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**Document Title:** Development of Modern Subadult Standards: Improved Age and Sex Estimation in U.S. Forensic Practice

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Summary of the project

Estimating the biological profile (i.e., age, sex, stature, and ancestry) from a set of unidentified remains is a primary objective for a forensic anthropologist. Unfortunately, when working with subadult material, an age at death estimate is the only parameter that can be provided to the requesting agency. A limited biological profile negatively impacts identification, as there are fewer parameters to narrow the list for potential matches. The limited abilities of the forensic anthropologist are a direct result of the paucity of modern, known subadult skeletal material. Without remains to work with, there is no way to create and/or validate methods. The majority of age estimation methods in use today were devised using historic growth studies or cemetery collections and/or relatively small sample sizes. Given documented secular changes in the growth and development of children (Beunen, Rogol, & Malina, 2006), methods derived from antiquated skeletal material are not appropriate to apply to modern subadult individuals in the medico-legal setting. Although recently developed radiographic databases attempt to mitigate the issues of sample size and modernity (Ousley, 2013), conventional X-rays present some limitations in data collection, such as magnification and 2D superimposition of anatomical...
structures. These limitations along with the lack of accessible skeletal material have hindered the development of subadult standards. Therefore, the aim of this project was to collect data from full-body computed tomography (CT) images generated at medical examiner’s offices in the United States to develop single and multiple variable methods to estimate subadult age and sex data for practical forensic applications.

Research Design, Methods, and Analytical Techniques

Materials

The final sample (n = 1327) stems from two geographically diverse medical examiner’s offices, the University of New Mexico Health Sciences Center, Office of the Medical Investigator (UNM) and the Office of the Chief Medical Examiner in Baltimore, Maryland (OCME) (Figure 1). The UNM sample (n = 1081, 81%) comprises a larger portion of the sample than the OCME sample (n = 246, 19% of total) (Figure 1). While working with full body CT images bolsters the analyses because all of the variables can be collected from each individual, there are also limitations with working with any type of imaging data generated at a medical examiner’s office. The biggest of which is the bimodal mortality distribution of subadults (Figure 1). The highest rates of death occur in the very young (< 3 years) and are usually associated with sudden infant death syndrome and congenital abnormalities (Bethesda, 2019; Ousley, 2013). The mortality is low through most of childhood until the mid-teens; deaths in teenagers is because of a rise in risk taking behavior and increased rates of homicide and suicide. Additionally, in all ages, males have a higher mortality rate than females (Bethesda, 2019; Ousley, 2013). Because of the bimodal distribution and different sex mortality rates, there is an unequal number of individuals per each chronological age (Figures 1; Table 1).

Even with the unequal age distribution, the sample of modern subadults, aged between birth and 20 years (n=1327), is large and diverse, capturing a wide range of variation (Tables 2 and 3; Figure 2). Diversity in the sample increases the generalizability of the models, which is crucial for practitioners. The three largest social race/ancestry groups in the sample are white (68%), American Indian (17%), and black (11%) (Figure 2). The OCME and UNM recorded Hispanics differently; the OCME considers it a social race group while UNM considers it an ethnicity (Tables 2 and 3). A total of 33% (n = 442) of the sample was recorded as Hispanic; the majority of this percentage was considered white ancestry within the UNM sample (Table 3).

Methods

The variables collected on each CT scan included: appearance of ossification centers, epiphyseal union from the long bones and pelvis, and diaphyseal dimensions from the long bones. Both left and right sides were collected for all individuals. Additionally, 34 three-dimensional (3D) landmarks were collected on the ossa coxae of each individual for interlandmark distances and geometric morphometric analyses. The greatest advantage of the sample, and extraordinarily unique feature compared to collections of skeletal remains, is that all variables (n = 140) were collected from all individuals, as long as the age of the individual enabled data collection. For example, in the oldest individuals, the diaphyseal data is no longer available once the epiphyseal fusion data has reached a specific stage (see below). Subsequently, the oldest individuals have far fewer variables to collect because of the interaction of the growth processes.

