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RESEARCH ARTICLE

# Facial soft tissue thickness in forensic facial reconstruction: Impact of regional differences in Brazil

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## Abstract

Forensic facial reconstruction aims to assemble and provide the appearance of a face over a skull, in order to lead to recognition of that individual, making possible the application of primary identification methods. The scientific literature presents facial soft tissue thickness (FSTT) tables for reference from a range of different geographic regions. However, the consensus on its importance or on how to use specific population data related to FSTT is not unanimous. Brazil is formed by geographic regions with diverse populations, which are reflected in facial features. This paper aimed to measure and compare FSTT of distinct Brazilian samples to ascertain the need for specific data sets for different regions. A specific protocol for cone beam computed tomography was used to standardize measurement, and it was applied in a sample of 101 subjects. The FSTT measurements of a Brazilian population from the Midwest Region was compared to a previous sample from Southeast, which was collected using the same protocol. High compatibility was observed when comparing the averages of FSTT among samples of these two different geographic regions. Regarding age groups, notable differences on the medium and inferior face were observed in females. Minor variances found are unlikely to affect the practice of forensic facial reconstruction. Facial features, such as eyes, lips, nose, and skin may also be relevant in the differentiation of people from these two areas in Brazil. Therefore, concerning the Southeast and Midwest Brazilian regions, the need to apply different data sets is unnecessary.

## Introduction

The forensic facial reconstruction (FFR) has an important role in recognizing individuals who are unable to be identified by other methods. Advanced postmortem decomposition process or the lack of antemortem information are its main indication for forensic purposes [1, 2]. The

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technique is based on the assembling of an approximated soft tissue topography over the unknown skull, with the intent of reconstructing its characteristics to be as similar as possible to the individual at the time of their death. Once the reconstruction is finished, a public campaign is issued, aiming to illicit a response from the public as to whom the remains might belong. Following this, a formal identification can then take place [1, 3, 4].

An approximated face is achievable by using various techniques. The 2D representation is based on a picture of the skull, whereas the 3D representation can be divided into manual or digital methods. The manual method involves direct modelling and sculpture, applying clay or Plasticine over the skull or a model of the skull [1]. More recently, a computerized face sculpture can be generated by using specialized modeling software, and different user interfaces can adapt the procedures to resemble manual modeling. Tablets and the assistance of haptic devices may be used in the 3D context to add soft tissues to a model of the skull [5]. However, independently of the method used, it is imperative to establish parameters and guidance such as the facial soft tissue thickness (FSTT), that serves as reference over specific craniometric landmarks [6], Those FSTT measurements are usually populational-based averages, but from this general information it is often expected to deliver a precise and reliable assistance during FFR [7].

FSTT charts in the scientific literature cover many craniometric landmarks, allowing it to be organized by sex, age, ancestry, BMI, among other facial features considered to be significant in an FFR. Many aspects of the method that seem to be related to the success rate or to the probability of recognition of the reconstructed faces. Studies have indicated that FSTT data are particularly influenced by the population groups under study [8–12], but the debate on the subject has far from achieved a consensus. Therefore, the expert should consider using of FSTT based on the population to which the body can be correlated by previous anthropological exams because specific FSTT charts could produce better results. However, in those studies, it is remarkable that many describe different methodologies, including the original measurement protocols, the source and type of imaging exam and the relation of regional charts, and the success of the FFR has not yet been established. Some researchers do not believe there is enough evidence to justify the use of different population data [13], but it is unclear how this might apply in countries like Brazil, with diverse and complex demographics.

The Brazilian territory has continental proportions, and it is divided into geographical regions (north, northeast, southeast, south, and Midwest), each one with its diverse environments and populations.

To understand the local context better, it is important to remember that the original population was based on several Indigenous Brazilian with different anthropological features among the groups found in the territory. The European colonization favored the concept of a mixed ancestry population, mostly influenced by the presence of Portuguese, Dutch and French. After the world wars of the 20th century, the presence of immigrants from Japan, Eastern Europe, the Middle East, Italy and Germany became important [14]. Their distribution was not even among the regions and, therefore, it is clear that each segment of Brazil has its own physical features that represent the dominant population groups, with different characteristics [15]. The increasing and continuous lack of ancestral uniformity, as it is observed in Brazil, raises the question whether or not the use of a single STT chart is appropriate for the estimation of facial morphology [16].

This study considers that the craniofacial morphology is determined by hereditary features and influenced by the environment [11], affecting the skull and the face. The combination of these anatomical differences makes the individual unique, leading to a wide variation of facial profiles across the country [17]. This justifies deeper investigation and the collection of data from different regions. With the intent of verifying the need for various FSTT charts on

reconstruction from each geographic region, this research aimed to measure the FSTT in a Brazilian sample from the Midwest region and to compare to a previous sample from the Southeast region [18] using the same cone-beam computed tomography (CBCT) measuring protocol.

### Materials and methods

The sample was composed of 101 CBCT exams of Brazilian subjects from the Midwest region, available in a previously established anonymous data bank in Digital Imaging Communication in Medicine (DICOM) format. Table 1 shows sample distribution.

