

## TITLE PAGE

TITLE: SEX ESTIMATION FROM DIMENSIONS OF THE FOURTH LUMBAR VERTEBRA IN NORTHERN FINNS OF 20, 30, AND 46 YEARS OF AGE

AUTHOR LIST: Petteri Oura<sup>1,2,3</sup>, Jaro Karppinen<sup>1,2,4</sup>, Jaakko Niinimäki<sup>1,3</sup>, Juho-Antti Junno<sup>1,5,6</sup>

## AFFILIATIONS

<sup>1</sup>Medical Research Center Oulu, Faculty of Medicine, University of Oulu and Oulu University Hospital, Oulu, Finland (PO Box 5000, FI-90014 University of Oulu, Finland)

<sup>2</sup>Center for Life Course Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland (PO Box 5000, FI-90014 University of Oulu, Finland)

<sup>3</sup>Research Unit of Medical Imaging, Physics and Technology, Faculty of Medicine, University of Oulu, Oulu, Finland (PO Box 5000, FI-90014 University of Oulu, Finland)

<sup>4</sup>Finnish Institute of Occupational Health, Oulu, Finland (Aapistie 1, FI-90220 Oulu, Finland)

<sup>5</sup>Department of Archaeology, Faculty of Humanities, University of Oulu, Oulu, Finland (PO Box 5000, FI-90014 University of Oulu, Finland)

<sup>6</sup>Cancer and Translational Medicine Research Unit, Faculty of Medicine, University of Oulu, Oulu, Finland (PO Box 5000, FI-90014 University of Oulu, Finland)

## CORRESPONDENCE

Mr. Petteri Oura, B.Med., Ph.D.

Center for Life Course Health Research, Faculty of Medicine, University of Oulu  
PO Box 5000, FI-90014 University of Oulu, Finland

## HIGHLIGHTS

- Northern Finns aged 20, 30, and 46 exhibit clear sex discrepancy in L4 dimensions
- The width, depth, and height of L4 estimate sex with an accuracy of > 80%
- Men show lower correct sex estimation rates than women
- Sex estimation accuracy is lower among those aged 46 than those aged 20 or 30

# SEX ESTIMATION FROM DIMENSIONS OF THE FOURTH LUMBAR VERTEBRA IN NORTHERN FINNS OF 20, 30, AND 46 YEARS OF AGE

## ABSTRACT

**Background:** Accurate sex estimation (sexing) is crucial for successful forensic identification. For the cases in which only a part of the skeleton or individual skeletal elements are available, we investigated the sex estimation potential of the fourth lumbar vertebra (L4) among 20-, 30-, and 46-year-old Northern Finns.

**Material and methods:** Magnetic resonance imaging scanned living subsamples of the Northern Finland Birth Cohort 1966 (scan at 46 years, n=1363) and the Northern Finland Birth Cohort 1986 (repeated scans at 20 and 30 years, n=375) provided the material for the study. After screening the scans for vertebral pathologies, we measured the maximum and minimum widths, depths, and heights of the L4 body. The mean vertebral width, depth and height were calculated together with vertebral cross-sectional area and volume. Sex estimations were performed using univariate and multivariate logistic regression analysis.

**Results:** We detected marked sex discrepancy in all the studied parameters of L4 ( $p < 0.001$ ). In the groups aged 20, 30, and 46 years, the regression models reached correct sex estimation rates of 86.4%, 87.7%, and 82.8%, respectively. At each time point, multivariate models proved more accurate than univariate models. Men showed consistently lower correct sex estimation rates than women.

**Conclusion:** Among 20-, 30-, and 46 year-old Finns, combining the measurements of width, depth, and height of the L4 body can be used to estimate sex with an accuracy of  $> 80\%$ . Vertebral width, depth, and height seem to yield as accurate sex estimates as more complicated vertebral parameters.

**KEY WORDS:** Sex estimation, L4, vertebral dimensions, magnetic resonance imaging, Forensic Anthropology Population Data

## INTRODUCTION

Successful identification of unknown deceased individuals is one of the primary aims of forensic medicine [1-7]. It is necessary not only as part of medicolegal investigations of crime but also in natural disasters and occasionally in cases of natural death when the remains are poorly preserved [1, 5]. Although molecular techniques have evolved considerably in recent years, anthropological methods of assessing skeletal remains play an established role in the identification process [6, 7]. An osteobiography, i.e., the biological profile of an unknown individual, is typically established first, using estimations of the individual's ancestry, sex, age, and stature [6, 8]. These data can then be accompanied by more specific investigations of the individual's identity.

Sex refers to the biological genotype of a human [9]. When profiling an unknown individual, sex estimation (i.e., sexing) is crucial for accurate subsequent estimation of age and stature [2, 5]. As bones typically express discrepancies between the sexes and are generally stable elements in the adult body, a wide range of skeletal features have been used for sexing [1, 8]. For example, morphologic characteristics of the cranium, pelvis, mandible, sternum, sacrum, femur, patella, humerus, radius, and small bones of the hand and foot have been used. Estimation accuracy has ranged from 64% to 100% [2], depending on skeletal region, the parameters used, the measurement method, the study population, and other methodological factors of the studies. An accuracy of  $\geq 80\%$  to  $\geq 95\%$  is commonly considered satisfactory [2, 10]. As only a few bones may be recovered or sufficiently preserved for profiling, further data is needed to describe the bones of other skeletal sites as potential indicators of sex.

