggesting possible differences in energy metabolism and insulin sensitivity. These findings highlight the utility of high-resolution plasma metabolomics in elucidating metabolic disturbances associated with NWO and provide insights into the underlying metabolic pathways contributing to this



condition. Although the study by Berg et al. (2015) was conducted in a Swedish population of women and men, we can observe that a wide range of body fat was present in normal weight individuals. Women with NWO had higher serum triglyceride levels, low density cholesterol, C-reactive protein, apolipoprotein B and apolipoprotein B/A-I ratio compared to the normal weight group.

This study has several limitations that should be acknowledged. First, due to the cross-sectional design, it is not possible to establish causal relationships between NWO and differences in lipid profile. Longitudinal studies would be necessary to determine causality. Second, the study population was limited to middle-aged Slovakian women, which may limit the generalizability of the findings to other populations and age groups. Third, the binary categorization of physical activity has its limitations as it does not take into account the duration, intensity, and frequency of physical activity, which are crucial factors in evaluating its influence on body composition and metabolic health. Finally, the use of volunteer-based recruitment can introduce biases and may not ensure population representativeness. Despite these limitations, this study provides valuable insights into the metabolic disturbances associated with NWO and underscores the importance of recognizing this phenotype in clinical practice to prevent related health issues. Further research is needed to explore the underlying mechanisms and to develop effective interventions for individuals with NWO.

## Conclusion

In conclusion, this study reveals significant differences in body composition and fat distribution between NWL and NWO

groups and highlights the importance of waist circumference as a measure of central adiposity. In addition, significant differences were found in serum uric acid levels between the aforementioned groups, with significantly higher levels observed in the NWO group.

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## Conflict of interests

The authors declare that there is no conflict of interest.

## Ethical approval

Each participant provided written informed consent for this study which adhered to the Declaration of Helsinki principles.

During the projects implementation, the Ethical approval for this type of project was not necessary in Slovakia. Our manuscript is based on three projects approved and financed by the Scientific Grant Agency (VEGA 1/0247/09, VEGA 1/0493/13) and by the Cultural and Educational Grant Agency (KEGA 015UK-4/2015) of the Ministry of Education, Science, Research and Sport of the Slovak Republic. All projects were evaluated by the Heads of these Agencies as successful.

## Authors' contribution

DF and SS contributed to the study conception, design, performance, and the

manuscript's writing. PŠ and AH participated in data collection and manuscript writing. All authors saw and approved the final version of the manuscript.

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## Revisiting the cranial variability of the Dmanisi hominins

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**ABSTRACT:** The Dmanisi specimens represent the most diverse contemporaneous hominin fossils found at one single site and are key in understanding the first out-of-Africa dispersal and the origins of *Homo erectus*. Due to these reasons, they have been the focus of many studies in paleoanthropology in the last 30 years. However, there has not been any consensus on how to classify these fossils, nor has it been clarified how many species were co-living at that site. In this article, we aim to revisit the subject and contribute further to the discussion.

**KEY WORDS:** *Homo erectus*, *Homo georgicus*, *Homo caucasi*, paleoanthropology.



Original article

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## Introduction

The Dmanisi site in Georgia is one of the oldest hominin sites outside of Africa (Garcia et al. 2010; Zhu et al. 2018). Located near the village of Patara Dmanisi, at an altitude of 915 m, the site is located on a basaltic spur formed at the confluence of the Pinezaouri and the Mashavera rivers. The fossiliferous deposits consist of sedimentary layers deposited on top of a volcanic basalt layer. The lowest layer in the site, which contains tools, faunal remains and human bones, was dated by several methods and recently has been contextualized by  $^{40}\text{Ar}/^{39}\text{Ar}$  geochronological dating to the age of  $1.81 \pm 0.03$  Ma (Garcia et al. 2010). Excavations of the fossiliferous strata in the site have provided five crania and four mandibles of hominins (Gabunia et al. 2000; Vekua et al. 2002; Lordkipanidze et al. 2006, 2013), which have been intensely discussed, given the importance of these fossils to the understanding of the early dispersal of *Homo* out of Africa. The site also has generated well-preserved non-hominin fauna fossils and thousands of Oldowan artifacts (Vekua et al. 2002; Mgladze et al. 2011; Lordkipanidze 2017).

The first hominin fossil (the D211 mandible) was unearthed in 1991 (Vekua and Lordkipanidze 2010). Its associated cranium, D2282, was found in 1999. The skull composed by D211 and D2282 probably belonged to a young female adult, and its gracile size is accentuated by its 680cc endocranial volume (Gabunia et al. 2002). The second cranium, D2280, was also found in 1999. It has no associated mandible and is considered to be a male, with an endocranial volume of 775cc.

In 2000, the D2600 mandible was discovered (Vekua et al. 2002), but its associated cranium, D4500, was only found

in 2005 (Lordkipanidze et al. 2013). This specimen is currently the earliest complete hominin skull ever found (Lordkipanidze et al. 2013), and presents an endocranial volume of 546cc, the smallest of the Dmanisi hominins. However, the robustness of the cranium and the mandible make it the largest skull on the site.

The D2700 cranium and its associated mandible D2735 (Vekua et al. 2002) were recovered in 2001. This skull, which presents an endocranial volume of 600cc, is considered to be from a subadult since its third molar is only partially erupted (Vekua et al. 2002).

The last skull, composed of the D3444 cranium, found in 2002, and its associated jaw, D3900, discovered in 2003 (Lordkipanidze et al. 2005; Lordkipanidze et al. 2006), is thought to have belonged to an older male, since its mandible and maxilla present no teeth (Lordkipanidze 2017). This individual, who presents an endocranial volume of 650cc (Lordkipanidze et al. 2006), possibly survived years without teeth before dying, suggesting that his group helped him with basic daily activities, such as food processing and eating (Lordkipanidze et al. 2005).

Since this sample is very diverse, not only in its endocranial volume but also in morphological features, this set of specimens has been classified in multiple ways. Even though it is clear that the fossils present a suite of characters typical of *Homo*, the number of species and which species are represented by the specimens are heated topics of debate (Rightmire et al. 2017).

Some claim that only one species is represented by the fossils: *Homo erectus* (Vekua et al. 2002; Ferring et al. 2011), or a species more closely related to *Homo habilis* (Lordkipanidze et al. 2007). Others, such as Rosas and Bermúdez de

Castro (1998), propose new taxonomic categories, classifying the specimens as *Homo sp. indet. (aff. ergaster)*. Similarly, Lordkipnidze et al. (2013) suggested the remains are part of a sub-subspecies unprecedentedly called *H. erectus ergaster georgicus*.

It is important to note that some studies that conclude that there is only one paleodeme represented in Dmanisi do not exclude peremptorily the possibility of multiple species being represented in the sample (Rightmire et al. 2018). Skinner et al. (2006) and Neves and Bernardo (2011), for instance, called attention to the fact that the Dmanisi sample presents more variation than ever seen in the hominin or ape lineages. Schwartz et al. (2014) suggested the specimens represent four different species, with D2600 mandible being the holotype of *Homo georgicus*. Scardia et al. (2020) also suggested that there are two different species on the site, naming D4500 as *Homo georgicus* and the other four skulls as “another species, which is not *H. erectus*” (p. 3). This naming convention follows what was first suggested by Gabunia et al. (2002), who discovered the D2600 mandible and suggested that it should be classified as *Homo georgicus*. Finally, Martinon-Torrez et al. (2008) suggested the possibility of the presence of two paleodemes at the site but does not define species names for them.

In this study, we aim to contribute further to the discussion about Dmanisi’s craniomorphological diversity. To do so, we analyzed the five fossils through Discriminant Function Analyses based on size and shape and only shape information. Finally, we compared the distribution of the Georgian specimens in the morphospace with the distribution of other Plio-pleistocene hominin species.

## Material and methods

This study is based on the contextualization of the Dmanisi fossils within the craniofacial morphospace defined by a comparative dataset of 87 linear craniometric dimensions of 267 specimens of Plio-Pleistocene hominins. The composition of the original database is detailed in Table 1. Given the expressive number of missing values in the database, we reduced the number of variables and specimens to be able to reconstruct the morphological diversity of hominins. This reduced database was used in our analyzes and comprises 23 craniometric variables of 121 specimens representing the following species: *P. boisei*, *P. aethiopicus*, *A. sediba*, *A. africanus*, *A. afarensis*, *H. rudolfensis*, *H. habilis*, *H. erectus*, *H. heidelbergensis*, *H. neanderthalensis* and *H. sapiens*. Table 2 details information about the specimens kept in the analyses. The final database includes variables represented in at least 70% of the specimens and specimens with at least 50% of the variables. Of the Dmanisi fossils, D3444 was not included, since we were unable to find enough craniometric data in the literature.

Before analyses, missing values were estimated using multiple linear regressions (see Hubbe et al. 2011). Analyses were done on the original data (size and shape), and on data with the effect of size corrected (shape alone). Size correction was achieved by dividing each measurement by the geometric mean of the individual (Darroch and Mosiman 1985). Morphological affinities of the Dmanisi specimens were assessed using Linear Discriminant Functions (LDA). The LDA functions were calculated for all the species in the data, excluding the Dmanisi specimens, which identify the axes of

variance that most discriminate the species represented in the comparative data. The Dmanisi specimens were then transformed to the coordinates of the first and second linear discriminant functions and the values of all the specimens in the data were plotted, allowing us to explore the

morphological affinities of the Dmanisi remains without having to assume any a priori species for them. All analyses were done in R (R Core Team 2023), complemented by packages MASS (Venables and Ripley 2002), ggplot2 (Wickham 2016), and ggrepel (Slowikowski 2023).