The exams were acquired from records of clinical diagnosis, and, therefore, no participant was exposed to ionizing radiation for this research. The image protocol had a 23 cm x 17 cm FOV field of view, allowing the visualization of all included anatomical structures and land-marks from the supraglabellare to menton in the vertical axis and posterior to the supraglenoid structure. The images were obtained with an i-CAT Next Generation tomograph with 17.8 second exposition and voxel of 0.3 mm, and they were exported on Digital Imaging and Communication in Medicine (DICOM) format. CBCT scans allow image acquisitions with the individual in a sitting or standing position. Despite containing stabilizing devices for the head, such as the cephalostat and the chin support, these were not used in our sample, in order to avoid the effects of compression of facial soft tissues.

The research was approved by the Committee of Ethics in Research of the School of Dentistry of University of São Paulo (CAAE 33433320.8.0000.007 and 33433320.8.0000.0075). The inclusion criteria were established by observing the facial integrity of the research subjects. Therefore, this project included the exams of adult patients (18 and older) with required presence of certain structures: the central incisors and second molars (including both natural and prosthetically rehabilitated elements), no visible facial syndromes (hard or soft tissue), with no severe asymmetry and that had not been submitted to orthognathic surgery. Furthermore, the research included exams of both male and female patients in balanced groups. Thus, the exclusion criteria rejected exams that did not fit all the inclusion criteria or had quality issues that could prejudice the measuring of soft tissues.

The selection and measurements were performed with the assistance of the Horos software (version 3.3.6, 64 bit, Horos Project, Purview, Annapolis, USA) installed on an iMac computer (macOS Catalina–version 10.25.2, a 3,4 GHz Intel Core i7 Quad-Core processor, 16 GB RAM) and the 3D reconstruction tool 3D volume rendering through the skin and bone filters. Horos software is an open access version of OsiriX (DICOM viewer software) and has demonstrated to be a good alternative for craniofacial anthropological analyses, both in hard and soft tissues [19].

The exams were divided by sex into three age groups (from 18 to 30 years old, from 31 to 40 years old, 41 and older) following the original research group divisions [18], whose control sample, from Southeast Brazilian region, was composed of 100 exams equally distributed among the groups.

Sex	Male	Female	Total	
Age group				
18 to 30 years	18	22	40	
31 to 40 years	13	14	27	
41 and older	14	20	34	
Total	45	56	101	

Table 1. Sample distribution by sex and age group.

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Fig 1. Craniometric landmarks measured.

The protocol established by Beaini et al. [18] was used to create a standardized measurements of FSTT in CBCT. In this guide, the skull images were positioned with the Frankfurt Horizontal Plane to avoid the influence of misplacement on multiplanar view. The measurements of FSTT were executed in the same craniometric landmarks studied by Rhine and Campbell [20]. Thirty-two craniometric landmarks were measured, ten located on the midline and eleven bilateral landmarks. Figs 1–3 present the craniometric landmarks and measurement direction, whereas Table 2 describes each landmark and the measurement directions according to the applied protocol.

The DICOM files from CBCT were imported into the Horos software, according to the protocol by Beaini et al. [18], a window level of 500 e window width of 3500 were applied to all exams to favor the visualization of both hard and soft tissue limits. The acquired volume was reconstructed on a 3D MPR window (multiplanar reconstruction) and visualized with the aid of a MIP tool (maximum intensity projection) to perform the volume repositioning to the Frankfurt Horizontal Plane, as previously mentioned.

An initial calibration was made in the form of inter- and intraobserver tests. The measurements for this research were collected only with a high concordance between observers. The evaluation and adjustment of inter and intra-examiners were achieved by testing the thirtytwo landmarks in fifteen exams (15% of the sample) and testing the intraclass correlation coefficient (ICC) for each point. Regarding intra-examiner assessment, the main examiner measured the fifteen exams twice, considering a one week time interval between the measurements. The main examiner's previous training of the method was consistent with the original examiner.

The data were organized in a spreadsheet, using the software Microsoft Excel, and statistical analysis was performed with aid of the SPSS statistic pack, establishing a reliability level of 95%.

Using the Shapiro-Wilk test, the normality distribution sample was checked for every landmark, with P < 0.05, which implies alteration of normality. In the nonnormal distribution landmarks, the bootstrap method was used (1000 resampling– 95% CI BCa) to obtain reliable average values.

To search for sex related differences, the *t*-test was applied on landmarks with normal distribution, whereas the Mann-Whitney test was used with nonnormal distribution data. The variance analysis from a one-way ANOVA was applied to investigate any differences among



Fig 2. Sagittal landmarks measurements.

the average FSTT, by landmark, on the three age groups (18 to 30 years, 31 to 40 years and 41 and older).

Concerning the regional differences, the research data were compared to the raw data of the Southeast region [18] to which full access was granted. A *t*-test was applied on the comparison of FSTT averages among the Southeastern [18] and Midwestern (this study) regions, for both sexes.

## Results

The concordance of the measurements verified by the ICC according to each landmark measurement revealed intra- and interexaminer agreement above 95% (data in <u>S1 Table</u>), demonstrating reliability among the measurements. Fig 4 presents the individual ICC records.

For male subjects, the Shapiro-Wilk test revealed that, from the thirty-two analyzed landmarks, two presented nonnormal distribution (lateral orbital and ectomolare<sup>2</sup>). Female subjects presented nonnormal distribution at the landmarks rhinion, mid-infraorbital, zygion and



Fig 3. Bilateral landmarks measurements.

