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# Comparative Analysis of Foot Arch Index between Yogic and Non-Yogic Female Practitioners

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## Abstract

**Introduction**
With advancing age in females, gait patterns change due to several factors, including nutritional and hormonal influences. These changes can lead to a lowering of the foot arch, resulting in mild valgus and varus deformities. Regular yoga practice may help tone the musculoskeletal system of the foot, promoting the maintenance of the medial longitudinal, transverse, and lateral arches.

**Study Aim**
This cross-sectional study aimed to compare the arch indices of female yoga practitioners to those of non-practitioners in order to ascertain how yoga affected female arch indices.

**Materials and Methods**
Fourteen female students from Banaras Hindu University in Varanasi, India, participated in the study, seven of which were regular yoga practitioners and seven were non-yoga practitioners. Their left and right arch indices were determined by taking measurements from their footprints. A Mann Whitney *U* test was used to compare the foot arch indices of the two groups.

**Results**
A statistically significant difference between the left as well as right foot arch indices of females was revealed.

**Conclusion**
The study concludes that females who practice yoga may experience foot arch plasticity which is not necessarily evident in non-yoga practitioners. Future studies should test larger sample sizes and different genders.

**Keywords:** foot biomechanics, foot health, physical activity, exercise, body adaptation

Original article

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## Introduction

### Importance of the Foot Arch and Its Role in Bipedal Locomotion

The human foot plays a vital role in supporting bipedal locomotion, with the medial longitudinal arch (MLA) serving as a key structural component in shock absorption, balance, and weight distribution. This arch allows the foot to adapt to uneven surfaces and absorb forces during gait. When the MLA is compromised, it can result in biomechanical dysfunctions, altered gait, and discomfort that may impair walking and posture (Holowka & Lieberman, 2018; Ker et al., 1987). The MLA is a unique feature in human evolution, enabling effective upright walking and running. Pathologies such as plantar fasciitis, Achilles tendinitis, and Morton's neuroma are known to cause foot pain and may limit mobility. Similarly, deformities like pes planus (flat feet) and pes cavus (high arches) often develop from prolonged weight-bearing or overuse and can be associated with secondary issues, including low back pain (Tsung et al., 2003). These facts emphasize the need for regular assessment of MLA using reliable, non-invasive tools to ensure early detection and correction.

### Tools for Assessing the Medial Longitudinal Arch

Several tools and indices have been developed to evaluate the structure and function of the MLA. Among these, the Foot Posture Index (FPI), and its revised version FPI-6, are widely used for visually assessing foot alignment in clinical and research settings. The FPI-6 has demonstrated moderate to high intra-rater reliability and moderate inter-rater reliability, and it has been validated in various age groups (Keenan et al., 2007; Morrison & Ferrari, 2009). In pediatric populations, the FPI-6 has shown good diagnostic accuracy when compared to radiographic assessments, with reported sensitivity and specificity values above 80% (Lee et al., 2020).

In addition to observational indices, footprint-based tools such as the Arch Index (AI) (Cavanagh & Rodgers, 1987) and the Staheli Index (Staheli et al., 1987) are frequently employed. These indices offer a non-invasive, cost-effective method for estimating MLA height. Although radiographic and anthropometric methods are more precise, footprint-based indices are widely used in field studies due to their simplicity. Importantly, Kanatli et al. (2001) reported significant correlations between footprint indices and radiographic measures of MLA, supporting their clinical relevance. Despite some limitations in accuracy, footprint indices remain a practical option, especially when radiological evaluation is not feasible.

### Influence of Body Composition and Lifestyle Factors on Medial Longitudinal Arch

The height and structure of the MLA are influenced not only by mechanical factors but also by body composition, gender, age, and lifestyle. Obesity has been identified as a major risk factor for pes planus, where increased body mass may cause excessive flattening of the arch, compromising foot function and resilience (Butler et al., 2008). This arch flattening can also lead to secondary problems in the knees, hips and lower back.

Several studies have explored the relationship between MLA parameters and body mass index (BMI). Stanković et al. (2018) observed a strong correlation between left foot arch angle and BMI, suggesting that this angle may serve as a more reliable predictor of BMI compared to other indices. Gilmour and Burns (2001) emphasized that body composition significantly affects arch index values, highlighting the importance of interpreting such indices alongside body composition data. Other variables, including gender, limb dominance, and age, have also been shown to influence MLA structure. In a study by Wearing et al. (2004), both AI and Navicular Height (NH) were found to be valid, non-invasive predictors of MLA across age groups. While NH appeared more sensitive to age-related changes, AI was slightly more accurate overall. Together, these findings support the use of such indices in large-scale population studies.

In light of the growing interest in the role of physical activity in foot structure, this study investigates whether regular yoga practice has an effect on the MLA in females. We test a hypothesis that the foot arch index of female yoga practitioners will differ from non-practitioners.

## Materials and Methods

A total of fourteen female participants were selected through simple random sampling from various sports specializations at the Department of Physical Education, Banaras Hindu University, India, aged between 19–23 years. Among them, seven participants reported engaging in regular yoga practice, while the remaining seven had no prior experience with yoga or related physical activities. Prior to data collection, all participants received both verbal and practical orientation regarding the purpose and procedures of the study. The study involved non-invasive procedures limited to the collection and analysis of static footprints. All participants were healthy adult volunteers who were informed in detail about the aims, procedures, and voluntary nature of the research. Written informed consent was obtained from each participant prior to data collection. As the study did not involve any clinical intervention, collection of sensitive personal data, or work with vulnerable populations, formal ethical clearance was not required by our institution. Nonetheless, the study adhered to the ethical principles outlined in the Declaration of Helsinki and followed standard academic practices for research involving human participants.

The primary variable selected for this study was the Foot Arch Index (Cavanagh & Rodgers, 1987; Ozer & Barut, 2012) (Figure 1), assessed separately for the left and right foot of each participant. This variable was chosen to evaluate the structure and characteristics of the MLA across both yogic and non-yogic groups. The Arch Index is the ratio of the area of the middle third of the toeless footprint to the overall toeless footprint area. A line is drawn between the center point of the second toe and the posterior-most point on the heel. Two parallel lines perpendicular to this line are drawn to divide the toeless footprint area into equal thirds. This was calculated from the foot imprints of the subjects, which were taken by using an inkpad on an A4 sheet with one leg standing position (Ozer & Barut, 2012).


Figure 1. Calculation of arch index of foot. The length of the footprint excluding the toes (L) is divided into equal thirds. The AI is then calculated as the area of the middle third of the footprint divided by the entire footprint area (Arch Index = B/(A + B + C))

The Foot Arch Index was calculated based on the method described by Ozer and Barut (2012), which has been established as a reliable and valid approach for assessing foot morphology in both athletic and general populations. This method enables a consistent and non-invasive measurement of the medial longitudinal arch using static footprint data. To classify the arch types into high arch, normal arch, and low arch (pes planus), categorization thresholds were adopted from established normative data presented in previous research (Menz et al., 2012). These classification criteria were used to interpret individual Foot Arch Index values and group participants accordingly. The procedure and classification system are illustrated in Figure 2.

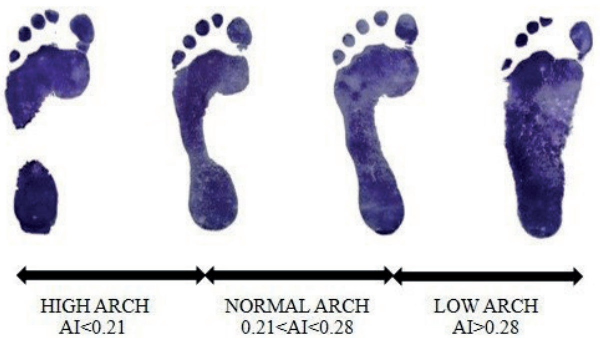


Figure 2. The visual categorization of foot arch index

## Statistical Procedures

Descriptive statistics, including the mean and standard deviation, were computed to summarize the arch index values of both yoga practitioners and non-practitioners. Given the small sample size (n = 14) and the non-parametric nature of the data, a Mann-Whitney *U* test was employed to examine differences in foot arch index between the two independent groups. All statistical analyses were performed using standard statistical software, with the level of significance set at $p < 0.05$.

## Results

The mean and standard deviation of the left and right foot arch index for both yoga practitioners and non-yoga practitioners are shown in Table 1. The data indicate a lower average arch index in the yoga group, suggesting a comparatively higher medial longitudinal arch.

Table 1. Descriptive statistics of left foot arch index in yoga and non-yoga female practitioners

| Group | N | Mean Arch Index | Standard Deviation |
|---|---|---|---|
| Yoga Practitioners: left foot | 7 | 0.264 | 0.052 |
| Non-Yoga Practitioners: left foot | 7 | 0.370 | 0.036 |
| Yoga Practitioners: right foot | 7 | 0.276 | 0.053 |
| Non-Yoga Practitioners: right foot | 7 | 0.360 | 0.037 |

Statistically significant differences were observed between the yoga practicing and non-practicing groups, indicating a potential influence of yoga practice on foot arch morphology. The p value was lower when the left foot was compared ($U = 1$; $p=0.001$), whereas it was higher ($U = 3$ ; $p=0.004$), but still statistically significant when comparing the right foot.

## Discussion

Our preliminary findings suggest that regular engagement in yoga may be associated with measurable alterations in medial longitudinal arch structure. The human foot, particularly the MLA, has long been considered an essential structure in the context of bipedal locomotion and postural stability—key traits in human evolutionary adaptation. This study explored whether regular engagement in yoga, a culturally rooted and functionally diverse physical practice, contributes to measurable differences in foot arch structure among females. The findings revealed statistically significant differences in the arch index of both the left and right foot between yoga practitioners and non-practitioners, suggesting that sustained functional loading through yoga may lead to subtle morphological adaptations in the foot.

From an anthropological perspective, these results add to the growing body of research that underscores the dynamic interaction between physical activity, cultural behavior, and human form. The consistent practice of yoga, involving postures that load the feet in varied planes and require proprioceptive feedback, may stimulate musculoskeletal adaptations over time—supporting the hypothesis that lifestyle factors can influence phenotypic variation in foot morphology (Kulthanan et al., 2004).

While previous research has emphasized the reliability of arch height index and footprint-based analysis in assessing MLA structure (McPoil et al., 2006; Ozer & Barut, 2012), the present findings highlight yoga as a potential behavioral modifier of foot form. The use of the arch index as a non-invasive measure aligns well with anthropological methods focused on external morphology and allows for cost-effective, reproducible data collection in field and community-based research.

The observed differences in arch index may reflect adaptive responses of the intrinsic and extrinsic foot musculature, possibly supporting arch elevation and alignment. These adaptations could reduce the risk of common foot pathologies such as pes planus, particularly in females, who are often more susceptible due to footwear choices and occupational load (Barton et al., 2009). Such findings are relevant in evolutionary anthropology and anatomy, where foot structure is not only examined for its role in past mobility patterns but also as a site of modern functional adaptation (e.g., Huang et al., 2019; Shibuya et al., 2010; Willems et al., 2012).

Nonetheless, this study is limited by its small, gender-specific sample size and cross-sectional design. Future studies should incorporate a broader demographic spectrum, including both sexes and varying age groups, to explore whether similar adaptations occur across populations. Longitudinal studies would also help determine whether observed changes are transient or structural.

## Conclusion

The present study provides preliminary evidence that regular yoga practice may influence the medial longitudinal arch structure in women, as reflected in lower arch index values compared to non-practitioners. These differences suggest that cultural practices like yoga—when performed consistently—can serve as functional stimuli capable of inducing measurable morphological variation in the human foot. With further evidence from other studies, implications for both clinical health and anthropological research can be achieved. Continued investigation into the effects of habitual activity patterns on skeletal and soft tissue structures may further illuminate the plasticity of human form in response to lifestyle, and deepen our understanding of the role behavior plays in shaping the body across both evolutionary and contemporary time scales.

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**Contributions from Individual Authors**

SM: conceptualized the initial scope of the paper and designed the study protocol, conducted a comprehensive literature review, collected the raw data and wrote the initial draft; BKD: collaborated on refining the paper's concept and scope, conducted in-depth analyses of the data; BCK: reviewed and edited the manuscript, providing critical revisions for the discussion and conclusion sections, supervised the overall development of the manuscript.

**Ethics Statement**

The present study involved non-invasive procedures limited to the collection and analysis of static footprints for the purpose of assessing the medial longitudinal arch. Written informed consent was obtained from each participant prior to data collection. Formal ethical clearance was not required by our institution. The study adhered to the ethical principles outlined in the Declaration of Helsinki.

**Data Availability Statement**

The dataset utilized for this study is not publicly available due to confidentiality clause promised to the participants. It can be obtained from the corresponding author upon request for reasonable academic purposes only.

**Financial Disclosure**

No funding has been provided by any agency at any stage of this study.

**Conflicts of Interest**

The authors declare that they have no competing financial interests.

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# Relationship between Psychomotor Abilities vs. the Declared and Actual Physical Activity Levels among Women over 65 Years of Age

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## Abstract

**Introduction**
Psychomotor performance is a key indicator of functional aging and may be influenced by physical activity. Understanding how different activity levels relate to psychomotor abilities in older women is important for supporting independence in later life.

**Study Aim**
To examine the association between reaction time, visuomotor coordination, movement anticipation, and both subjective and objective physical activity levels in women aged 65+.

**Material and Methods**
A total of 30 women from the University of the Third Age in Rzeszów (70.87 ± 6.07 years) participated. Psychomotor abilities were assessed using standardized computerized tasks, while physical activity was measured using a self-report questionnaire and objective monitoring.

**Results**
Higher engagement in household physical activities was associated with faster simple reaction time. Conversely, a greater number of steps and higher low-intensity activity co-occurred with slower responses in choice-based and anticipation tasks. Increased sedentary time was related to poorer visuomotor reaction speed.

**Conclusions**
Although the observed associations were not fully consistent, the findings indicate that maintaining regular physical activity may help preserve psychomotor functioning and functional autonomy in aging women. Further research is needed to clarify the direction and mechanisms of these relationships.

**Keywords**: ageing, older women, psychomotor performance, physical activity

Original article

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## Introduction

Demographic realities in the populations of developed areas, as well as forecast projections for the coming years, indicate an increasing proportion of older people regardless of the accepted age limits for these subpopulations. In Poland in 2018, the number of people aged 60 and over accounted for nearly 25% of the total population, while in 2010, their share was 19.6%. According to a forecast by the Central Statistical Office of Poland (GUS), in 2050, the number of people of this age will be approximately 40% of the population (Statistic Poland, 2020).

The process of population ageing in Poland and worldwide is a consequence of increasing life expectancy, exacerbated by low fertility rates. United Nation projections state that the share of the population aged 65 and over worldwide will increase from 9% in 2020 to around 16% in 2050 (United Nation DESA, 2020). Europe is currently the major area in the world with the highest ratio of seniors and is projected to remain so for at least the next 50 years. The systematically increasing share of older people in the total population both in Europe and worldwide is also confirmed by other reports from abroad of Poland (Eurostat, 2020; DG ECFIN and AWG, 2020; WHO, 2015).

Considering these developments, the demographic changes of modern Europe and the world call for a deeper analysis of the broader issues of ageing and old age, as well as their consequences to maintain the independence, autonomy and activity of seniors. Increasingly, the term 'healthy ageing' is being used in the literature and includes the process of developing and maintaining functional capacity for successful ageing (WHO, 2020). Functional abilities of older people mean physical abilities, mental abilities, and the interaction abilities of visual-motor functions. Of particular importance is functional fitness, the physiological ability of performing daily activities in a safe and independent way, without excessive fatigue for seniors to maintain their mobility and independence (Rikli & Jones, 1999). Declining levels of physical fitness with deteriorating eye-hand coordination may lead to a rapid decline in physiological ageing parameters, which – coupled with civilisation diseases – may restrict the mobility in seniors, their functional fitness, and last but not least, their quality of life (Ignasiak et al., 2020). Against the backdrop of a progressively ageing population, it becomes important to determine the level of functional fitness as to be able to assess it regionally and globally, followed by application of the measures necessary to maintain or even improve the physical fitness of senior citizens. Various tools are now used in research to assess physical fitness in older people (Jones & Rikli, 1999; Lemmink et al., 2001; Osness et al., 1990; Rikli & Jones, 1999). Functional fitness tests for seniors provide an opportunity to analyse the individual motor skills required to perform daily activities and maintain independence among seniors.

Physical activity should also be of interest, which must be adapted to age and health status and can mitigate the course of ageing and delay involution processes. Maintaining physical activity among seniors contributes to better intellectual performance, higher self-esteem and emotional balance. However, it first helps to maintain physical fitness, which increases independence in old age (Langhammer et al., 2018).

According to the latest WHO recommendations, people over 65 years of age should undertake regular physical activity. Moderate activity of at least 150–300 minutes/week, or vigorous activity of at least 75–150 minutes/week, or an equivalent combination of the two, is recommended. In addition, seniors should do muscle-strengthening exercises involving all major muscle groups twice a week. Functional balance exercises and strength training are recommended three times a week. For additional health benefits, moderate activity can be increased to more than 300 minutes per week, with intense activity increased to more than 150 minutes. The WHO also recommends limiting time spent sitting, replacing it with any physical activity, even of low intensity, as any activity is better than no activity (WHO, 2021).