Table 1. Species included in the original databank

Species	N	Source
<i>Homo sp.</i> (Dmanisi)	4	Wood (1991), Laird et al. (2017), Rightmire et al. (2017), Ni et al. (2021)
<i>S. tchadensis</i>	1	Zollikofer et al. (2005)
<i>A. afarensis</i>	16	Wood (1991), Kimbel et al. (2004)
<i>A. africanus</i>	17	Wood (1991), Laird et al. (2017)
<i>A. sediba</i>	1	Berger et al. (2010), Laird et al. (2017)
<i>P. aethiopicus</i>	2	Berger et al. (2010), Kimbel et al. (2004), Wood (1991)
<i>P. boisei</i>	8	Wood (1991)
<i>P. robustus</i>	11	Wood (1991), Laird et al. (2017)
<i>H. habilis</i>	9	Wood (1991), Laird et al. (2017)
<i>H. erectus</i>	53	Wood (1991), Rightmire (1996), Laird et al. (2017), Ni et al. (2021), Rightmire et al. (2017), Kaifu et al. (2008), Weidenreich (1943), Rightmire (1990), Stringer cranial data
<i>H. heidelbergensis</i>	27	Ni et al. (2021), Stringer cranial data, Laird et al. (2017), Rightmire (1996), Guipert et al. (2014), Stringer et al. (1979), Arsuaga et al. (1997), Howell (1960), Rak et al. (2021), Young (1938), Marston (1937)
<i>H. floresiensis</i>	1	Kaifu et al. (2011)
<i>H. antecessor</i>	1	Arsuaga et al. (1999)
<i>H. naledi</i>	3	Laird et al. (2017)
<i>H. neanderthalensis</i>	27	Ni et al. (2021), Stringer cranial data, Howell (1960), Martin and Saller (1957), Guipert et al. (2014), Guipert (2010), Sergi (1991)
<i>H. sapiens</i>	21	Ni et al. (2021), Stringer cranial data
<i>H. longi</i>	1	Ni et al. (2021)

Table 2. Species and specimens included in this study

Species	N	Specimens	Source
<i>Homo sp.</i> (Dmanisi)	4	D2280, D2282, D2700, D4500.	Wood (1991), Laird et al. (2017), Rightmire et al. (2017), Ni et al. (2021)
<i>Au. afarensis</i>	1	A.L. 444-2.	Wood (1991)
<i>Au. africanus</i>	2	Sts 5, Sts 71.	Wood (1991)

Species	N	Specimens	Source
<i>Au. sediba</i>	1	MH1	Berger et al. (2010), Laird et al. (2017)
<i>P. aethiopicus</i>	1	KNM-WT 17000.	Wood (1991), Berger et al. (2010)
<i>P. boisei</i>	4	KNM-ER 406, KNM-ER 407, KNM-ER 732, OH 5.	Wood (1991)
<i>H. habilis</i>	3	KNM-ER 1805, KNM-ER 1813, OH 24.	Wood (1991), Ni et al. (2021)
<i>H. rudolfensis</i>	1	KNM-ER 1470.	Wood (1991)
<i>H. erectus</i>	15	Hexian, KNM-ER 3733, KNM-ER 3883, KNM-WT 15000, Nanjing 1, Ngandong 7, Ngandong 11, Ngandong 12, OH 9, Sambungmacan 1, Sambungmacan 3, Sangiran 2, Sangiran 17, Zhoukoudien X, Zhoukoudien XI.	Wood (1991), Weidenreich (1943), Rightmire (1990), Kaifu et al. (2008), Ni et al. (2021), Stringer personal data
<i>H. heidelbergensis</i>	13	Arago, Broken Hill, Bodo, Ceprano, Dali, Kabwe, Petralona, Sima de los Huesos 4, Sima de los Huesos 5, Sima de los Huesos 6, Steinheim, Narmada, Jinniushan.	Stringer et al. (1979), Wood (1991), Rightmire (1996), Arsuaga et al. (1997), Lubsen e Corrucini (2011), Guipert et al. (2014), Rightmire et al. (2017), Ni et al. (2021), Stringer personal data
<i>H. neanderthalensis</i>	14	Amud 1, Forbes Quarry, Gibraltar 1, Guattari 1, La Chapelle-Aux-Saints, La Ferrassie 1, Saccopastori 1, Saccopastori 2, Shanidar 1, Shanidar 5, Spy 1, Spy 2, Saint Cesaire, Tabun.	Stringer et al. (1979), Guipert et al. (2014), Ni et al. (2021), Stringer personal data
<i>H. sapiens</i>	62	Arene Candide 1, Arene Candide 4, Arene Candide 5, Afalou 9, Afalou 10, Afalou 29, Afalou 32, Brno3, Chancelade, Cohuna, Cro-Magnon 1, Cro-Magnon 2, Dolní Vestonice 3, Elyie Springs, Eroel 2, Fish Hoek, Gough 1, Gramat, Grotte des Enfant 6, Hayonim, Hohlenstein I, Hohlenstein 2, Hotu, Jebel Irhoud 1, Jebel Irhoud 2, Kafi, Kanalda, Kaylor, Kostenki14, Ks 1, L Nitch, Liujiang, Minat 1, Mladec 1, Mladec 5, Nahal E, Nahal Oren, Oberkassel M, Oberkassel F, Omo 2, Ona1938, Ona1933, Ortucchio 1, Pataud 2, Paviland 1, Predmostí 3, Predmostí 4, Qfzeh 6, Qfzeh 9, Skull 5, Skull 9, Taforalt XI, Taforalt 17, Teodoro 3, Teviec I, Teviec 11, Upper Cave 101, Upper Cave 103, Wadjak 1, Ndutu, LH 18, Oase.	Ni et al. (2021), Stringer personal data

## Results

Figure 1 presents the distribution of individuals and species when size and shape are taken into consideration. As can be seen, two main groups are formed in the morphospace: the left upper quadrant of the graph is occupied by *P. boisei*, *P. aethiopicus*, *A. sediba*, *A. africanus*, *A. afa-*

*ensis*, *H. rudolfensis*, and *H. habilis*. The right lower quadrant of the graph is occupied by *H. erectus*, *H. heidelbergensis*, *H. neanderthalensis* and *H. sapiens*. The material from Dmanisi occupies an intermediate position between these two main groups. D4500 lies within the distribution of *P. boisei*. D2700 lies within the distribution of *H. habilis*, while D2280

and D2282 approach the distribution of *H. erectus*. Table 3 shows the correlation between each linear discriminant function and the original variables in the analysis, which facilitates the interpretation of the morphological differences observed in the data. The first discriminant function is strongly correlated with measurements associated with neurocranium height (basion-bregma distance), breadth (minimum frontal breadth, maximum parietal breadth), and length (glabella-bregma chord, parietal sagittal length chord, occipital sagittal length chord), showing

smaller individuals on the left side of the plot and larger individuals on the right. The separation of the Dmanisi specimens on this axis then follows closely their reported braincase sizes. The second discriminant function does not have strong correlations with variables, but moderate negative correlations are observed with occipital breadth (biasterionic breadth chord) and upper facial and nasal breadths (superior facial breadth, maximum nasal width). Therefore, in this axis, specimens with narrower faces, noses, and occipitals tend to occupy a higher position.

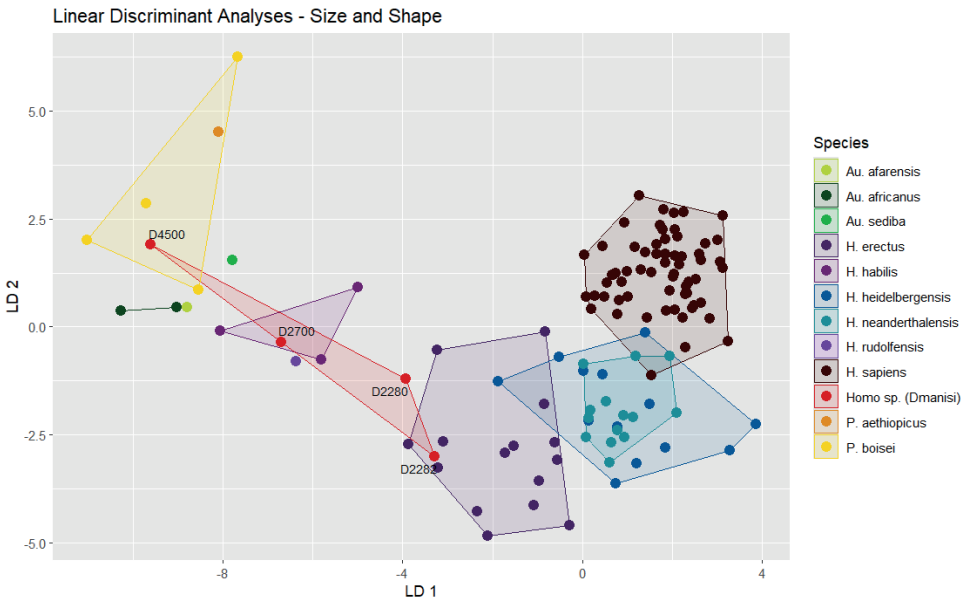


Fig. 1. Morphological affinities of hominin species based on the coordinates of the first two Linear Discriminant Functions (LDF), using size and shape information

Table 3. Correlations between the original variables and the linear discriminant functions calculated in the size and shape, and shape only analyses\*

Measurement	LD1	LD2	LD1	LD2
Basion bregma	0.751	0.253	0.491	0.467
Basion nasion	0.146	-0.244	-0.490	0.140
Mastoid length	-0.076	0.251	-0.319	0.393
Minimum frontal breadth	0.793	-0.281	0.774	-0.108



Measurement	LD1	LD2	LD1	LD2
Maximum parietal breadth	0.808	-0.375	0.786	-0.230
Biporionic breadth	0.407	-0.264	0.044	0.014
Supramastoid breadth	0.488	-0.467	-0.022	-0.206
Glabella bregma chord	0.701	-0.157	0.494	0.143
Parietal sagittal length chord	0.824	0.421	0.628	0.655
Lambda inion chord	0.627	-0.055	0.438	0.165
Occipital sagittal length chord	0.836	0.295	0.638	0.489
Biasterionic breadth chord	0.587	-0.535	0.376	-0.414
Superior facial height	-0.441	-0.324	-0.755	-0.167
Superior facial length	-0.157	-0.289	-0.540	-0.027
Superior facial breadth	0.145	-0.499	-0.379	-0.345
Biorbital breadth	0.496	-0.445	0.174	-0.306
Bimaxillary breadth	-0.216	-0.343	-0.730	-0.078
Orbital breadth	0.518	-0.203	0.229	0.076
Orbital height	-0.102	-0.447	-0.580	-0.211
Vertical thickness of supraorbital torus	0.080	-0.456	-0.077	-0.386
Maximum nasal width	0.020	-0.513	-0.315	-0.382
Nasal height	-0.031	-0.348	-0.445	-0.161
Maxillo alveolar breadth	-0.337	-0.248	-0.788	0.013

\* In the spirit of the academic freedom the Editors recognize the right of the Authors to express their opinions and conclusions, irrespective of the opinions of the Editors.

Figure 2 depicts the distribution of individuals and species when only shape information is taken into consideration. The australopithecines (*P. aethiopicus*, *P. boisei*, *A. africanus*, *A. afarensis* and *A. sediba*) occupy the left side of the morphospace, while the right half is occupied only by species of the genus *Homo* (*H. rudolfensis*, *H. habilis*, *H. erectus*, *H. heidelbergensis*, *H. neanderthalensis*, and *H. sapiens*). The relative position of the Dmanisi specimens is very similar to the previous analysis, occupying an intermediate space between these two groups. D4500 appears close to the distribution of *P. boisei*, D2700 lies very near to the distribution of *H. habilis*, while D2280 and D2282 are completely integrated

within the distribution of *H. erectus*. Table 3 shows the correlations between these linear discriminant functions and the original size-corrected variables. The first discriminant function shows high positive correlation with relative cranial breadth (minimum frontal breadth, maximum parietal breadth) and high negative correlations with superior facial height and breadth (superior facial height, and bimaxillary breadth). As these variables are size corrected, the specimens to the left of the plot show relatively wider neurocrania and shorter and narrower faces, which supports that the differentiation observed among the Dmanisi specimens in the previous analysis is not only a reflection of their distinct sizes. As with

the previous analysis, the second discriminant function does not show any particularly high correlations with the original size corrected data, but it shows

moderate positive correlations with posterior neurocranium length (parietal sagittal length chord, and occipital sagittal length chord).