	Landmark	Description	Measurement direction
	Median points		
1	Supraglabellare	Deepest part of the supraglabella fossa in the median plane	Perpendicular to the bone surface
2	Glabella	Most projecting median point on lower edge of the frontal bone	Perpendicular to the bone surface
3	Nasion	Intersection of the nasofrontal sutures in the median plane	Nasal soft tissue fold
4	Rhinion	Most rostral point on the internasal suture	Perpendicular to the bone surface
5	Mid-Philtrum	Deepest point of the maxillary alveolar bone	Mid-Philtrum or subnasale
6	Prosthion	Median point between the central incisors on the anterior margin of the maxillary alveolar rim	Upper vermilion border
7	Infradentale	Median point at the superior tip of the septum between the mandibular central incisors	Lower vermilion border
8	Supramentale	Deepest median point in the groove superior to the mental eminence	Chin fold
9	Pogonion	Most anterior median point on the mental eminence of the mandible	Perpendicular to the bone surface
10	Menton	Most inferior median point of the mental symphysis	Vertical direction or to neck fold
	<b>Bilateral Points</b>		
11	Frontal Eminence	Most projecting point of the frontal bone, at a line that vertically bisects the orbit	Perpendicular to the bone surface
12	Mid- supraorbital	Point on the anterior aspect of the superior orbital rim, at a line that vertically bisects the orbit	Orthoradial direction
13	Mid-infraorbital	Point on the anterior aspect of the inferior orbital rim, at a line that vertically bisects the orbit	Orthoradial direction
14	Malar	Intersection of the maxillary alveolar process and zygomatic process, at a line that vertically bisects the orbit	Perpendicular to the bone surface
15	Lateral Orbital	On the center of the zygomatic bone, at a line that vertically crosses the lateral orbital margin	Orthoradial direction
16	Zygion	The most lateral point on the zygomatic arch	Orthoradial direction
17	Supraglenoid	Most rostral point on the superior rim of the glenoid cavity	Orthoradial direction
18	Gonion	Point on the rounded margin of the angle of the mandible, bisecting two lines one following vertical margin of ramus and one following horizontal margin of the corpus of mandible	Perpendicular to the bone surface
19	Ectomolare <sup>2</sup>	Most lateral point on the buccal alveolar margin, at the center of the superior second molar position	Orthoradial direction
20	Occlusal Line	Most anterior point on the mandibular ramus, where the occlusal line meets de mandible	Orthoradial direction
21	Ectomolare <sub>2</sub>	Most lateral point on the buccal alveolar margin, at the center of the inferior second molar position	Orthoradial direction

#### Table 2. Craniometric landmarks, descriptions, and measurement direction.

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gonion. In nonnormal distribution landmarks, the bootstrap method (1000 resamples– 95% CI BCa) was used to obtain reliable mean values. Such statistical procedure assigns consistent results, through the process of random resampling, because the process tends to correct normality deviations of the sample [21].

Table 3 exhibits the FSTT averages as the main result of this research. It has been divided by sex with SD, SE and *P* values, corresponding to each test, verifying statistically significant differences between the female and male averages, after bootstrapping procedure.

The FSTT analysis indicates the male sample exhibited higher FSTT values compared to the female sample, except for the orbital lateral, which demonstrated a difference among the averages of 0.8 mm. The major thickness discrepancies between the sexes were found on land-marks the gonion, ectomolare<sup>2</sup>, ectomolare<sub>2</sub> and occlusal line, in which mean differences were above 3 mm.

Regarding age, an ANOVA was used, on both sexes, to compare averages between the age groups (18 to 30 years, 31 to 40 years and 41 and older). A direct relation between FSTT and ageing process can be seen, particularly among females. The mid-philtrum, prosthion an ecto-molare<sup>2</sup> landmarks presented a decrease of FSTT as the age advanced.





Figs 5 and 6 (data in S2 Table) show the comparison by sex among age groups. Quantitatively, a direct relation is noted between age and divergence of FSTT specifically for females, on mid-philtrum, prosthion and ectomolare<sup>2</sup> landmarks, which showed a decrease of FSTT as the age advanced.

Table 4 presents FSTT averages, on each landmark in comparison to the Southeastern sample (SE) [18] and the Midwestern (MW) (this paper), by sex, as well as *P* values (*t* test).

#### Discussion

FSTT is one of the most studied subjects in the FFR field [22]. It provides quantitative data for the technique [13], but there are still some geographical regions that have not been studied. Studies have confirmed that FSTT measures obtained by using CBCT result in reliable and reproducible data [7, 18, 23, 24].

Several studies have verified the presence of FSTT variances among different populations, suggesting the importance of using specific values for each one [8, 10–12, 25, 26]. Some of these studies compared data of FSTT averages that were obtained with different measurement protocols with inevitable bias to those conclusions [13]. This may explain why there are other studies that contradict this hypothesis and do not support that specific FSTTs are needed for different populations for the FFR [27–29]. The FSTT measurement method is important to the analysis of the specificity among different studies [29]. Our study was able to compare regional FSTT using the same method from a previous study, eliminating the method variant.