A seven-stage scoring system was used for the six long bone epiphyses and calcaneal tuberosities. The stages are defined as: 0) the epiphysis has not ossified (or appeared) (i.e., absent); 1) the epiphysis has appeared but is characterized by the lack of any bony attachments (i.e., present); 1/2) "early active union" is used when bony bridging exists, but is between 0 and 25% of the entire surface; 2) "active union" is used when bony bridging is equal or slightly less than half the length of the epiphyseal growth; 2/3) "active/advanced union" is used when bony
bridging is approximately at 50%; 3) “advanced union” is characterized by bony bridging greater than half the length of the growth plate, with no or minor radiolucent gaps retained throughout; and 4) complete fusion, as demonstrated by homogenous radiodensity. Scoring in seven stages offers a high level of precision in data collection and retains the ability to collapse stages. The collapsed stage system is defined as: 0) absent, 1) unfused, 2) fusing, and 3) fused. In the collapsed system, stages 1/2, 2, 2/3, and 3 are all collapsed into stage 2 (fusing).

A three-stage scoring system was used for the pelvis and is defined as: 0) unfused, 1) fusing, and 2) fused. Ossification of the carpals and tarsals was assessed by their number, and a binary (present/absent) scoring system was used for the patella and the different elements composing the proximal humeral head and the distal humerus. Table 4 has the complete list of epiphyseal fusion variables and their stages.

Diaphyseal measurements, specifically lengths and breadths, were taken in Amira software from the filled smoothed generated surface using the 3D measurement tool to the nearest hundredth of a millimeter. Diaphyses of the humerus, radius, ulna, femur, tibia, and fibula were rotated into anatomical position and viewed from their anterior surfaces when they were measured based on the definitions provided by Stull and collaborators (Stull, L’Abbé, & Ousley, 2014), which were modified from Fazekas and Kosa’s dry bone measurements (Fazekas & Kósa, 1978). The measurements to be taken include:

- Humerus – diaphyseal length, midshaft breadth, proximal breadth, distal breadth
- Ulna – diaphyseal length, midshaft breadth
- Radius – diaphyseal length, midshaft breadth, proximal breadth, distal breadth
- Femur – diaphyseal length, midshaft breadth, distal breadth
- Tibia – diaphyseal length, midshaft breadth, proximal breadth, distal breadth
- Fibula – diaphyseal length

If the epiphyseal fusion score was greater than or equal to two (“active union”), the diaphyseal length and midshaft breadth for that bone cannot be recorded as these measurements will be obscured at this stage of development. If the fusion score for a distal or proximal epiphysis is four (“completely fused”), then the corresponding distal or proximal diaphyseal breadth was not measured.

All variables and single variable, multiple variable, and Bayesian statistical approaches were used to develop subadult age and sex estimation standards.
Figure 1. The age distribution of the total sample separated by sex (top) and then the distribution separated by collaborating institution (bottom).
Table 1 – Sex and age distribution for the entire sample (pooled UNM and OCME).

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Table 2 - Demographics and the associated sample size: Office of the Chief Medical Examiner, State of Maryland

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Table 3 - Demographics and the associated sample size: University of New Mexico, Office of the Medical Investigator

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Figure 2. Ancestry/race data for the pooled sample.
Table 4 – Detailed information on how each site was scored for the epiphyseal appearance/fusion variables.

<table>
<thead>
<tr>
<th>Bone</th>
<th>Epiphyses</th>
<th>Abbreviation</th>
<th>Scoring system</th>
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<td>Humeral Head Ossification</td>
<td>HH_Oss</td>
<td>2-stage scoring system</td>
</tr>
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<td>Humeral Greater Tubercle Ossification</td>
<td>HGT_Oss</td>
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<tr>
<td></td>
<td>Humeral Lesser Tubercle Ossification</td>
<td>HLT_Oss</td>
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<td>Humeral Proximal Epiphyseal Fusion (PE=fused head, GT and LT). If PE not fused, score 0</td>
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<td>7-stage scoring system</td>
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<td>Capitulum Ossification</td>
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<td>Trochlea Ossification</td>
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<td>Lateral Epicondyle Ossification</td>
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<td>Composite Epiphysis 1 (fusion of capitulum and trochlea) Epiphyseal Fusion</td>
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<td>Composite Epiphysis 2 (fusion of CE1 and lateral epicondyle) Epiphyseal Fusion</td>
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Participants and other collaborating organizations

- University of New Mexico Health Sciences Center, Office of the Medical Investigator (UNM)
- Office of the Chief Medical Examiner, State of Maryland (OCME)

Outcomes

Activities/accomplishments

One of the most substantial impacts of this funded proposal was the development of a large virtual database of CT images of modern subadults. While the development of the Subadult Virtual Anthropology Database (SVAD) was not a primary goal of the proposal, the research protocol directly led to its development and it now offers a repository of anonymized CT images that have freely available biological/skeletal (diaphyseal dimensions, epiphyseal fusion, and dental development data), demographic (social race, age, sex), and mortality (cause of death, manner of death) data.