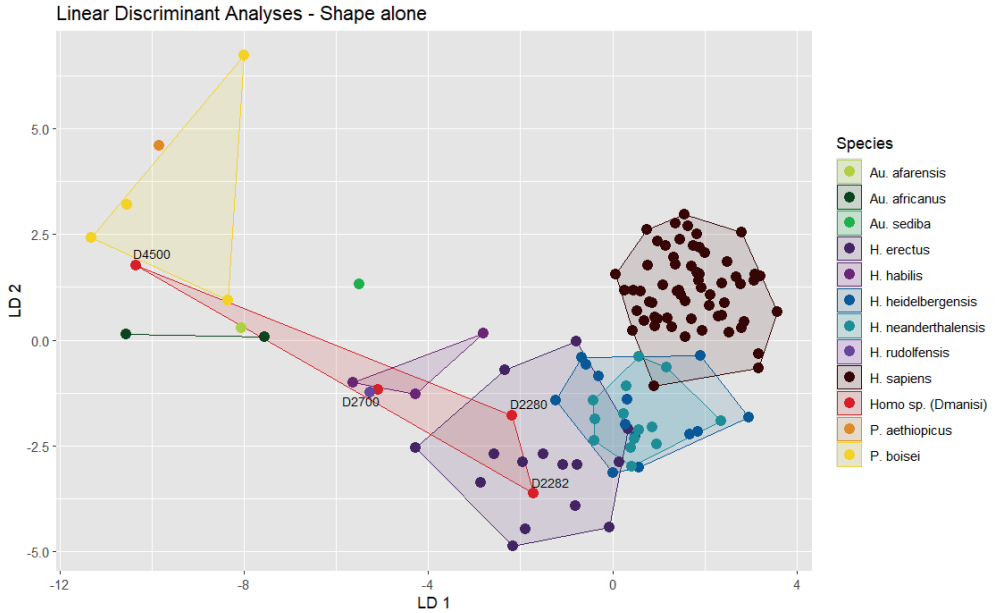


Fig. 2. Morphological affinities of hominin species based on the coordinates of the first two Linear Discriminant Functions (LDF), using only shape information

## Discussion and conclusions

Our results illustrate the magnitude of the variation observed among the Dmanisi specimens when they are placed within the context of the morphological variation of hominins. The four crania show morphological affinities that spread across the observed variation that separates two different hominin genera (*Paranthropus*, and *Homo*) and three different species (*Paranthropus boisei*, *Homo habilis* and *Homo erectus*). This high level of variance is particularly striking when we take into consideration that the fossils were retrieved from the same

site and present very close ages ( $1.81 \pm 0.03$  Ma; Garcia et al. 2010, Ferring et al. 2011). No other species included in our analysis share these same characteristics, which further accentuates the relative morphological variance among the Dmanisi fossils.

Our results are in agreement with several previous analyses. Skinner et al. (2006), who analyzed the mandibular size and shape of the Georgian specimens, emphasized that the variation of the Dmanisi individuals does not resemble that of any living species. Neves and Bernardo (2011) stated that the “Georgian specimens constitute the most variable

regional sample of Early *Homo*" (p. 109). Their analyses were based on Principal Components applied to a dataset of 17 Plio-pleistocene mandibles, including *Homo antecessor*, *Homo ergaster*, *Homo erectus*, *Homo habilis*, *Homo rudolfensis*, and three Georgian mandibles (D211, D2600, and D2735). Scardia et al. (2020) claimed that Skull 5 (D4500 and D2600) is completely different from the other four skulls found at Dmanisi. Their results are replicated in our analyses, which show that D4500, the smallest of the Dmanisi fossils, shows higher affinities with *Paranthropus* than with *Homo*, and has the most distinct position of all the Dmanisi specimens. Accordingly, the authors suggest that there are two species at the site: *Homo georgicus*, represented by Skull 5, and another non-*erectus* species, represented by the remaining specimens. Lordkipanidze et al. (2013) claimed that Dmanisi's variation can be explained by sexual dimorphism and the biological age of the individuals, defining the smaller skulls as female and/or sub-adult and the larger skulls as male adults. Similarly, Rightmire et al. (2018) stated that the variation among the Dmanisi group is not extraordinary, and could be well explained by sexual dimorphism, since Skull 5 "shares with the four other Dmanisi individuals a total morphological pattern" (p. 490). Surprisingly, the authors affirm in the same study that "patterning of sex dimorphism at Dmanisi may differ from that in extant apes, living humans, and mid Pleistocene hominins" (p. 492). Although this view of taxa homogeneity is supported by Zollikofer et al. (2014), the hypothesis of sexual dimorphism explaining the variance seen in Dmanisi is not strongly supported by our analyses, since the affinity of D4500 with *Paranthropus* would presume a level

of sexual dimorphism unseen among the hominins included in our dataset. While it is not impossible that the species could show more dimorphism than observed among early *Homo*, this would go against the general trend of reduced dimorphism seen in the *Homo erectus*, and we argue this is not a parsimonious explanation for the variance observed.

Ultimately, our study shows that the variability displayed by the Georgian fossils is not found in any other Plio-Pleistocene hominin, even when analyzing *Homo erectus* and *Homo sapiens*, which have the largest number of specimens of all hominins included in our study. Once more, this level of variance is particularly noteworthy when we consider that *H. erectus* spans more than 1.7 million years and shows a much more stable morphological pattern than the one observed in Dmanisi.

It is important to note that defining what species are present at Dmanisi is another difficult task. Many authors argue that the Georgian specimens are a transitory clade between *H. habilis* and *H. erectus*. As stated by Rightmire et al. (2018) "the Caucasus hominins share features with African *H. habilis* but had not yet evolved a full suite of characters diagnostic for later *H. erectus*" (p. 492). The answer to this question also affects another important discussion, which is defining what species first left Africa. Vekua et al. (2002), for instance, argued that the first humans to leave Africa were similar to *H. habilis*, and had not yet developed the full *H. erectus* suite. Other authors (e.g., Ferring et al. 2011 and Scardia et al. 2020) believe that an early *Homo* species first differentiated in Africa, left the African continent, gave rise to the Georgian fossils, and then returned to Africa as *H. erectus*. Our

results show that with the exception of D4500, the Dmanisi specimens present a transitory morphology between *H. habilis* and *H. erectus*, concurring with Ferring et al. (2011) and Scardia et al. (2020) that a transitory species evolved into *H. erectus* in Dmanisi, then returned to Africa.

Even though we tend to avoid “species inflation”, when, for example, “subspecies are raised to species” (Isaac et al. 2004; p. 464), we concur with the statement of Schwartz et al. (2014) that ignoring Skull 5’s “distinct identity is effectively to deny the utility of morphology in systematics” (p. 360). In this respect, we suggest classifying D4500 as *Homo georgicus*, taking into account the precedence of Gaburnia et al. (2002), and the remaining skulls as *Homo caucasi*, an intermediary species between *Homo habilis* and *Homo erectus*, taking into account our results and the observations above. However, we feel at odds to classify a hominin specimen (in this case D4500) that displays only 546 cm<sup>3</sup> of cranial capacity and a large dentognathic apparatus in the genus *Homo*. Irrespective of their taxonomic classification, it is important to emphasize that these fossils cannot be easily grouped into a single paleodeme, unless extreme levels of sexual dimorphism for hominins are considered possible. As such, while the discussion about their taxonomic classification continues, we suggest that these species should not be grouped a priori into one single paleodeme, especially when D4500 is considered. The distinct characteristics of the latter can be more informative to general discussions about the trends of evolution in *Homo* when it is not integrated and subsumed into the variation of the remainder hominins from Dmanisi.

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### Conflict of interests

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

### Authors’ contribution

WA conceived the study; WA and MS designed the study; LV and MS compiled the data used for the study; MH and MS conducted the experiments; WA, LV, MH and MS analyzed the data; WA, LV, MH and MS wrote the manuscript.

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## A review on association between menopausal symptoms and cardiovascular risk factors

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**ABSTRACT:** Menopausal transition and post-menopausal periods can have short-term and long-term effects on mid-life health of women. The short-term effects include the possibility of experiencing of menopausal symptoms, while the long-term effects include cardiovascular diseases (CVD) risk. The occurrence of menopausal symptoms varies widely within and between populations. Studies indicate that the frequency and severity of menopausal symptoms are linked to CVD risk factors, but the existing literature is divergent and somewhat limited. Thus, women belonging to different populations are likely to be at a different risk of CVD, but the exact physiological mechanism behind this relationship remains unclear. The present narrative review aimed to synthesize the available evidence of menopausal symptoms in association with various conventional CVD risk factors such as blood pressure, total cholesterol and blood glucose levels and obesity, as well as to determine the potential link between these two processes. We undertook a rigorous data base search to identify, examine, and critically assess the existing literature on the associations between menopausal symptoms and CVD risk factors. We applied inclusion and exclusion criteria to filter the retrieved articles and classified the literature into eight major categories. The risk of CVD is higher among women who experience vasomotor, psychological, and urogenital symptoms compared to those who do not experience these symptoms. Our review indicates that menopausal symptoms can be used as markers in assessing CVD risk factors during midlife. Thus there is a need for larger-scale research to support these findings and identify the potential mediators that are controlling this association.

**KEY WORDS:** cardiovascular disease, menopausal symptoms, vasomotor symptoms, psychological symptoms, postmenopausal women, insulin resistance, lipid profile, blood pressure level.



Original article

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## Introduction

An estimated 1.5 million women pass through the menopausal transition every year (Santoro et al. 2015). The phases of menopausal transition and postmenopause have profound effects on women's mid-life health. The period between perimenopausal and postmenopausal stages involves a biopsychosocial process, where the majority of women experience some short-term and long-term physiological changes. The short-term changes may include experiencing of vasomotor, urogenital, and psychological symptoms, decreased libido, insomnia, fatigue, as well as joint pain (Dennerstein et al. 2000; Sherman et al. 2005; Cohen et al. 2006). It has been argued that these short-term changes are related to the decline in the levels of estrogen and progesterone, one of the most important hormones in female's physiology.

Cardiovascular disease (CVD) continues to be the leading cause of mortality for women (Townsend et al. 2016). Menopausal transition is identified as a significant risk factor of CVD, which increases independently of the effects related to aging (Matthews et al. 2017; El Khoudary et al. 2020). The decline in the estrogen levels during and following the menopausal transition has been reported to be associated with menopausal symptoms and CVD risk factors (Carr et al. 2003). Studies show that the alternations in the estrogen levels not only affects glucose and insulin metabolism, but also changes body fat distribution, introduces dyslipidemia, coagulation, fibrinolysis, and vascular endothelial dysfunction (Carr 2003; Cakmak et al. 2015; Son et al. 2015).