The concordance results from the interexaminer ICC analysis showed the Beaini protocol [18] can provide reproducibility and that it is recommended for future research. The intraexaminer ICC analysis demonstrated high concordance on the measurements made by the main examiner, in accordance with other studies that utilized the CBCT with the same objective [18, 30]. Nevertheless, it was a result of practical experience gained on previous training, which may be necessary and well demonstrated in future studies.

	Female		Male						
	Mean	SD	SE	Mean	SD	SE	Mean difference (mm) **	Mean difference (%) ***	P value
Supraglabella	4.5	0.97	0.14	5.5	1.14	0.23	-1	18.1	0.00* T
Glabella	4.9	0.77	0.1	5.9	0.95	0.14	-1	16.9	0.00* T
Nasion	6.2	0.97	0.13	8.1	1.24	0.18	-1.9	23.4	0.00* T
Rhinion	1.7	0.43	0.06	2.3	0.58	0.09	-0.5	26.0	0.00* M
Mid-Philtrum	13.1	1.82	0.24	15.7	2.22	0.33	-2.7	16.5	0.00* T
Prosthion	10	1.58	0.21	13.3	2	0.3	-3.3	24.8	0.00* T
Infradentale	9.8	1.34	0.18	11.9	1.5	0.22	-2.1	17.6	0.00* T
Supramentale	11.9	1.53	0.2	12.9	1.85	0.28	-1.1	7.7	0.00* T
Pogonion	9.8	2.18	0.29	11.4	2.25	0.34	-1.6	14.0	0.00* T
Menton	7	1.73	0.24	9	2.08	0.33	-2	22.2	0.00* T
Frontal Eminence R	4.1	0.89	0.14	5	1.23	0.33	-0.9	18.0	0.00* T
Frontal Eminence L	4.2	1.11	0.18	4.9	1.2	0.32	-0.7	14.2	0.04* T
Mid-supraorbital R	6.5	1.27	0.17	8.6	1.33	0.2	-2.1	24.4	0.00* T
Mid-supraorbital L	6.4	1.37	0.18	8.7	1.33	0.2	-2.4	26.4	0.00* T
Mid-infraorbital R	5.3	1.47	0.2	5.8	1.51	0.23	-0.5	8.6	0.07 M
Mid-infraorbital L	5.5	1.58	0.21	5.9	1.65	0.25	-0.4	6.7	0.07 M
Malar R	21.4	2.57	0.34	22.8	2.75	0.41	-1.4	6.1	0.01* T
Malar L	21.7	2.38	0.32	22.9	2.8	0.42	-1.2	5.2	0.02* T
Lateral Orbital R	9.1	1.73	0.23	8.3	1.46	0.22	0.8	-9.6	0.02* M
Lateral Orbital L	9.2	1.74	0.23	8.3	1.36	0.2	0.8	-10.8	0.02* M
Zygion R	7.8	1.79	0.24	9.2	1.85	0.28	-1.4	15.2	0.00* M
Zygion L	7.8	1.9	0.25	9.1	1.87	0.28	-1.3	14.2	0.00* M
Supraglenoid R	10.7	1.9	0.25	12.8	1.57	0.23	-2.1	16.4	0.00* T
Supraglenoid L	10.7	1.84	0.25	12.8	1.6	0.24	-2.1	16.4	0.00* T
Gonion R	12.8	3.65	0.49	18.3	5.54	0.83	-5.5	30.0	0.00* M
Gonion L	13	3.88	0.52	17.8	5.49	0.82	-4.8	26.9	0.00* M
Ectomolare <sup>2</sup> R	27	3.62	0.48	30.3	3.29	0.49	-3.3	10.8	0.00* M
Ectomolare <sup>2</sup> L	27.2	3.64	0.49	30.2	3.44	0.51	-2.9	9.9	0.00* M
Occlusal Line R	20.3	2.82	0.38	24.6	3.17	0.47	-4.4	17.4	0.00* T
Occlusal Line L	20.4	2.86	0.38	24.3	3.21	0.48	-3.8	16.0	0.00* T
Ectomolare <sub>2</sub> R	24.9	3.06	0.41	28.2	3.96	0.59	-3.4	11.7	0.00* T
Ectomolare <sub>2</sub> L	24.8	3.2	0.43	28.4	3.48	0.52	-3.6	12.6	0.00* T

Table 3. Mean FSTT (millimeters and percentage), SD, and SE divided by sex.

R-right; L-left; T-t-test; M-Mann Whitney.

\* P < 0.05.

\*\*; \*\*\*Differences were calculated between female means compared to male means.

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Compared to the previous research [18], that produced a FSTT chart from a Brazilian Southeastern sample, it was clear that the heterogeneity of the Brazilian population is marked by a diverse population affinity [2]. Analyzing the average of the differences among the Southeastern and Midwestern samples, the negative variation shows a predominance of deeper FSTTs among the Midwestern sample when compared to the Southeastern sample.