Research analyses are increasingly using accelerometer-based data outputs to reliably estimate the physical activity, sedentary lifestyle and sleep levels in older people. A study by Sasaki et al. (2017), using the ActiGraph wGT3X-BT, suggests that at least five days of monitoring are required to reliably estimate physical activity levels and sedentary lifestyle. Monitoring physical activity in a specific subpopulation is crucial not only for assessing physical activity levels but also trends and directions for physical activity among seniors. It is important to consider that information on physical activity is highly dependent on accelerometer data processing criteria and the application of different physical activity recommendations – these may impact estimates and tracking of prevalence and physical activity patterns in a given population (Sagelv, 2019). Complementary to objective methods of assessing physical activity and fitness levels are self-descriptive methods (meaning, 'declared physical activity'). To subjectively assess seniors' physical activity levels, the Physical Activity Scale for the Elderly (PASE) is used, among other research tools. Seniors assess the duration, frequency, level of exercise and amount of physical activity undertaken over a seven-day period using a PASE questionnaire (Washburn et al., 1993; Wiśniowska-Szurlej et al., 2020).

In situations involving decision-making, error correction as well as situations that are new to seniors (and which are accompanied by involution processes), the speed and appropriateness of reaction is a priority; in selected tasks, visual-motor coordination determines the accuracy and speed of whole-body movements as well as at the level of fine motor skills. Above 60–70 years of age, limb muscle strength decreases by 20–40%, which translates into the interaction ability of visual-motor functions (Doherty et al., 1993).

Note that the limited opportunity for seniors to engage in physical activity results in reduced performance of proprioceptive sensitivity and eye-hand coordination, thereby impairing the postural stability of seniors, a common cause of injury in old age (Famuła et al., 2012). People over 65 years of age need more time to perform a precise movement which negatively affects motor economy and efficiency in performing daily activities (Rand et al., 2011; 2012). Studies have shown that the value of choice reaction time correlated with motor and cognitive exercise has significantly improved which means seniors performing many day-to-day activities with higher efficiency (León et al., 2015). Burton et al. (2009) indicated that reaction time is a predictor of older people's ability to solve everyday problems (in an Everyday Problem Test, EPT). The efficiency of the EPT was significantly related to the age and cognitive status in the seniors. Slower and inconsistent reaction times were associated with poorer skills in solving daily problems based on the EPT (Burton, 2009). It can be predicted that psychomotor involution, the implication of which is a decrease in fluidity and speed of movement, can be reduced in severity by appropriate physical activity for seniors. A lifestyle which involves regular physical activity in seniors is indirectly associated with a reduction in neuromuscular loss, using two different measures of neuromuscular integrity, the coincidence-anticipation time (Christensen et al., 2003). Physical activity has a significant impact on the psychomotor abilities of seniors, especially the reaction time as an indicator of quality of life in older people. Therefore, special attention should be paid to motor activation, motor skills and visual-motor functions to optimise the ageing process (counteracting involution, the deterioration of cognitive and physical functions, and psychomotor abilities, or dementia).

Psychomotor abilities are an important indicator of functional aging, as they reflect both the speed of information processing and the integration of cognitive and motor processes. In older adults, particularly women, declines in reaction time and visuomotor coordination are associated with a higher risk of falls, reduced independence, and slower performance of activities of daily living (Lord et al., 2003; Muir et al., 2010). Psychomotor performance is also strongly linked to executive functions and processing speed—domains that deteriorate with age (Salthouse, 2000; Verhaeghen & Salthouse, 1997). At the same time, numerous studies suggest that physical activity may enhance or slow the decline of psychomotor functions by influencing neuroplasticity, cognitive efficiency, and motor control (Colcombe & Kramer, 2003; Voelcker-Rehage et al., 2011). Therefore, analyzing the association between physical activity levels and psychomotor abilities provides valuable insights into potential protective mechanisms that support mobility and functional autonomy in aging female populations.

Results obtained with the Test2Drive system can be directly compared with findings generated using other psychomotor assessment tools commonly applied in gerontology. Test2Drive evaluates fundamental components of psychomotor performance—simple and choice reaction time, hand–eye coordination, and spatial anticipation—which correspond to the domains measured in computerized systems such as the Vienna Test System and CANTAB (Tarnowski, 2016). Unlike manual assessments, it enables a more precise separation of reaction time from movement time, which is crucial in older adults who often present concurrent cognitive and motor slowing. Its brief procedures and user-friendly interface make it suitable for field studies while maintaining comparability with standard gerontological instruments. The consistency of our findings with previously reported results supports the measurement validity of Test2Drive for assessing psychomotor functioning in older populations.

Thanks to the multifaceted approach to physical activity in older people in connection with psychomotor abilities, this research may contribute to further in-depth scientific analyses of selected determinants of quality of life for seniors to maintain their fitness and physical activity and independence. The study posed the following research question: What is the relationship between psychomotor abilities and both self-reported and objectively measured physical activity levels among women aged 65 years and older?

The aim of the study was therefore to assess the relationship between psychomotor abilities (simple reaction time, choice reaction time, and hand-eye coordination and anticipation) and the declared (PASE) and actual (accelerometer-tested) physical activity levels among women over 65 years of age.

## Material and Methods

### Subjects

The study was conducted on a group of 30 Polish women aged ≥ 65 years (x=70.87±6.07) living in the Podkarpacie region (70% of the respondents reside in urban areas and 30% come from rural areas). Among the study population, 50% of the women had a tertiary education and about 33% of the respondents declared that they lived alone. In addition, the vast majority of women were not in employment (approximately 87%). See Table 1 for detailed data. The research was conducted in September 2022. Each participant was in generally good health, reporting no musculoskeletal or cognitive complaints. The ethics protocol was approved by the Bioethics Committee at the University of Rzeszow, Approval No. 19/03/2020. All the subjects signed a written informed consent form before participating in the study.

Body composition was assessed using The Tanita Body Composition Analyzer type DC 430 S MA. Parameters including body mass (kg), MM (muscle mass) (kg), BM (bone mass) (kg), FAT (fat mass) (%, kg), FFM (fat free mass) (kg), TBW (total body water) (%, kg), and visceral fat were analysed. Body height was measured using a Seca 217 stadiometer and the BMI was calculated (Kyle et al., 2004).

Table 1. Subjects background

| n (%) | |
|---|---|
| Education | |
| basic | 5 (16,6) |
| vocational | 5 (16,6) |
| secondary | 5 (16,6) |
| higher | 15 (50) |
| Current occupation | |
| yes | 4 (13.33) |
| no | 26 (86.66) |
| Household composition | |
| alone | 10 (33.3) |
| with someone | 20 (66.6) |
| Marital status | |
| married | 16 (53.33) |
| not married | 14 (46.66) |
| Material situation | |
| very good | 1 (3.33) |
| good | 18 (60) |
| average | 10 (33.3) |
| bad | 1 (3.33) |
| Living situation | |
| rural | 9 (30) |
| urban | 21 (70) |

### Psychomotor Abilities

The Test2Drive computer system (ALTA, Siemianowice Slaskie, Poland) was used to evaluate psychomotor abilities. Using Test2Drive, the following parameters were assessed: simple reaction time (SIRT), choice reaction time (CHORT), hand-eye coordination (HECOR) and spatial anticipation (SPANT). They were tests during which reaction (RT) and movement time (MT) were assessed. The subjects performed each test in a standing position and with the dominant hand, with a touchscreen display laid flat on a table. Before the actual test was performed, each woman was given detailed instructions on each test. This was followed by the exercise phase as guided the instructions in Test2Drive, after which the proper test began. A board showing circular boxes was presented to the subject on the touchscreen display. At the bottom of each displayed screen was a rectangular resting box labelled "START". The subjects' task was to respond as quickly as possible to the stimuli in all four tests. Each test took approximately three minutes to complete. In the SIRT, HECOR and SPANT test, 20 stimuli were displayed, with the stimulus exposure time of 3 seconds. The stimuli were presented at time intervals changing every 1s; 1.5s or 2s. The subject's task was to respond as quickly as possible to the stimuli that appeared on the display.

The study included the following tests:

- SIRT – this test was aimed to assess the speed of the reaction and its stability. The displayed stimulus was visual: in the top row of the screen, the central, bright red box occasionally lit up, changing the colour to fiery red. The required reaction was to move the finger from the START resting field to the reaction field highlighted in blue, and then quickly return the finger to the START resting field.
- CHORT – this test was aimed to assess the speed and adequacy of the response in a complex situation. The displayed stimulus was visual: from time to time, different patterns comprising vertical, horizontal and diagonal lines appeared in the boxes in the top row of the screen. Horizontal and vertical patterns were stimuli that required a response, a diagonal pattern was a neutral stimulus that did not require a response. For the response-required stimuli, the subject was to move the finger from the START resting field to the highlighted response field as quickly as possible. For the neutral pattern, the task was to refrain from reacting and keep the finger on the START resting field. In the CHORT test, 24 stimuli were displayed, including 16 critical stimuli (8 stimuli with a horizontal pattern and 8 stimuli with a vertical pattern), along with 8 neutral stimuli (diagonal pattern).
- HECOR – this test was aimed to assess eye-hand coordination. The displayed stimulus was visual: in the top row of the screen, one of the bright red boxes occasionally lit up, changing the colour to fiery red. The required reaction was to move the finger from the START resting field to the corresponding reaction field, located exactly below the displayed stimulus.
- SPANT – this test was aimed to assess visual-motor coordination but compared to the HECOR test (analogous to the Piórkowski apparatus) the task was much more complex and taxing on cognitive processing. The blue reaction boxes were displayed visible in the centre of the board on screen. For the SPANT test, there was a double stimulus – from time to time, two of the bright red boxes at the periphery of the board (at the top and to the right or left) lit up simultaneously, turning bright red. The required reaction was to move the finger from the START resting field to the reaction box located exactly at the intersection of the row and column indicated by the fiery-red boxes, followed by the fastest possible return to the start resting field (Core 2023).

### Physical Activity (Accelerometer)

The GT3X accelerometer (Actigraph, Pensacola, Florida, USA) was worn by the participants on an elastic belt over the right hip for a duration of seven days, ensuring usage during waking hours and removing the device only for activities involving water, such as swimming or bathing. The recorded accelerometer data were transferred to a PC workstation using the ActiLife 6.0 software. Data processing followed standardized procedures, where raw movement signals detected along the vertical axis were aggregated into 60-second epoch intervals. The ActiGraph wGT3X-BT, a compact and lightweight device with adjustable sampling frequencies between 30 and 100Hz, increasing in 10Hz increments. For this study, the device was set to record data at an 80Hz sampling rate. During an in-person session, participants received instructions to place the accelerometer on the iliac crest, aligned with the anterior axillary line of the right hip, and secure it using the provided elastic belt. A day was considered valid if the device was worn for at least 600 minutes, and participants meeting the criterion of three or more valid days were included in the analysis, in accordance with established methods for assessing habitual physical activity (Davis et al., 2011; Harris et al., 2008; Hart et al., 2011). To categorize daily activity, cut-off thresholds were applied: sedentary behavior was defined as periods with fewer than 100 counts per minute, light physical activity (LPA) ranged between 100 and 2019 counts per minute, while moderate-to-vigorous physical activity (MVPA) was classified as exceeding 2019 counts per minute, following the methodology outlined in prior research (Troiano et al., 2008).

### Physical Activity Scale for the Elderly (PASE)

The PASE is a 12-item scale that assesses the level of physical activity over the past seven days in three life spheres: leisure time, household and work-related activities. The following leisure time activities were rated by the respondents based on weekly frequency and daily duration: walking outside; light, moderate and strenuous activities; and muscle strengthening. In terms of any activities bound to a household activities, the respondents were asked about: housework that is light and/or heavy, repairs around the house, work in the garden (such as mowing the lawn or outdoor gardening), and caring for others. When analysing paid or volunteer work-related activities, the number of hours per week and the type of work performed was asked. Each activity was scored by multiplying the activity frequency value by the weight given according to the score. After the calculations, a total PASE score (the sum of all activities) and partial scores for each sphere of life were obtained: leisure time activities, household activities and work-related activities. The activity partial scoring for actions related to these activities was calculated by totalling the activities corresponding to each sphere of life. For greater reliability, the PASE questionnaire survey was administered by an investigator (Washburn et al., 1993).

## Statistical Analysis

The study calculated basic statistical measures: arithmetic mean, standard deviation, coefficient of variation, and the minimum and maximum value. The strength and direction of the relationship between the two groups was calculated using the Pearson correlation coefficient.

In addition, a determination coefficient was calculated according to the formula $R^2 = (r)^2$, where r is the value of the correlation coefficient. The distribution of the variables was assessed using the Shapiro–Wilk test; all variables were consistent with a normal distribution, allowing the use of Pearson's correlation. All statistical calculations were performed using R Statistical Software (v4.3.3) at a significance level of $\alpha = 0.05$

## Results

Table 2 lists the somatic parameters, body composition, psychomotor abilities, as well as the declared (PASE) and actual (accelerometers) physical activity levels of the female subjects. The mean body height of the study population was 161.73cm±5.06 and the mean body weight was 72.74kg±12.19. The BMI (27.84) indicated that the women surveyed were overweight, but the actual body fat content was within the normal range (31.88%). In addition, the body composition analysis revealed that the MM was 46.66kg±8.40; the FFM was 49.07kg±8.88 and the BM was 3.15kg±3.59. The visceral fat index was 10.23±3.37, with TBW 44.68%.

Analysing the individual psychomotor abilities, it was noted that the SIRT performed best, where the female subjects achieved the shortest RT (458.87ms±106.32) as well as MT (365.43ms±123.66) compared to the other tests. The longest RT was recorded for CHORT (751.73ms±266.78). Also in the SPANT, RT was more than 700 ms.

The mean PASE score was 192.27±60.47. It was also noted that household activities accounted for approximately 60% of the total PASE score, with leisure time activities of around 38%. With the majority of these women no longer in employment at the time of the study, the percentage of work-related activities in the overall PASE score was low.

Accelerometer data indicated that the moderate level of physical activity of the women surveyed averaged around 160 min/week. In the case of vigorous physical activity, the situation was markedly different, as only about 2 min per week of physical activity for women was qualified as 'intensive'. In addition, it was noted that the subjects' average step count was around 6392 per day, which means more than 44.500 steps per week.

In the study, sleep efficiency averaged 95.4 per cent and sleep duration averaged 410 minutes; both were within normal values (Li et al., 2022). For people over 65, sleep efficiency should be above 85% and sleep duration should average 420 minutes.

By analysing the relationship between the psychomotor abilities and the declared level of physical activity (PASE) (Table 3), it was found that only SIRT RT was significantly correlated with the total PASE score ($r=-0.42$; $p=0.026$) and household activities ($r=-0.45$, $p=0.016$). The determination coefficient for household activities exceeded 20%, while the total PASE score was approximately 18%. In case of the actual measurement of physical activity (accelerometer) (Table 3) more significant relationships were found: CHORT RT was correlated with sedentary time ($r=0.039$, $p=0.040$), LIGHT ($r=-0.40$, $p=0.035$) and step counts ($r=0.42$, $p=0.026$); SPANT RT was significantly correlated with LIGHT and step counts ($r=0.38$, $p=0.046$). The strongest predictor of CHORT RT was steps ($R^2=17.6\%$). See Tables 3 and 4 for detailed data.