The validity of the research methods was dependent on the establishment of the reference sample. The results and findings discussed below offer a breadth of analyses that when previously explored, if ever done, were hindered by inappropriate samples in size and/or modernity. Therefore, the findings offer substantive and transformative knowledge of the subadult skeletal system and its utility in estimating age and sex. The results directly impact the capacity of biological and forensic anthropologists when faced with subadult skeletal remains and ultimately the best practices in the field.

Results and Findings

Reliability of Segmentation/Thresholding

One of the additional considerations when working with advanced imaging is the accuracy and precision of the image, as this directly impacts the validity and applicability of the methods. Colman et al., (2019) and Colman, Dobbe, Stull, & Ruijter (2017) explored the effects of imaging parameters on the precision of the virtual rendering and on the impact of soft tissue on the accuracy of the measurements on the virtual rendering. Therefore, as part of the current research, the impact of post-imaging protocols on the virtually rendered images were quantified. Four observers segmented the ossa coxae from eleven randomly selected individuals from the sample. Root mean square error, the average distance deviation, and maximum deviation distances demonstrated that thresholding values of ~50 Hounsfield units (HU) have minimal impact on the rendered image. Remarkably, even the largest inter-observer difference in thresholding values (130 HU) in this study resulted in a model RMSE values < 0.5 mm (Stock et al., 2020).

Subadult Age Estimation

The majority of efforts associated with subadult age estimation was the development of a Bayesian model that could incorporate both the ordinal and continuous age indicators into a single estimate that considers conditional dependence, missing data, and the heteroscedastic nature of age indicators. The mixed cumulative probit (MCP) model has four different algorithms with two factors that vary: conditional independence/dependence and homoscedasticity/heteroscedasticity. Based on our data, the log-likelihoods and root mean squared errors indicate the appropriate model is one that considers the correlations and heteroscedasticity (Figure 3). While the PIs have focused their efforts on developing an
algorithm that can analyze all ordinal and continuous variables at one time, the age indicators (i.e., dental development, epiphyseal fusion, and diaphyseal dimensions) and variables (i.e., femur diaphyseal length, humerus distal breadth, etc.) can also be individually evaluated. The MCP is recommended for age estimation as numerous studies (as well as our own) have demonstrated that the incorporation of more variables leads to less biased and more precise age estimates (De Tobel et al., 2019; Štern, Payer, Giuliani, & Urschler, 2019; Stull & Armelli, submitted)). However, the individual analyses of each variable will provide useful in evaluating secular trends in age indicators as well as exploring inter-population variation.

The model was presented at the 72nd Annual Meeting of the American Academy of Forensic Sciences (Stull, Price, Corron, & Chu, 2020) both in a presentation and a workshop. The model is being fine-tuned prior to publication; we expect the manuscript to be submitted by June 2020. In an effort to maximize applicability and bridge the gap between researchers and practitioners, the algorithm will be available through two different means. First, if one is interested in estimating subadult age, then practitioners can use the graphical user interface, KidStats. The algorithm can also be used to answer any type of research question as long as the data has a similar structure (i.e., continuous outcome). Therefore, the algorithm will also be made freely available via a R package that is expected to be released in the next year.