Considering the differences related to sex in the samples of the Southeastern [18] and Midwestern regions (this paper), statistical differentiation was observed. In males, differences were more evident in five landmarks on the midline and eight bilateral points. Quantitatively, that variance did not surpass 2.5 mm, except the ectomolare<sub>2</sub> with 3.1 mm. Proportionally, it varied





between 10 to 15% in most cases with a few landmarks showing higher values. Within females, studies indicate fewer divergent landmarks (three on the midline and eight bilateral) where only malar showed a difference above 2.5 mm (3.0 mm). These results reveal high



Fig 6. FSTT means: Comparison among age groups in females.

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		Female					Male				
Landmark	SE	MW	Mean Difference (mm)**	Mean Difference (%)***	T Test Sig.	SE	MW	Mean Difference (mm)**	Mean Difference (%)***	T Test Sig.	
Supraglabellare	3.4	4.5	-1.1	-24.4	0.00*	4.3	5.5	-1.2	-21.8	0.00*	
Glabella	5	4.9	0	0.0	0.94	5.8	5.9	-0.2	-3.4	0.45	
Nasion	5.9	6.2	-0.3	-4.8	0.11	7.2	8.1	-1	-12.3	0.00*	
Rhinion	1.7	1.7	0	0.0	0.61	1.9	2.3	-0.3	-13.0	0.01*	
Mid-Philtrum	12.3	13.1	-0.7	-5.3	0.02*	15.1	15.7	-0.6	-3.8	0.16	
Prosthion	9.5	10	-0.5	-5.0	0.17	12.4	13.3	-0.9	-6.8	0.02*	
Infradentale	11.3	9.8	1.5	15.3	0.29	11.2	11.9	-0.8	-6.7	0.05*	
Supramentale	10.8	11.9	-1.1	-9.2	0.00*	11.4	12.9	-1.5	-11.6	0.00*	
Pogonion	9.4	9.8	-0.4	-4.1	0.42	10.8	11.4	-0.7	-6.1	0.21	
Menton	6.9	7	-0.1	-1.4	0.87	8.6	9	-0.4	-4.4	0.38	
Frontal Eminence R	3.5	4.1	-0.6	-14.6	0.00*	4.4	5	-0.6	-12.0	0.09	
Frontal Eminence L	3.4	4.2	-0.8	-19.0	0.00*	4.5	4.9	-0.5	-10.2	0.21	
Mid-supraorbital R	6.2	6.5	-0.3	-4.6	0.22	7.3	8.6	-1.3	-15.1	0.00*	
Mid-supraorbital L	6.1	6.4	-0.2	-3.1	0.33	7.2	8.7	-1.6	-18.4	0.00*	
Mid-infraorbital R	5	5.3	-0.3	-5.7	0.32	5.4	5.8	-0.4	-6.9	0.16	
Mid-infraorbital L	4.8	5.5	-0.6	-10.9	0.03	5.4	5.9	-0.5	-8.5	0.07	
Malar R	19.4	21.4	-2	-9.3	0.00*	20.3	22.8	-2.5	-11.0	0.00*	
Malar L	18.7	21.7	-3	-13.8	0.00*	20.3	22.9	-2.6	-11.4	0.00*	
Lateral Orbital R	9	9.1	-0.1	-1.1	0.76	7.5	8.3	-0.8	-9.6	0.01*	
Lateral Orbital L	10.4	9.2	1.2	13.0	0.38	7.3	8.3	-1	-12.0	0.00*	
Zygion R	7.4	7.8	-0.4	-5.1	0.27	8.1	9.2	-1.1	-12.0	0.01*	
Zygion L	7.5	7.8	-0.3	-3.8	0.43	7.8	9.1	-1.4	-15.4	0.00*	
Supraglenoid R	10	10.7	-0.7	-6.5	0.06	11.4	12.8	-1.4	-10.9	0.00*	
Supraglenoid L	9.9	10.7	-0.8	-7.5	0.04*	11.1	12.8	-1.6	-12.5	0.00*	
Gonion R	13.2	12.8	0.3	2.3	0.66	16.9	18.3	-1.4	-7.7	0.21	
Gonion L	13.2	13	0.2	1.5	0.73	17.2	17.8	-0.6	-3.4	0.55	
Ectomolare <sup>2</sup> R	26	27	-1	-3.7	0.11	28.2	30.3	-2.2	-7.3	0.01*	
Ectomolare <sup>2</sup> L	26.3	27.2	-0.9	-3.3	0.17	28.2	30.2	-1.9	-6.3	0.01*	
Occlusal Line R	20.1	20.3	-0.2	-1.0	0.73	22.8	24.6	-1.8	-7.3	0.01*	
Occlusal Line L	20.4	20.4	0	0.0	0.93	23	24.3	-1.3	-5.3	0.07	
Ectomolare <sub>2</sub> R	23.5	24.9	-1.3	-5.2	0.03*	25.1	28.2	-3.1	-11.0	0.00*	
Ectomolare <sub>2</sub> L	24	24.8	-0.8	-3.2	0.18	25.5	28.4	-2.9	-10.2	0.00*	

#### Table 4. FSTT (in millimeters) comparison among SE and MW regions, in both sexes.

 $^{*}P < 0.05.$ 

\*\*; \*\*\* Differences calculated between the averages of SE sample compared to MW sample.

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compatibility among the samples, considering that errors lower than 2.5 mm, may result in high correspondence on a facial pool [31] and differences between  $2.5 \le 2.9$  mm have minimal practical impact [32].