Table 2. Group characteristics

| | x | sd | v | min | max |
|---|---|---|---|---|---|
| Somatic parameters | | | | | |
| body height [cm] | 161.73 | 5.06 | 3.13 | 152.00 | 173.00 |
| body mass [kg] | 72.74 | 12.19 | 16.76 | 51.90 | 98.10 |
| BMI | 27.84 | 4.61 | 16.55 | 21.00 | 39.80 |
| Fat [%] | 31.88 | 10.61 | 33.29 | 3.00 | 47.50 |
| Fat [kg] | 23.67 | 9.96 | 42.08 | 1.80 | 46.60 |
| FFM [kg] | 49.07 | 8.88 | 18.10 | 34.40 | 75.50 |
| MM [kg] | 46.66 | 8.40 | 18.01 | 32.60 | 71.80 |
| TBW [kg] | 32.61 | 4.59 | 14.08 | 25.40 | 45.00 |
| TBW [%] | 44.68 | 4.66 | 10.42 | 38.00 | 53.80 |
| BM [kg] | 2.49 | 0.42 | 16.89 | 1.80 | 3.70 |
| Visceral fat | 10.23 | 3.27 | 31.92 | 3.00 | 17.00 |
| Psychomotor abilities | | | | | |
| SIRT RT [ms] | 458.87 | 106.32 | 23.19 | 302.00 | 691.00 |
| SIRT MT [ms] | 365.43 | 123.66 | 33.84 | 187.00 | 709.00 |
| CHORT RT [ms] | 751.73 | 266.78 | 35.49 | 0.00 | 990.00 |
| CHORT MT [ms] | 380.20 | 196.02 | 51.56 | 0.00 | 819.00 |
| HECOR RT [ms] | 509.97 | 133.10 | 26.10 | 0.00 | 719.00 |
| HECOR MT [ms] | 413.10 | 127.87 | 30.95 | 0.00 | 686.00 |
| SPANT RT [ms] | 709.57 | 225.89 | 31.84 | 0.00 | 980.00 |
| SPANT MT [ms] | 465.93 | 186.70 | 40.07 | 0.00 | 873.00 |
| Physical activity (PASE) | | | | | |
| Leisure time activities | 72.86 | 50.65 | 69.52 | 5.50 | 197.99 |
| Household activities | 114.73 | 46.46 | 40.85 | 0.00 | 171.00 |
| Work-related activities | 4.68 | 13.06 | 278.88 | 0.00 | 53.97 |
| Total PASE score | 192.27 | 60.47 | 32.40 | 73.78 | 302.13 |
| Physical activity (accelerometer measurement) | | | | | |
| SPA [min] | 1280.52/day<br>8663.6/week | 1181.24 | 92.25 | 805.00 | 7503.00 |
| LPA [min] | 315.52/day<br>2208.6/week | 113.35 | 35.92 | 150.43 | 590.57 |
| MPA [min] | 22.86/day<br>160.02/week | 21.94 | 96.01 | 0.86 | 86.43 |
| VPA [min] | 0.30/day<br>2.1/week | 0.70 | 234.07 | 0 | 2.00 |
| MVPA | 21.71/day<br>151.9/week | 20.18 | 92.94 | 0.75 | 75.88 |
| step counts | 6391.18/day<br>44738.2/week | 2941.49 | 46.02 | 2411.43 | 12549.70 |
| Average Efficiency [%] | 95.40 | 1.73 | 1.82 | 91.44 | 98.57 |
| Average Total Sleep Time [min] | 410.22 | 86.57 | 21.20 | 221.27 | 593.71 |

x – mean; sd – standard deviation; v – variation; min – minimum; max – maximum

Table 3. Correlation between psychomotor abilities and the declared level of physical activity (PASE)

| | SIRT | | CHORT | | HECOR | | SPANT | |
|---|---|---|---|---|---|---|---|---|
| | RT | MT | RT | MT | RT | MT | RT | MT |
| Leisure | ns | ns | ns | ns | ns | ns | ns | ns |
| Household | -0.45*<br>$R^2$=20.2% | ns | ns | ns | ns | ns | ns | ns |
| Work-related | ns | ns | ns | ns | ns | ns | ns | ns |
| Total PASE score | -0.42*<br>$R^2$=17.6% | ns | ns | ns | ns | ns | ns | ns |

*Statistical significance at α≤0.05;
ns – not statistically significant

Table 4. Correlation between psychomotor abilities and the actual measurements of physical activity (accelerometer measurement)

| | SIRT | | CHORT | | HECOR | | SPANT | |
|---|---|---|---|---|---|---|---|---|
| | RT | MT | RT | MT | RT | MT | RT | MT |
| SPA | ns | ns | -0.39*<br>$R^2$=15.2% | ns | ns | ns | ns | ns |
| LPA | ns | ns | 0.40*<br>$R^2$=16% | 0.40*<br>$R^2$=16% | ns | ns | 0.38*<br>$R^2$=14.4% | ns |
| MPA | ns | ns | ns | ns | ns | ns | ns | ns |
| VPA | ns | ns | ns | ns | ns | ns | ns | ns |
| MVPA | ns | ns | ns | ns | ns | ns | ns | ns |
| STEPS | ns | ns | 0.42*<br>$R^2$=17.6% | ns | ns | ns | 0.38*<br>$R^2$=14.4% | ns |
| Average Efficiency | ns | ns | ns | ns | ns | ns | ns | ns |
| Average Total Sleep Time | ns | ns | ns | ns | ns | ns | ns | ns |

*Statistical significance at α≤0.05
ns – not statistically significant

## Discussion

The aim of the study was to assess the relationship between selected psychomotor abilities (SIRT, CHORT, HECOR, SPANT) and the declared and actual levels of physical activity among women over 65 years of age. For the PASE, the results show a significant negative relationship between SIRT RT and household activities and the total PASE score. In contrast, analysing the actual level of physical activity (accelerometery) revealed a significantly positive relationship between CHORT and SPANT RT vs. step counts and LPA. A significant negative correlation with CHORT RT was found for SPA.

For older people, the WHO recommends at least 150 min MPA (Moderate Physical Activity) or 75 min VPA (Vigorous Physical Activity) per week (WHO, 2010; 2021). According to this study, women had approximately 160 min of MPA per week, so they met the recommendations. In terms of step counts, at least 7.000 steps/day is recommended for older people (Hsueh et al., 2021; Tudor-Locke et al., 2011). The accelerometer data revealed that the women surveyed made more than 6.300 steps a week. This value deviates slightly from the recommendations. The number of 7000–8000 steps may provide better health benefits later in life, including those related to depression and all-cause mortality (Lee et al., 2019; Tudor-Locke et al., 2011) and 7000 steps per day is also comparable to the physical activity guidelines (Chen et al., 2019; Hsueh et al., 2021).

For LPA there are no recommended levels. However, many authors emphasise that increasing LPA may contribute to improved cardiometabolic outcomes (Buman et al., 2015). LPA has also been found to have a beneficial association with diastolic blood pressure (Buman et al., 2010). Other studies, such us in US older adults, show that LPA is associated with lower depression levels (Buman et al., 2010). In Hart et al. (2011), participants engaged in an average of 12.0–15.7 min.day of MVPA whereas in this study, it was 21.7 min. A recent meta-analysis showed a maximum reduction in risk of all-cause mortality for 24 min of MVPA a day (Sagelv et al., 2019).

Although the study group met the WHO recommendations for MPA, this did not translate into higher levels of psychomotor ability. The research by these authors shows that MVPA, MPA and VPA are not significant for psychomotor abilities. In contrast, it was found that CHORT (RT and MT) and SPANT (RT) time increases with higher levels of LPA and number of steps, with a consequent deterioration in the speed of these psychomotor abilities. Different conclusions were reached by Johnson et al. investigating the relationship between LPA and cognitive ability among older adults (Johnson et al., 2016). In this study, higher levels of LPA were associated with higher levels of cognitive function. It was therefore concluded that exercise is a good neuroprotective measure. The results from Rosano et al. (2010) also tend towards similar conclusions. A group of individuals with higher LPA levels simultaneously showed higher brain activation in areas important for processing speed (Rosano et al., 2010). Better associations of LPA — in contrast to MVPA — with cognitive function were also shown by Gothe (2021).

Current physical activity recommendations for older people also revolve around reducing SPA (Sedentary Physical Activity). These authors' analysis, however, showed that as SPA increases, reaction time decreases for CHORT RT (improvement in reaction speed). Fanning et al. completed a study on a group of older people to assess self-regulatory and executive functioning effects by substituting SPA for LPA, MVPA and sleep. The analysis revealed that replacing SPA with sleep was associated with significantly faster reaction times in some of the more difficult tasks. In contrast, the replacement of SPA by LPA and MVPA had no significant effects (Fanning et al., 2017).

For the declared physical activity score using the PASE questionnaire, the study revealed that the higher the total PASE sore and the more physical activities related to housework, the significantly faster the reaction time, SIRT (RT), was for the simplest task.

## Conclusions

The results of the present study indicate the presence of moderate associations between physical activity levels and selected components of psychomotor performance in older women. For the PASE, the findings demonstrated a significant negative relationship between SIRT RT and both household activities and the total PASE score, suggesting that greater engagement in physically demanding housework may be associated with faster reaction speed in the simplest psychomotor task. In contrast, an analysis of objectively measured physical activity using accelerometers revealed significant positive associations between step counts and LPA with CHORT and SPANT reaction times (RT) – indicating slower psychomotor responses – while a significant negative correlation was observed between SPA and CHORT RT, pointing to improved reaction speed with reduced sedentary behavior.

However, these associations do not allow for causal inference. Although previous studies have shown that MVPA, MPA, and VPA are not strong determinants of psychomotor abilities in older women (≥65 years), the present results suggest that both higher sedentary behavior and lower levels of low-intensity activity may co-occur with less favorable values of specific psychomotor parameters. Still, some contradictory patterns emerged – for instance, increased SPA was linked to decreased CHORT RT (better reaction speed), whereas higher LPA and a greater number of steps corresponded with longer RT and MT values in CHORT and SPANT tasks, indicating deterioration in the speed of these psychomotor abilities.

Taken together, these findings imply that the magnitude and direction of associations between activity behaviors and psychomotor performance remain unclear and may vary depending on the type and measurement method of physical activity. Therefore, longitudinal and interventional research is needed to determine whether modifying sedentary time and specific intensities of physical activity can exert a meaningful impact on psychomotor functioning in aging populations.

The study is limited by a small sample size, a single-sex cohort, and its cross-sectional design. The analysis did not account for possible factors that may influence both physical activity levels and psychomotor abilities, including chronic health conditions, medication use, educational attainment, socioeconomic status, cognitive engagement, or participation in social activities.

**Contributions from Individual Authors**

KS, RG, JH, KM, DK designed the research, developed the project concept research plan. KM developed the data collection plan and provided study oversight of the trial in the field. KS, RG, JH, KM analysed the data, wrote the paper and had responsibility for the final content. All authors read and approved the final manuscript.

### Ethics Statement

The ethics protocol was approved by the Bioethics Committee at the University of Rzeszow, Approval No. 19/03/2020. All the subjects signed a written informed consent form before participating in the study.

### Data Availability Statement

The data presented in this study are available from the corresponding author upon reasonable request.

### Financial Disclosure

No funding was received for this study.


### Conflict of Interest

The authors have no conflict of interest to declare.


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# Frequency of Basic Types of Dorsal Hand Vein Patterns in the Slovak Population

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## Abstract

**Introduction**
Dorsal hand vein pattern represents a unique morphological feature of the human body which may serve as a biometric tool for forensic identification.

**Study Aim**
The primary aim of this study was to determine the frequency and distribution of dorsal hand vein patterns in a Slovak adult population, with respect to sex and laterality of the hand.

**Material and Methods**
This study provides a morphological analysis of dorsal hand vein patterns in a sample of 70 healthy adults from the Slovak population. Vein configurations were classified using the 1951 system developed by Suchý, distinguishing four main types: branched, double-branched, simple, and composite.

**Results**
The most frequent patterns were branched and double-branched, while the composite form was rare. No statistically significant differences were found between sexes or between hands, suggesting a high degree of bilateral and intersexual symmetry. A rare morphological subtype, labelled 2N4, appeared exclusively in females on the left hand, potentially reflecting sex-linked vascular variation.

**Conclusion**
The results support the hypothesis that dorsal venous architecture is largely determined by early developmental and genetic factors. Given the pattern stability and inter-individual variability, dorsal hand veins remain a promising biometric marker. Despite limitations related to imprinting technique and assessment subjectivity, the study offers a valuable anatomical reference for future biometric, forensic, or anthropological research.

**Keywords:** venous morphology, biometric identification, sex differences, vascular symmetry

Original article

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## Introduction

The dorsal hand vein pattern represents a unique morphological feature of the human body, which has become a subject of interest in recent decades for researchers in the fields of biometric systems and clinical practice. This pattern is formed by a network of metacarpal and dorsal veins located beneath the skin, creating an individual-specific pattern (Elmegarhi et al., 2018). Due to its high level of uniqueness and stability over a lifetime, dorsal hand vein patterns have emerged as a valuable tool in biometric identification, forensic analysis, and medical diagnostics (Hsu et al., 2011). The dorsal hand vein pattern shows significant individual variability, resulting from a combination of genetic predispositions and external factors. The characteristic pattern is formed by the main metacarpal veins, often complemented by finer branches known as minutiae. These small veins may connect the main branches, encircle them, or form independent structures, contributing to the uniqueness of individual's vein pattern (Elmegarhi et al., 2018; Suchý, 1951a).

Research suggests that the basic type of vein pattern is genetically determined, with individuals possessing a thicker subcutaneous fat layer more frequently exhibiting a less complex and simpler vein pattern (Hung et al., 2022; Suchý, 1951b). However, the genetic basis of variability in dorsal hand vein patterns remains underexplored. The first study was conducted by Suchý (1951b) on a sample of 66 people, including 13 complete families. The results showed similarities in vein patterns not only among siblings but also across generations.

The visibility of the vein pattern is influenced by both external and individual factors. The prominence of veins increases with age due to the natural reduction of subcutaneous fat on the dorsal hand, making both veins and tendons more visible (Hung et al., 2022). Additionally, medical conditions such as long-term intravenous infusions can lead to thrombosis and subsequent vein occlusion, further altering the pattern over time (Fiala et al., 2015). Physical labor and sports activities also increase vein visibility, especially in simple vein patterns, while environmental temperature causes veins to appear bluish in cold and more prominent in warm conditions (Suchý, 1951b; Hung et al., 2022). Sexual dimorphism has been observed, with males generally exhibiting more pronounced veins, particularly in physically active individuals, but these differences diminish with age. The visibility of vein relief is also influenced by factors that affect vasoconstriction or vasodilatation. Vasoconstriction is the process of contracting smooth muscle tissue in the blood vessel walls, narrowing the vessels and increasing the blood pressure, making veins less visible. Factors with vasoconstrictive effects include hormones (norepinephrine, vasopressin), medications and drugs (pseudoephedrine, corticosteroids, nicotine) and dietary substances (caffeine, salt, licorice). On the contrary, vasodilatation causes relaxation of smooth muscles in the vein walls, widening their diameter and increasing blood flow, making veins more prominent and visible. Such factors include prostaglandins, adenosine, histamine, and dietary substances such as garlic, dark chocolate and regular physical activity (Charkoudian, 2010; Hung et al., 2022; Suchý, 1951b; Zrzavý, 1957).

Asymmetry of the vein pattern is another important feature, initially linked to motor dominance, although later studies did not confirm this view (Černáček, 1994; Kaczeńska & Dilling-Ostrowska, 1961; Minor, 1931). Freerksen (1938) found asymmetry between the right and left hands of monozygotic twins, and Suchý (1961) defined four degrees of relative symmetry, with absolute bilateral symmetry occurring in only 0.2% of cases.

In recent years, research on vein patterns has expanded to their use in biometric applications, identification systems, and forensic sciences (Hartung et al. 2020; Wilson 2010). Hartung et al. (2020) demonstrated that dorsal hand vein patterns are sufficiently individual for reliable person identification, with the probability of a random match between two people being less than 1:1000. Their study analyzed vein patterns of 30 participants using standardized grid analysis, quantifying the number of vein crossings and branching in different hand regions. The results indicated a high stability of vein patterns, supporting their use in identification systems. Furthermore, the study highlighted the existence of a "region with reduced vein density" between the proximal halves of the second and third metacarpal bones, where vein density is lower, which may affect identification accuracy in certain areas. Hartung et al. (2020) also noted that factors such as age, sex, and health condition can influence vein visibility and pattern, with veins being more prominent in older individuals and those with low subcutaneous fat.

The vein pattern is immutable, non-transferable, and characterized by high accuracy (Ahmed et al., 2013). In contrast to other biometric features, such as papillary terrain or the iris, which may be sensitive to age-related structural changes, vein pattern identification offers higher security since veins lie deeper under the skin and are less prone to potential forgery (Harchana et al., 2021). Vein pattern imaging systems use infrared diodes and scanners with weather shields, allowing for accurate visualization of veins as the warmest objects in the image. The procedure is non-invasive and easy to perform under standardized conditions with an error rate of just 0.01% (Rak et al., 2018; Wilson, 2010). Unlike face or iris scanning, which may be influenced by external factors such as lighting or wearing glasses, vein patterns provide more stable and reliable results in various operational conditions. Vein pattern technology thus meets all the main characteristics of a good biometric trait: universality, uniqueness, permanence, ease of collection, high accuracy, and wide acceptance worldwide (Harchana et al., 2021).

The primary aim of this study was to determine the frequency and distribution of dorsal hand vein patterns in a Slovak adult population, with respect to sex and laterality of the hand. The research aims to deepen the understanding of anatomical variability and assess the potential forensic and biometric applications of vein pattern analysis. By enhancing knowledge in this field, the study seeks to contribute to the development of more accurate and secure identification systems for forensic and security purposes.

## Material and Methods

This study examined a total of 70 participants from Slovakia, consisting of 33 females and 37 males, with an age range of 18 to 83 years and an average age of 39.49 years. Participation was voluntary and written informed consent was obtained. The consent included details about the study, such as its aims, methodology and other participation information. Withdrawal from the study was possible at any time. For each participant, the following information was recorded: identification number, sex, age, and any familial relationship with other participants. Imprints of the dorsal hand veins were obtained from both the right and left hands of each participant. To document the dorsal hand vein patterns, a method described by Suchý (1951b) was applied. This method involves taking imprints of the superficial veins on the dorsal surface of the hand and is practical, fast, and applicable in various settings. However, it may be influenced by subjective error, especially in individuals with a thicker layer of subcutaneous fat.

The equipment used included an Esmarch bandage, oily cream, a fine brush, stamp ink diluted with water at a ratio of 1:50, filter paper (135 mm x 85 mm), a pencil, as well as cotton and cleaning agents such as benzine and warm water. The procedure to enhance vein visibility included several techniques: compression of the veins on the palmar side of the forearm using the middle three fingers with the thumb resting on the ulna approximately 5 cm from the *olecranon ulnae* compression at the level of the *processus styloideus radii* and *processus styloideus ulnae* with simultaneous dynamic finger exercises (alternating finger spreading and fist clenching); application of an Esmarch bandage above the elbow combined with wrist movements; application of an Esmarch bandage below the elbow with finger exercises; immersion in warm water; and rapid immersion in cold water (Suchý, 1951b).

Subsequently, the dorsal surface of the hand was coated with oily cream in the metacarpal and carpal areas, and ink was applied along the visible veins using a brush in the direction from the fingers. The vein paths were marked while the participant's hand was clenched into a fist to minimize the influence of factors such as physical activity, sports, or age. The vein patterns were then transferred by pressing filter paper onto the inked dorsal surface of the clenched hand. The paper was placed perpendicular to the longitudinal axis of the hand, with the label positioned on the radial side. Finally, the interphalangeal joint spaces were numbered with a pencil directly on the hand, starting from the thumb (marked as one) to the little finger (marked as five).