There is a clear sexual dimorphism in vertebral size [11-15]. For example, the vertebral cross-sectional area is 20% to 30% larger among men than women, indicating that vertebral morphology may prove useful in sex estimation [16]. Ostrofsky et al. [17] recently studied various morphological parameters of the lumbar vertebrae (L1—L5) of 98 South African blacks from the Raymond A. Dart Collection. Their successful sex estimation rates varied from 57.7% to 88.7%, with diameters of the vertebral body yielding the highest accuracies. Correspondingly, several studies [18-22] investigating lower thoracic and upper lumbar vertebrae in various ethnic groups have reached estimation accuracies of up to 94.2%. However, their sample sizes have been mostly small, and conclusions have been drawn from cadaveric material from skeletal collections and/or material with a broad age range. Conclusions drawn from skeletal collections may be inaccurate due to secular changes in environmental, societal and lifestyle factors over the past century [23]. Moreover, male vertebrae in particular are subject to age changes in their shape and size [11], which implies that sexing accuracy may depend on age; using samples with a broad age range may thus be problematic in this regard.

Here, we report the sex estimation potential of three easily obtainable dimensions of the L4 body (width, depth, height) in three age groups (20, 30, 46 years) of the general living Northern Finnish population. We obtained the L4 dimensions from recent lumbar magnetic resonance imaging (MRI) scans, and used univariate and multivariate logistic regression models to assess them in terms of sex estimation.

## MATERIAL AND METHODS

### Study samples

We used two living Finnish birth cohort populations as our material. The younger population was a subsample of the Northern Finland Birth Cohort 1986 (NFBC1986) [24] with lumbar MRI scans from the ages of 20 and 30 years ( $n = 375$  individuals with no vertebral pathologies). The older population was constituted by a subsample of the Northern Finland Birth Cohort 1966 (NFBC1966) [25] with lumbar MRI scans available from the age of 46 ( $n = 1363$  individuals with no vertebral pathologies). We selected these samples and time points in accordance with our aim of investigating the sex estimation potential of healthy adult vertebrae before they are affected by osteoporosis or degeneration. We have previously shown that the MRI samples are representative of the general, contemporary Northern Finnish population [26, 27].

### Vertebral measurements

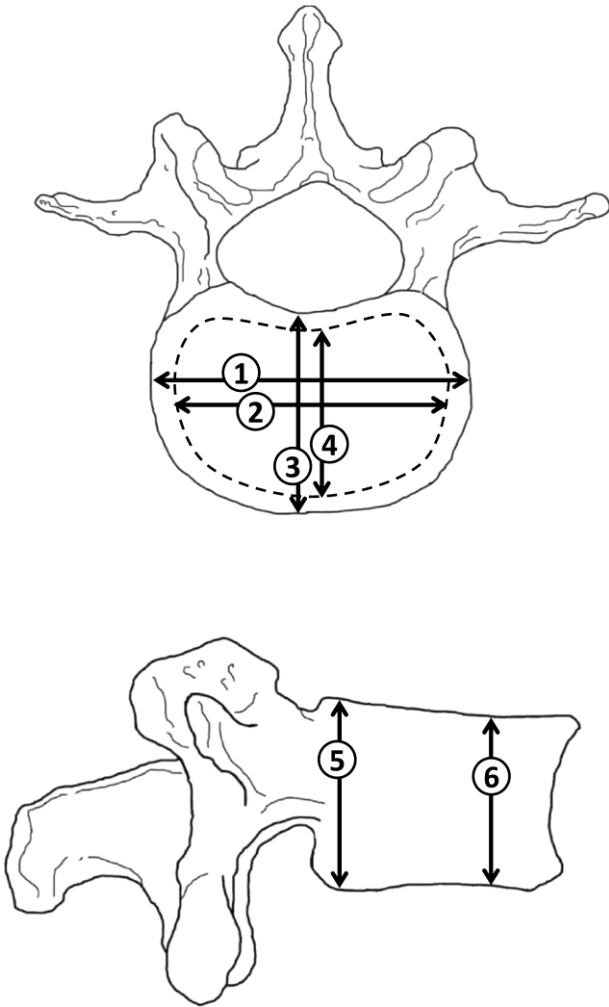
The 20-year and 46-year lumbar MRI scans were obtained in 2005–2008 and 2012–2014, respectively, using 1.5 T Signa HDxt (General Electric, Milwaukee, Wisconsin, USA). The 30-year scans were obtained in 2015–2017, using 1.5 T Optima MR450w (General Electric, Milwaukee, Wisconsin, USA). At each stage, a routine lumbar spine imaging protocol was used, with T2-weighted fast-recovery fast spin-echo images in sagittal and transverse planes. The specific imaging parameters are presented in earlier publications [26, 28]. MRI scanners were subject to quality assurance, including checks for geometric accuracy, on a weekly basis. Before measuring vertebral dimensions, we excluded scans with vertebral pathologies (segmentation error, endplate erosion, severe disc degeneration, spondylodesis, Schmorl's nodes).