Some researchers have posited that the frequency and severity of menopausal symptoms are linked to CVD risk factors

(Gast et al. 2008, 2011; Gallicchio et al. 2010; Szmuiłowicz et al. 2011; Kagitani et al. 2014; Thurston 2018) while several studies demonstrated the relationship between the frequency and severity of these symptoms with subclinical CVD, including increased intima media thickness (Thurston et al. 2012), aortic calcification (Thurston et al. 2008), and endothelial dysfunction (Bechlioulis et al. 2010). A systematic review and meta-analysis conducted by Muka et al. (2016), which included 213,976 middle aged women revealed a significant association of vasomotor, psychological, and urogenital symptoms with an elevated risk of CVD. Although several studies have documented the association between menopausal symptoms and CVD, the resulting findings have not been consistent (Svartberg et al. 2009; Tuomikoski and Peltonen 2017). Menopausal symptoms vary greatly across populations so women belonging to different population groups are likely to be at a differential risk of CVD, thus the exact physiological mechanism linking menopausal symptoms and CVD risk factors remain unclear (Gast et al. 2008).

In the present review, we aimed to synthesize all available evidence regarding the association between menopausal symptoms (e.g., vasomotor, urogenital, and psychosocial) and various conventional CVD risk factors, such as blood pressure, total cholesterol and total glucose levels, and obesity, and to determine the potential link between these two events.

## Methodology

### Data sources and Search strategy

In this review, we undertook a rigorous data base search in order to identify, examine, and critically assess the studies conducted on associations between men-

opausal symptoms and CVD risk factors, such as hypertension, diabetes, serum total cholesterol, and central obesity. The search was conducted using the Google search engine, PubMed, and Medline websites during the period of May 2023 to January 2024 using specific keywords: "Menopause", "Cardiovascular disease", "Vasomotor symptoms", "Menopausal Symptoms", "Psychological symptoms", "Hypertension", "Estrogen", "Lipid" etc. While performing the search we included some specific combinations of terms, such as vasomotor symptoms and CVD, psychological symptoms and CVD, hot flush and hypertension, the physiology of vasomotor symptoms, the frequency and severity of VMS among midlife women, the severity of menopausal symptoms and cardiovascular risk factors. Initially, we categorized the published literature based on studies that independently linked VMS, psychological, and urogenital symptoms to CVD risk factors. Subsequently, we thoroughly investigated the association between these symptoms and manifestations of CVD risk factors. Finally, we attempted to identify and discuss the research gaps within the Indian context.

### **Selection criteria**

We used the following inclusion and exclusion criteria to filter the retrieved articles. Articles published in peer-review journals or by any reputed organization related to the topic of women's midlife health and/or on perimenopausal and postmenopausal women, based on both field based, and hospital based studies and observational and interventional studies that reported an association between menopausal symptoms and CVD were considered in this study. A few systematic reviews, meta-analysis, pooled analysis, cohort stud-

ies were also included in this review. Only articles that have been published or translated into English were considered in this review, spanning between January 2000 and December 2023.

### **Data extraction**

602 articles were retrieved following the article-retrieval protocol described above. A predesigned data extraction form (Franco et al. 2015) was used to extract pertinent information from each eligible study. This incorporated information on the sample size, baseline population, study design, age at baseline, duration of follow-up (for cohort studies), degree of adjustments for confounders (like age, sex, smoking status, ethnicity and BMI), and the outcome and limitations of the study. 313 articles were retrieved following this process. The authors sorted the selected articles to eliminate similar studies with diverging results and conclusions, non-English literatures, and editorial letters or book reviews. We finally considered 156 full length articles exploring the physiology of menopausal symptoms and its association with CVD risk factors. When multiple publications by the same author were considered, we incorporated the most recent publications of that author.

### **Classification of the literature**

We classified the literature after completion of the article retrieval process. The full text articles were divided into eight major categories: (1) 29 articles on the severity and frequency of menopausal symptoms, (2) 15 on the physiology of vasomotor symptoms, (3) 12 on the physiology of urogenital symptoms, (4) 18 on the psychological symptoms of menopause, (5) 33 on the association between VMS and CVD, (6) 23 on association between

psychological symptoms and CVD, (7) 9 on association between urogenital symptoms and CVD, and (8) 17 articles on the association between menopause and CVD risk factors. We examined the reference lists of the selected articles to identify the potential articles for cross-referencing and later cited in this review. We

did not find any Indian study examining the association between menopausal symptoms and CVD risk factors. However, we included in our study some Indian studies that only explored the severity and frequency of menopausal symptoms. A flowchart depicting the article retrieval process is presented below (Fig. 1).

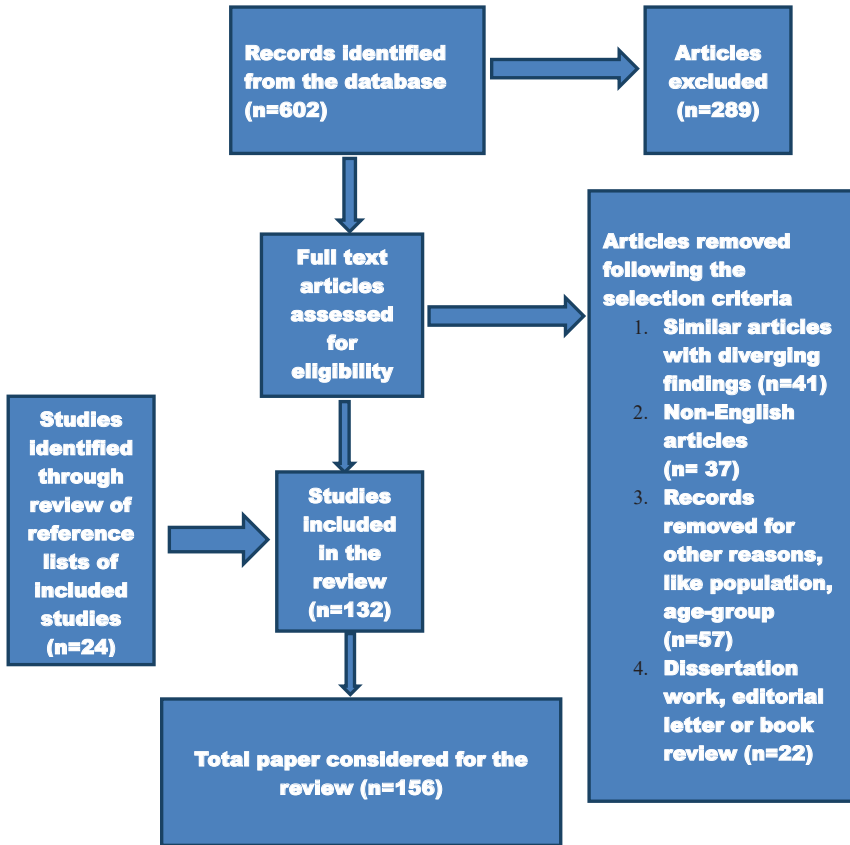


Fig. 1. Flowchart of narrative review of literature

## Discussion

### Vasomotor symptoms and CVD

Vasomotor symptoms (VMS) are one of the most prevalent symptoms experienced by 80 % women worldwide

during the menopausal transition. The symptoms include hot flushes and night sweats; the severity and duration of these symptoms also vary across populations (Franco et al. 2015). Previous studies have indicated that the median duration



of VMS is 7.4 years, while other studies have reported much longer duration (Avis et al. 2015). Hot flush refers to a sudden sensation of heat and sweating, most notably on the upper part of the body, while night sweats are the manifestation of heat during nighttime. Although estrogen has been used to treat vasomotor symptoms, the exact mechanism through which estrogen induces this phenomenon inside the body has not been investigated. VMS originates from the changes in the neurotransmitters located at the brain. It brings instability in the hypothalamic thermoregulatory center and causes decrease in estrogen levels. Hot flushes, a type of VMS result from a reduced thermoneutral zone (Freedman and Krell 1999). This reduction seems to be linked directly to the elevated norepinephrine (NE) levels in the central nervous system, partly through alpha 2 adrenergic receptors reducing the width of the thermoneutral zone (Avis et al. 2015; Thurston 2018). Estrogen modulates the alpha 2 adrenergic receptors (Thurston 2018). The fluctuation in estrogen levels affects the alpha 2 receptors and lead to increased NE levels, which ultimately cause inappropriate heat loss mechanisms and increase the likelihood of VMS (Freedman 2005).

A growing body of evidence suggests that women who reported VMS are likely to have higher cholesterol, triglyceride, low density lipoprotein cholesterol (LDL) and systolic and diastolic blood pressure levels, a higher body mass index (BMI), and insulin resistance (Gast et al. 2008; Thurston et al. 2012; Cagnacci et al. 2015; Franco et al. 2015), although the reported results are not consistent (Gallicchio et al. 2010; Dam et al. 2020). Studies also have demonstrated the association of VMS with hypertension

(Gast et al. 2008; Jackson et al. 2016), insulin resistance (Gray et al. 2018), adverse lipid profiles (Gast et al. 2008), poor endothelial dysfunction (Thurston et al. 2008; Bechlioulis et al. 2010), high proinflammatory profile (Huang et al. 2017), and incidences of subclinical CVD (Özkaya et al. 2011; Thurston et al. 2016). For example, two epidemiological studies conducted among middle aged women in Japan (Kagitani et al. 2014) and the Netherlands (Gast et al. 2008) found that, compared to women without VMS, women with VMS are more likely to have a higher CVD risk factor, including high blood pressure and total cholesterol levels. Similarly, four cohort studies, some of which included American women, revealed increased risk factors of CVD (high blood pressure and total cholesterol levels) for those who reported a higher incidence of VMS compared to women who did not (Gast et al. 2008; Kagitani et al. 2014; Franco et al. 2015). Some studies suggested that percent body fat and VMS are positively correlated (Thurston et al. 2013; Da Fonseca et al. 2013; Herber-Gast et al. 2013; Gallicchio et al. 2014). For example, a study conducted by Cagnacci et al. (2015) showed that women who experienced hot flushes had a higher body fat percentage and increase in the severity of VMS was associated with higher levels of body fat. Similarly, a recent study conducted among 2533 women aged between 42 and 52 years showed that lean body mass is inversely associated with the incidence of VMS (Woods et al. 2020). The underlying mechanism of this association is not yet clear due to the incomplete understanding of the physiology of VMS. Some scholars argue that the sympathetic overactivity that exists in both VMS and metabolic syndrome

is a common pathway linking these two processes (Schlaich et al. 2015; Gava et al. 2019). Earlier studies have hypothesized that body fat is protective against VMS due to the process of peripheral aromatization of androgens to estrogen in adipose tissues (Kershaw et al. 2004). The 'thermoregulatory model' suggests that adiposity inhibits heat dissipation and raises the core body temperature, thereby increasing the incidence of VMS (Duffy et al. 2013). On the contrary, a recent 20-year of follow-up study by Dam et al. (2020) reported no significant association between vasomotor symptoms and CVD, coronary heart disease, or cerebrovascular disease. However, the same study reported that women who had night sweats are at 18% increased risk of CVD compared to those who did not, although this increase was statistically nonsignificant. A study among perimenopausal women conducted by Thurston et al. (2012) showed that higher VMS is associated with a derangement of the lipid profile. Gast et al. (2008, 2010) have replicated findings of positive associations between VMS, body fat, total cholesterol, systolic and diastolic blood pressure levels from population-based studies conducted in the Netherlands and Sweden. Gallicchio et al. (2010) observed a non-significant association between hot flush and blood pressure level after adjustment for confounders such as smoking, alcohol consumption, ethnicity and the use of hypertensive medicines. Similarly, after controlling the confounders like educational attainment, smoking status, and BMI, Gast et al. (2008) reported no significant association between blood pressure level and hot flushes.