## Evaluation of Vein Patterns

The dorsal hand vein system was assessed according to the methodology of Suchý (1951b). The evaluation included classification based on the ratio of radioulnar branch distribution and the branching type of the ulnar segment. The venous formula was determined according to Suchý's numerical system (1961). The radioulnar segments of the dorsal venous network are typically composed of an ulnar segment and a radial segment (Figure 1). The ulnar segment usually originates from the second, third, or fourth interphalangeal spaces and contributes significantly to the venous pattern. The radial segment predominantly drains the thumb and radial side of the index finger and often includes a prominent branch arising from the second interphalangeal space. Each segment has a principal vein trunk. In the ulnar segment, this is typically a branch from one of the mentioned interphalangeal spaces, while in the radial segment, the main trunk most commonly originates from the second interphalangeal space or the radial side of the index finger if no connection to this space is present. In rare cases, the pattern also includes the *vena basilica* when it connects to the ulnar trunk of the *vena cephalica* before crossing the limiting line defined by the so-called limiting line of the dorsal venous pattern, which runs between the *processus styloideus radii* and *processus styloideus ulnae* (Suchý 1951b).

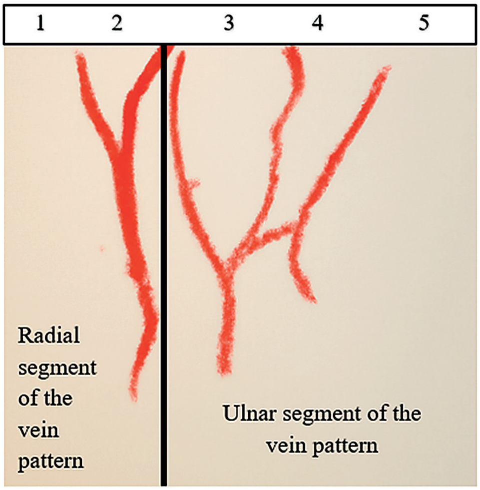


Figure 1. Original imprint of the dorsal hand vein pattern on the left hand (1–5 indicate the numbers of the interphalangeal spaces)

Vein patterns were classified visually by one rater according to the criteria described by Suchý (1951). The classification was based on direct visual comparison of the imprints with defined morphological types. The assessment was not blinded, as the rater had access to demographic and hand-side information during evaluation. Demographic data are essential for interpreting responses accurately, as the data access to demographics allows to assess whether responses align with expected norms. Interobserver reliability was not tested, as all patterns were evaluated by a single examiner, trained to recognize and minimize personal biases. These aspects may represent methodological limitations and should be considered when interpreting the results.

The decision to use the classification system described by Suchý (1951) was based on its historical relevance and frequent application in Central European anthropological studies. Despite the existence of modern, image-based classification systems, the Suchý method (1951) remains suitable for visual evaluation from ink-based imprints, which was the chosen technique in this study.

Additionally, the use of ink transfer may have introduced minor artifacts such as smearing, uneven pressure, or incomplete contact with the skin, potentially affecting the accuracy of recorded vein patterns. These limitations were minimized through standardized procedures but cannot be entirely excluded.

Vein pattern types were determined based on Suchý's (1951b) classification system (Figure 2), which divides dorsal hand vein patterns into two primary categories: single-trunk vein patterns and composite vein patterns.

Single-trunk vein patterns include three subtypes (Figure 3):

1. Branched pattern – three veins converge: the main trunk arises from the third space and receives branches from the second and fourth spaces. Variants include: Type A–the branch from the fourth space drains into the main trunk more distally than the branch from the second space; Type B–branches from the second and fourth spaces drain at approximately the same level; and Type C– the branch from the fourth space drains proximally relative to the branch from the second space.
2. Double-branched pattern (Type Y) – the main trunk splits into two venous arms, each forming a Y-shaped bifurcation, resembling the letters "YY." Variations such as VY, YV, and VV may occur.
3. Simple pattern (Type N) – composed of two branches originating from either the second and third, third and fourth, or second and fourth spaces. The overall pattern resembles the letter "N" or "H".

The second main category is the composite vein pattern (Type S), where branches originate from the radial trunk of the *vena cephalica*, often extending into the area of the second interphalangeal space.

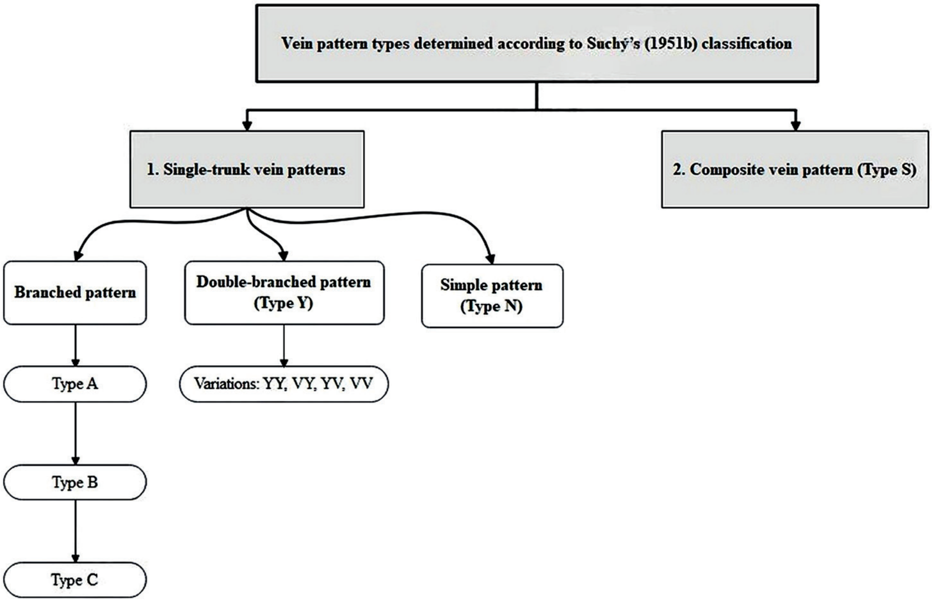


Figure 2. Classification of dorsal hand vein patterns according to Suchý (1951b)

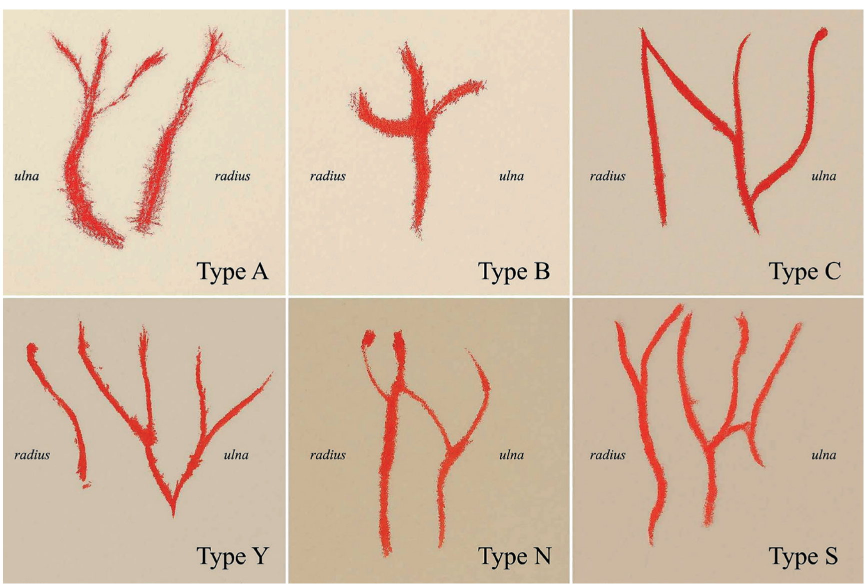


Figure 3. Basic types of dorsal hand vein patterns according to Suchý (1951b): Type A–C (branched variants), Type Y (double-branched), Type N (simple), and Type S (composite pattern with radial contribution)

## Statistical Analysis

The observed vein pattern types were evaluated as qualitative traits, and their frequencies in the study population were expressed as percentages (p). For each percentage value, the standard error (sp) was calculated using the formula:

$$sp = \frac{\sqrt{p \times q}}{N(\%)}$$

Where p = observed percentage, q = 100 − p, N = total number of observations.

To assess the significance of differences between frequencies, Fisher's exact test and the chi-square test were used. Statistical calculations were performed using online calculators available at www.graphpad.com and www.quantpsy.org. The results were compared with critical values at a 5% significance level. A p-value of less than 0.05 was considered statistically significant.

## Results

A total of 140 dorsal hand vein imprints (from 70 individuals; 66 female and 74 male hands) were analyzed, with imprints taken from both the left and right hands. Radioulnar segments of the dorsal venous network were generally well developed. In 98.57% of individuals, both the radial and ulnar parts of the vein pattern were simultaneously present on the dorsal surface of the hands. Only one participant (1.43%) exhibited a vein pattern consisting solely of the ulnar component.

Across both hands and sexes, the most frequently observed pattern was the branched configuration (A–C), found in 37.14% of all cases. This was followed by the double-branched (Type Y) pattern with a frequency of 31.43%, and the simple (Type N) pattern, occurring in 24.29% of cases. The composite (Type S) vein pattern was rare, comprising only 7.14% of the total sample (Table 1).

Vein pattern frequencies varied slightly between sexes. For the right hand, the simple pattern (N) occurred in 33.33% of females and 21.62% of males (p = 0.30), while the branched pattern (A–C) appeared in 39.39% of females and 29.73% of males (p = 0.86). On the left hand, the double-branched pattern (Y) was present in 30.30% of females and 32.43% of males (p = 0.20). Across all types, inter-sex differences were not statistically significant (Table 1, Figure 4).

No statistically significant bilateral differences were observed between the right and left hands for any vein pattern type in either sex. In males, the most noticeable asymmetry appeared in the branched pattern, which was more frequent on the left hand, while in females, the double-branched pattern showed a slightly higher prevalence on the left. For the composite pattern, minor side differences were noted in both sexes. However, none of these differences reached statistical significance, with bilateral *p*-values ranging from 0.23 to >0.99 (Table 1), indicating an overall symmetry in vein pattern distribution.

Vein formulae were established for each hand using Suchý's classification system (1951b, 1961), taking into account the origins and connections of individual vein branches. Most individuals showed symmetrical or near-symmetrical formulae between their right and left hands, although complete symmetry (identical formulae) was rare.

Table 1. Frequency of basic dorsal hand vein pattern types by sex and hand side

| Sex | Male (M) | | *p | Female (F) | | **p | Total | p-value (M vs. F) |
|---|---|---|---|---|---|---|---|---|
| Hand | right | left | | right | left | | | |
| Branched (A–C) | 11 | 17 | 0.23 | 13 | 11 | 0.80 | 52 | 0.863 |
| % | 29.73 | 45.95 | | 39.39 | 33.33 | | 37.14 | |
| Double-branched (Y) | 15 | 12 | 0.63 | 7 | 10 | 0.57 | 44 | 0.204 |
| % | 40.54 | 32.43 | | 21.21 | 30.30 | | 31.43 | |
| Simple (N) | 8 | 7 | >0.99 | 11 | 8 | 0.59 | 34 | 0.324 |
| % | 21.62 | 18.92 | | 33.33 | 24.24 | | 24.29 | |
| Composite (S) | 3 | 1 | 0.61 | 2 | 4 | 0.67 | 10 | 0.516 |
| % | 8.11 | 2.70 | | 6.06 | 12.12 | | 7.14 | |
| Total | 37 | 37 | | 33 | 33 | | 140 | |

*p-value: bilateral differences of basic dorsal hand vein pattern types in males

**p-value: bilateral differences of basic dorsal hand vein pattern types in females

p-value (M vs. F): differences in vein pattern types between males and females (combined data from both hands)

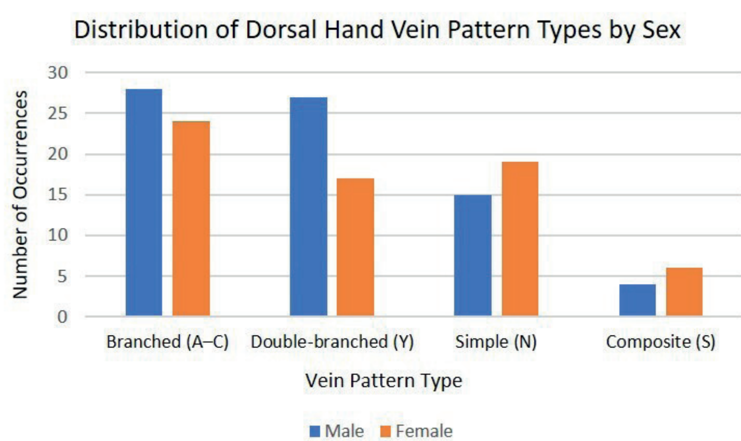


Figure 4. Frequency distribution of dorsal hand vein pattern types in males and females

## Discussion

This study provides a detailed overview of the frequency and distribution of basic dorsal hand vein pattern types in a sample of the Slovak adult population. The most common patterns observed were the branched type (A–C) and the double-branched type (Y), followed by the simple type (N). The composite pattern (S) was found to be the least prevalent. These findings are consistent with the notion that the venous architecture of the dorsal hand typically follows a branching morphotype, allowing for efficient drainage through multiple interconnected pathways.

Although slight differences in the frequency of certain patterns were observed between males and females, none of these differences reached statistical significance. For example, the simple pattern (N) appeared more frequently in females than in males, particularly on the right hand (33.33% vs. 21.62%; $p = 0.30$), while the branched pattern was more common in males. However, Fisher's exact test revealed that these sex-related differences were not statistically significant ($p = 0.204–0.863$). These findings suggest that sex may not play a major role in determining the morphotype of dorsal hand vein patterns, at least within this sample.

Interestingly, a specific morphological variant of the simple type, labelled as 2N4 (indicating origin from the second and fourth interphalangeal depressions), was observed exclusively in females and only on the left hand. While the frequency of this pattern was low, its localized appearance may suggest an underlying anatomical or developmental predisposition, potentially influenced by sex-linked vascular variation. These finding merits further investigation in larger or age-stratified cohorts.

The high occurrence of the 2N4 type in the analyzed Slovak population may be related to specific developmental, genetic, or population-based factors. Several studies suggest that dorsal hand vein patterns are stable, unique, and likely influenced by early vascular development and inherited traits (Alashik & Yildirim, 2021; Hsu et al., 2011).

Some infrared imaging studies indicate that dorsal vein morphology exhibits inter-individual variability, which may also reflect underlying demographic or population-level differences (Hartung et al., 2020; Wang et al., 2021).

From a biometric perspective, the high frequency and localized presence of the 2N4 type may enhance recognition accuracy if included in algorithmic templates. Recent studies using deep learning and vein segmentation techniques, for example graph-based pattern matching or U-Net variants, have confirmed that regular, well-defined vein configurations significantly improve biometric performance (Lefkovits et al., 2022; Lajevardi et al., 2014).

Regarding bilateral distribution, no significant differences were found between the right and left hands within individuals. Frequencies of vein pattern types on both sides were similar across the population. The branched and double-branched types together accounted for more than 68% of patterns on each hand, with minor variations. Statistical analysis did not reveal significant bilateral asymmetry (e.g., $p = 0.23$ for branched type in males; $p = 0.80$ in females), with bilateral p-values ranging from 0.23 to >0.99 in males and from 0.57 to 0.80 in females (Table 1).

Vein pattern formulae were established for each hand using Suchý's classification system (1951b; 1961), taking into account the origins and connections of individual vein branches. Most individuals showed symmetrical or near-symmetrical formulae between their right and left hands, although complete symmetry (identical formulae) was rare. This variability may reflect functional adaptation or minor individual anatomical variation.

The high prevalence of both radial and ulnar components in 98.57% of participants further confirms the typical bipartite organization of dorsal hand venous networks. The single case exhibiting only the ulnar component could represent a natural anatomical variant or an underdeveloped radial segment, but given its rarity, no general conclusion can be drawn from this observation.

Although this study focused on a morphological classification using anatomical imprint analysis, several recent works have demonstrated the utility of imaging-based and biometric approaches. For instance, Hsu et al. (2014) applied Gaussian directional filters to infrared images to enhance vein patterns for biometric recognition, achieving robust performance (Hsu et al., 2014). Similarly, Huang et al. (2015) combined vascular and subcutaneous tissue features for hand dorsum identification using multi-source key points and vein textures (Huang et al., 2015). While these approaches differ methodologically, they reinforce the anatomical assumption of individual uniqueness and bilateral symmetry in dorsal venous architecture. This supports the relevance of our findings, even when interpreted through modern biometric frameworks.

In recent years, research in the field of dorsal hand vein biometrics has focused on the use of advanced technologies such as infrared (IR) imaging and deep learning. These methods allow for more precise capture and analysis of vein patterns, thereby increasing the reliability of identification systems.

For example, the study by Hsu et al. (2011) presents an approach to personal authentication using dorsal vein patterns through infrared imaging, achieving high identification accuracy. Further research by Nayebi and Turgut (2021) demonstrates the effectiveness of deep neural networks in classifying vein patterns, reaching an accuracy of up to 99.64%. These studies confirm the potential of modern technologies in the field of vein pattern biometrics.

There are several limitations that should be acknowledged. First, the sample size, while sufficient for exploratory analysis, may not allow the detection of small but meaningful differences, especially in less common pattern types such as the composite form. It should also be noted, that the data analyzed in the study were not collected with regard to factors that could affect the visibility of veins. Second, the sample was limited to healthy adults from a single geographic region, which may reduce the generalizability of findings, although the rater has been trained to recognize and minimize personal biases. Third, although the ink method used for imprint collection was effective for visualizing superficial vein patterns, it may not have captured deeper venous structures or minor vessels with less prominence. Fourth, the classification of vein patterns was conducted visually by a single rater without blinding or interobserver reliability testing, which may introduce potential observer bias. Future research should include digital infrared imaging techniques and larger, more diverse populations to validate the distribution of dorsal hand vein patterns. In addition, the use of ink transfer may have introduced artifacts such as smudging or incomplete imprinting, which could have affected the clarity or accuracy of some recorded vein patterns. Furthermore, confidence intervals were not reported for frequency comparisons, which limits the precision of estimated differences and reduces the statistical interpretability of the results.