One blinded researcher (P.O.) took the measurements using NeaView Radiology software version 2.31 (Neagen Oy, Oulu, Finland). The maximum and minimum widths, depths, and heights of the L4 body were measured from each applicable scan (**Figure 1**). We measured the widths, depths and heights of L4 because they were easily obtained and thus potentially applicable in a forensic context. Previous research has also suggested that they yield the highest estimation accuracies [17]. Each dimension was documented to the accuracy of 0.1 mm with high intra-rater reliability (intraclass correlation coefficient = 0.963) [28]. We measured L4 as it was located in the centre of the MRI scans and was therefore most often accessible with appropriate axial and sagittal slices. L4 is known to represent the other lumbar vertebrae well [28, 29] and has been used in a number of previous studies [28, 30–33]. We have previously used cadaveric vertebrae to compare MRI-based vertebral measurements to direct measurements taken with standard osteometric calipers and concluded that they are highly equivalent (correlation coefficient = 0.985) [15].

We based our sex estimations on the following L4 parameters: 1) maximum width, depth and height; 2) minimum width, depth and height; 3) mean width, depth and height (i.e., the averages of maximum and minimum values). Mean values were investigated in order to control for the natural variety in vertebral shape [34, 35]. As additional variables, we assessed the usability of vertebral cross-sectional area (CSA) and volume in sex estimation. CSA and volume were calculated using the acknowledged [36] ellipsoid formulae

$$\text{CSA} = \pi \times \frac{a}{2} \times \frac{b}{2}$$
$$\text{Volume} = \pi \times \frac{a}{2} \times \frac{b}{2} \times c$$

where  $a$  = mean width,  $b$  = mean depth, and  $c$  = mean height of the L4 body. The results of these additional variables are given as supplementary data.



**Fig. 1** Measured dimensions of the L4 body: Maximum width (#1) and minimum width (#2); maximum depth (#3) and minimum depth (#4); maximum height (#5) and minimum height (#6).

#### Statistical analysis

We used SPSS software version 24 (IBM, Armonk, NY, USA) to analyse our data. We calculated frequencies and percentages for the categorical variables, and means and standard deviations (SD) for the continuous variables. The sex differences in vertebral dimensions were assessed using independent-samples t test. We also illustrated our data by showing the distributions of vertebral CSA across the age groups as box plots and whiskers which were drawn as follows: minimum, quartile 1, median, quartile 3, maximum.

We used SPSS's Binary Logistic Regression tool to conduct the sex estimations and analyse their accuracy. We chose logistic regression over discriminant function analysis as it allows more variation in the structure of the data without compromising the accuracy of the models [37]. Our binary outcome variable (i.e., sex) was coded so that 0 = female and 1 = male, and the predictor variables (i.e., vertebral dimensions) were continuous. We performed both univariate and multivariate analyses which are specified in the results section. In each analysis, the regression model was first fitted on the basis of the sex and vertebral dimension data, and the numerical regression parameters were documented ( $\alpha$  is the constant term of the model and  $\beta$  is the coefficient of the predictor variable). A predicted sex was then assigned to each individual according to the highest posterior group membership probability (male/female), and prediction accuracy was assessed by calculating the percentage of correct predictions.

### Ethical approval

Approval was obtained from the Ethical Committee of Northern Ostrobothnia Hospital District. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The article does not contain any studies with animals performed by any of the authors.

The datasets generated and analysed during the study are not made publicly available. The dataset is administered by the NFBC Project Center but restrictions apply to the availability of these data due to local privacy regulations.

## RESULTS

The 20- and 30-year-old samples consisted of 375 individuals (60.8% women), and the 46-year-old sample of 1363 individuals (54.7% women). Their general characteristics are shown in **Table 1**. The sex-specific reference values for each L4 parameter are presented in **Table 2** (width, depth, height) and **Supplementary Table 1** (CSA, volume). The sex estimation accuracies and the corresponding regression parameters for potential future applications are presented in **Table 3** (width, depth, height) and **Supplementary Table 2** (CSA, volume). Generally, all the measured dimensions of L4 were larger among men than women at all time points ( $p < 0.001$ ). As an example, **Figure 2** presents the box plots of vertebral CSA across the age groups of 20, 30, and 46 years. The multivariate regression analyses which included the mean width, depth and height of L4 yielded the highest sex estimation accuracies in all age groups (86.4%, 87.7%, and 82.8% at the ages of 20, 30, and 46, respectively). CSA and volume provided slightly less accurate estimations, and the univariate analyses of width, depth, and height yielded markedly lower accuracies. In general, the sex estimation accuracies were consistently higher for females than males, and the models which were based on the 46-year-old sample showed lower sex estimation accuracies than the corresponding 20- and 30-year-old sample models.

**Table 1.** General characteristics of the samples.

	20-year-old sample	30-year-old sample	46-year-old sample
Mean age at imaging (years $\pm$ SD)	21.3 $\pm$ 0.6	30.7 $\pm$ 0.6	46.8 $\pm$ 0.4
Men (n, %)	147 (39.2%)	147 (39.2%)	618 (45.3%)
Women (n, %)	228 (60.8%)	228 (60.8%)	745 (54.7%)
Representativeness of Northern Finnish population (reference)	High [26]	High [26]	High [27]