A couple of studies reported that severity of VMS, rather than frequency, is associated with an increased risk of CVD

(Muka et al. 2016 and Zhu et al. 2020). The severity of menopausal symptoms among postmenopausal women, as evaluated by the Green Climacteric Scale, is related to endocrine and metabolic modifications, possibly leading to an increased risk of CVD (Cagnacci et al. 2011, 2012). In these studies, hot flush and night sweats were combined thus, it was not possible to evaluate the impact of each symptom independently. A cross-sectional study found night sweats rather than hot flush is associated with an elevated risk of coronary heart disease; the association persisted even after controlling for the traditional CVD risk factors like BMI, blood pressure, and total cholesterol levels (Gast et al. 2011). Some studies have reported that hot flushes and night sweats have different etiologies for CVD, but the exact mechanism is not well understood (Hitchcock et al. 2012; Herber-Gast et al. 2013). Some evidence indicates that the combined effect of hot flush and night sweats on the risk of CVD was higher than the independent effect of each symptom (Zhu et al. 2020).

Studies suggest that age of occurrence of VMS may influence the association between VMS and CVD risk factors (Szmuiłowicz et al. 2011; Thurston et al. 2016). It has been hypothesized that the predictive value of vasomotor symptoms for CVD risk factors may vary with the onset of VMS at different stages of menopause (Szmuiłowicz et al. 2011; Thurston et al. 2016). For example, a study of Women's Ischemia Syndrome Evaluation reported that women who experienced menopausal symptoms at an early phase of their lives (before 42) had endothelial dysfunction and elevated CVD related mortality compared to those who experienced menopausal symptoms later in life. The association persisted even after the

adjustment for BMI, smoking status, and age at menopause (Thurston et al. 2016). On the contrary, the Women's Health Initiative Observational Study (WHI-OS) conducted in the New York City demonstrates that although early onset of VMS is not associated with CVD risk factors, the late-onset of VMS increases the risk of CVD and all-cause mortality (Szmuiłowicz et al. 2011). A recent systematic review conducted by Armeni et al. (2023) showed VMS increases the risk of CVD for those women aged less than 60 years suggesting that age might be a mediating factor in the association between these two processes. A pooled analysis of six prospective studies reported both early and late-onset of VMS are associated with an increased risk of CVD (Zhu et al. 2020). The two contrasting findings probably result from the use of two different definitions used in defining the onset of VMS. In the WHI-OS study, early-onset of VMS was defined as VMS at the onset of menopause, while late-onset was defined as VMS at the time of enrollment in the study, which was around 63.3 years at the time of interview. On the other hand, in the pooled analysis, VMS, which first occurred before menopause was classified as early-onset, while the VMS that first occurred after menopause was classified as late-onset. Therefore, the difference in definition could account for the differential outcomes. It has been hypothesized that the mechanisms at play in the occurrence of early and late VMS are different. For instance, Szmuiłowicz et al. (2011) argued that the chance of early-onset of VMS before the menopausal transition is a physiological response to typical hormonal fluctuations, while late-onset VMS could be a manifestation of vascular instability. Therefore, both early and late-onset of

VMS exhibit different associations with CVD. However, further research is needed for a better understanding of this association.

A possible mechanism linking women with VMS and an increased risk of CVD is a potential up-regulation in the sympathetic nervous activity (Gastet et al. 2008). Some scholars are of the opinion that the narrowing of thermoneutral zone results in hot flushes, which appears to be closely related to elevated central nervous system levels of nor-epinephrine (Freedman 1999). Epinephrine and nor-epinephrine secreted from the sympathetic nervous system are the possible mediators for various vascular and metabolic abnormalities, including hypertension and increased total cholesterol levels (Engler and Engler 1995). The significant increase in body fat percentage in older women with VMS, as observed in previous studies may also be due to the secretion of nor-epinephrine from the sympathetic nerves (Gast et al. 2008). The association of VMS with increased catecholaminergic activity and modifications in calcitonin-related peptides, may increase the risk of CVD (Gupta et al. 2008). A study by Cagnacci et al. (2011) showed hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and increased cortisol concentration among those who experience VMS; this may represent another mechanism linking VMS with CVD risk factors such as insulin resistance and abnormal lipid metabolism. Since VMS is associated with thermoregulatory dysfunction, it alters the activity of the autonomic nervous system (ANS). The altered activity of both ANS and HPA may serve as a common link between VMS and cardiovascular risk factors (Zhu et al. 2020). However, whether vasomotor symptoms

precede the CVD risk factors or are symptomatic manifestations underlies adverse changes in a woman's vasculature warrants further investigation. For example, studies show that increased BMI and blood pressure levels are associated with an increased secretion of NE from sympathetic nerves, which in turn leads to the incidence of VMS (Cagnacci et al. 2015; Thurston 2018). However, other studies show conflicting results (Mendelsohn et al. 1999; Randolph et al. 2005). Thus, there is a need for further research to establish the exact link between these two events.

### **Psychological symptoms and CVD**

Women during midlife may also experience psychological symptoms, such as depression, mood disturbance, loss of energy, loss of interest, forgetfulness, anxiety, panic attacks, irritability and lack of concentration that may impede coping and decrease the quality of life (Ohayon et al. 2006; Thurston et al. 2017; Gavaet al. 2019). Freeman (2015) reported that midlife women in their late-perimenopausal phase are three times more likely to develop depressive symptoms compared to premenopausal women. A decline in the estrogen levels during menopausal transition may trigger psychological symptoms by inhibiting the production of major neuroprotective factors and altering neurotransmission. This decline may interfere with the synthesis of catecholamines and increase the level of serotonin potentially leading to the development of psychological symptoms (Santoro et al. 2015; Gava et al. 2019).

Psychological disorders, including depression, anxiety, and panic attacks, are already known to be CVD risk factors in both men and women of any age (Walters et al. 2008; Player et al. 2011; Brun-

ner et al. 2014). Studies revealed that bothersome psychological symptoms are associated with increased CVD risk factors among midlife women (Ward et al. 1994; Smoller et al. 2003; Collins et al. 2007; Low et al. 2010; Im et al. 2016). Lack of sleep, depression and panic disorder that are indicative of poor physical and mental health, have been associated with an increased risk of CVD (Cappuccio et al. 2011; Laughsand et al. 2014). For example, a multi-ethnic study reported that postmenopausal women with severe psychological symptoms experience an increased risk of CVD related mortality (Im et al. 2016). Research indicates that both psychological symptoms and CVD risk factors could be influenced by the hormonal changes that occur during the menopausal transition. These hormonal alterations may bring changes in the hypothalamic-adrenal axis, serotonergic transmission, and the renin-angiotension-aldosterone system, which in turn increases the risk of CVD (Im et al. 2016; Muka et al. 2016). Scholars are of the opinion that depression and anxiety result in autoimmune activation, leading to increased blood pressure levels, decreased endothelial dysfunction, and increased platelet activity; all of which contribute to the development of CVD (Carney et al. 2005; Mittleman and Mostofsky 2011). The sympathetic nervous activation following the psychological symptoms could also mediate the increased risk of CVD (Esler et al. 2004). Another plausible mechanism explains that the co-occurrence of psychological symptoms and CVD risk factors is because of altered systematic inflammatory response; and this is influenced by the hormonal changes during menopause (Im et al. 2016).

It is postulated that menopausal symptoms affect cardiovascular health through established risk factors such as elevated BMI and central obesity (De Wit et al. 2009; Lallukka et al. 2012). Previous studies show a significant association between obesity and mood dysregulation (Glaus et al. 2019). Adipokines produced by adipose tissues activate systemic inflammation. This affects the brain and leads to various psychological symptoms (Ali et al. 2020). On the other hand, there are assumptions that psychological symptoms related to menopause increase the number of cytokines and free radicals, resulting in more fat deposition (Elavsky and Gold 2009); thus, the association can perhaps be explained to be bi-directional. Obesity may also induce low self-esteem among midlife women, resulting in mood dysregulation to some extent (Yaylali et al. 2010). The Epic-Norfolk study reported central obesity to be significantly associated with psychological symptoms, rather than general obesity (Myint et al. 2006). However, conflicting findings have also been reported (Ward Ritacco et al. 2015; Glaus et al. 2019).

Sleep disturbance is also associated with increased CVD risk factors like hypertension (Capuccio et al. 2011), obesity (Li 2021), type 2 diabetes mellitus (Capuccio et al. 2010), and atherogenic lipid profile (Kaneita et al. 2008; Tsiptsios et al. 2022). The exact mechanism underlying these associations is not fully understood. The possible mechanisms linking short durations of sleep to adverse health outcomes include reciprocal changes in circulating levels of leptin and ghrelin (Taheri et al. 2004); these changes would increase appetite, caloric intake, reduce energy expenditure and facilitate the development of obesity, and impair glycaemic control,

which ultimately increases the risk of CVD (Khakpash et al. 2023).

The association between psychological symptoms and CVD risk factors could be mediated by other factors, such as ethnicity. For example, a multi-ethnic study found association between psychological symptoms and CVD risk factors varies across different ethnic groups, with Asian women showing the strongest association compared to the other ethnic groups (Im et al. 2016). The studies interpreted the strongest association among Asian women in the context of Asian culture, which prohibits the expression of psychological symptoms due to the stigmatization of mental illness (Kramer et al. 2002; Im et al. 2016). On the contrary, there are studies that reveal no ethnic variances in the association between psychological symptoms and CVD in midlife women (Ferketich et al. 2000; Franco et al. 2015). Researchers are of the opinion that the association may not be greatly influenced by social-cultural factors, although women's socio-economic position, reproductive history, cultural beliefs, attitudes, knowledge, and perception could affect psychological symptoms (Ali et al. 2020; Weidner et al. 2020) and CVD risk factors independently (Tedesco et al. 2015; Beussink-Nelson et al. 2022).