Despite these limitations, the present study provides a valuable contribution to the documentation of dorsal venous patterning in the Slovak population and establishes a useful reference for future anatomical, biometric, or forensic research.

The observed stability of major pattern types and lack of sex-based differences suggest a relatively conserved morphological structure that may be applicable across broader populations.

## Conclusion

The present study provides a detailed characterization of dorsal hand vein pattern types in a sample of the Slovak adult population. Among the basic morphotypes analyzed, the branched (A–C) and double-branched (Y) patterns were the most prevalent, while the composite type (S) occurred rarely. No statistically significant differences were found between sexes or between hands, suggesting a stable and bilaterally symmetrical venous structure. The rare occurrence of specific variants, such as the 2N4 pattern found exclusively in females, highlights the need for further research into individual and sex-linked morphological differences. These findings contribute to the growing body of knowledge on superficial venous anatomy and may serve as a useful reference for anatomical classification, biometric identification, and forensic applications. The results may be used in the development of biometric systems or as an auxiliary identification criterion in forensic anthropology.

### Contributions from Individual Authors

Petra Švábová: Methodology, Investigation, Writing – original draft, Writing – review & editing, Project administration. Zuzana Kozáková: Writing – review & editing. Investigation, Methodology. Stela Orosová: Writing – review & editing, Mária Chovancová: Investigation, Data curation, Writing – review & editing. Zuzana Matušíková: Investigation, Data curation. Radoslav Beňuš: Methodology, Project administration, Data curation. All authors have read and approved the final version of the paper.

### Ethics Statement

This project was reviewed and approved at the department level in accordance with internal regulations. Informed Consent was obtained from the participants prior to their participation in the study. The study was conducted in accordance with the Declaration of Helsinki.

### Data Availability Statement

Data are available from the corresponing author upon reasonable request.

### Financial Disclosure

None to declare.

### Conflict of Interest

The authors declare that there is no conflict of interest regarding this manuscript.

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# Sex Estimation using Footprint Ridge Density – A Mini Review

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## Abstract

**Introduction**

The epidermal level of human footprint possesses raised ridges and furrows, which form unique patterns. Ridge density quantification, involving counting the ridges within a predefined unit area, emphasizes variability due to differences in epidermal ridge thickness. Analyses of these ridges exhibit sex-based differences, making it important for forensic identification purposes.

**Study Aim**

The present mini review was conducted to showcase a targeted synthesis of seven research articles related to sex estimation using Footprint Ridge Density (FPRD).

**Methods**

Relevant studies were identified through the search engines Google Scholar, PubMed, Scopus and ResearchGate, using Boolean operators and/or, between 2010 and August 2025.

**Results**

Higher FPRD were observed in females and statistically significant sexual dimorphism was inferred across plantar and toe regions, particularly at the medial ball and selected toe areas.

**Conclusions**

This review provides key inferences from the existing literature and points out the potentiality of using FPRD as a reliable parameter in sex estimation studies to improve forensic procedures.

**Keywords:** Human morphology, Forensic podiatry, Human identification, Footprints, Ridge density analysis, Sexual dimorphism

Original article



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## Introduction

Human body impressions serve as an important element in understanding human morphology. These include imprints on the external ears, lips, fingers, palms and foot. While each type exhibits different aspects of individuality, fingerprints, palm prints and footprints, in particular, contain raised lines on the dermal layers of the skin, forming complex ridges and furrows (Naffah, 1977). The development of these dermal papillary ridges of a human fetus usually starts taking shape after the 10th week of the intrauterine period of the gestation phase and continues till approximately to the 17th or 18th week (Babler, 1991). The pattern development is primarily determined by genetic and hereditary factors (Patnaik et al., 2024), coupled by several environmental factors such as variation in pressure distribution of amniotic fluid, movements that happen due to nutritional and hormonal factors, changes in womb's temperature and placental oxygen levels. However, these factors predominantly affect the formation of the papillary ridges only until the end of the third month of intrauterine development (Babler, 1991; Schaumann & Alter, 1976). Following the development and maturation of the primary and the secondary epidermal layers, the ridges remain unaltered throughout the life of an individual and even persist in the post-mortem phase until the onset of decomposition of the body. This renders the analyses of epidermal ridges as a significant marker in forensic perspectives (Champod & Evett, 2001; Gutiérrez-Redomero et al., 2013).

Bare footprints, as a form of evidence, fall within the field of forensic podiatry. They are frequently reported at floor surfaces and insoles of shoes in various crime sites like suicides, burglaries, physical assaults, thefts and murders. The visualization of dermal ridge patterns from footprints often depends on the surface type and the location in which the evidence is recovered, equivalent to fingerprinting (Liu, 2025; Malik & Bashir, 2023). Deformable surfaces require techniques like impression casting with plaster or dental stones, whereas smooth surfaces like glass require the use of powders like magnesium and aluminum or chemical reagents like ninhydrin, iodine solution and silver nitrate. Further, methods like Vacuum Metal Deposition (VMD) or Alternative Light Sources (ALS) like ultraviolet (UV), infrared (IR) and blue lights facilitate in-depth visualization and analysis by adhering vaporized metals to residues for ridge enhancement, ensuring accurate analyses of plantar ridges (Campo, 2018; Dhaneshwar et al., 2021; Reichardt et al., 1978).

Two important parameters are usually considered in the forensic analysis of ridge density at the plantar epidermis: the thickness of a ridge and the distance between two ridges. Analysis of the two aspects exhibit sex-based differences across individuals and populations. However, it is equally important to analyze footprints beyond forensic and legal perspectives, since forensic podiatry limits itself on the morphological and metric features of footprints that can be used for personal identification. Understanding specific dermatoglyphic patterns can also be deemed critical to encompass anthropological contexts, particularly to assess biological variability (Cummins & Midlo, 1926; Kanchan et al., 2012; Naffah, 1977).

As Fingerprint Ridge Density (Acree, 1999; Das et al., 2024; Dhall & Kapoor, 2016; Gutiérrez-Redomero et al., 2008;

Kapoor & Badiye, 2015; Nayak et al., 2010; Polcerová et al., 2022; 2023; Qi et al., 2022; Soanboon et al., 2016) and Palmprint Ridge Density (Ali & Ahmed, 2020; Gutiérrez-Redomero & Alonso-Rodríguez, 2013; Kanchan et al., 2013; Krishan et al., 2014; Mohamed et al., 2020) have been widely studied, revealing consistent sexual dimorphism across various palmar regions, the present mini review identified a significant gap in research- very few studies have explored the forensic applicability of sex estimation using Footprint Ridge Density (FPRD). This mini review aims to provide a detailed evaluation of methodological approaches, key findings and the significance of FPRD on the basis of existing studies conducted among different populations. The inferences may add up to the existing information on the relevance of footprints as forensic evidence and beyond.

## Methodology

For the present mini review, the terms „Footprint Ridge Density", „Sex Estimation" and „Forensic Podiatry" were searched on Google Scholar, PubMed, Scopus and ResearchGate as part of literature identification and screening using Boolean operators and/or. The inclusion criteria required articles that were written in English language and centered around FPRD, addressing its applicability in determining sex-based variability. Following the eligibility assessment, seven articles were reviewed that met the inclusion criteria between 2010 and August 2025, while two articles were excluded due to unavailability of the texts (Moorthy and Hairunnisa 2020, Moorthy et al. 2022). A flowchart depicting a schematic understanding of selecting the research articles is presented below (Fig. 1).

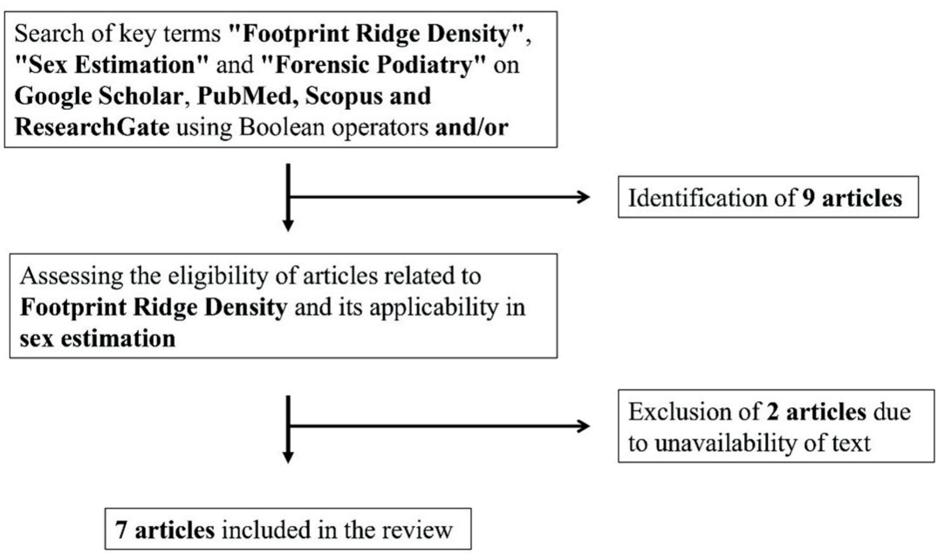


Figure 1. Schematic representation of selection of research articles for the present review on sex estimation from Footprint Ridge Density (FPRD)

**Footprint Ridge density (FPRD) analysis: Methodological overview**

Ridge density quantification entails counting dermal ridges within predefined anatomical regions. It includes methodologies like measuring ridge counts between the core of the fingerprint patterns and the triradius (delta) (de Jongh et al., 2024) and between individual interdigital triradii in palmprints (Jerković et al., 2021). Similarly, Acree (1999) introduced a methodology to count ridges in fingerprints, which has become widely accepted in similar studies worldwide. He selected a predefined topological area of 5 × 5 mm outside the central core region of the radial side in bilateral fingers and diagonally counted the ridges to emphasize its variability due to differences in epidermal ridge thickness. This methodology was incorporated in the evaluation of FPRD by Kanchan et al. (2012) in a controlled environment. They examined four specific regions on the plantar surface of the foot to measure ridge density: at the medial side of the 1st toe, at the ball region of the 1st toe (also referred to as the medial ball), at the lateral ball region located beneath the triradius of the 5th toe, and at the central region of the heel. Moorthy and Hairunnisa (2018a) followed Acree's (1999) methodology, but further expanded the number of measurable regions by incorporating the medial regions of each toe. Traditional studies analyzing ridge density patterns in footprints have focused on the depicted anatomical regions (see Fig. 2), with their statistical evaluations employing Receiver Operating Characteristic (ROC) and Area Under the Curve (AUC) analyses for the purpose of identifying robust indicators and their sex classification accuracy. More recently, Budka et al. (2021) revolutionized this traditional ridge count technique by replacing manual analysis with convolutional neural networks (CNNs) application to estimate sex from 2D footprint images. They formulated 10 mm$^2$ sampling squares from the entirety of a footprint, and developed learned models based on size, shape and texture of footprints that included incomplete or noisy ridge segments typical of ideal prints recovered from a crime scene.

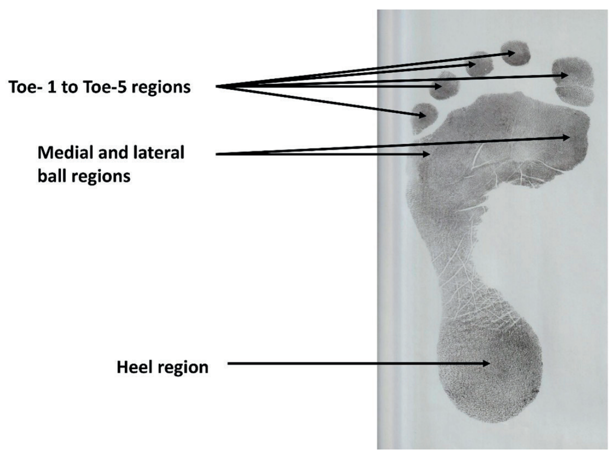


Figure 2. Illustration of the anatomical regions in a footprint used for sex estimation from Footprint Ridge Density (FPRD)

**Review of literature on sex estimation from FPRD**

Kanchan et al. (2012) assessed the potential use of FPRD for sex estimation among 106 subjects from a medical college in Mangalore, Southern India, who were between the ages of 20 and 25. Findings inferred mean FPRD was 11.9 ridges/25 mm$^2$ or more for females and 10.4 ridges/25 mm$^2$ or less for males for medial ball region on the right side, and deemed most accurate region for sex determination, out of 212 footprints that were examined. Conversely, the heel area showed the least variation, with a FPRD value of 10.4 ridges/25 mm$^2$ or less was likely to be a male, and 11.0 ridges/ 25 mm$^2$ or more was considered as a female. The analyzed areas demonstrated statistically significant ($p < 0.05$) mean ridge density values, confirming the sex-based variation. Further, ROC curve analysis predicted medial ball region had the highest potential for sex discrimination, with an AUC value of 77.8% for the left foot and 86.5% for the right, followed by medial region of 1st toe, lateral ball and heel region. The researchers concluded that females have a higher ridge density than males, perhaps as a result of their finer ridges and narrower valleys in their dermatoglyphic patterns, showcasing the applicability of FPRD in estimating sex.

Sex differences from FPRD were examined by Krishan et al. (2015) among 160 college students of Shimla town in North India, aged 18 to 25 (39 males and 121 females). Based on the outcomes, females possessed a higher mean ridge density than males in each defined region of the footprints. The highest FPRD differences were observed in the medial ball region, where males showed a density value of 7.3 ridges/25 mm$^2$ or less, and females recorded a ridge density of 8.7 ridges/25 mm$^2$ or more. On the other hand, the heel region showed the least amount of variation between the sexes; for males, the discriminative accuracy was approximately 6.7 ridges/25 mm$^2$ or less, while for females, it was likely 7.5 ridges/ 25 mm$^2$ or more. On evaluating the sexing potential of ridge density through the ROC curve, right footprints demonstrated greater discriminatory power, and the medial ball region possessed the highest AUC of 85.3%, followed by 1st toe (81.7%), lateral ball (80.1%) and heel region (69.2%). The sexing potentials of the left and right footprints were 77.7% and 91.5%, respectively, according to ROC analyses of the total FPRD value.

Moorthy and Hairunnisa (2018a) conducted an investigation on the Bidayuh population of Malaysia (100 males and 100 females). They examined sex differences from FPRD in eight different areas among 400 footprints- medial regions of each toe, medial and lateral ball regions and heel region. Highest mean ridge density in males was found in 3rd and 4th toes ($\leq$ 13.71 ridges/25 mm$^2$), whereas females were identified by values of $\geq$ 14 ridges/ 25 mm$^2$. ROC curve analysis demonstrated strong sexing potential, with the right 1st toe yielding the highest AUC of 97.3%, followed by left 3rd toe (97.0%) and right medial ball (96.8%).

Moorthy and Hairunnisa (2018b) further evaluated toeless FPRD from the Iban ethnic group of Malaysia. The sample consisted of 200 individuals (100 males and 100 females) of age group 29–69 years. They analyzed ridge density in three regions: medial ball region, lateral ball region and heel region. Mean FPRD values showed medial ball region showed the highest discriminatory power for sex determination in the right side (male – 10.75 ridges/ 25 mm$^2$ or less; female – 12.18 ridges/ 25 mm$^2$ or more), followed by lateral ball region, while heel region demonstrated the lowest predictive strength. ROC analyses confirmed these findings, inflicting high sexing potential of FPRD in medial ball region (AUC – 94.8% for right side, 92.1% for left side), followed by lateral ball region (AUC – 95.7% for right side, 88.6% for left side) and heel region (AUC – 87.2% for right side, 92.6% for left side).

Moorthy and Hairunnisa (2019) examined sex differences from toeprint RD among 100 males and 100 females of the Melanau indigenous population in Malaysian Borneo Island. They found female ridge densities to be significantly higher in comparison to male ridge densities, indicating gender variation in all bilateral toes. For the left side, 3rd toe RD showed higher gender variation ($t = 6.782$), while for the right side, variations were recorded the highest in 4th toe ($t = 8.689$), followed by 3rd toe ($t = 8.101$).

Moorthy et al. (2021) analyzed sex variations from toeprint RD among the Kagay-Anon population of Cagayan de Oro city in Philippines; examining 4000 toe prints from 201 females and 199 males. ROC analyses determined the 3rd toe of the left side (AUC – 68.8%) and 4th toe of the right side have the highest sexing potential (AUC – 59.3%).

Budka et al. (2021) studied the sex estimation of the British population, aged 16 to 81, using texture density, size and shape of footprints. The researchers used CNNs to analyze two datasets: a pilot dataset of 196 footprints and a larger dataset of 2677 footprints. Their research highlighted the heel region, and the region between the 1st and 2nd toe as important contributors in discriminating sex. These regions were further validated by Grad-CAM heatmaps as being significant to the model's decision-making process. The study demonstrated the potential of implementing algorithms trained with machine learning to identify sex by achieving an accurate prediction of nearly 83% on the larger dataset for texture analysis and ~ 90% upon including all the variables.

