A RE-EVALUATION OF THE IMPACT OF RADIOGRAPHIC ORIENTATION ON THE IDENTIFICATION AND INTERPRETATION OF HARRIS LINES

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ABSTRACT

The identification of Harris lines through radiographic analysis has been wellestablished since their discovery in the late 19th century. Most commonly associated with stress, the study of Harris lines has been fraught with inconsistent identification standards, high levels of intra- and inter-observer error, and the inevitability of skeletal remodelling. Despite these methodological challenges, the use of Harris lines remains an important contributor to studies of health in archaeological populations. This research explores the radiographic process, specifically orientation and how Harris lines are initially captured for study. Using the Black Friars (13th - mid 17th centuries) skeletal sample from Denmark, 157 individuals (134 adults; 23 subadults) were radiographically analyzed in both an anterior-posterior (A-P) and medial-lateral (M-L) view for the left and right radii and tibiae. Based on the current methodological standards within the literature, it was hypothesized that the A-P view would provide the best resolution and visualization of Harris lines. The results, however, show that the number of lines visible in the M-L view were significantly higher than those visible in the A-P view; inferring that the M-L view is superior for the study of Harris lines.

The use of Harris lines as an osteological indicator of stress has been well-established since their initial explorations over a century ago (e.g. Wegner 1874; Gies 1877; Ludoff 1903). Though studied well before the work of their namesake H.A. Harris, it was his pioneering studies (Harris 1926; 1931; 1933) on their formation and causative factors that 'Harris' lines became the colloquial terminology for these osteological changes.

The radiographic study of Harris lines has unquestionably contributed to the understanding of growth in the human skeleton, but has been continually criticized for a lack of standardized methodology (e.g. Goodman and Clark 1981; Mays 1995; Suter et al. 2008), intra- and inter-observer error (MacChiarelli et al. 1994; Grolleau-Raoux et al. 1997; Suter et al. 2008), the impact of remodelling over time (Garn and Schwager 1967; Garn et al. 1968; Hummert and Van Gerven 1985) and the difficulty in consistently associating these lines with other indicators of stress or ill-health (e.g. McHenry and Schulz 1976; Maat 1984; Mays 1995; Ribot and Roberts 1996; Papageorgopoulou et al. 2011).

Born out of these challenges, researchers have begun to question the validity of correlating these lines with episodes of childhood ill-health (i.e. Alfonso et al. 2005; Alfonso-Durruty 2011; Papageorgopoulou et al. 2011). Alfonso and colleagues argue that because skeletal growth does not occur along a linear continuum but rather has periods of "saltation and stasis" (2005:393), Harris lines represent normal growth fluctuations where specifically timed hormonal signals dictate the formative process of these radiopaque lines (Alfonso et al. 2005; Alfonso-Durruty 2011). However, the same argument can be made for the previous interpretations that Harris lines form during periods of duress as clinical studies have continually shown that glucocorticoid release

during prolonged or chronic periods of stress disrupt hormone secretion and function which can lead to skeletal changes (Charmandari, Kino and Chrousos 2004). From this perspective then, whether growth hormones are being controlled by the dictates of normal skeletal maturation or influenced by periods of ill-health, the argument can be made that Harris lines may form as the result of two distinct circumstances – normal growth or in response to stress. While the latter has been the primary interpretation in previous osteological studies, the formation of Harris lines as the result of normal growth also presents an opportunity to better explore skeletal maturation patterns. While the scope of this paper does not encompass the arguments for or against either position, we assert that the most important step for the continued use of Harris lines in osteological research is a re-consideration of issues related to radiographic acquisition -- the importance of which was stressed half a century ago by Garn and colleagues (1968). As the debate continues around the formative process and interpretative value of Harris lines, an accurate quantification of their presence or absence is arguably the most important consideration.

The goal of this research is to examine how radiographic positioning contributes to the visualization and identification of Harris lines. To date, virtually no osteological studies have compared line visualization between the anterior-posterior (A-P) and medial-lateral (M-L) orientations, with the majority of studies focusing on the A-P orientation alone. In omitting this second orientation, however, researchers may effectively be impeding their ability to visualize all Harris lines (Garn et al. 1968; Garn and Braunstein 1986; Hughes et al. 1996), leading to loss of data and therefore interpretive power.