Depressive symptom is a greater risk factor for CVD compared to other psychological symptoms (Smoller et al. 2003; Wright et al. 2014). Pathophysiological alterations that occur due to depression may affect the cardiovascular system by increasing variability in heart rate and platelet aggregation (Nemeroff 2008). Wright et al. (2014) posited that hormonal fluctuations experienced during menopause affect the systematic inflammatory process, which, in turn, increases the frequency of psychological

symptoms and CVD risk factors. Heart rate and blood pressure increase during psychological stress (tension or anxiety) due to the activation of the sympathetic nervous system and adrenal medullary release of epinephrine, which, in turn, alter insulin resistance and lipid metabolism (Steptoe et al. 2006); some inconsistent findings have also been reported (Ward et al. 1994). Literature reveals an association of emotional distress with CVD risk factors, such as central obesity (Elavsky and Gold 2009). Emotional distress increases the production of cytokines and free radicals, resulting in the increase in central deposition of body fat (Elavsky and Gold 2009). Some studies have demonstrated that obesity and metabolic syndrome, characterized by impaired glucose and lipid metabolism, are independently associated with depression (Schmitz et al. 2018; Moazzami et al. 2019). The underlying mechanism linking obesity and depression is a complex pathway. Cerebral insulin resistance develops in obese women due to the depletion of adiponectin, which is a hormone derived from the adipose tissue and regulates the metabolism of glucose and lipids (Nguyen 2020). Obesity might also trigger major oxidative and inflammatory pathways, leading to neuroinflammation, which is a crucial factor in the development of mood and cognitive disorders, such as anxiety and depression (Kiecolt-Glaser et al. 2015). These are the possible pathways linking psychological symptoms of menopause and CVD risk factors among women (Schmitz et al. 2018; Moazzami et al. 2019). Future studies should address whether psychological symptoms precede the CVD risk factors or whether the CVD risk factors induce depression and anxiety among midlife women.

### **Urogenital symptoms and CVD**

Urogenital symptoms also show a significant association with CVD. For example, a recent study conducted by Cagnacci et al. (2022) among 504 postmenopausal women revealed genito-urinary symptoms are more likely to be associated with CVD risk factors rather than hot flashes. Some earlier studies have also investigated the association between sexual symptoms and metabolic problems (Lee et al. 2015; Russo et al. 2015; Lee 2019; Semczuk-Kaczmarek et al. 2021). For example, a systematic review conducted by Hunskaar (2008) showed that central obesity, higher BMI, higher low density lipoprotein cholesterol, and fasting blood glucose levels are significantly associated with genito-urinary problems like urinal incontinence. Kilinc et al. (2017), on the other hand, reported that the incidence of severe coronary artery disease to be higher in patients with urinal incontinence, Park et al. (2018) showed that abdominal obesity is more likely to be associated with urinal incontinence compared to general obesity. However, studies investigating the link between sexual symptoms and obesity are limited and show inconsistent results. It is postulated that central obesity is associated with increased pressure on the urinary bladder, and adipose tissue acts, such as a neuroendocrine organ that produces inflammatory factors and induces the sympathetic nervous system (Subaket et al. 2009; Tang et al. 2022); this, in turn, leads to the development of some urinary symptoms like urinal incontinence. It is postulated that metabolic co-morbidity, such as obesity and dyslipidemia, could increase the severity of urogenital symptoms, possibly by causing inflammation, oxidative stress, hormonal disruptions, and impaired blood vessel formation (Zhu et al. 2024).



In addition, there is evidence showing that women with an altered lipid profile exhibit activation of inflammatory pathways, a decrease in neuroprotective factors, damage to DNA, and apoptosis ultimately leading to the development of urogenital symptoms (Zhu et al. 2024). The Boston Area Community Health (BACH) survey reported moderate to severe urogenital symptoms to be associated with an increased risk of diabetes mellitus (Lin et al. 2013; Hwang et al. 2015). The exact mechanism linking urogenital symptoms and CVD risk factors is less understood. Previous studies show that some common factors such as estrogen deficiency, endothelial dysfunction, increased Rho-kinase activation, impaired nitric-oxide synthase pathway in the endothelium, autonomic hyperactivity with sympathetic dysregulation are likely to be linked with urogenital symptoms and CVD (Fusco et al. 2013; Semczuk-Kaczmarek et al. 2021). Age-related changes in the bladder structure and function seem to play a central role in the occurrence of urogenital symptoms. For example, endothelial dysfunction in the

pelvic vascular system is considered to play a role in urinal incontinence. The common pathway linking hypertension and urogenital symptoms could be explained by increased sympathetic activity and altered alpha 2-adrenoreceptor activity. Furthermore, diabetes mellitus can increase the severity of urogenital symptoms through neurogenic bladder dysfunction (Russo et al. 2015). However, these associations cannot be explained as a cause-effect relation. For example, it appears from a recent study that patients with coronary artery disease have a higher severity of urogenital symptoms; this may indicate a significant role of CVD in the pathophysiology of urogenital symptoms (Semczuk-Kaczmarek et al. 2021). The same study further reported association of increasing severity of urogenital symptoms with age, metabolic biomarkers and arterial hypertension. It is argued that a healthy lifestyle, antihypertensive therapy with angiotensin II receptor blockers, and lipid lowering medicines like statins could reduce both CVD and the severity of urogenital symptoms (Semczuk-Kaczmarek et al. 2020).

Table 1. General findings of the studies included in the review

Association	Researchers	Outcomes
<b>VMS and CVD risk factors</b>		
VMS and hypertension	Bechlioulis et al. 2010; Kagitani et al. 2014; Jackson et al. 2016	Incidence of VMS increases the likelihood of hypertension compared to those without VMS. VMS is associated with a derangement of the lipid profile.
VMS and total cholesterol level	Gast et al. 2008; Thurston et al. 2012	Incidence of VMS increases the likelihood of derangement of the lipid profile.
VMS and body fat percentage	Herber-Gast et al. 2013; Gallichio et al. 2014	Greater the degree of severity of VMS, higher is the likelihood of body fat percentage.
VMS and insulin resistance	Szmulowicz et al. 2011; Cagnacci et al. 2011	Early onset of VMS is not associated with CVD risk factors such as insulin resistance, but late-onset of VMS increases the risk of CVD and all-cause mortality.

<b>Psychological symptoms and CVD</b>		
Psychological symptoms, blood glucose and lipid profile	Cappucio et al. 2010; Wright et al. 2014; Tsiftis et al. 2022	Psychological symptoms increase the activity of sympathetic nervous system and adrenal medullary release of epinephrine, leading to insulin resistance and abnormal lipid metabolism.
Psychological symptoms and hypertension	Im et al. 2016; Muka et al. 2016	Depression and anxiety result in autoimmune activation, leading to increased blood pressure levels, decreased endothelial dysfunction, and increased platelet activity.
Psychological symptoms and obesity	Taheri et al. 2004; Glaus et al. 2019; Khakpash et al. 2023	Psychological distress would increase appetite, caloric intake, reduce energy expenditure and facilitate the development of obesity.
<b>Urogenital symptoms and CVD</b>		
Urogenital symptoms and insulin resistance	Lin et al. 2013; Hwang et al. 2015	Moderate to severe levels of urogenital symptoms is associated with increased risk of insulin resistance.
Urogenital symptoms and hypertension	Fusco et al. 2013; Semczuk-Kaczmarek et al. 2021	Incidence of hypertension increases the likelihood of the severity of urogenital symptoms.
Urogenital symptoms and central obesity	Park et al. 2018; Tang et al. 2022	Abdominal obesity is more likely to be associated with urinal incontinence compared to general obesity.

## Future direction and conclusion

Our review suggests that the decline in the estrogen levels remains the common origin for menopausal symptoms and CVD risk factors during mid-life affecting the quality of life of women. This narrative review revealed the following unmet gaps in research. (1) How different is the association between menopausal symptoms and CVD risk factors across different ethnic groups? (2) Are menopausal symptoms associated with the CVD risk factors and, if so, determine the degree of the CVD risk factors? (3) Does the age of onset of menopausal symptoms has an association with the CVD risk factors? (4) To what extent VMS, psychological, and urogenital symptoms independently or in combi-

nation play roles in predicting CVD risk factors? (5) Does women with hormone replacement therapy have reduced risk of menopausal symptoms and CVD risk factors? (6) The existing literature has rarely reported studies from low-middle-income countries where the menopausal age is advancing (Mozumdar et al. 2015). Are, thus, women from these parts of the world more likely to be at a risk of CVD?

Our review suggests that women who experience vasomotor, psychological, and urogenital symptoms are likely to have a higher risk of CVD compared to those who have not experienced such symptoms. Our review also indicates the potential usefulness of menopausal symptoms as marker for CVD risk factors during midlife.

### Conflict of interests

The authors declares that they have no conflict of interest.

### Authors' contribution

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

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## Association between expression level of the miR-320, miR-182, miR-223 and miR-486 and body composition among young Polish female volleyball players

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**ABSTRACT:** The expression of circulating microRNAs appears to be a promising indicator of physical strength. The objective of this study was to determine whether there is an association between the expression level of four selected microRNAs and body composition over time among young female volleyball players. Blood samples and body composition measurements were taken from 7 females who are Polish volleyball players before and after 5 matches played out between the years 2017 and 2018. The blood spots were used to assess the expression of four microRNAs: miR-320, miR-182, miR-223, and miR-486. Fat mass, PFB% and BMI were positively correlated with expression level (exp.l) of miR-182. The miR-320 the exp.l was positively correlated with muscle mass and TBW. There were inverse correlations between miR-486 exp.l and PBF%, as well as between miR-486 exp.l and body mass, muscle mass, TBW, FFM, and BMR. Conversely,

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there were positive correlations between miR-486 exp.l and body mass and fat mass. The miR-182 may be positively correlated with fat tissue, miR-320 was positively correlated with muscle mass, and miR-486 was negatively correlated with fat mass. Overall, our study shows that the expression of miR-182, miR-320, and miR-486 is associated with body composition. The results of our study also suggest that exercise may decrease the level of miR-486.

**KEY words:** body composition, microRNA, epigenetic, volleyball.

## Introduction

MicroRNAs are small noncoding molecules which may influence expression of various genes causing various phenotypic changes (Großhans and Filipowicz 2008). From a methodological perspective, microRNAs appear to be stable, reliable sources of biological material for epigenetic analysis (Jung et al. 2010).

To the best of our knowledge, there is a lack of research that addresses the relationship between microRNAs and physical activity over an extended period of time. The longitudinal effect of microRNAs on IGF1 and muscle tissue is not yet known. The miR-320, miR-182, miR-223 and miR-486 seem to be important molecules associated directly and indirectly with IGF1 and muscle tissue. The regulation of IGF is important for sport achievements due to maintaining processes of cells proliferation and differentiation, energy metabolism and glucose homeostasis (Jung and Suh, 2015). Consequently, alterations in selected miRNAs may prove pivotal in monitoring physical condition and athletic performance.

The microRNA (miRNA) miR-320 is associated with glucose levels. Increased levels of glucose concentration are associated with decreased level of miR-320 expression. The downregulation of miR-320 has been observed in individuals with diabetes (Zampetaki et al. 2010). Moreover, there is a link between the expression of miR-320 and muscle tissue.

In diabetic rats, the expression level of miR-320 was increased in cardiac microvascular cells (Wang et al. 2009). Furthermore, Yerlikaya and Mehmet (2019) demonstrated that high-fat and high-sucrose diets resulted in the down-regulation of miR-320. In a 2018 study, Munetsuna and colleagues (2018) observed a correlation between miR-320 expression and fat tissue and BMI. They also noted a significant decrease in miR-320 levels among Japanese adults with excess body fat.