## Discussion

Forensic assessments utilizing dermatoglyphic prints from the human body offer a cost-effective and non-invasive tool for human identification purposes. These prints exhibit inherent variations in ridge configurations, which form the basis for FPRD analysis (Kanchan et al., 2012). All the reviewed literature analyzed FPRD of populations with varied demographic backgrounds. While, Kanchan et al. (2012) studied young adults aged 20–25 years from Southern India, Krishan et al. (2015) considered students aged 18–25 years from Northern India. In contrast, Moorthy and Hairunnisa (2018a;b) researched on indigenous groups of Malaysia aged 29–69. Budka et al. (2021) further included British volunteers spanning a wide age range of 16–81 years, all demonstrating the validity of FPRD across diverse life stages while taking into consideration demographics that differ both genetically and environmentally.

The reviewed literature consistently highlighted significant sex-based differences in ridge density of footprints. For example, Kanchan et al. (2012) found females exhibited a mean FPRD of ≥ 11.9 ridges/25 $mm^2$ and males displayed a value of ≤ 10.4 ridges/25 $mm^2$ in the medial ball region of the footprint. Similarly, Moorthy and Hairunnisa (2018b) reported higher mean FPRD values in females (≥ 12.18 ridges/25 $mm^2$) compared to males (≤ 10.75 ridges/25 $mm^2$) also in the medial ball region. These outcomes are deemed similar to Acree's (1999) preliminary research related to ridge density study of fingers, which established sex-specific ridge density thresholds of ≥ 12 ridges/25 $mm^2$ for females and ≤ 11 ridges/25 $mm^2$ for males. An analogous outcome was also observed in a study on the hypothenar region of palmprints, with females recording a mean ridge density of ≥ 13.5 ridges/$cm^2$, compared to ≤ 11.8 ridges/$cm^2$ for males (Kanchan et al., 2013). This parallel observation of higher ridge densities in females can be attributed to their finer ridge detailing and narrower valleys, as a characteristic of dermatoglyphic patterns across footprints, fingerprints and palmprints.

Moreover, variations in predictive capabilities across footprint regions highlights the need to generate unique models for sex estimation using FPRD. For example, medial ball region was identified as having higher discriminatory power for sex estimation in three reviewed studies (Kanchan et al., 2012; Krishan et al., 2015; Moorthy & Hairunnisa, 2018b), while Budka et al. (2021) highlighted heel regions as accurate most sex predictor. Heathfield et al. (2016) further supported the significance of heel region in their study to find out ethnic differences, comparing FPRD between two South African populations. The disparity indicates the population-specific nature of FPRD and the limitations of applying generalized prediction models universally, affirming one of the core principles of forensic anthropology. It also highlights the need of including every region in FPRD-based studies since it is very unlikely to predict which region of the footprint might be recovered from a crime scene. Table 1 summarizes the reviewed studies and lists accurate most regions of sex estimation from FPRD.

Table 1. Overview of the demographic backgrounds, sample size considered, footprint regions studied and their most discriminatory sex prediction region of the selected Footprint Ridge Density (FPRD) literature

| Study reference | Demographic background | Sample size | Footprint regions | Most accurate sex predictor |
|---|---|---|---|---|
| Kanchan et al. (2012) | South Indian population | 56 males and 50 females | 1st toe, medial ball, lateral ball, heel | Medial ball |
| Krishan et al. (2015) | North Indian population | 39 males and 121 females | 1st toe, medial ball, lateral ball, heel | Medial ball |
| Moorthy and Hairunnisa (2018a) | Bidayuh ethnicity, Malaysian population | 100 males and 100 females | 1st–5th toe, medial ball, lateral ball, heel | Heel |
| Moorthy and Hairunnisa (2018b) | Iban ethnicity, Malaysian population | 100 males and 100 females | Medial ball, lateral ball, heel | Medial ball |
| Moorthy and Hairunnisa (2019) | Melanau ethnicity, Malaysian population | 100 males and 100 females | 1st–5th toe | 3rd toe, 4th toe |
| Budka et al. (2021) | British population | Pilot – 101 males and 132 females; Larger dataset – 1194 males and 1483 females | Toe regions, medial arch, lateral arch, ball of the foot, and heel | Heel, region between toes |
| Moorthy et al. (2021) | Kagay-Anons, Philippine population | 201 males and 199 females | 1st–5th toe | 3rd toe, 4th toe |

It is also evident from the studies reviewed that FPRD have shown promising percentages of accuracy in sex estimation, since Kanchan et al. (2012), Krishan et al. (2015) and Moorthy and Hairunnisa (2018b) reported medial ball region's high discriminatory power with ROC analyses revealing AUC values of 86.5%, 85.3% and 94.8% respectively. These robust prediction rates align with similar investigations done on finger and palmar ridge densities. For instance, Qi et al. (2022) reported over 90% accuracy with supervised learning settings, while Das et al. (2024) achieved an accuracy exceeding 80% using traditional methods, both from fingerprints. A success rate over 80% was also reported in research on palmprint ridges analysis (Gutiérrez-Redomero & Alonso-Rodríguez, 2013). Comparisons thus infer FPRD can be seen as a reliable and consistent biometric parameter to establish sex differences.

Technological advancements like the utilization of Artificial Intelligence (AI) algorithms underscore the potential to extract meaningful footprint patterns and establish sex-based variability from ridge analyses (Budka et al., 2021). This progress aligns with the broader biometric application of AI in ridge density analyses of other body prints, as noted by the use of autoencoder networks on fingerprints (Qi et al., 2022).

**Limitations and future applications**

Numerous studies exist that have examined morphometric characters of footprints to estimate sex. However, research related to quantification of plantar ridges is still at an initial level. A key limitation in the majority of existing studies till date is the uptake of smaller sample size and lacking consideration of environmental variations in previous studies, which may restrict the generalizability of the findings. Thus, the present mini review voices for potential use and standardization of FPRD as a parameter for sex identification. It highlights the necessity to develop population and region-specific sex estimation algorithms using larger samples in different forensic casework scenarios, as existing literature indicates substantial threshold values of FPRD (Kanchan et al., 2012; Krishan et al., 2015).

Traditional dermatoglyphic techniques for analyzing FPRD provide direct and interpretable observations while being comparatively inexpensive to capture fine-scale ridge features without computational requirements. However, these methods are susceptible to inter-observer variability, labor-intensiveness, and also challenging to scale for larger repositories. Thus, the present study suggests for complementing the integration of AI in sex estimation studies on FPRD, rather than replacing traditional methodologies, as generating supervised models using Machine Learning (ML) and Deep Learning (DL) algorithms can reduce observer bias, improve reproducibility and enhance predictive capability for sex estimation to analyze complex datasets (Budka et al., 2021; Kanchan et al., 2012).

Future studies can investigate the relationship between FPRD and other biological profiling factors, such as body weight and stature, and broaden its application beyond sex estimation. Examining these relationships may yield profound understanding of the trends in ridge count variability and how it relates to physical characteristics of the human body. Further explorations on tracing ancestry through FPRD is also recommended. Although previous studies have demonstrated the value of ethnicity profiling from FPRD

(Heathfield et al., 2016), carrying out comparable studies in other populations could contribute to an improved understanding of this aspect.

Since research within the domain of fingerprinting has established correlations between ridge density and epidemiological attributes like diabetes, hypertension and genetic anomalies (Sharma et al., 2021), implementing these outcomes can open opportunities for investigations into the variables affecting sexual variations in FPRD. Expanding this research area thus can help establish links between dermatoglyphic traits of footprints and underlying genetic or hormonal factors. Additionally, studying the influence of occupational factors might offer another direction for future investigations, as in many circumstances, people of rural and environmentally secluded communities travel barefooted for socio-economic and occupational reasons, it can impact the pattern of plantar ridges over time (Krishan, 2008).

## Conclusion

The present mini review concludes that FPRD is a relatively novel parameter that has the potential of contributing to sex estimation studies. Its application not only offers an upgradation on the existing value of footprints in forensic podiatry, but also promises valuable contributions to broader fields of human genetics and anthropological sciences.

**Contributions from individual authors**

SS: Conceptualization, data collection, drafting of manuscript, data analysis, data interpretation, final version approval; MS: data collection, drafting of manuscript, data analysis, data interpretation, final version approval; MC: drafting of manuscript; review & editing, final version approval; PŠ: drafting of manuscript, review & editing, final version approval, manuscript supervision; RB: drafting of manuscript, review & editing, final version approval, manuscript supervision

**Ethics statement**

Not applicable as this is a literature review.

**Data availability statement**

No raw data were generated in this study. The reviewed articles are referenced in text.

**Financial disclosure**

None to declare.

**Conflict of Interest**

None to declare.

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# Innate and adaptive immune parameters and adiposity in non-obese adults

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## Abstract

**Introduction**
Excess body weight, mainly resulting from high body fat mass, is known to negatively affect immune system functioning due to the proinflammatory and prooxidative roles of adipose tissue. In general, most studies have documented the immunosuppressive role of adiposity by comparing selected immune markers between individuals of normal weight and those with obesity.

**Study aims**
This study investigates whether the amount of adipose tissue observed in overweight individuals may already negatively affect immune function.

**Methods**
We examined a broad panel of immunological parameters – both innate (WBC, neutrophil count, phagocytic uptake, respiratory burst, complement activity, lysozyme activity) and adaptive (total IgA, total IgG, CD3 count, CD19 count, the strength of antibody response to flu vaccination), in 85 women and 98 men aged 18–37 years, with a BMI between 18.5 and 29.99. As measures of adiposity, we used BMI on a continuous scale, BMI categorized as normal weight or overweight, and body fat mass percentage.

**Results**
In women, CD3 count was positively associated with continuous BMI ($p = 0.03$) and categorized BMI ($p = 0.006$). CD19 was positively associated with continuous BMI ($p = 0.03$), categorized BMI ($p = 0.01$), and fat mass percentage ($p = 0.02$). In men, categorized BMI was positively associated with phagocytic uptake ($p = 0.04$). However, none of these relationships would remain statistically significant after applying corrections for multiple comparisons.

**Conclusion**
Our results suggest that there is no relationship between adiposity and the analyzed immunity markers in non-obese (normal weight and overweight) adults.

**Keywords**: BMI, body fat percentage, waist to height ratio, innate immunity, adaptive immunity

Original article

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## Introduction

Obesity, defined as excessive fat deposition that can impair health (WHO, 2006), is a known risk factor for various infectious diseases, including influenza (Karki et al., 2018; Louie et al., 2011; Maier et al., 2021), COVID-19 (Alberca et al., 2021; Stefan et al., 2020), cellulitis (Cheong, 2019; Taira et al., 2024), and urinary tract infections (Alhabeeb et al., 2021; Nseir et al., 2015; Semins et al., 2012). Obesity is also correlated with greater severity and/or worse outcomes of influenza (Fezeu et al., 2011; Martin et al., 2013; Ren et al. 2013; Ribeiro et al., 2015; Vaillant et al., 2009;) and COVID-19 (Arulanandam et al., 2023; Gao et al., 2020; Tamara & Tahapary, 2020; Poly et al., 2021; Yang et al., 2021a). It is additionally associated with more frequent surgical site infections (Koutsoumbelis et al., 2011; Mehta et al., 2012; Olsen et al., 2008; Waisbren et al., 2010), as well as to more frequent carriage of *Staphylococcus aureus* (Befus et al., 2015; Campbell et al., 2013, Erikstrup et al., 2019; Herwaldt et al., 2004; Olsen et al. 2013) which is one of the most common causes of such infections (Bucataru et al., 2023; Saadatian-Elahi et al., 2008; Zarb et al., 2012).

The relationship between adiposity and infection rate appears to be U-shaped – not only obesity, but also underweight, is associated with an increased risk compared to normal weight (Dobner & Kaser, 2018). In studies on adults, this pattern was observed in relation to the risk of hospitalization from influenza (Moser et al., 2019), the risk of influenza-related pneumonia (Phung et al., 2013), respiratory infections in general *(*Harpsøe et al., 2016; Yang et al., 2021b), the risk of septicemia (Yang et al., 2021b), and the risk of death from COVID-19 (Guglielmi et al., 2022; Wu et al., 2021).

Human defense mechanisms against pathogens are categorized into two groups: innate (specific) immunity, which serves as the body's initial line of defense, and adaptive (non-specific) immunity, which functions as the secondary line of defense (Wang et al., 2024). Among leukocytes (white blood cells, WBC), neutrophils are the predominant subtype, comprising 50–70% of leukocytes (Leliefeld et al., 2016). Neutrophils kill pathogens through phagocytosis (Boero et al., 2021). The killing of engulfed microbes can be performed by using antibacterial proteins or reactive oxygen species (Kolaczkowska & Kubes, 2013), the latter process being called respiratory burst (Dahlgren & Karlsson, 1999). While neutrophils are cellular components of innate immunity, humoral components include the complement system and lysozyme (Wang et al., 2024). The other subtype of WBC, lymphocytes, participate in adaptive immune mechanisms (Alberts et al., 2002). T lymphocytes drive the cellular immune response by reacting directly against foreign antigens, while B lymphocytes are involved in the humoral immune response by secreting immunoglobulins (Ig) (Alberts et al., 2002). For quantitative analysis of lymphocyte subsets, cell surface proteins termed cluster of differentiation (CD) are used; CD3 is a marker for T lymphocytes and CD19 is a marker for B lymphocytes (Hongbao et al., 2015).

Elevated leukocyte levels are crucial markers of inflammatory conditions, including obesity-related inflammation (Dixon & O'Brien, 2006). In adult studies, higher WBC counts were found in obese individuals relative to nonobese individuals (Al-Sufyani & Mahassni, 2011;

Ilavská et al., 2012; Kullo et al., 2002; Marzullo et al., 2013; Nieman et al., 1999; Womack et al., 2007), with the exception of two studies conducted on women, which found no difference (Mahassni, 2020; Mahassni & Bashanfar, 2019). Neutrophil count was also higher in obese than in non-obese subjects (Al-Sufyani & Mahassni, 2011; Ilavská et al., 2012; Marzullo et al., 2013; Nieman et al., 1999), with the exception of studies by Scully et al. (2017) and Kullo et al. (2002). In the study on women, Nieman et al. (1999) showed that obese women had higher levels of phagocytic uptake and respiratory burst compared with non-obese women, while in the study on both sexes, no difference in phagocytosis was observed (Scully et al., 2017). The complement system is considered to be one of the key factors contributing to inflammation and obesity-associated metabolic disorders (Ahmad & Al-Domi, 2017; Moreno-Navarrete & Fernández-Real, 2019). Studies on complement and adiposity yielded mixed results. Some studies observed that serum complement factors (i.e., C3 protein) rise in parallel with BMI ( (Hernandez-Mijarez et al., 2007; Muscari et al., 1998; 2000), while others found that C3 levels were lower in obese patients than in non-obese ones (Gottschlich et al., 1993), or no differences in selected serum complement components were observed between lean, overweight, and obese individuals (Błogowski et al., 2013).

Lymphocytes also play an important role in obesity-associated inflammation (Chatzigeorgiou et al., 2012). In the study by Nieman et al. (1999), obese women had higher CD3 and CD19 levels than non-obese women. Ilavská et al. (2012) also observed a significant positive correlation between BMI and CD3 and CD19 in women but not in men. Tanaka et al. (1993) analyzed the proliferation of mitogen-induced B- and T-cells in obese subjects and non-obese controls. Proliferation was significantly lower in obese participants. Furthermore, T lymphocyte responses to mitogen increased in these individuals after weight reduction (in B lymphocytes increase was not statistically significant) (Tanaka et al., 1993). Reduced lymphocyte proliferation among obese women, relative to nonobese women, was also documented by Nieman et al. (1999). Total levels of immunoglobulins IgG (Gottschlich et al., 1993; Marzullo et al., 2013) and IgA (Marzullo et al., 2013) were compared between obese and non-obese subjects, with no difference found. Studies have demonstrated a reduced response to the hepatitis B vaccine (Averhoff et al., 1998; Fan et al., 2016; Liu et al., 2017; Young et al., 2013) and the rabies vaccine (Banga et al., 2014) in obese adults compared to non-obese individuals, whereas mixed results were found regarding the response to the influenza vaccination (Callahan et al., 2014; King et al., 2024; Neidich et al., 2017; Sheridan et al., 2012).

In most studies examining the relationship between adiposity and immunity, adiposity is measured using BMI. BMI is recommended as a costless, easy, and reliable tool to assess adiposity in young adults and middle-aged people (Adab et al., 2018; Kato et al., 1996; Mohajan & Mohajan 2023; NHLBI, 1998); however, it has some limitations. It does not measure the amount of body fat directly, as it is based not on fat tissue mass but on total body weight, which is also influenced by muscle mass. As a result, people with high muscle mass may be misclassified as overweight or even obese (Ode et al., 2007; Witt & Bush, 2005).

In the study by Alasagheirin et al. (2011), men with similar average BMI fell into a different category of adiposity based on fat mass percentage. Moreover, misclassification in both directions was observed – some participants were overclassified as obese, while others were underclassified as normal-weight. Additionally, while BMI serves as a measure of total body fat, it does not provide information about fat distribution. However, the biggest health risk was found to be associated with fat excess in the abdominal region, even in individuals classified as non-obese based on their BMI (Emery et al., 1993; Eyben et al., 2003; Lukács et al., 2019; Matsuzawa et al., 1995; Silva et al., 2009; Von Kawamoto et al., 2005). The waist-to-height ratio (WHtR) has been proposed as an indicator of abdominal obesity (Ashwell & Clarke, 2009; Ashwell & Lejeune, 1996; Hsieh & Yoshinaga, 1995).