SD = Standard deviation



**Table 2.** Dimensions of L4 among the 20-, 30-, and 46-year-old samples.

L4 parameter	Reference value (mean $\pm$ standard deviation)		
	Male	Female	P for sex difference
Age 20			
Width (mm)			
Maximum	49.4 $\pm$ 4.0	44.2 $\pm$ 2.9	< 0.001
Minimum	42.9 $\pm$ 3.2	37.8 $\pm$ 2.6	< 0.001
Mean	46.2 $\pm$ 3.5	41.0 $\pm$ 2.6	< 0.001
Depth (mm)			
Maximum	34.5 $\pm$ 2.5	30.9 $\pm$ 2.2	< 0.001
Minimum	32.3 $\pm$ 2.2	28.8 $\pm$ 1.9	< 0.001
Mean	33.4 $\pm$ 2.3	29.8 $\pm$ 2.0	< 0.001
Height (mm)			
Maximum	29.8 $\pm$ 1.9	27.7 $\pm$ 1.7	< 0.001
Minimum	25.4 $\pm$ 2.1	23.8 $\pm$ 1.7	< 0.001
Mean	27.6 $\pm$ 1.7	25.8 $\pm$ 1.5	< 0.001
Age 30			
Width (mm)			
Maximum	51.2 $\pm$ 3.9	45.9 $\pm$ 2.9	< 0.001
Minimum	43.9 $\pm$ 3.3	38.6 $\pm$ 2.7	< 0.001
Mean	47.6 $\pm$ 3.4	42.3 $\pm$ 2.7	< 0.001
Depth (mm)			
Maximum	36.1 $\pm$ 2.5	32.1 $\pm$ 2.1	< 0.001
Minimum	33.9 $\pm$ 2.3	30.0 $\pm$ 2.0	< 0.001
Mean	35.0 $\pm$ 2.3	31.0 $\pm$ 2.0	< 0.001
Height (mm)			
Maximum	30.5 $\pm$ 2.0	28.4 $\pm$ 1.8	< 0.001
Minimum	25.6 $\pm$ 2.1	24.2 $\pm$ 1.7	< 0.001
Mean	28.1 $\pm$ 1.8	26.3 $\pm$ 1.5	< 0.001
Age 46			
Width (mm)			
Maximum	51.3 $\pm$ 4.1	46.1 $\pm$ 3.4	< 0.001
Minimum	41.8 $\pm$ 3.2	37.0 $\pm$ 2.9	< 0.001
Mean	46.5 $\pm$ 3.3	41.5 $\pm$ 2.9	< 0.001
Depth (mm)			
Maximum	37.7 $\pm$ 2.8	33.6 $\pm$ 2.4	< 0.001
Minimum	34.6 $\pm$ 2.5	31.0 $\pm$ 2.3	< 0.001
Mean	36.1 $\pm$ 2.5	32.3 $\pm$ 2.3	< 0.001
Height (mm)			
Maximum	30.3 $\pm$ 1.7	29.0 $\pm$ 1.7	< 0.001
Minimum	25.5 $\pm$ 1.8	24.3 $\pm$ 1.7	< 0.001
Mean	27.9 $\pm$ 1.5	26.7 $\pm$ 1.5	< 0.001

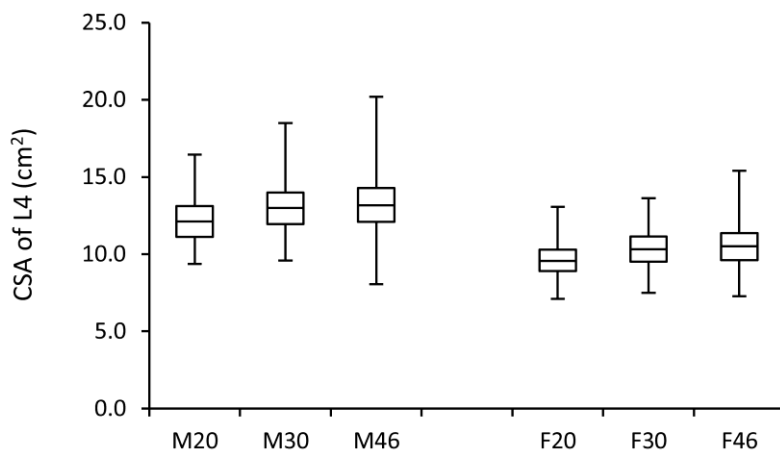
'Mean' = mean of maximum and minimum values