Harris line formation

Harris lines can form within any of the endochondral bones of the skeleton, however the distal tibia is most favourable for visualization (Follis and Park 1952; Park and Richter 1953; Park 1964; Garn et al. 1968). This amplified formation in the tibia is likely associated with better circulation which improves osteoblast function and bone formation (Hughes 1996). During endochondral growth primary ossification centres are separated from their epiphyses by growth plates, made up of chondrocyte cells (Nilsson et al. 2005; Giustina et al. 2008). As chondrocytes cells multiply and pass through their life cycle they become stacked in vertical columns and as the oldest chondrocyte cells begin to die this allows osteoblast cells to infiltrate these columns (Acheson 1959; Park 1964; Garn et al. 1968). If chondrocyte cessation occurs due to the influence or stress or normal growth hiatus, the growth plate becomes impenetrable to the osteoblast cells which begin to mineralize the thin horizontal layer of chondrocytes located at the terminus of the growth plate, forming the "primary stratum" that reaches across the medullary cavity (Park 1964; Steinbock 1976). However, this "primary stratum" is not radiopaque until recovery has occurred and ossification is greater than 25 percent (Garn and Braunstein 1986). Depending on the period of recovery and the intensity of the growth disruption the Harris line will become thickened (Park 1964). Therefore, Harris line formation is a two-step process facilitated by chondrocyte cessation and a decrease in osteogenesis (Follis and Park 1952; Acheson 1959; Park 1964).

MATERIALS AND METHODS

For this study, the Medieval Danish Black Friars cemetery sample (AD1240-1660) curated at the University of Southern Denmark was examined. A sample of 134 adults and 23 subadults was analyzed with digital radiographs taken of both the left and right

tibiae and radii when possible. Each bone was radiographed in standard clinical anterior-posterior (A-P) and medial-lateral (M-L) orientations by radiography technicians at the Odense University Hospital (Denmark). All radiographs were taken on a Siemens Ysio machine with a standard 115cm source to bone distance. Maximum exposure ranged between 49-52 kVp with radiographic density ranging between 3.0-3.2 mAs for adults, and 46-52 kVp and 1.3-3.1 mAs for subadults. Radiographs were captured sequentially for each individual to ensure consistency between both orientations. Radiographs were saved as DICOM files and analyzed using Materialise MIMICSTM software. All data were visually assessed by the first author. Identification followed standard osteological protocols with Harris lines only being scored if they: 1) were at least half way across the diaphysis and 2) had a predominantly transverse orientation (Mays 1985: Clark 1981; Mays 1995). All lines visible along the diaphysis that met these criteria were scored. For each individual, all A-P orientations were scored first and then scored in the M-L view with the A-P data kept blind so as to not bias the M-L scores. All files were set to the same resolution and magnification to standardize the view. The MIMICS thresholding function, based on a calibrated Hounsfield scale for CT images used to demarcate regions of increased bone density, was used to enhance the visibility of faint Harris lines during initial identification. Compared to static plain film radiographs, this function allows the observer to manually manipulate regions of poor radiographic visibility, increasing quantification accuracy. However, the final count was conducted with this function turned off and was repeated to ensure the count remained consistent.

Once all data were collected, intra-observer error was estimated from a second round of scoring in a resampled set of 25 and 10 randomly selected adults and subadults, respectively. The second round of scoring was conducted blind of the original

counts three weeks after the primary scoring was complete. Asymmetry has been shown to be minimal when assessing Harris lines in the standard A-P view (Hughes et al. 1996); therefore, there was a need to validate this assumption for the M-L orientation with differences between the left and right side counts assessed. Following this, comparisons of the number of identified Harris lines between A-P and M-L views were assessed using paired samples t-tests. In addition to these raw counts, individuals were also categorized into four groups: none (0 lines); low (1-3 lines); moderate (4-8 lines); and high (9 or more lines), to further assess the patterns emerging from the A-P and M-L counts. As a final step, cortical thickness of the anterior, posterior, medial, and lateral aspects of the left adult tibia (right side when left was absent) were also collected to compare between both orientations.