Correlations between adiposity and immunological parameters are usually presented as comparisons between two groups: obese vs. non-obese (Gottschlich et al., 1993; Nieman et al., 1999; Tanaka et al., 1993) or obese vs. normal weight (Al-Sufyani & Mahassni, 2011; Ilavská et al., 2012; Scully et al., 2017; Sheridan et al., 2012; Young et al. 2013). Studies where BMI was analyzed on a continuous scale often included BMI values classified as obesity (Ilavská et al., 2012; Nieman et al., 1999; Sheridan et al., 2012; Quach et al., 2023). As a consequence, there is limited data on the correlation between adiposity and immunity within the non-obese group.

The aim of this paper is to analyze the relationship between adiposity and various parameters of innate and adaptive immunity in men and women with a BMI range of 18.5–29.99. In addition to BMI, body fat percentage and WHtR were included as measures of adiposity.

## Materials and Methods

### Participants

We recruited a total of 126 women aged 18.6–36.1 years and 134 men aged 18.9–36.7 years. The criteria for participation in the study included: willingness to receive an influenza vaccine, absence of chronic diseases or hormonal problems, and, for women, no hormonal contraception. On the day of the first visit, participants confirmed the absence of any symptom of an ongoing infection, and an additional current health assessment was performed by a physician to qualify participants for vaccination. When preparing the database for analyses, the following exclusion criteria were applied: incomplete immunological data, use of antibiotics (including between two visits), and elevated inflammation markers (WBC > $10^3/\mu l$ or/and CRP > 5 mg/l). From the remaining sample, we also excluded participants with a BMI value indicating underweight, i.e., <18.49 (eight women, four men), or obesity, i.e., ≥ 30.00 (one woman, two men). The final sample comprised 85 women aged 18.6–36.1 years (mean=25.2, SD=4.4) and 98 men aged 19.0–36.7 years (mean=27.4, SD=4.7).

### Study procedure

The study protocol received approval from the Bioethics Commission of the Lower Silesian Chamber of Physicians and Dentists (2/PB/2013). All participants provided written informed consent.

The study included influenza vaccination; therefore, it was conducted during the "flu season" – that is, during the months of high influenza prevalence when influenza vaccination is recommended, i.e., from September to February (with the last follow-up appointments in March), across two consecutive years.

For each participant, the study protocol included two visits with a 28-day interval. Blood sampling procedures necessitated scheduling both visits between 7:30 and 9:00 AM in a private healthcare facility. The first visit included a medical examination (to confirm eligibility for vaccination), blood sampling, body measurements, questionnaire completion, and administration of the influenza vaccine (Vaxigrip, Sanofi Pasteur).

The second visit included blood sample collection and completion of questionnaires.

On each day of the study, blood samples were transported within two hours of collection to a certified analytical laboratory (for morphological and biochemical tests), and to the university laboratory to immediately perform tests on whole blood. Additionally, serum was separated and and stored at -80°C for further analyses.

### Adiposity measures

Adiposity was assessed using three indicators: BMI, body fat percentage (BF%), and WHtR. Height was measured to the nearest 0.1 cm with a Martin anthropometer, and weight to the nearest 0.01 kg with a Radwag® electronic scale. BMI values of 18.0–24.99 were classified as normal weight, and 25.0–29.99 as overweight. A bioimpedance analyzer (Bodycomp MF; Akern®) was used to measure body fat percentage (BF%) and calculated to 0.1 accuracy using the manufacturer's software (BodyGram 1.2; Akern®). WHtR was calculated as the ratio of waist circumference (cm) to body height (cm), with waist circumference measured with a flexible tape to an accuracy of 0.1 cm.

### Immunological measures

The immunological parameters assessed included:

1) Innate immunity measurements: neutrophil count, phagocytic uptake, respiratory burst, complement activity, lysozyme activity;
2) Adaptive immunity measurements: total IgA level, total IgG level, CD3 (marker of T lymphocytes), CD19 (marker of B lymphocytes), strength of response to the influenzavaccine, and seroconversion after the vaccine.

Additionally, WBC was measured, comprising cells that participate in both innate and adaptive immune responses.

Postvaccination response strength was calculated as the fold rise in antiinfluenza IgG antibody titer from baseline (day of vaccination) to day 28. A fold increase of $\geq 4$ was classified as positive (seroconversion), while a fold increase $< 4$ was classified as negative (lack of seroconversion) (Cauchemez et al., 2012). The immunological procedures were described in detail in a previous paper (Nowak et al., 2018).

### Control variables

There is sufficient evidence that human immune functioning changes with age (Kumar & Burns, 2008; Shaw et al., 2014); for example, a decline in phagocytic uptake (Emanuelli et al., 1986; Wenisch et al., 2000) and in respiratory burst (Di Lorenzo, et al. 1999; Fülöp et al., 1985; Nagel et al., 1982) was observed (although the samples had a different age range than in this study – they included individuals >60 years old).

Since testosterone is considered to be an immunosuppressant (Furman et al., 2014; Trigunaite et al., 2015), free testosterone level (fT) was included as a potential confounding factor. Free testosterone was measured in serum using a commercial ELISA kit (Demeditec®). Considering that our study was conducted over two consecutive flu seasons, we checked if there were differences in immunological measures between seasons. Taken together, we used age, fT, and season as potential cofounders.

### Statistical analyses

Statistical analyses were performed in Statistica 13.3 (TIBCO Software Inc., USA). A significance threshold of $p < 0.05$ was set. Normality of data distribution was checked using the Shapiro–Wilk test. Because most variables did not show normal distribution, nonparametric tests were performed. A Mann–Whitney test was applied for group comparisons and a Spearman's rank correlation for associations. To account for potential confounders, we applied multiple regression analyses, treating immunity markers (excluding seroconversion) as dependent variables and adiposity measures as predictors. Variables lacking normal distribution were used in regression analyses transformed using the Box-Cox formula.

BMI was analyzed both as a continuous and categorical variable, with normal weight coded as 0 and overweight as 1. The strength of the post-vaccination response was used as both a continuous and categorical variable, with a distinction between an absence of seroconversion (coded as 0) and seroconversion (coded as 1). Among the control variables, study season was categorical: the first season was coded as 0 (47 women, 30 men) and the second was coded as 1 (38 women, 68 men).

Because seroconversion was a categorical variable, the Mann–Whitney test was used to examine its associations with continuous adiposity measures, and the chisquare test was applied to assess its relationship with categorized BMI.

## Results

Table 1 presents descriptive statistics for adiposity measures, immune parameters, and control variables analyzed on a continuous scale. Men and women differed significantly in age, fT, BMI, BF%, WHtR, phagocytic uptake, respiratory burst, and CD19 (Mann-Whitney test: $p < 0.001$). Seventy-one women and 69 men had a BMI within the normal range, while 14 women and 29 men were overweight. Seroconversion was observed in 57 women and 76 men, and no seroconversion was found in 28 women and 22 men.

Table 1. Descriptive statistics of the studied variables and comparisons between women and men

| | Women (N = 85) | | Men (N = 98) | | Mann-Whitney test | |
|---|---|---|---|---|---|---|
| | Mean (SD) | Min-max | Mean (SD) | Min-max | Z | p |
| Age [years] | 25.25 (4.36) | 18.64–36.10 | 27.36 (4.68) | 18.97–36.72 | –2.98 | 0.003 |
| fT [pg/ml] | 5.67 (8.20) | 0.19–52.95 | 24.79 (11.01) | 2.53–65.23 | –10.72 | < 0.001 |
| BMI [kg/m$^2$] | 22.39 (2.63) | 18.55–28.94 | 23.79 (2.66) | 18.71–29.88 | –3.60 | <0.001 |
| BMI in normal weight group (N = 140) | 21.52 (1.83) | 18.55–24.93 | 22.40 (1.60) | 18.71–24.90 | –3.02 | 0.003 |
| BMI in overweight group (N = 43) | 26.81 (1.21) | 25.00–28.94 | 27.09 (1.51) | 25.05–29.88 | –0.38 | 0.71 |

| | Women (N = 85) | | Men (N = 98) | | Mann-Whitney test | |
|---|---|---|---|---|---|---|
| | Mean (SD) | Min-max | Mean (SD) | Min-max | Z | p |
| BF [%] | 27.25 (5.83) | 14.8–41.5 | 20.70 (4.77) | 9.4–32.5 | 7.00 | <0.001 |
| WHtR | 0.43 (0.04) | 0.30–0.59 | 0.46 (0.04) | 0.39–0.59 | –5.28 | <0.001 |
| WBC [$10^3/\mu l$] | 5.88 (1.46) | 3.2–9.6 | 5.84 (1.40) | 3.4–9.8 | 0.14 | 0.89 |
| Neutrophil count [$10^3/\mu l$] | 3.14 (1.12) | 1.5–6.3 | 2.94 (1.00) | 1.2–6.5 | 1.08 | 0.28 |
| Phagocytic uptake[1] | 237.6 (113.3) | 63.3–583.7 | 174.4(64.9) | 54.3–430.6 | 4.05 | <0.001 |
| Respiratory burst[2] | 6.43 (4.2) | 1.39–24.56 | 9.11 (9.10) | 2.67–60.08 | –3.66 | 0.001 |
| Complement activity [ng/ml] | 187506 (72014.7) | 50082.2–341366.7 | 186901.7 (52583.1) | 98462.2–287527.8 | 0.52 | 0.60 |
| Lysozyme activity[3] | 0.38 (0.08) | 0.20–0.54 | 0.37 (0.08) | 0.08–0.64 | 1.39 | 0.16 |
| IgA [g/l] | 1.79 (1.05) | 0.55–7.01 | 1.82 (1.01) | 0.57–7.34 | –0.37 | 0.71 |
| IgG [g/l] | 11.20 (3.72) | 4.23–23.41 | 11.99 (4.47) | 4.16–26.95 | –0.94 | 0.34 |
| CD3 [cells/$\mu l$] | 1472.62 (639.10) | 412.08–4481.33 | 1476.17 (525.33) | 538.13–3422.81 | –0.34 | 0.74 |
| CD19 [cells/$\mu l$] | 195.9 (95.39) | 39.5–459.4 | 237.78 (119.26) | 45.33–639.93 | –2.58 | 0.01 |
| Strength of post-vaccination response [fold increase] | 7.05 (7.21) | 1–32 | 8.23 (9.66) | 1–64 | –0.99 | 0.32 |

[1] Mean fluorescence intensity measured in blood phagocytes following phagocytosis of fluorescently labelled bacteria.
[2] Mean area under the chemiluminescence curve (AUC) for the stimulated sample, expressed relative to the control AUC.
[3] The absorbance difference between control samples (bacterial suspension lacking lysozyme) and test samples (bacteria exposed to serum containing lysozyme).

All three measures of adiposity were correlated with each other (BMI and BF%: women: $r_s = 0.75$, $t_{(83)} = 10.44$, $p < 0.001$; men: $r_s = 0.50$, $t_{(96)} = 5.66$, $p < 0.001$; BMI and WHtR: women: $r_s = 0.72$, $t_{(83)} = 9.56$, $p < 0.001$; men: $r_s = 0.87$, $t_{(96)} = 16.96$, $p < 0.001$; WHtR and BF%: women: $r_s = 0.63$, $t_{(83)} = 7.39$, $p < 0.001$; men: $r_s = 0.59$, $t_{(96)} = 7.08$, $p < 0.001$).

Zero-order correlation tests showed no significant relationship between adiposity measures and immune parameters in women. Among the controlled factors, only fT was related to neutrophil count ($r_s = 0.25$, $t_{(83)} = 2.31$, $p = 0.02$), phagocytic uptake ($r_s = -0.21$, $t_{(83)} = -1.99$, $p = 0.0497$), and lysozyme activity ($r_s = 0.23$, $t_{(83)} = 2.15$, $p = 0.03$) (Table 2). In men, only post-vaccination response was negatively corelated to BMI ($r_s = -0.23$, $t_{(96)} = -2.28$, $p = 0.02$) and WHtR ($r_s = -0.21$, $t_{(96)} = -2.15$, $p = 0.03$). Free testosterone was positively associated with the strength of the post-vaccination response ($r_s = 0.31$, $t_{(96)} = 3.13$, $p = 0.002$), age was negatively correlated with CD3 ($r_s$ = -0.30, $t_{(96)}$ = -3.03, p = 0.003), CD19 ($r_s$ = -0.22, $t_{(96)}$ = -2.19, p = 0.03), and the strength of the post-vaccination response ($r_s$ = -0.30, $t_{(96)}$ = -3.06, p = 0.003) (Table 3).

Table 2. Correlation of immunological parameters with adiposity measures and continuous control variables in women – $r_s$ values

| | Adiposity measures | | | Controlled factors | |
|---|---|---|---|---|---|
| | BMI | BF% | WHtR | Age | fT |
| WBC | -0.04 | -0.001 | -0.002 | 0.11 | 0.20 |
| Neutrophil count | 0.05 | 0.06 | 0.11 | 0.18 | **0.25*** |
| Phagocytic uptake | 0.003 | -0.06 | -0.02 | -0.04 | **-0.21*** |
| Respiratory burst | 0.005 | -0.03 | -0.13 | -0.08 | -0.18 |
| Complement activity | -0.02 | -0.02 | -0.07 | 0.08 | -0.08 |
| Lysozyme activity | 0.05 | 0.13 | -0.03 | -0.01 | **0.23*** |
| IgA | -0.09 | -0.04 | -0.01 | 0.22 | 0.04 |
| IgG | -0.02 | 0.03 | -0.13 | -0.14 | 0.12 |
| CD3 | 0.12 | 0.06 | 0.09 | 0.12 | 0.01 |
| CD19 | 0.12 | 0.14 | 0.10 | 0.06 | 0.03 |
| Strength of post-vaccination response | -0.18 | -0.15 | -0.14 | -0.02 | -0.02 |

* $p < 0.05$

Statistically significant relationships ($\alpha = 0.05$) are bolded.

Table 3. Correlation of immunological parameters with adiposity measures and continuous control variables in men – $r_s$ values

| | Adiposity measures | | | Controlled factors | |
|---|---|---|---|---|---|
| | BMI | BF% | WHtR | Age | fT |
| WBC | -0.03 | 0.04 | 0.02 | -0.19 | 0.12 |
| Neutrophil count | -0.03 | 0.08 | 0.07 | -0.10 | 0.09 |
| Phagocytic uptake | 0.20 | 0.03 | 0.16 | -0.001 | -0.14 |
| Respiratory burst | 0.02 | -0.06 | -0.08 | -0.03 | 0.16 |
| Complement activity | -0.08 | 0.04 | -0.09 | 0.07 | -0.03 |
| Lysozyme activity | -0.16 | -0.06 | -0.10 | -0.07 | 0.04 |
| IgA | 0.04 | -0.05 | 0.06 | 0.18 | 0.0002 |
| IgG | -0.05 | -0.05 | -0.13 | -0.11 | 0.16 |
| CD3 | -0.06 | 0.04 | -0.09 | **-0.30**** | -0.0002 |
| CD19 | 0.08 | -0.01 | 0.04 | **-0.22*** | -0.04 |
| Strength of post-vaccination response | **-0.23*** | 0.03 | **-0.21*** | -0.30** | 0.31** |

* $p < 0.05$; ** $p < 0.01$

Statistically significant relationships ($\alpha = 0.05$) are bolded.

A simple comparison between two BMI categories showed that overweight women had lower CD3 ($p = 0.03$) and CD19 ($p = 0.04$) levels compared to normal-weight women. Meanwhile, overweight men had lower phagocytic uptake ($p = 0.03$) and higher lysozyme activity ($p = 0.04$) compared to normal-weight men (Table 4). There was also a trend ($p = 0.06$) toward a weaker post-vaccination response in overweight men compared to normal-weight men.

Table 4. Results of Mann-Whitney's test with immunological parameters as dependent variables and categorized BMI (normal weight vs. overweight) as independent variable

| | categorized BMI | | | |
|---|---|---|---|---|
| | Women | | Men | |
| | Z | P | Z | P |
| WBC | -0.63 | 0.53 | 0.75 | 0.45 |
| Neutrophil count | -1.10 | 0.27 | 0.74 | 0.46 |
| Phagocytic uptake | -0.82 | 0.41 | -2.20 | **0.03*** |
| Respiratory burst | 0.67 | 0.50 | 0.32 | 0.75 |
| Complement activity | 0.34 | 0.74 | 0.67 | 0.50 |
| Lysozyme activity | 1.49 | 0.14 | 2.02 | **0.04*** |
| IgA | 0.21 | 0.84 | -0.85 | 0.40 |
| IgG | -0.28 | 0.78 | 0.51 | 0.61 |
| CD3 | -2.14 | **0.03*** | -0.36 | 0.72 |
| CD19 | -2.08 | **0.04*** | -0.57 | 0.57 |
| Strength of post-vaccination response | 0.66 | 0.51 | -1.88 | 0.06 |

*p < 0.10

Statistically significant results ( = 0.05) are bolded.

In order to conduct multiple regression analyses, we assessed which of the potential cofounders should be included in the model. For each immune parameter, we conducted a series of three simple regression analyses, with age, fT, and season as independent variables. Variables found to be significant predictors were included in multiple regression models (Table 5 and Table 6).