**Table 3.** Sex estimation accuracy of the dimensions of L4 among the 20-, 30-, and 46-year-old samples.

L4 parameter	Sex estimation accuracy (%)			Regression parameters	
	Male	Female	All	$\alpha$	$\beta$
Age 20					
Width					
Maximum	68.7	86.0	79.2	-21.57	0.45
Minimum	70.7	87.3	80.8	-25.17	0.62
Mean	72.1	86.4	80.8	-25.49	0.58
Depth					
Maximum	71.4	89.0	82.1	-22.74	0.68
Minimum	72.8	87.7	81.9	-24.56	0.79
Mean	73.5	89.0	82.9	-24.73	0.77
Height					
Maximum	52.4	83.8	71.5	-18.82	0.64
Minimum	50.3	85.1	71.5	-11.88	0.47
Mean	56.5	85.5	74.1	-19.24	0.71
Multivariate model with all means <sup>a</sup>	78.2	91.7	86.4	-43.26	0.35; 0.49; 0.45
Age 30					
Width					
Maximum	68.0	85.1	78.4	-23.82	0.48
Minimum	74.8	88.2	82.9	-25.77	0.62
Mean	72.1	87.3	81.3	-27.36	0.60
Depth					
Maximum	74.1	88.2	82.7	-26.96	0.78
Minimum	76.9	89.0	84.3	-27.43	0.85
Mean	75.5	89.0	83.7	-28.77	0.86
Height					
Maximum	54.4	84.6	72.8	-18.45	0.61
Minimum	42.2	84.2	67.7	-10.97	0.42
Mean	53.7	83.8	72.0	-18.44	0.66
Multivariate model with all means <sup>a</sup>	81.6	91.7	87.7	-45.93	0.34; 0.59; 0.40
Age 46					
Width					
Maximum	71.4	80.4	76.3	-18.54	0.38
Minimum	76.9	83.2	80.3	-21.03	0.53
Mean	77.5	83.4	80.7	-23.39	0.53
Depth					
Maximum	74.4	83.8	79.5	-22.42	0.63
Minimum	72.7	83.6	78.7	-20.50	0.62
Mean	76.2	83.5	80.2	-23.17	0.67
Height					
Maximum	56.0	74.0	65.8	-13.35	0.44
Minimum	52.6	73.3	63.9	-10.47	0.41
Mean	56.3	74.4	66.2	-15.69	0.57
Multivariate model with all means <sup>a</sup>	79.6	85.5	82.8	-35.12	0.30; 0.39; 0.31

<sup>a</sup>'Mean' = mean of maximum and minimum values

<sup>a</sup>Regression coefficients are given in the following order: mean width, mean depth, mean height.



**Fig. 2** Box plots illustrating the cross-sectional area (CSA) of L4 in men and women among the 20-, 30-, and 46-year-old samples. M = Male, F = Female

## DISCUSSION

This study assessed the sex estimation potential of vertebral width, depth, and height, measured from the L4 body using recent MRI scans, in three specific age groups of representative Northern Finns. In the groups aged 20, 30, and 46 years, all these dimensions showed marked sex discrepancy and their most accurate combinations reached correct estimation rates of 86.4%, 87.7%, and 82.8%, respectively (male and female accuracies pooled).

Whereas dimensions of the pelvis and cranium have proven most accurate in sexing, with reported multivariate accuracies of virtually up to 100%, the dimensions of the bones of the extremities and vertebrae have mostly yielded accuracies of 75% to 95% [2, 17]. In particular, analyses of the lumbar vertebrae have produced accuracies of 79% to 92% [17, 19, 22]. Thus, our results concerning L4 were of a similar magnitude to previous reports. Importantly, our sex estimation rates in all age groups clearly exceeded 80%, which is considered an acceptable threshold for accurate sexing tools [10].

In their comprehensive analysis of all five lumbar vertebrae, Ostrofsky et al. [17] previously detected a decreasing trend in estimation accuracy with increasing vertebral segment (L1 → L5). L4- and L5-based sex estimations were correct in 80.2% and 81.3% of cases, respectively, when all the eleven studied parameters or their most optimal combination were analysed (cranial width and depth of body, anterior and posterior height of body, width and height of pedicle, thickness of spinous process, two diameters of zygapophysis, interfacet width, wedging angle). This finding was potentially due to the more prominent physiological variation in the morphology of L4-L5 than that of L1-L3. Surprisingly, our approach, which utilized only the rather easily measurable maximum and minimum widths, depths, and heights of L4 showed higher estimation accuracies in all age groups, i.e., 20, 30, and 46 years, than the analysis by Ostrofsky et al. Furthermore, our results concerning L4 nearly matched the previously reported higher correct estimation rates of the upper lumbar vertebrae [2, 19, 22]. It thus seems that L4 can also be equally accurately used for sexing. However, we acknowledge the methodological differences between the studies (e.g., assessment of skeletal collections vs. living individuals, ancestry, sample size, direct vs. imaging-based measurements, no age stratification vs. specific age strata, data analysis method), and their effect on the comparability of results.

We compiled our data using three distinct age groups, i.e., 20, 30, and 46-year-olds, due to the age-related increase in vertebral size [11]. These distinct samples enabled us to assess the sex and age interplay in L4-based sex estimation. Interestingly, men showed consistently lower correct sex estimation rates than women, and the sex estimation accuracy was generally lower among 46-year-olds than 20- or 30-year-olds. The male/female discrepancy in sex estimation accuracy has been previously described by Ostrofsky et al. [17]. Potential explanations for this may be, firstly, the fact that males express greater variation in vertebral morphology overall, or secondly, the statistical properties of the models that were constructed for making the sex estimations. Notably, in our data the male and female dimensions of L4 were considerably overlapping, as illustrated by CSA in **Figure 2**. Men also showed a higher level of dispersion in vertebral dimensions which is likely to explain the lower accuracies among men. Curiously, however, the sex discrepancy in sex estimation accuracy has been described as concerning mostly L4 and L5, whereas the variation in vertebral morphology seems to be of similar magnitude regardless of lumbar segment [17]. Moreover, we analysed our data using logistic regression, not discriminant factor analysis, and still detected this clear trend. As our data is not able to address other vertebral segments than L4, the basis of this finding remains obscure and requires further study.