RESULTS

Intra-observer error results show differing levels of consistency between the A-P and M-L views. For the A-P orientation in adults there was a statistically significant difference seen between repeated counts in the left proximal tibia, the right distal tibia, and the right proximal radius. However, there were no statistically significant differences between scoring rounds in the M-L orientation (Table 1). For the subadults in the A-P orientation there was a statistically significant difference between the left and right distal tibia, the left proximal radius, and the right distal radius and in the M-L orientation the left and right distal tibia, left and right proximal tibia, and the left distal radius. Asymmetry in Harris line counts between matched left and right sides observed no statistically significant differences between any of the adult bones in either orientation and only one comparison in the subadults (Tables 2 and 3).

Pairwise comparisons of the number of Harris lines in the A-P versus M-L orientations revealed statistically significant differences for adults, with the M-L orientation typically having higher counts than in the A-P view (Table 4). In subadults, only three comparisons demonstrated a statistically significant difference between the A-P and M-L orientations (right proximal tibia, left distal radius, right distal radius). Cross tabulations of the adult categorical data also showed significant differences between the A-P and M-L orientations with the M-L view having more individuals in the higher count categories relative to the A-P view (Table 5). For example, Table 5 shows that for adults in the A-P view when the observed Harris lines are low, in the M-L view more than half of the individuals are observed as moderate or high. Similar to the raw counts, the subadult cross tabulations data demonstrated inconsistency between the A-P and M-L orientations (Table 6).

Comparisons were also made between A-P and M-L midshaft cortical bone thickness in the adults between orientations. The mean M-L cortical thickness of 18.6mm was significantly thinner than the mean A-P cortical thickness of 27.3mm (t=-21.677; df=118; p<0.001).

DISCUSSION

Based on this analysis, the M-L orientation results in the identification of more Harris lines than the standard A-P orientation in adults. Further, there were no statistically significant differences in intra-observer error, suggesting more clarity and therefore consistency in the M-L orientation. This contrast between orientations, however, was not as distinct within the subadult sample where the pattern is less consistent and likely influenced by size and shape differences due to age. While the division of the subadult data into age specific categories might reduce the

inconsistences, the small sample size did not allow for this. However, given the strong evidence suggesting an M-L orientation provides better visualization of Harris lines, there would be future value in seeing whether there is a consistent pattern present in subadults across age categories where growth-related factors such as cortical thickness and size can be controlled for.

This study stands in contrast to the earlier work of Clark and Mack (1988) who found no differences between the two orientations when scored by experienced observers. The same study did however observe some inconsistencies regarding intra-observer reliability, as the second round of scoring for both the A-P and M-L orientations produced higher line counts by both observers. While Clark and Mack (1988) argued that many factors, including aspects of the radiographic process, must be standardized, they failed to account for key aspects of this visualization process, specifically the two primary variables of radiographic quality: kilovoltage (kVp) and milliampere second (mAs).

The kVp value determines the energy of the photons that produce the radiographic image and the degree to which these photons penetrate the object being analyzed (Kirberger 1999; Farquharson and Brickley 2000), while the mAs value determines the density of the radiograph, essentially controlling the 'blackness' of the image (Yochum and Rowe 2004). In general, the thicker the tissue the higher the kVp value (Farquharson and Brickley 2000); however, if the kVp value is too high an excess of photons will reach the film producing a low contrast, or dark image (Yochum and Rowe 2004). Consideration of these values is critical when translating clinical standards for studies of archaeological dry bone, as soft tissue interference becomes irrelevant and dosage values can be lowered. However, just as clinical studies use a range when