Whether reconstructions should match local facial traits is not under debate, and the use of FSTT tables of a specific ancestry FSTT charts may provide better FFR. However, local changes should not be exclusively related to FSTT [33]. Eyes, lips, nose, and skin features could also be relevant in the differentiation of people from these two different areas in Brazil.

Investigating further into our results, they support that previous to the FFR, a careful anthropological evaluation of the skull regarding sex, age and ancestry variance remain necessary [6] because it provides important information to the forensic professional. However, the use of FSTT mean values divided by sex, age, and ancestry is no guarantee of better recognition rates, and awareness of the complexity of soft tissue displacement caused by aging [34]. The assessment of the age variant was originally made to reduce sample distribution bias when compared to the Southeastern sample [18] but it presented interesting results. Our results show that attention to this matter should not be neglected on reconstructed faces [27].

The same was planned to verify sex differences because the Southeastern research showed statistically significant changes in STT related to sex. In this research, in agreement with previous studies [18, 35-37], the analysis among sexes revealed males tend to possess larger depths than females, excepting the lateral orbital landmark, in which it has been observed a mean difference of 0.8 mm.

However, such statistical difference may not be the only reason for sexual dimorphism features [9, 36, 38]. Landmarks showed FSTT averages variate thickness within a small quantitative interval (<6% average), leaving sex variance among the individuals to skull formation [38].

The variation between males and females was no greater than 3 mm in most points. Only four landmarks exhibited greater discrepancies and those were located at anatomical regions most influenced by the BMI [37], such as gonion (5.5 mm), ectomare<sup>2</sup> (3.3 mm), ectomloare<sub>2</sub> (3.6 mm) and occlusal line (4.4 mm). Hence, sexual dimorphism was not clearly evidenced in the variation of depths of soft tissue. Despite the statistical differences, the authors found that male and female individuals appear to share similar averages [38]. The human skull is the third best reference for determining sex, after the pelvic bone and postcranial dimensions [39]. Therefore, the differences verified in this study indicate that sexual dimorphism is probably based, mainly, on the skull morphology.

Multiple facial morphological and physiological alterations act on the ageing process of the face. The initiation and progression vary between individuals, sexes, and populations [40]. In this study, meaningful differences were observed in the FSTT among the age groups, notably in females, at the landmarks located on the medium and inferior face. The FSTT averages of the landmarks mid-philtrum, prosthion and ectomolare<sup>2</sup> tend to decrease progressively with age, reproducing the morphological alteration patterns that occur in facial ageing, demonstrating a coherence among the data. With the advancing of age, the average face volume is permanently lost from both from superficial and deep fat tissue [41], and a redistribution of this volume occurs that causes slenderizing of the superior lip more than the inferior [42]. Bone remodeling in that region results in a decrease of the overlying soft tissue support area. This phenomenon, along with the effect of gravity, characterizes the average ageing of the face [34, 43].

#### Conclusions

The measurements analyzed in this research, when compared to those collected in the Southeast region of Brazil, are statistically different at many landmarks. However, observing the averages in a specific FSTT chart, indicated that regional differences between the two samples are quantitatively small and they should have minimal influence on facial reconstructions. Therefore, the use of specific FSTT charts is not necessary when performing FFR of individuals from Southeast and Midwest Brazil.

#### Supporting information

**S1 Table. ICC intra- and interexaminer.** (PDF)

**S2 Table. FSTT means in age groups in both sexes.** (PDF)

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#### References

- 1. Wilkinson C. Facial reconstruction–anatomical art or artistic anatomy? J Anat. 2010; 216: 235–250. https://doi.org/10.1111/j.1469-7580.2009.01182.x PMID: 20447245
- Wilkinson C. Computerized forensic facial reconstruction: A review of current systems. Forensic Sci Med Pathol. 2005; 1: 173–177. https://doi.org/10.1385/FSMP:1:3:173 PMID: 25870042
- Wilkinson C. Facial identification of the dead. J Anat. 2008 [cited 29 Jun 2020]. https://doi.org/10.1111/j. 1469-7580.2008.00972.x PMID: 19141110
- 4. Fernandes CMS, da Costa Serra M, da Silva JVL, Yoshito Noritomi P, de Sena Pereira FDA, Melani RFH. Tests of one Brazilian facial reconstruction method using three soft tissue depth sets and familiar assessors. Forensic Sci Int. 2012; 214: 211.e1–211.e7. https://doi.org/10.1016/j.forsciint.2011.08.017 PMID: 21937172
- Stephan CN, Caple JM, Guyomarc'h P, Claes P. An overview of the latest developments in facial imaging. Forensic Sci Res. 2019; 4: 10–28. https://doi.org/10.1080/20961790.2018.1519892 PMID: 30915414
- de Almeida NH, Michel-Crosato E, de Paiva LAS, Biazevic MGH. Facial soft tissue thickness in the Brazilian population: new reference data and anatomical landmarks. Forensic Sci Int. 2013; 231: 404.e1–7. https://doi.org/10.1016/j.forsciint.2013.05.024 PMID: 23791380
- Fourie Z, Damstra J, Gerrits PO, Ren Y. Accuracy and reliability of facial soft tissue depth measurements using cone beam computer tomography. Forensic Sci Int. 2010; 199: 9–14. <a href="https://doi.org/10.1016/j.forsciint.2010.02.018">https://doi.org/10.1016/j.forsciint.2010.02.018</a> PMID: 20236780
- Cavanagh D, Steyn M. Facial reconstruction: Soft tissue thickness values for South African black females. Forensic Sci Int. 2011; 206: 215.e1–215.e7. https://doi.org/10.1016/j.forsciint.2011.01.009 PMID: 21288672
- Tedeschi-Oliveira SV, Melani RFH, de Almeida NH, de Paiva LAS. Facial soft tissue thickness of Brazilian adults. Forensic Sci Int. 2009; 193: 127.e1–127.e7. https://doi.org/10.1016/j.forsciint.2009.09.002 PMID: 19781879