The lower sex estimation accuracy among 46-year-olds may result from several potential underlying factors. Firstly, vertebral size is influenced by lifestyle-related factors, and the effects seem to be more prominent among women than men (e.g., body mass index [32], physical activity [28, 31]). The lifestyle-

related modifications in vertebral dimensions are likely to accumulate with age, thus reducing the sex-related discrepancy in vertebral size. In contrast, the vertebral size of 20- and 30-year-olds is largely the result of skeletal growth which continues beyond peak bone mass until the third to fourth decade of life [38]; lifestyle-related factors are likely to have a more prominent (and less sex-dependent) effect on vertebral dimensions thereafter. Secondly, our analyses were not adjusted for body size or skeletal size which have been associated with vertebral dimensions; however, these data are not always available in forensics. Thirdly, although we excluded individuals with pathological findings in their MRI, it is possible that mild degenerative changes in the spine may affect the vertebrae either directly or via altered biomechanics. All these factors lead to a higher level of variation in vertebral size (which can be clearly seen in our data; **Figure 2**) and thus lower sex estimation accuracy towards the age of 46 years. However, we emphasize that even among 46-year-olds, the sex estimation accuracy of L4 was high (up to 82.8%).

Another curious finding of our study was that vertebral height yielded rather low correct estimation rates (e.g., 66.2% in 46-year-olds). This phenomenon was displayed from another angle by vertebral CSA, calculated as the mathematical combination of vertebral width and depth, which yielded estimation accuracies almost as high as vertebral volume. Vertebral height strongly correlates with stature [15], which is markedly variable across individuals; this physiological variety may reduce its potential in the sex estimation process. It was also the smallest dimension that we measured, indicating that measurement accuracy may play a part in this finding.

Our study has several strengths. First, we were able to investigate living individuals. This is important, because conclusions drawn from past populations may be inaccurate due to marked secular changes in environmental, societal and lifestyle factors over the past century [23]. Second, our sample size was large compared to those of previous studies and, importantly, our sample was representative of the general Northern Finnish population. Studies of Finnish individuals have been lacking in this regard. Third, we were able to assess vertebral dimensions in several coeval age groups, which increased the accuracy of our investigation. We provided reference values and regression coefficients for each age group separately to increase the applicability of our data in both future studies and biological profiling. The samples consisted of adults with healthy vertebrae, which increased the accuracy of our results. Utilizing  $\leq 46$ -year-old individuals minimized the effect of vertebral osteoporosis or degeneration on the sex estimation process. Fourth, we used easily obtainable vertebral measurements (i.e., width, depth, and height) and showed their high potential to provide accurate sex estimations. We also chose an alternative statistical method to analyse our data (i.e., logistic regression instead of discriminant function analysis), in order to approach the study question from a different statistical perspective and confirm the previous findings in this regard.

Our study also had limitations. First, as the study focused on living individuals, direct measurement of L4 was beyond our reach and we consequently obtained the dimensions from MRI scans. We validated this method against standard osteometric calipers as part of an earlier study [15]. We also obtained results that were very similar to previous studies, which supports the validity of our approach. Second, we could only measure L4, as it was most often accessible in the MRI scans with appropriate axial and sagittal slices. However, the dimensions of L4 can be extrapolated over the other lumbar vertebrae rather well [29].

In this paper, we have reported the high sex estimation potential of three easily obtainable dimensions of the L4 body (width, depth, and height) in 20-, 30-, and 46-year-old Northern Finns. In comparison to 1) other lumbar vertebrae and 2) more complicated measurement parameters, the use of the L4 body and its width, depth, and height seems justified in terms of sex estimation accuracy. This may especially be the case when other skeletal material is poorly preserved or otherwise not available. Nevertheless, our findings add to the growing evidence that vertebrae can be utilized as part of the sexing process in an accurate manner. Further studies are needed to investigate whether applying only these simple parameters to other lumbar and spinal segments may yield equally high results; whether combining vertebral dimensions from

several spinal segments may add to sex estimation accuracy; and what the underlying factors of the poorer sex estimation accuracy among men are.

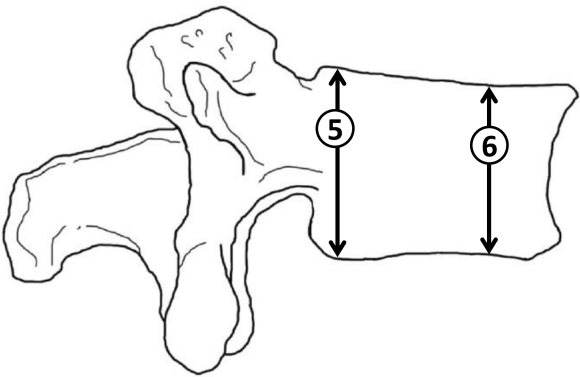
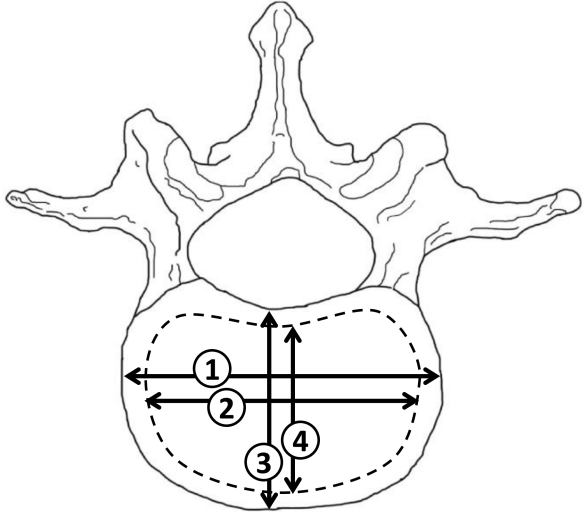
## REFERENCES