determining proper kVp and mAs values, so too must archaeological studies. As Yochum and Rowe (2004) discuss, in clinical cases of decreased bone density, there must also be a reduction of the mAs value to ensure a similar level of radiograph resolution regardless of any underlying pathological conditions. Because Clark and Mack (1988) failed to compensate for differing levels of bone density within their study, radiographic resolution would have been compromised in some individuals, especially when comparing individuals as young as 20 years to those in their 9th decade of life. Further, their use of elevated kVp and mAs values more akin to clinical standards, would have led to an over exposure of their radiographs. While this over exposure may not have been visually obvious at the time of image capture, even a slight decrease in contrast between potential Harris lines and surrounding boney tissues may have obscured their ability to visualize the thinner and partially remodelled lines in their study sample. Confounding this issue, the technological limitations of the plain film used by Clark and Mack (1988), and many osteological studies of Harris lines, also hinder the accuracy of their results, as imaging tools that allow for post-acquisition image manipulation and better visualization for Harris line detection is limited with plain film radiographs.

Based on the current analysis using contemporary digital radiography, it appears that there is a significant discrepancy in Harris line counts between the A-P and M-L views. When re-examining the adult individuals with the highest level of inconsistency between the A-P and M-L counts, two patterns emerged. First, in the M-L view more lines exceeded 50 percent of the diaphyseal width (Fig. 1), and second, in the M-L view more lines were visible towards the midshaft (Fig. 2). One of the primary explanations for the discrepancy between the A-P and M-L visualization is the distortion that occurs

from compressing a 3D object into a 2D radiograph. Arguably, the standard A-P orientation is favoured due to the ease of data collection, but this effectively halves the data available upon which to make interpretations.

Garn and Braunstein (1986) argue that Harris lines are better visualized when cortical bone is thinner, as the increased density of these lines makes distinguishing them from surrounding cortical bone more difficult. We have demonstrated here that in the tibia cortical thickness is greater on average in the A-P orientation than the M-L orientation which may explain why M-L raw counts were significantly higher than the A-P raw counts. However, increased cortical thickness did not predict greater discrepancy between the views as was expected. A possible explanation for this lack of correlation may be due to using the midshaft cortical measurements as proxies for cortical thickness at the distal end of the tibia. Despite this, there is evidence that cortical thickness plays a role in the identification of Harris lines. Discussed by Garn and colleagues (1968), as bone remodels in the adult years and Harris lines disappear, the process does not occur at an equal pace around the perimeter of the bone, but rather the process is directed across the M-L or transverse plane. As bone is being created along the lateral border of the bone it is being equally removed along the medial aspect, resulting in Harris lines being slowly obliterated across the transverse aspect (Garn et al. 1968). Therefore, as this directed remodelling occurs, Harris lines will become consistently obliterated and less visually apparent in the standard A-P orientation. However, in an M-L orientation as the lateral aspect remodels, the Harris line will still be present across the entire width of the bone until complete remodelling has occurred up to the medial aspect (Fig. 3). This may help to explain why in some individuals there are many more lines visible in the M-L orientation especially towards the midshaft where

these lines have undergone the longest period of remodelling due to their formation earlier in life. Even though Harris lines in the distal tibia have been shown to persist into the 9th decade of life (Garn and Schwager 1967), as the remodelling process occurs, these oldest lines may be nearly obliterated in the A-P view, but at least partially visible in the M-L orientation. Of course there are many factors that influence the remodelling process and the persistence of Harris lines, but an understanding of the underlying mechanism provides an important insight into why this difference between A-P and M-L visualization may be occurring.

Future studies may benefit from age-specific analysis to assess whether individuals demonstrating the greatest discrepancies between views are those in the later decades of life who have been exposed to longer periods of remodelling. Further, cortical thickness measurements taken at the site of these Harris lines would perhaps provide a clearer understanding of the relationship between cortical thickness and the visualization of Harris lines in both an A-P and M-L view. There would also be valueadded in matching Harris lines visible in both the A-P and M-L views to determine the proportion of lines that overlap in both orientations. By understanding the pattern of where these lines are forming during bone growth, and the methodological limitations for visualizing them, we may be better able to predict why this discrepancy is occurring and most importantly how that may be accounted for through radiographic standardization. Finally, the use of high resolution three-dimensional CT imaging may also help elucidate the differences observed between the M-L and A-P orientations by providing visual confirmation of the remodelling process as shown in Fig. 3, and the impact this has on the accurate scoring of Harris lines in both views.