The regression analyses in women showed that none of the innate immunity parameters, nor the antibody variables (IgA and IgG) or post-vaccination response were predicted by any of the morphological measures. There was only a positive trend for phagocytic uptake in relation to categorized BMI ($\beta = 0.18$, $t_{(81)} = 1.87$, $p = 0.06$), but not to continuous BMI ($\beta = 0.08$, $t_{(81)} = 0.82$, $p = 0.42$) (Table 5). CD3 was positively associated with BMI, both continuous ($\beta = 0.22$, $t_{(82)} = 2.16$, $p = 0.03$) and categorized ($\beta = 0.28$, $t_{(82)} = 2.83$, $p = 0.006$), but not with fat mass percentage ($\beta = 0.11$, $t_{(82)} = 1.09$, $p = 0.28$) or WHtR ($\beta = 0.13$, $t_{(82)} = 1.22$, $p = 0.23$). CD19 was positively associated with all quantitative measures of adiposity: continuous BMI ($\beta = 0.23$, $t_{(82)} = 2.24$, $p = 0.03$), categorized BMI ($\beta = 0.27$, $t_{(82)} = 2.63$, $p = 0.01$), and BF% ($\beta = 0.24$, $t_{(82)} = 2.30$, $p = 0.02$), but not to WHtR ($\beta = 0.16$, $t_{(82)} = 1.52$, $p = 0.13$).

In men, phagocytic uptake was significantly predicted only by categorized BMI in relation to phagocytic uptake ($\beta = 0.20$, $t_{(95)} = 2.06$, $p = 0.04$); there was also a positive trend for continuous BMI ($\beta = 0.18$, $t_{(95)} = 1.83$, $p = 0.07$) (Table 6). For lysozyme activity, there was a negative trend in relation to categorized BMI ($\beta = -0.18$, $t_{(95)} = -1.78$, $p = 0.08$) but not with continuous BMI ($\beta = -0.14$, $t_{(95)} = -1.42$, $p = 0.16$). There was also a positive trend for the relationship between CD19 and WHtR

($\beta = 0.20$, $t_{(94)} = 1.87$, $p = 0.06$), but not between CD19 and any other measure of adiposity (BMI continuous: $\beta = 0.16$, $t_{(94)} = 1.55$, $p = 0.13$, BMI categorised: $\beta = 0.11$, $t_{(94)} = 1.10$, $p = 0.27$, BF%: $\beta = 0.06$, $t_{(94)} = 0.59$, $p = 0.56$).

Table 5. Beta-coefficients (with Standard Errors) of regression analyses in women (N = 85). BMI categorical means comparison between normal weight and overweight

| | continuous BMI | categorized BMI | BF% | WHtR |
|---|---|---|---|---|
| WBC | -0.06 (0.11) | 0.07 (0.11) | -0.04 (0.11) | 0.03 (0.11) |
| Neutrophil count[1] | 0.003 (0.11) | 0.08 (0.11) | 0.04 (0.11) | 0.10 (0.11) |
| Phagocytic uptake[2] | 0.08 (0.10) | 0.18^ (0.10) | 0.03 (0.10) | 0.06 (0.10) |
| Respiratory burst[3] | 0.08 (0.11) | -0.02 (0.11) | 0.05 (0.11) | -0.08 (0.11) |
| Complement activity | -0.02 (0.11) | -0.03 (0.11) | -0.03 (0.11) | -0.08 (0.11) |
| Lysozyme activity | 0.04 (0.11) | -0.13 (0.11) | 0.13 (0.11) | -0.04 (0.11) |
| IgA | -0.09 (0.11) | -0.01 (0.11) | -0.05 (0.11) | -0.02 (0.11) |
| IgG | -0.02 (0.11) | 0.03 (0.11) | 0.05 (0.11) | -0.11 (0.11) |
| CD3[3] | **0.22*** **(0.10)** | **0.28**** **(0.10)** | 0.11 (0.10) | 0.13 (0.10) |
| CD19[3] | 0.23* (0.10) | 0.27* (0.10) | 0.24* **(0.10)** | 0.16 (0.10) |
| Strength of post-vaccination response | -0.16 (0.11) | -0.05 (0.11) | -0.12 (0.11) | -0.11 (0.11) |

bold = p < 0.10; bold* p < 0.05; bold** p < 0.01

[1] controlled for fT; [2] controlled for season and fT; [3] controlled for season.

Statistically significant results (α = 0.05) are bolded.

Although the Spearman correlation analysis showed associations between the strength of the post-vaccination response with continuous BMI and WHtR, it did not remain significant after controlling for age, fT, and season (BMI continuous: $\beta = -0.09$, $t_{(92)} = -0.93$, $p = 0.36$, WHtR: $\beta = -0.06$, $t_{(92)} = -0.56$, $p = 0.58$), while the models including these confounders were significant (model with BMI continuous: adj $R^2 = 0.15$, $F_{(4,92)} = 5.09$, $p < 0.001$, model with WHtR: adj $R^2 = 0.14$, $F_{(5,91)} = 4.92$, $p = 0.001$).

The Mann-Whitney test showed no difference in continuous measures of adiposity between individuals with positive and negative responses to vaccination (i.e., with seroconversion and without seroconversion), in either women (BMI: $Z = 1.44$, $p = 0.15$, BF%: $Z = 0.84$, p = 0.40, WHtR: Z = 0.98, p = 0.33) or men (BMI: Z = 1.72, p = 0.09, BF%: Z = -1.22, p = 0.22, WHtR: Z = 1.06, p = 0.29). The chi-square test also showed no statistically significant association between seroconversion and categorized BMI – seroconversion was not less frequent in overweight individuals (women: $\chi2 = 0.06$, $p = 0.81$, men: $\chi2 = 1.74$, $p = 0.19$).

Table 6. Beta-coefficients (with Standard Errors) of regression analyses in men (N = 98). BMI categorical means comparison between normal weight and overweight

| | continuous BMI | categorized BMI | BF% | WHtR |
|---|---|---|---|---|
| WBC[1] | 0.07 (0.11) | -0.01 (0.10) | 0.07 (0.10) | 0.16 (0.11) |
| Neutrophil count | -0.04 (0.10) | -0.09 (0.10) | 0.05 (0.10) | 0.03 (0.10) |
| Phagocytic uptake[2] | 0.18^ (0.10) | **0.20* (0.10)** | 0.05 (0.10) | 0.16 (0.10) |
| Respiratory burst[2] | 0.13 (0.09) | 0.07 (0.09) | 0.01 (0.09) | 0.06 (0.10) |
| Complement activity[2] | -0.14 (0.10) | -0.13 (0.10) | 0.01 (0.10) | -0.15 (0.10) |
| Lysozyme activity[2] | -0.14 (0.10) | -0.18^ (0.10) | -0.04 (0.10) | -0.08 (0.10) |
| IgA[3] | -0.07 (0.11) | -0.002 (0.10) | -0.05 (0.10) | -0.01 (0.11) |
| IgG[2] | -0.01 (0.09) | 0.01 (0.09) | -0.01 (0.09) | -0.06 (0.09) |
| CD3[1] | 0.09 (0.10) | 0.14 (0.10) | 0.12 (0.10) | 0.11 (0.11) |
| CD19[1] | 0.16 (0.10) | 0.11 (0.10) | 0.06 (0.10) | 0.20^ (0.11) |
| Strength of post-vaccination response[4] | -0.09 (0.10) | -0.06 (0.10) | 0.13 (0.10) | -0.06 (0.11) |

bold: $p < 0.10$; bold* $p < 0.05$;

[1] controlled for age and season; [2] controlled for season; [3] controlled for age; [4] controlled for age, fT, and season

Statistically significant results ($\alpha = 0.05$) are bolded.

## Discussion

None of the adiposity measures used in our study were related to white blood cell count or neutrophil count. The lack of difference in WBC and neutrophil count between normal weight and overweight individuals is consistent with the results obtained in the series of studies on Saudi women (Al-Sufyani & Mahassni, 2011; Mahassni, 2020; Mahassni & Bashanfar, 2019), in the study on men (Kullo et al., 2002), and in the study on both sexes combined (Ilavská et al., 2012). On the contrary, Womack et al. (2007) observed higher WBC in overweight women compared to normal-weight ones. A positive correlation between continuous BMI and WBC was observed in some studies; however, the study sample also included much older participants than in our study – the age range was 45–64 years (Neto et al., 1992), 30–74 years (Schwartz & Weis, 1991), and 18–89 years (Panagotiakos et al., 2005).

A positive correlation between body fat percentage and both WBC and neutrophil count, after adjusting for sex, was observed in a study by Marzulo et al. (2013). Their sample consisted of a non-obese group and an obese group (categorized by BMI), the latter with a mean BF% of 45.1% (SD 6.8); thus, their data included higher values of BF% compared to the present study (see Table 1). It is possible that the correlation of WBC and neutrophil count with fat mass may be observed only over a wider range of body fat mass, when obese individuals are also included. In other words, an excess of body fat does not affect leukocyte count until it reaches a certain threshold, with values above this threshold being typical of obesity.

The absence of a correlation between WHtR and WBC aligns with findings by Mahassni and Bashanfar (2019), who likewise reported no differences in WBC across groups defined by low, moderate, and high waistcircumference–based healthrisk categories. In the study on women by Mahassni (2020), a significant difference in WBC was observed only between extreme categories of waist circumference, i.e., between low and high risk but not between low and moderate risk.

After controlling for confounders, phagocytic uptake of *E. coli* by neutrophils was positively associated with BMI both in men (continuous and categorized BMI) and in women (only categorized BMI). Meanwhile, lysozyme activity was negatively associated only with categorized BMI in men. The positive association found between BMI and phagocytic uptake aligns with the findings by Nieman (1999), who reported enhanced phagocytic uptake of *S. aureus* by granulocytes in obese women compared to their non-obese counterparts. These findings indicate that overweight and obese women both show differences from normal-weight women in this immune measure. Although Scully et al. (2017) found no difference in phagocytic uptake of *S. aureus* by neutrophils between non-obese healthy controls and obese subjects, it is worth noting that they compared only 12 controls (4 males) with 20 obese subjects without MetS (12 males), and 20 obese subjects with MetS (14 males) of different ethnicity.

We found no association between measures of adiposity and complement activity or respiratory burst. Our results contrast with general remarks that complement activity (measured as the levels of its components, C3 and C4) is positively associated with adiposity measures in adults (Gabrielsson et al., 2003; Nilsson et al., 2014; Qin et al., 2014), as well as in children (Cianflone et al., 2005) and adolescents (Wärnberg et al., 2006) , when comparing obese and lean individuals. However, to our knowledge, this is the first study in which total serum complement activity was compared between normal-weight and overweight individuals. In previous research examining complement C3 levels in different BMI categories, comparisons were made between non-obese versus obese individuals (Gottschlich et al., 1993).

No correlation between BMI and respiratory burst in peripheral blood mononuclear cells (i.e., phagocytes other than neutrophils) was observed in the study by Pangrazzi et al. (2020), where the BMI range was 20.2–43.5. It needs to be mentioned, however, that the age of partcipants differed from our sample – the range was 31–89 years with a mean of 69.7 years (SD: 12.9). Contrary to the study by Panrazzi et al. (2020), Nieman et al. (1999), who compared middle-aged non-obese and obese women, found that the respiratory burst of granulocytes was significantly higher in obese women. Thus, as with WBC and neutrophil count, we can conclude that complement activity and respiratory burst are likewise altered mainly in obese subjects while remaining unaffected or only slightly affected in overweight subjects.

After adjusting for confounding variables, Tlymphocyte count (CD3) showed a positive association with BMI in women but not in men. B lymphocyte count (CD19) in women was positively correlated with BMI and BF% but not with WHtR. In men, there was a positive correlation with WHtR but not with BMI or BF%. The observed sex difference regarding the correlation of CD3 and CD19 with BMI is consistent with the study by Ilavská et al. (2012), where significant positive correlations were found in women (CD3: $r = 0.23$; CD19: $r = 0.38$) but not in men. It is worth noting that although both our study and the study by Ilavská et al. (2012) showed a linear correlation of BMI with CD3 and CD19 in women, BMI classified as underweight was not included in the analyses. Thus, it is possible that across a wider range of BMI, with values lower than those categorized as normal weight, the relationship would no longer be linear. This has been shown in some studies comparing individuals with a BMI outside the normal range (18.5–24.99), which indicated an increased susceptibility to infection in individuals with both higher and lower than normal weight (Harpsøe, et al. 2016; Moser et al., 2019; Phung et al., 2013; Yang et al., 2021b).

CD3 and CD19 levels were higher in overweight compared to normal-weight women. Differences in these parameters between normal weight and other BMI categories were also analyzed in Slovakian men and women aged 40–45 years (Ilavská et al., 2012), Pakistani men aged 18–29 years (CD19 only) (Alam et al. 2012), Saudi women aged 24–52 years (Mahassni, 2019), and Saudi women aged 17–26 years (Al-Sufyani & Mahassni, 2011). Ilavská et al. (2012) found a significant difference in CD3 in obese women but not in overweight women, while the difference in CD19 was significant in both overweight and obese women. No difference was observed in men. In the study on men, there was no difference in CD19 between normal weight and other BMI categories (underweight, overweight, obese) (Alam et al., 2012). In the study by Mahassni (2019), obese women had a significantly higher T lymphocyte count compared to normal-weight women, while overweight women demonstrated a positive trend ($p = 0.068$). For CD19, no difference was found. In the study on women by Al-Sufyani and Mahassni (2011), no difference in T lymphocyte count was observed between five BMI categories (from underweight to highly obese), while for B lymphocyte count, the differences were marginally significant ($p = 0.057$). Prechtl et al. (2023) found no association between total body fat and visceral fat with B lymphocyte count, but they observed a positive correlation with one subset of B lymphocytes ($IgD^+$ B cells).

Although the results are mixed, some of the findings (including ours) suggest that being overweight may lead to an increase in CD3 and CD19 levels.

No relationship was found between any measure of adiposity and total immunoglobulin levels, including IgA and IgG. This agrees with the results of Mahassni (2019) in women, where no difference was found in total IgG or total IgA levels between underweight, normal weight, overweight and obese groups, and between waist circumference ranges classified as low, moderate, and high health risk. In the study by Marzullo et al. (2013), neither IgA nor IgG differed between obese versus non-obese, and no correlation was found with BF%.

No measure of adiposity was related to influenza vaccination effectiveness, regardless of whether effectiveness was assessed via the fold increase in anti-influenza IgG antibody titers or by seroconversion status. Although simple correlations in men showed negative associations between BMI and WHtR and the strength of the postvaccination response, these relationships were not significant after adjusting for age, fT, and season. Our results agree with Neidich et al. (2017), where the risk of influenza and influenza-like illness among vaccinated subjects was significantly lower in normal-weight individuals compared to obese individuals, but not compared to overweight individuals. Moreover, there was no difference in seroconversion rates between four BMI categories (from underweight to obese). Callahan et al. (2014) found no difference in the rate of seroconversion between five categories of BMI (from underweight to morbid obesity) in the analysis of influenza vaccination effectiveness in adults. Thus, being overweight appears to not be a risk factor for the non-effectiveness of the influenza vaccine.

Considering the immunomodulatory role of adipose tissue (Grant & Dixit, 2015; Pond et al., 2005), we might expect a relationship between the analyzed immune parameters and body fat mass percentage rather than with BMI. Our results, however, showed otherwise. We did not find any correlation between immune parameters and body fat distribution (measured as WHtR), either. Existing evidence may suggest to use various measures of adiposity in future studies. For example, Yoshimura et al. (2015) observed no correlation of WBC or neutrophil count and body fat percentage; however, they found a positive correlation with the visceral fat thickness measured by ultrasonography. Prechtl et al. (2023) measured adiposity using BMI, total body fat mass percentage, visceral fat mass percentage (assessed by bioelectrical impedance), and WHtR. They obtained mixed results regarding correlations with B lymphocytes, depending on the cell subset and method of measurement (gated vs. ungated B lymphocytes).

Owing to the distinct anatomical and functional characteristics of visceral versus subcutaneous fat depots (Ibrahim 2010), the immunomodulatory effects of adipose tissue—especially in overweight individuals—would likely be mediated predominantly by visceral fat (VAT), a parameter not captured in our study. Some studies showed that waist circumference (an anthropometric measure of VAT amount) is the best measure of physiological disturbances associated with fat amount, including immunity changes (Mahassini, 2020).

Considering the evolutionary aspect and hypotheses related to the signaling of biological condition in the mate market, our results do not suggest that overweight status in young and middle-aged adults affects the biological quality related to immunity. Our results may also support the Immunity Priority Hypothesis (Pawlowski et al., 2017). This is because, despite various factors affecting biological condition (and attractiveness – at least within the broad range of normality), an organism should always secure enough energy for immune function. It is, however, likely that being overweight may impair some immune parameters in older individuals compared to those studied here, as the organism typically has a lower biological condition and is not able to compensate for the physiological costs of being overweight. Furthermore, our study does not support the 'good genes' hypothesis, which postulates that physical attractiveness can be an honest signal of underlying biological quality in the studied age range. We should also remember that in the past, to secure energy reserves for pregnancy and lactation, selection may have favored a tendency toward excess weight in women (during the reproductive period of life), and therefore also may have driven the selection of adequate immune function within this BMI range.

It should be noted that among the relationships identified through regression analyses, only one—BMI categorized with CD3 in women—was significant at α 0.01. For the others, *p* -values ranged between 0.01 and 0.04. This means that after applying corrections for multiple comparisons, no relationship would remain significant.

In conclusion, our results imply that associations between adiposity and immunological parameters, which were observed in the studies using comparisons between obese and non-obese individuals, are weak or not present within a group of non-obese adults.

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None.

## Contributions from individual authors

All authors participated in designing the study. BB: participants recruitment, anthropometric data collection, statistical analysis, writing of major parts of manuscript. NJ: hormonal and immunological data collection, critical revision and writing of minor parts of manuscript; PB: conception of the study, critical revision and writing of minor parts of manuscript.

## Data availability statement

Data are available from the corresponding author upon request.

## Financial disclosure

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## Conflict of Interest

All authors have no competing interests to declare.

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