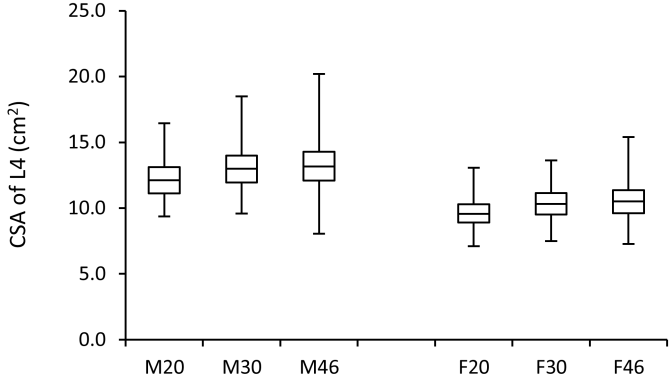
- (1) Krogman W. The Human Skeleton In Forensic Medicine. Springfield, Illinois: Charles C Thomas; 1962.
- (2) Krishan K, Chatterjee P, Kanchan T, et al. A review of sex estimation techniques during examination of skeletal remains in forensic anthropology casework. *Forensic Sci Int* 2016 Apr;261:165.e1-165.e8.
- (3) Mundorff A. Integrating forensic anthropology into Disaster Victim Identification. *Forensic Sci Med Pathol* 2012 Jun;8(2):131-139.
- (4) Ciaffi R, Gibelli D, Cattaneo C. Forensic radiology and personal identification of unidentified bodies: a review. *Radiol Med* 2011 Sep;116(6):960-968.
- (5) Franklin D. Forensic age estimation in human skeletal remains: current concepts and future directions. *Leg Med* 2010 Jan;12(1):1-7.
- (6) Dirkmaat D, Cabo L, Ousley S, et al. New perspectives in forensic anthropology. *Am J Phys Anthropol* 2008(Suppl 47):33-52.
- (7) Cattaneo C. Forensic anthropology: developments of a classical discipline in the new millennium. *Forensic Sci Int* 2007 Jan 17;165(2-3):185-193.
- (8) Blau S, Briggs C. The role of forensic anthropology in Disaster Victim Identification (DVI). *Forensic Sci Int* 2011 Feb 25;205(1-3):29-35.
- (9) Goodfellow P, Darling S. Genetics of sex determination in man and mouse. *Development* 1988 Feb;102(2):251-258.
- (10) Rogers T. A visual method of determining the sex of skeletal remains using the distal humerus. *J Forensic Sci* 1999 Jan;44(1):57-60.
- (11) Mosekilde L. The effect of modelling and remodelling on human vertebral body architecture. *Technol Health Care* 1998 Dec;6(5-6):287-297.
- (12) Mosekilde L, Mosekilde L. Sex differences in age-related changes in vertebral body size, density and biomechanical competence in normal individuals. *Bone* 1990;11(2):67-73.
- (13) Taylor J, Twomey L. Sexual dimorphism in human vertebral body shape. *J Anat* 1984 Mar;138(Pt 2):281-286.
- (14) Taylor J. Growth of human intervertebral discs and vertebral bodies. *J Anat* 1975 Sep;120(Pt 1):49-68.
- (15) Junno J, Niskanen M, Nieminen M, et al. Temporal trends in vertebral size and shape from medieval to modern-day. *PLoS One* 2009;4(3):e4836.
- (16) MacLaughlin S, Oldale K. Vertebral body diameters and sex prediction. *Ann Hum Biol* 1992 May-Jun;19(3):285-292.
- (17) Ostrofsky K, Churchill S. Sex determination by discriminant function analysis of lumbar vertebrae. *J Forensic Sci* 2015 Jan;60(1):21-28.