![Figure 3. Predicted point age estimates against the true age with color indicating the model implemented. The model that considers correlations and heteroscedasticity is considered the appropriate model.](image)
Subadult Sex Estimation

**Long Bones**

The preliminary results used models developed with 18 variables, flexible discriminant analysis (FDA), downsampled subsets for a balanced sex distribution, and unique training (n = 270) and testing (n = 88) samples. Classification accuracies of 75% were achieved and display no sex bias and have no systematic misclassification patterns by age. Furthermore, age as a covariate does not improve classification accuracy. The first explorations of sex estimation using multiple long bone dimensions were published using a South African sample that is freely available (Stull, L’Abbe, & Ousley, 2017). In an effort to evaluate the performance of the model when more observations were included, the South African and US data were pooled. The South African data does not have a large number of individuals with all measurements, therefore only a subset of variables was selected (n = 8) to compromise sample and multiple variables. Subsequently, the analyses have more individuals in the sample, but less variables for differentiating the sexes. Downsampling was implemented for balanced classes, and unique subsets were used to train (n = 390) and test (n = 128) the model. The pooled US and SA model achieved a slightly lower classification accuracy (73%), but still presented without age or sex biases. Additional statistical analyses beyond FDA are being implemented to see if the accuracy can be improved. Upon completion of the analyses, the manuscript will be submitted.

The methodological aspects (e.g., downsampling and training and testing subsets) as well as additional reference samples are incorporated into a ‘Sex Estimation’ module in KidStats to facilitate subadult sex estimation.

**Ossa Coxae: Outline Analyses**

Ilium outlines and basic metric data (component breadths and lengths, as well as greater sciatic notch measurements) were collected on 394 individuals displaying unfused acetabula. The resultant age distribution was from birth to 14 years of age. Metric data included: ilium blade breadth, minimum iliac breadth, ischial length, and pubic breadth and length. Previous research and preliminary analyses suggested that greater sciatic notch (GSN) morphology could also be informative of subadult sex differences, so GSN length, depth, angle, and position of greatest depth were also collected. Finally, in case the GSN metrics were not capturing the full GSN morphology, GSN outlines were also collected.

Statistically significant sex differences were found in all the measurements when age was a covariate in the MANCOVA. When all ages were combined in a step-wise discriminant function analysis (DFA), however, the model only achieved classification accuracies of 60%. If analyses were limited to individuals older than 5 years of age, the classification accuracy increased to 73%.

Outline data were analyzed with elliptical Fourier analysis and principal component analysis (PCA) and the resulting principal components were used in a step-wise DFA (Figure 4). While some variables demonstrated significant sex differences in the MANCOVA/ANOVA, the classification accuracies were consistently below 69%. Limiting analyses to only an older subset of individuals did not increase classification accuracy. A MANCOVA on the GSN PCs revealed no significant effects of sex, although age was a significant covariate (p<0.001).
Figure 4. Example of a 3D pelvic model, in which the ilium was isolated, orientation was standardized, and an outline with a scale was extracted for elliptical Fourier analysis.

Ossa Coxae: Geometric Morphometrics

A total of 34 3D landmarks were collected from the ossa coxae of the entire sample (Figure 5), resulting in a significant amount of data that can be utilized to answer a multitude of questions. Preliminary analyses to assess the overarching question of the utility of 3D landmarks in subadult sex estimation used only 19 of these landmarks; this was reduced to eliminate landmarks with higher interobserver variability and those that could not be collected across the full range of the age sample. The resultant sample size for this analysis was 720 individuals, between birth and 20 years. General Procrustes analysis (GPA) and PCA were performed on the data to evaluate the range of shape variation. MANCOVA revealed both statistically significant ($p < 0.05$) age and sex effects and an ANCOVA revealed statistically significant sex differences in 10 of the 20 PCs. The PCs were then used in a stepwise DFA, resulting in a 80% correct classification with slight sex bias (M=80.4%, F=76.2%). The PCs with the highest coefficients have the greatest contributions to the discriminant function and related to greater sciatic notch morphology and relative pubic and ischial lengths. In the pooled sample, it is possible that the older individuals are driving the classifications. When restricted to individuals less than 5 years of age, only a 65.1% classification was obtained. Those 5 to 15 years resulted in a 75.7% classification. If the age of the sample is restricted to those over the age of 10 years, a 92.7% correct classification is achieved. The clearest separation of males and females follows acetabular fusion and puberty at ~15 years of age (95.2% classification); this is also when the sample size increases (see Limitations) (Figure 6).
Figure 5. Thirty-four landmarks were placed on segmented ossa coxae. Because of the changes associated with ontogeny, visualizations are provided for when the ilium, ischium, and pubis are separate (top) and when the bones are fused (bottom).
Figure 6. Discriminant scores plotted on age reveals a stronger divergence between the sexes after ~5 years of age. The separation between the sexes increases as age increases.