Olivieri et al. (2014) reported significantly lower levels of miR-182 and miR-223 in muscle samples during periods of enhanced IGF-1 signaling among monozygotic twin pairs. As reported by Zhang et al. (2016), miR-182 is associated with glucose metabolism and muscle tissue. The authors showed that in murine models, a reduction in miR-182 expression results in alterations in muscle fiber composition and glucose metabolism.

Aoi et al. (2013) showed that a reduction in miR-486 may be associated with metabolic changes during physical activity, which are influenced by training. The miR-486 is encoded by ankyrin and myosin heavy chain, which are muscle-specific genes (McCarthy et al. 2009; Small et al. 2010). It has been observed that exercise may alter the concentration of miR-486, which may result in phenotypic changes (Safdar et al. 2009; Nielsen et al. 2010). Furthermore, Prats-Puig et al. (2013) demonstrated that miR-486



was upregulated in prepubertal obese children, which may be associated with a reduction in physical activity. It is important to note that the expression of certain microRNAs can be influenced by the presence or absence of physical activity. This underlying aspect forms the foundation of our investigation.

The objective of this pilot study was to assess the association between the expression levels of four microRNAs (miRNAs) that have been linked to insulin-like growth factor I (IGF-I) factor, strength, and muscle structure. In addition, we aimed to determine the association between the selected microRNAs and body composition among young Polish female volleyball players during the seasonal matches of the volleyball academic league.

## Material and methods

Blood samples were taken from 7 Polish volleyball female players of volleyball academic league before and after 5 matches played between November 2017 and November 2018. The blood spots were taken from fingertips and collected using the Whatman™ FTA™ classic cards from each player, approximately 2 hours before and after each match, by a professional medical nurse. In addition, all players underwent body composition

analysis before each of five matches (11.11.2017, 18.11.2017, 2.12.2017, 16.12.2017, 10.11.2018) using the In-Body 230 BIA analyzer. Several parameters were recorded: body mass, muscle mass in kilograms, fat mass in kilograms, total body water (TBW), fat free mass in kilograms (FFM), percentage of body fat (%PBF), waist-to-hip ratio (WHR) and basic metabolic rate (BMR). The BMI was calculated based on the measure of height and weight [ $\text{kg}/\text{m}^2$ ]. In total, 70 measurements were taken by a professional anthropologist.

The study was approved by Senate's Ethic Committee of Scientific Research at University School of Physical Education in Wroclaw 4/2020.

### Laboratory work

Expression of selected microRNAs was associated with IGF1 and muscle tissue according to current literature (Tab. 1).

To isolate microRNAs RNA/miRNA the Purification kit was used (EURx) according to modified protocol by Skonieczka et al. (2016). To perform reverse transcription miRCURY LNA RT Kit was used. The analyses were done according to protocol supplied by the producer using in every sample 6 ng/ml of RNA. The reactions were performed in thermocycler Labcycler 48 (SensoQuest) according to reaction parameters supplied by

Table 1. Characteristic of the included microRNAs

MicroRNA	Description
miR-182	diminishing miR-182 expression enhances IGF-1 signaling in skeletal muscle (Olivieri et al. 2016)
miR-320	inhibition of miR-320 expression significantly increased IGF-1 and IGF-1R mRNA levels (Song et al. 2016)
miR-486	Correlation with VO <sub>2</sub> max R=0.58 (Aoi et al. 2013), downregulation during exercise (Xu et al. 2014)
miR-223	Up-regulation during exercise (Xu et al. 2014)

kit producer. Further, we diluted the obtained cDNA according to protocol 1:50 using RNase DNase free water. During the final step qPCR was performed for each sample in duplicates and each microRNAs (using proper starters: hsa-miR-223-3p miRCURY LNA miRNA PCR Assay; hsa-miR-182-3p miRCURY LNA miRNA PCR Assay; hsa-miR-320-3p miRCURY LNA miRNA PCR Assay, hsa-miR-486-3p miRCURY LNA miRNA PCR Assay) using Real-Time PCR Rotor-Gene Q 5-plex HRM (Qiagen) containing reaction mix miRCURY LNA SYBR Green PCR kit (Qiagen) according to the protocol. The final results were obtained using the Rotor-Gene Q Series Software 2.1.0 which calculated Ct for each sample. The sample over Ct=39 cycles were precluded from the further analyses. The spike in kit was used to normalize obtained microRNAs expression level. The dCt method was used to make final calculation using the following formula:  $2^{-(\text{mean Ct}_{\text{miR}} - \text{mean Ct of reference})}$  (Livak and Schmittgen, 2001). Due to insufficient quality of the analysis, 8 probes (3 of miR-182 and 5 of miR-320) were unaccounted for in further calculations.

### Statistical analysis

Differences in the level of expression of each microRNAs before and after each of five subsequent matches in 7 players were assessed by the Wilcoxon matched pair test, nonparametric equivalent to t-student test for dependent samples. The Spearman Rank correlation was applied to examine the relationship between each body composition parameter and level of expression of microRNAs before and after five subsequent matches. All the calculations were performed using the Statistica 13.1 software (Dell Inc. 2016).

### Results

Descriptive statistics of microRNA before and after five successive matches are presented in Table 2. Changes of all body composition parameters in five successive matches are shown in Table 3. The Spearman Rank test showed (Tab. 4) that fat mass was positively correlated with expression level of miR-182 after the 3rd match ( $R=0.857$ ;  $p=0.0137$ ). Moreover, before the 5th match the level of miR-182 was positively correlated with BMI ( $R=0.786$ ;  $p=0.0362$ ) and after the match positively correlated with PBF% ( $R=0.829$ ;  $p=0.0416$ ). In the case of miR-320 the expression level after 5th match was positively correlated with muscle mass ( $R=0.786$ ;  $p=0.0362$ ) and TBW ( $R=0.786$ ;  $p=0.0362$ ).

There were statistically significant and negative correlations between miR-486 expression level before the 3rd match and PBF% ( $R=-0.857$ ;  $p=0.0140$ ); between miR-486 expression level after the 3rd match and body mass ( $R=-0.786$ ;  $p=0.0362$ ), muscle mass ( $R=-0.929$ ;  $p=0.0025$ ), TBW ( $R=-0.929$ ;  $p=0.0025$ ), FFM ( $R=-0.929$ ;  $p=0.0025$ ) and BMR ( $R=-0.929$ ;  $p=0.0025$ ). Moreover, after the 4th match there were statistically significant and positive correlations between miR-486 expression level and body mass ( $R=0.786$ ;  $p=0.0362$ ) and fat mass ( $R=0.786$ ;  $p=0.0362$ ).

The Wilcoxon test showed that the level of expression of miR-320 after 1st match was significantly (although marginally) lower ( $Z=2.028$ ;  $p=0.0425$ ). In the case of miR-486 the level of expression was statistically significantly lower after the 4th match ( $Z=2.028$ ;  $p=0.0425$ ).

The micro-RNA 486 had, although nonsignificant, lower level after each match ( $Z=1.769$ ;  $p=0.0769$ ) (Fig. 1).

None of the forward stepwise multiple regression models for muscle mass were statistically significant.

Table 2. Descriptive statistics of each microRNA expression level among players before and after successive matches

microRNA	Players	DeltaCT before			DeltaCT after		
		N	mean	SD	N	mean	SD
182	P1	4	-12.55	4.28	5	-12.45	7.19
	P2	5	-13.06	1.51	5	-13.00	7.20
	P3	5	-10.99	3.32	5	-13.89	4.15
	P4	5	-13.41	2.74	5	-13.29	6.09
	P5	5	-11.18	5.32	5	-11.03	2.63
	P6	4	-16.87	4.11	5	-11.44	7.73
	P7	5	-10.47	7.26	4	-11.02	5.86
223	P1	5	-9.54	2.12	5	-10.14	2.09
	P2	5	-8.98	2.80	5	-10.76	2.59
	P3	5	-10.62	0.79	5	-9.62	0.77
	P4	5	-9.39	0.85	5	-10.07	1.01
	P5	5	-9.01	1.26	5	-8.01	1.46
	P6	5	-12.53	7.00	5	-10.28	2.70
	P7	5	-10.90	2.57	5	-13.95	4.38
320	P1	5	-14.45	2.74	4	-15.94	3.55
	P2	5	-11.91	2.69	5	-14.48	3.47
	P3	5	-13.14	1.68	5	-14.11	1.81
	P4	5	-15.78	2.31	4	-13.17	1.93
	P5	5	-11.24	2.57	5	-12.21	1.80
	P6	4	-15.82	5.31	4	-16.51	5.91
	P7	5	-14.36	3.47	4	-14.01	1.08
486	P1	5	-11.18	1.02	5	-14.79	2.67
	P2	5	-12.44	3.98	5	-12.14	5.68
	P3	5	-11.82	2.14	5	-12.75	1.98
	P4	5	-11.33	1.45	5	-12.99	2.44
	P5	5	-12.54	1.86	5	-10.27	3.38
	P6	5	-10.16	5.60	5	-13.67	2.50
	P7	5	-13.54	2.15	5	-14.73	2.84

Table 3. Descriptive statistics of parameters of body composition by five successive matches in all players

matches	Body mass (kg)		Muscle mass (kg)		Fat mass (kg)		TBW		FFM		BMI		%PBF		WHR		BMR		
	N	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
I	7	70.67	15.62	29.60	5.83	17.43	5.77	38.89	7.19	53.24	9.94	23.23	2.55	24.26	2.54	0.86	0.04	1520.00	215.17
II	7	70.49	16.60	30.56	6.20	15.74	6.41	39.96	7.66	54.74	10.67	23.13	2.80	21.71	3.89	0.86	0.04	1552.71	230.20
III	7	69.84	16.74	30.66	6.18	15.01	7.37	40.03	7.60	54.83	10.59	22.91	2.87	20.70	6.20	0.85	0.05	1554.29	228.62
IV	7	69.90	16.72	30.73	6.51	14.94	6.47	40.13	7.87	54.94	10.91	22.94	2.88	20.71	4.21	0.85	0.04	1556.86	235.56
V	7	69.44	17.54	31.54	8.33	13.40	7.99	41.04	9.81	56.04	13.42	22.74	3.15	18.80	7.83	0.84	0.03	1524.00	242.87
		F=0.01; n.s.		F=0.08; n.s.		F=0.32; n.s.		F=0.06; n.s.		F=0.06; n.s.		F=0.03; n.s.		F=1.00; n.s.		F=0.39; n.s.		F=0.04; n.s.	