- (18) Amores A, Botella M, Aleman I. Sexual Dimorphism in the 7th Cervical and 12th Thoracic Vertebrae from a Mediterranean Population. *J Forensic Sci* 2014 March;59(2):301-305.
- (19) Pastor R. Sexual dimorphism in vertebral dimensions at the T12/L1 junction. *Proceedings of the 57th Annual Meeting of the American Academy of Forensic Sciences*; 2005 2005;11:302-303.
- (20) Hou W, Cheng K, Tian S, et al. Metric method for sex determination based on the 12th thoracic vertebra in contemporary north-easterners in China. *J Forensic Leg Med* 2012 Apr;19(3):137-143.
- (21) Yu S, Lee U, Kwak D, et al. Determination of Sex for the 12th Thoracic Vertebra by Morphometry of Three-dimensional Reconstructed Vertebral Models. *J Forensic Sci* 2008 May;53(3):620-625.
- (22) Zheng W, Cheng F, Cheng K, et al. Sex assessment using measurements of the first lumbar vertebra. *Forensic Sci Int* 2012 Jun 10;219(1-3):285.e1-285.e5.
- (23) Blau S, Ubelaker D. *Handbook of Forensic Anthropology and Archaeology*, Second Edition. New York: Routledge; 2016.
- (24) Jarvelin M, Hartikainen-Sorri A, Rantakallio P. Labour induction policy in hospitals of different levels of specialisation. *Br J Obstet Gynaecol* 1993 Apr;100(4):310-315.
- (25) Rantakallio P. The longitudinal study of the northern Finland birth cohort of 1966. *Paediatr Perinat Epidemiol* 1988 Jan;2(1):59-88.
- (26) Takatalo J, Karppinen J, Niinimäki J, et al. Prevalence of degenerative imaging findings in lumbar magnetic resonance imaging among young adults. *Spine (Phila Pa 1976)* 2009 Jul 15;34(16):1716-1721.
- (27) Oura P. Search for lifetime determinants of midlife vertebral size : emphasis on lifetime physical activity and early-life physical growth. *Acta Univ Oul D* 1418. Oulu: Oulun yliopisto; 2017.
- (28) Oura P, Paananen M, Niinimäki J, et al. Effects of Leisure-Time Physical Activity on Vertebral Dimensions in the Northern Finland Birth Cohort 1966. *Sci Rep* 2016;6:27844.
- (29) Brinckmann P, Biggemann M, Hilweg D. Prediction of the compressive strength of human lumbar vertebrae. *Clin Biomech (Bristol, Avon)* 1989;4(Suppl 2):3-27.
- (30) Oura P, Paananen M, Niinimäki J, et al. Effect of occupational physical activities on vertebral dimensions in midlife in the Northern Finland Birth Cohort 1966. *Occup Environ Med* 2016 Nov 18;74(5):351-356.
- (31) Oura P, Paananen M, Niinimäki J, et al. High-impact exercise in adulthood and vertebral dimensions in midlife - the Northern Finland Birth Cohort 1966 study. *BMC Musculoskelet Disord* 2017 Nov 06;18(1):433.
- (32) Oura P, Paananen M, Ojaniemi M, et al. Effect of early life physical growth on midlife vertebral dimensions - The Northern Finland Birth Cohort 1966 study. *Bone* 2017 Aug;101:172-178.
- (33) Oura P, Paananen M, Auvinen J, et al. Gravity, Parity and Vertebral Dimensions in the Northern Finland Birth Cohort 1966. *Spine (Phila Pa 1976)* 2018 [Epub ahead of print].
- (34) Griffith J. Identifying osteoporotic vertebral fracture. *Quant Imaging Med Surg* 2015 Aug;5(4):592-602.



- (35) Masharawi Y, Salame K, Mirovsky Y, et al. Vertebral body shape variation in the thoracic and lumbar spine: characterization of its asymmetry and wedging. *Clin Anat* 2008;21(1):46-54.
- (36) Peel N, Eastell R. Diagnostic value of estimated volumetric bone mineral density of the lumbar spine in osteoporosis. *J Bone Miner Res* 1994 Mar;9(3):317-320.
- (37) van Belle G, Fisher L, Heagerty P, et al. *Biostatistics - A Methodology for the Health Sciences*, Second Edition. Hoboken, New Jersey: John Wiley & Sons; 2004.
- (38) Heaney R, Abrams S, Dawson-Hughes B, et al. Peak bone mass. *Osteoporos Int* 2000;11(12):985-1009.





**Supplementary Table 1.** Additional L4 parameters among the 20-, 30-, and 46-year-old samples.

L4 parameter	Reference value (mean $\pm$ standard deviation)		
	Male	Female	P for sex difference
Age 20			
Cross-sectional area (cm <sup>2</sup> )	12.17 $\pm$ 1.57	9.63 $\pm$ 1.09	< 0.001
Volume (cm <sup>3</sup> )	33.61 $\pm$ 5.20	24.98 $\pm$ 3.52	< 0.001
Age 30			
Cross-sectional area (cm <sup>2</sup> )	13.10 $\pm$ 1.66	10.33 $\pm$ 1.15	< 0.001
Volume (cm <sup>3</sup> )	36.84 $\pm$ 5.60	27.22 $\pm$ 3.77	< 0.001
Age 46			
Cross-sectional area (cm <sup>2</sup> )	13.26 $\pm$ 1.72	10.56 $\pm$ 1.32	< 0.001
Volume (cm <sup>3</sup> )	37.08 $\pm$ 5.68	28.19 $\pm$ 4.13	< 0.001

**Supplementary Table 2.** Sex estimation accuracy of the additional L4 parameters among the 20-, 30-, and 46-year-old samples.

L4 parameter	Sex estimation accuracy (%)			Regression parameters	
	Male	Female	All	$\alpha$	$\beta$
Age 20					
Cross-sectional area	76.2	90.8	85.1	-16.83	1.53
Volume	77.6	92.1	86.4	-14.84	0.50
Age 30					
Cross-sectional area	77.6	88.2	84.0	-18.54	1.57
Volume	80.3	91.2	86.9	-15.76	0.49
Age 46					
Cross-sectional area	77.3	85.5	81.8	-14.34	1.20
Volume	77.7	85.6	82.0	-12.49	0.38

## AUTHOR CONTRIBUTION STATEMENT

**Petteri Oura:** Conceptualization, methodology, investigation, formal analysis, Writing – Original Draft, Writing – Review and Editing

**Jaro Karppinen:** Resources, Writing – Review and Editing

**Jaakko Niinimäki:** Resources, Writing – Review and Editing

**Juho-Antti Junno:** Conceptualization, methodology, Writing – Review and Editing