Limitations
The biggest limitation of the final sample is the bimodal distribution and subsequent unequal number of individuals per chronological age. The distribution is problematic considering the numerous growth and development processes active during the chronological ages where the samples are the smallest.

Artifacts
The current proposal has laid the foundation for the development of at least four doctoral research topics, two federally funded grants (NIJ Awards 2017-DN-BX-0144 and 2019-DU-BX-0039), and is being used in numerous other dissertations.

Databases and Software
- The development of the Subadult Virtual Anthropology Database, which is housed at the University of Nevada, Reno. All data that were collected is freely available. Additionally, the anonymized CT images collected from UNM are available for research.


Stull, L’Abbe, & Ousley (2014 and 2017) introduced KidStats to facilitate estimation of subadult age and sex using upwards of 18 dimensions collected from long bones from South African subadults. The GUI has been updated to include all age indicators (i.e.,
epiphyseal fusion, dental development, and diaphyseal dimensions) and the United States reference sample. The improvements now allows users to estimate age using a specific population, South Africa (Stull et al., 2017) or United States (i.e., current award), or a pooled sample that includes all data. While the age estimation algorithm has been updated to implement the MCP, the sex estimation has not been updated yet. The PIs were waiting to disseminate the results prior to updating the GUI.


KSCollect was developed to standardize data collection and minimize downstream and integrity issues with data storage. Essentially, this is a protected database that stores the data in a .rds file, which is a R data format, and preserves data structures. By saving the file as a RDS file, a serialized version of the dataset is created and then saved with gzip compression. Therefore, KSCollect allows researchers, students, and professionals a standardized approach to subadult data collection of variables commonly employed in analyses.

Dissemination Activities

- Workshop at the American Academy of Forensic Sciences, February 17, 2020
  KidStats: Improving the Subadult Biological Profile. Chair: Kyra Stull, Co-Chair: Heather Garvin, Faculty: Alexandra Klales, Louise Corron, Michael Price

- Workshop at Texas State University, April 2019
  KidStats and Subadult Age Estimation

Publications and Conference Papers

All resulting publications, presentations, or workshops are provided in the table below. Any author with a * indicates they are/were a student.

<table>
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<th>Status</th>
<th>Authors</th>
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<tr>
<td>In preparation</td>
<td>Stull, KE, Wolfe, CA, Corron, LK, Heim, K, Hulse, CN, Pilloud, MA.</td>
<td>“A Comparison of Subadult Skeletal and Dental Development Based on Living and Deceased Samples”</td>
<td>To be submitted to American Journal of Physical Anthropology.</td>
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<tr>
<td>Submitted</td>
<td>Stull, KE and Armelli, KA* (submitted) “Combining Variables to Improve Subadult Age Estimation.”</td>
<td>Forensic Anthropology.</td>
<td>FORENSIC ANTHROPOLOGY.</td>
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<tr>
<td>2019</td>
<td>Stull, KE, Corron, LK, Hulse, CN*, Yang, Y</td>
<td>“Exploring the relationship between dental development, population variation, and environment.”</td>
<td>Annual Meeting of the American Association of Physical Anthropologists, Cleveland, OH.</td>
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Corron, LK, Stull, KE, Yang, Y. “Variation of Skeletal Growth and Development Patterns in Populations with Diverse Socio-Economic Backgrounds.” Annual Meeting of the American Association of Physical Anthropologists, Cleveland, OH.

Mohamed, M*, Garvin, HM. “Sex Estimation from the Greater Sciatic Notch Outline of Subadult Human Pelves.” Poster presentation at DMU Winter Research Symposium.


Corron, LK and Stull, KE. “The predictive ability of subadult age indicators according to life history stages: a preliminary study” Annual Meeting for the Forensic Anthropology Society of Europe, Marseille, France.


Severa K*, Garvin HM. Subadult pelvic sex estimation from a modern multi-slice computed tomography sample. Poster presentation at DMU MSRP Summer Research Symposium.

Ternent E*, Garvin HM. Assessing the utility of greater sciatic notch measurements in subadult sex estimation. Poster presentation at DMU Research Symposium. *Graduate Award in Anatomy/Paleontology


References