- Bulut O, Sipahioglu S, Hekimoglu B. Facial soft tissue thickness database for craniofacial reconstruction in the Turkish adult population. Forensic Sci Int. 2014; 242: 44–61. https://doi.org/10.1016/j.forsciint. 2014.06.012 PMID: 25023216
- 11. Codinha S. Facial soft tissue thicknesses for the Portuguese adult population. Forensic Sci Int. 2009; 184: 80.e1–80.e7. https://doi.org/10.1016/j.forsciint.2008.11.011 PMID: 19124207
- De Donno A, Sablone S, Lauretti C, Mele F, Martini A, Introna F, et al. Facial approximation: Soft tissue thickness values for Caucasian males using cone beam computer tomography. Leg Med. 2019; 37: 49– 53. https://doi.org/10.1016/j.legalmed.2019.01.002 PMID: 30660658
- Stephan CN, Simpson EK. Facial Soft Tissue Depths in Craniofacial Identification (Part I): An Analytical Review of the Published Adult Data\*. J Forensic Sci. 2008 [cited 26 Jun 2020]. https://doi.org/10.1111/ j.1556-4029.2008.00852.x PMID: 18783476
- Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SDJ. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci. 2003; 100: 177–182. <u>https://doi.org/10.1073/pnas.0126614100</u> PMID: 12509516
- Gonzales PS, Machado CEP, Michel-Crosato E. Photoanthropometry of the Face in the Young White Brazilian Population. Braz Dent J. 2018; 29: 619–623. <u>https://doi.org/10.1590/0103-6440201802027</u> PMID: 30517486
- Jurda M, Urbanová P. Sex and ancestry assessment of Brazilian crania using semi-automatic mesh processing tools. Leg Med. 2016; 23: 34–43. https://doi.org/10.1016/j.legalmed.2016.09.004 PMID: 27890100
- Cunha E, Lopez-Capp TT, Inojosa R, Marques SR, Moraes LOC, Liberti E, et al. The Brazilian identified human osteological collections. Forensic Sci Int. 2018; 289: 449.e1–449.e6. <u>https://doi.org/10.1016/j.</u> forsciint.2018.05.040 PMID: 29921482
- Beaini TL, Miamoto P, Duailibi-Neto EF, Tedeschi-Oliveira SV, Chilvarquer I, Melani RFH. Facial soft tissue depth measurements in cone-beam computed tomography: A study of a Brazilian sample. Leg Med. 2021; 50: 101866. https://doi.org/10.1016/j.legalmed.2021.101866 PMID: 33667933
- 19. Dias PEM, Beaini TL, Melani RFH. Evaluation of osifix software with craniofacial anthropometric purposes. J Res Dent. 2013; 1: 351. https://doi.org/10.19177/jrd.v1e42013351-367
- Rhine JS, Campbell HR. Thickness of facial tissues in American blacks. J Forensic Sci. 1980; 25: 847– 858. PMID: 7430993
- Haukoos JS. Advanced Statistics: Bootstrapping Confidence Intervals for Statistics with "Difficult" Distributions. Acad Emerg Med. 2005; 12: 360–365. https://doi.org/10.1197/j.aem.2004.11.018 PMID: 15805329
- Stephan CN, Munn L, Caple J. Facial soft tissue thicknesses: Noise, signal, and P. Forensic Sci Int. 2015; 257: 114–122. https://doi.org/10.1016/j.forsciint.2015.07.051 PMID: 26295929
- Hwang H-S, Kim K, Moon D-N, Kim J-H, Wilkinson C. Reproducibility of Facial Soft Tissue Thicknesses for Craniofacial Reconstruction Using Cone-Beam CT Images: FACIAL SOFT TISSUE THICKNESS USING CBCT IMAGES. J Forensic Sci. 2012; 57: 443–448. <u>https://doi.org/10.1111/j.1556-4029.2011</u>. 02007.x PMID: 22150152
- 24. Hwang H-S, Choe S-Y, Hwang J-S, Moon D-N, Hou Y, Lee W-J, et al. Reproducibility of Facial Soft Tissue Thickness Measurements Using Cone-Beam CT Images According to the Measurement Methods. J Forensic Sci. 2015; 60: 957–965. https://doi.org/10.1111/1556-4029.12766 PMID: 25845397
- Phillips VM, Smuts NA. Facial reconstruction: utilization of computerized tomography to measure facial tissue thickness in a mixed racial population. Forensic Sci Int. 1996; 83: 51–59. https://doi.org/10.1016/ 0379-0738(96)02010-5 PMID: 8939013
- Baillie LJ, Mirijali SA, Niven BE, Blyth P, Dias GJ. Ancestry and BMI Influences on Facial Soft Tissue Depths for A Cohort of Chinese and Caucasoid Women in Dunedin, New Zealand. J Forensic Sci. 2015; 60: 1146–1154. https://doi.org/10.1111/1556-4029.12799 PMID: 26260028
- Lee W-J, Wilkinson CM, Hwang H-S, Lee S-M. Correlation Between Average Tissue Depth Data and Quantitative Accuracy of Forensic Craniofacial Reconstructions Measured by Geometric Surface Comparison Method. J Forensic Sci. 2015; 60: 572–580. <u>https://doi.org/10.1111/1556-4029.12726</u> PMID: 25739646
- Thiemann N, Keil V, Roy U. In vivo facial soft tissue depths of a modern adult population from Germany. Int J Legal Med. 2017; 131: 1455–1488. https://doi.org/10.1007/s00414-017-1581-y PMID: 28417258
- Guyomarc'h P, Santos F, Dutailly B, Coqueugniot H. Facial soft tissue depths in French adults: variability, specificity and estimation. Forensic Sci Int. 2013; 231: 411.e1–10. https://doi.org/10.1016/j.forsciint. 2013.04.007 PMID: 23684263
- Baccarin LS. Análise da relação entre as espessuras médias de tecidos moles da face de crianças e adultos para reconstrução facial forense. Doutorado, Universidade de São Paulo. 2019.