Table 4. The Spearman Rank correlation showing relationship between each body composition parameters and level of expression of microRNAs before and after five subsequent matches

	Match I		Match II		Match III		Match IV		Match V	
	Before	After	Before	After	Before	After	Before	After	Before	After
microRNA - 182										
Body mass (kg)	0.257	-0.286	0.214	-0.250	-0.143	0.714	0.571	-0.071	0.571	
Muscle mass (kg)	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.001	0.714	0.429
Fat mass (kg)	0.600	-0.286	0.001	0.036	-0.371	0.857*	0.500	-0.107	0.001	0.725
TBW	0.257	-0.378	0.143	-0.179	-0.257	0.500	0.607	0.000	0.714	0.429
FFM	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.000	0.679	0.429
BMI	0.714	-0.464	-0.054	-0.036	-0.600	0.679	0.393	0.000	0.786*	0.600
%PBF	0.600	-0.250	0.143	-0.036	-0.714	0.750	0.536	-0.500	0.107	0.829*
WHR	0.231	-0.200	0.382	-0.509	0.200	0.679	0.234	-0.018	0.727	0.579
BMR	0.257	-0.286	0.143	-0.179	-0.257	0.500	0.607	0.000	0.679	0.429
microRNA - 223										
Body mass (kg)	-0.143	-0.179	-0.429	-0.429	-0.286	-0.036	-0.250	0.214	-0.143	0.179
Muscle mass (kg)	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.143	0.464
Fat mass (kg)	-0.107	-0.107	-0.357	-0.643	-0.143	0.107	-0.107	0.179	-0.450	-0.234
TBW	-0.054	-0.288	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.143	0.464
FFM	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.036	0.286
BMI	-0.036	-0.286	-0.270	-0.577	-0.143	-0.071	-0.321	0.357	0.286	0.321
%PBF	-0.321	-0.071	-0.357	-0.714	0.250	0.000	0.143	-0.036	-0.214	-0.214
WHR	-0.273	0.327	-0.346	-0.309	0.214	0.428	0.162	0.234	0.036	0.091
BMR	-0.143	-0.179	-0.536	-0.357	-0.607	-0.250	-0.429	0.179	0.036	0.286

	Match I		Match II		Match III		Match IV		Match V	
	Before	After	Before	After	Before	After	Before	After	Before	After
microRNA - 320										
Body mass (kg)	0.143	0.000	-0.250	-0.100	-0.107	-0.371	0.086	0.086	0.321	0.643
Muscle mass (kg)	0.143	0.000	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.143	0.786*
Fat mass (kg)	0.321	0.214	-0.214	-0.300	-0.214	-0.314	0.143	0.143	0.432	0.054
TBW	0.054	-0.091	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.143	0.786*
FFM	0.143	0.000	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.214	0.678
BMI	0.071	0.036	-0.036	0.154	0.071	-0.371	-0.257	0.371	0.357	0.643
%PBF	0.286	0.250	-0.036	0.100	-0.250	-0.258	-0.257	-0.200	0.571	-0.143
WHR	0.436	0.164	0.000	0.410	-0.107	-0.143	0.290	0.087	0.618	0.509
BMR	0.143	0.001	-0.429	-0.200	0.036	-0.086	0.086	0.143	0.214	0.679
microRNA - 486										
Body mass (kg)	-0.036	-0.286	-0.429	0.071	-0.143	-0.786*	-0.429	0.786*	-0.286	0.143
Muscle mass (kg)	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.179	0.393
Fat mass (kg)	0.000	-0.250	-0.357	0.000	-0.393	-0.714	-0.571	0.786*	-0.162	-0.342
TBW	0.036	-0.306	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.179	0.393
FFM	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.214	0.250
BMI	0.107	-0.179	-0.270	0.000	-0.536	-0.607	-0.536	0.500	0.143	0.179
%PBF	-0.036	-0.393	-0.357	0.000	-0.857**	-0.179	-0.464	0.750	0.036	-0.536
WHR	-0.309	0.200	-0.346	0.236	-0.143	-0.393	-0.652	0.739	-0.109	-0.091
BMR	-0.036	-0.286	-0.536	-0.071	0.036	-0.929**	-0.321	0.679	-0.214	0.250

\* p<0.05; \*\* p < 0.01

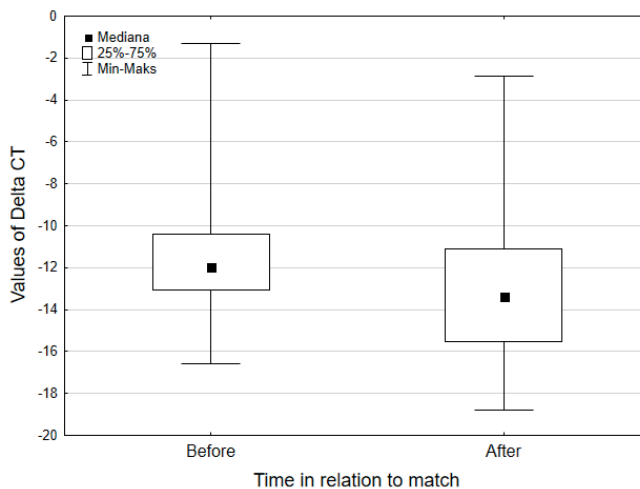


Fig. 1 The micro-RNA 486 expression before and after each match (Z=1.769; p=0.0769)

## Discussion

The objective of our study was to evaluate the role of microRNA expression in body composition, with a particular focus on the muscle tissue in athletes. MicroRNAs, which are small, non-coding RNA molecules, perform essential regulatory functions in a variety of fundamental biological processes.

Despite the small sample size, we presented in this study a novel approach to longitudinal changes of the microRNAs connected with IGF1 and muscle mass and revealed some correlations and tendencies.

The association between microRNA expression as well as the composition of skeletal muscle and adipose tissue has been documented. Recent studies have demonstrated a considerable utility of microRNA analyses in a variety of contexts, including clinical, forensic studies (Barreiro et al. 2019; Svingos et al. 2019; Zampetaki et al. 2010) and the assessment of their role in competitive sport (Aoi et al. 2013). It is evident that body composition is responsive to physical exercise. Consequently, the question arises as to whether a modified level of selected microRNAs in the bloodstream translates into more favorable parameters of body composition in athletes. MicroRNAs are characterized by significant stability and may be introduced into sports practice as biomarkers (Jung et al. 2010). The utility of microRNA methodologies and analyses in the context of sport can be employed to elucidate their functions within the human body, particularly in relation to body composition (muscle tissue, adipose tissue, TBW). It is possible that further research will demonstrate the value of the advanced genetic biomarker methods in conjunction with the

currently employed physiological methods utilized in preparing individuals for competitive sport. As such, our research represents a considerable advancement in the field of diagnostics, as well as contributes to the growing body of knowledge regarding the expression function of selected microRNAs.

The results of our study showed a positive correlation between miR-182 and fat mass following the third match. Furthermore, a positive correlation was observed between miR-182 and both before the 5th BMI and with percent body mass (PBF%) after the 5th match. Given that muscle and fat mass are inversely correlated, our results align with those reported by Olivieri et al. (2014), who observed significantly lower miR-182 levels in muscle samples. Furthermore, a study conducted on rats indicated that miR-182 levels were responsive to exercise in muscle tissue (Song et al. 2017). It was hypothesized that the level of miR-182 may serve as an indicator of the composition of adipose and skeletal muscle tissue in the body. Moreover, Zhang et al. (2016) demonstrated that miR-182 regulates glucose utilization in skeletal muscle, influencing blood glucose levels.

Our findings showed a positive correlation between miR-320 and muscle mass and TBW following the fifth match. In contrast, Munetsuna et al. (2018) identified a negative association between miR-320 expression and excess body fat. In addition, Yerlikaya and Mehmet (2019) demonstrated that high-fat conditions downregulated miR-320. Therefore, it can be postulated that the level of miR-320 is positively correlated with muscle mass and negatively correlated with fat mass. However, there is few studies that addresses the issue of miR-320 expression and total body water. Nevertheless,



the proper metabolism and body condition are hallmarks of organism hydration (Chumlea et al. 1999).

In contrary to clinical and forensic research, we are the first who tackled the problem of longitudinal effect of the circulation microRNAs expression level among athletes (Zampetaki et al. 2010; Barreiro et al. 2019; Svingos et al. 2019; Di Pietro et al. 2017; Goljanek-Whysall et al. 2020; McCrae et al. 2016; Woo et al. 2018). As stated by Nielsen et al. (2010), coordinated expression of selected microRNAs and non-single microRNA expression is crucial for skeletal muscle in endurance and speed training and adapts to the level of physical activity. The authors emphasized that the details on which the selected microRNA assembly can directly affect human physiology in response to physical exercise remain unknown. In addition, McCarthy et al. (2009) highlighted the role of several microRNAs (MyomiRs) as regulators of skeletal muscle cell function. Conversely, Small et al. (2010) observed that microRNA-486 exhibited particularly high levels in skeletal muscle cells and heart muscle, reaching ten to twenty times the concentration observed in other tissues. Aoi et al. (2013) suggested that microRNA-486 may regulate insulin-dependent glucose uptake in skeletal muscle tissue and may facilitate glucose uptake by activating insulin signaling during exercise. In the studies conducted by the authors among 10 healthy men, they observed that after a 4-week workout, the level of microRNA-486 decreased significantly. However, 24 hours after exercise, the microRNA returned to the baseline level and increased with insulin. Thus, microRNA can be considered to mediate adaptive muscle responses to physical exercise following training. In addition, we

showed that PBF% is negatively correlated with miR-486; in contrast, Prats-Puig et al. (2013) showed that among obese prepubertal children the level of miR-486 was elevated.

The heterogeneous picture of body composition compounds and selected microRNAs suggests the need for further research on this problem. At the same time, we underline that the research conducted during the series of match games could also be associated with the level of sports advancement in subsequent matches. Specifically, in the case of the 3rd, 4th and 5th matches we observed the highest number of microRNA associations with body composition parameters.

It should be emphasized that the majority of studies on the role and functions of microRNA expression have been conducted on animals or through clinical observations and forensic analyses, thus applicative use in the sport industry may be limited. In this context, our research represents a novel approach to the application of microRNAs in the field of sport. Our findings show a correlation between body composition and microRNA expression levels. This suggests that the selection of microRNAs may be applied as a useful tool in identifying young athletes who are predisposed to different types of sports.

The study's limitations include a relatively small sample size thus further studies with larger sample sizes are needed to validate our results. In addition, further studies should include both sexes and other age groups. Nevertheless, we showed a longitudinal effect of each of the players. Moreover, a machine error in the case of one measurement of PBF% due to outliers' detection cannot be discarded.

We showed that expression levels of miR-182, miR-320 and miR-486 are associated with body composition. We also showed that the miR-182 is positively correlated with fat tissue, miR-320 is positively correlated with muscle mass while miR-486 is negatively correlated with fat mass. In addition, the results of our study indicated that exercise may result in a reduction in the level of miR-486.

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### Ethical approval statement

The study was approved by Senate's Ethic Committee of Scientific Research at University School of Physical Education in Wroclaw 4/2020.

### Conflict of interests

The authors declare no potential conflict of interest.

### Authors' contributions

PPP – participating in designing the study, performed laboratory work, analyzed the data, prepared the draft and final version of the manuscript, SK – designed the study, conducted the analysis and prepared the draft and checked the final version; MK – collected the blood samples, prepared samples for analysis, build the database, EŻ, ZF, AR, IC, AD, MŚ, KK, AS – recruited and instructed participants, conducted measurements and prepare database, prepare first draft.

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