- Stephan CN. Facial Approximation-From Facial Reconstruction Synonym to Face Prediction Paradigm. J Forensic Sci. 2015; 60: 566–571. https://doi.org/10.1111/1556-4029.12732 PMID: 25703265
- Briers N, Steyn M. Re-assessment of South African juvenile facial soft tissue thickness data for craniofacial approximation: A comparative analysis using central tendency statistics. Forensic Sci Int. 2018; 291: 280.e1–280.e13. https://doi.org/10.1016/j.forsciint.2018.08.008 PMID: 30213707
- Wilkinson C, Neave R, Smith D. How important to facial reconstruction are the correct ethnic group tissue depths? Proceedings of the 10th Conference of the International Association of Craniofacial Identification. Bari, Italy: University of Bari; 2002. pp. 111–21.
- Buchanan DR, Wulc AE. Contemporary Thoughts on Lower Eyelid/Midface Aging. Clin Plast Surg. 2015; 42: 1–15. https://doi.org/10.1016/j.cps.2014.09.003 PMID: 25440737
- Farias Gomes A, Moreira DD, Zanon MF, Groppo FC, Haiter-Neto F, Freitas DQ. Soft tissue thickness in Brazilian adults of different skeletal classes and facial types: A cone beam CT–Study. Leg Med. 2020; 47: 101743. https://doi.org/10.1016/j.legalmed.2020.101743 PMID: 32659706
- Dong Y, Huang L, Feng Z, Bai S, Wu G, Zhao Y. Influence of sex and body mass index on facial soft tissue thickness measurements of the northern Chinese adult population. Forensic Sci Int. 2012; 222: 396.e1–396.e7. https://doi.org/10.1016/j.forsciint.2012.06.004 PMID: 22738738
- De Greef S, Vandermeulen D, Claes P, Suetens P, Willems G. The influence of sex, age and body mass index on facial soft tissue depths. Forensic Sci Med Pathol. 2009; 5: 60–65. <u>https://doi.org/10.1007/s12024-009-9085-9 PMID: 19437147</u>
- Stephan CN, Norris RM, Henneberg M. Does sexual dimorphism in facial soft tissue depths justify sex distinction in craniofacial identification? J Forensic Sci. 2005; 50: 513–518. PMID: 15932080
- Nikita E, Michopoulou E. A quantitative approach for sex estimation based on cranial morphology. Am J Phys Anthropol. 2018; 165: 507–517. https://doi.org/10.1002/ajpa.23376 PMID: 29265191
- Cotofana S, Gotkin RH, Ascher B, Morozov SP, Gombolevsky VA, Laipan AS, et al. Calvarial Volume Loss and Facial Aging: A Computed Tomographic (CT)-Based Study. Aesthet Surg J. 2018; 38: 1043– 1051. https://doi.org/10.1093/asj/sjy096 PMID: 29635393
- Boehm LM, Morgan A, Hettinger P, Matloub HS. Facial Aging: A Quantitative Analysis of Midface Volume Changes over 11 Years. Plast Reconstr Surg. 2021; 147: 319–327. <u>https://doi.org/10.1097/PRS.000000000007518 PMID: 33165293</u>
- Ko AC, Korn BS, Kikkawa DO. The aging face. Surv Ophthalmol. 2017; 62: 190–202. <u>https://doi.org/10.1016/j.survophthal.2016.09.002</u> PMID: 27693312
- Mendelson B, Wong C-H. Changes in the Facial Skeleton With Aging: Implications and Clinical Applications in Facial Rejuvenation. Aesthetic Plast Surg. 2012; 36: 753–760. <u>https://doi.org/10.1007/s00266-012-9904-3</u> PMID: 22580543