CONCLUSIONS

While the criteria for identifying Harris lines can be re-evaluated to minimize the inconsistency between the A-P and M-L orientations, the fact that the M-L view in many individuals clearly shows additional lines is an important consideration. While individual variability in bone shape and curvature in addition to taphonomic changes can make standardization difficult, we argue that two key elements must be considered during future radiographic acquisition: 1) the use of clinical standard positioning, capturing the true transverse orientation of the bone and; 2) the use of supportive materials (e.g. foam) to ensure a consistent view is being achieved. If Harris lines are being underestimated in osteological research because of the common approach of only taking radiographs in the A-P orientation, then this is likely contributing to the disconnect and growing unease with the interpretation of these lines. As such, radiograph orientation must be considered first and foremost in future studies of Harris lines.

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Figure 1: Tibial radiographs showing more Harris lines exceeded 50 percent of the diaphyseal width in the M-L view (right).

Figure 2: Tibial radiographs showing more Harris lines visible towards the midshaft in the M-L view (right).

Figure 3: Illustration of a tibia cross section, showing the effect of orientation on radiographic visualization of Harris lines during directed remodelling (after Garn et al. 1968, Figure 30).



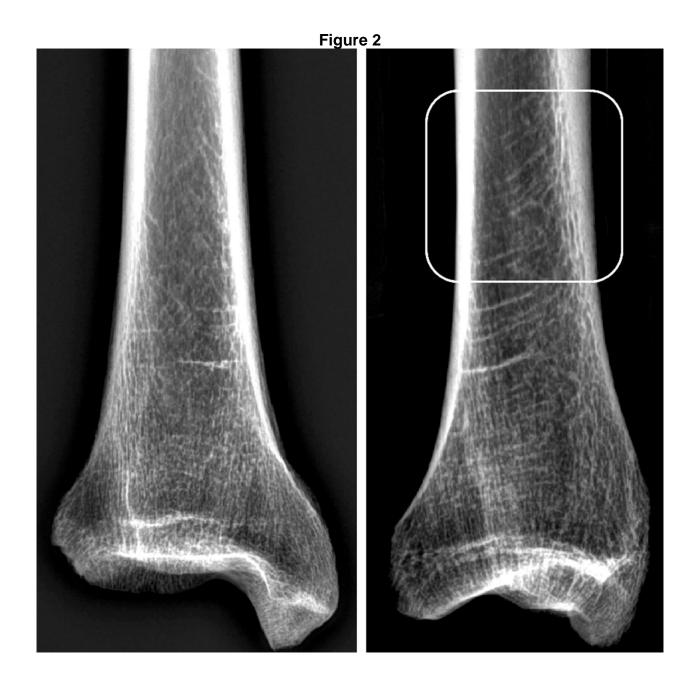


Figure 3

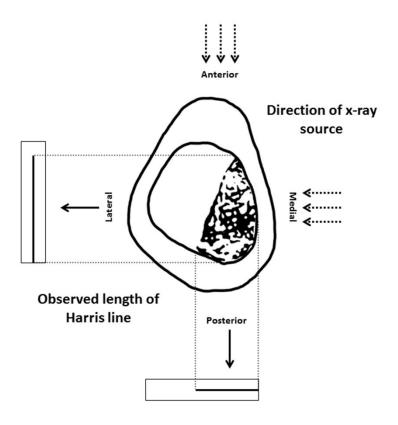


Table 1: Intra-observer error for adults

Paired Differences Run 1 - Run 2

95% Confidence Interval of the

Difference

Orientati	on	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
A-P	Left Distal Tibia	3200	2.3043	.4609	-1.2712	.6312	694	24	.494
	Left Proximal Tibia	9600	1.7673	.3535	-1.6895	2305	-2.716	24	.012*
	Right Distal Tibia	8800	1.6411	.3282	-1.5574	2026	-2.681	24	.013*
	Right Proximal Tibia	4400	1.6603	.3321	-1.1253	.2453	-1.325	24	.198
	Left Distal Radius	1667	.7614	.1554	4882	.1548	-1.072	23	.295
	Left Proximal Radius	2500	.8470	.1729	6077	.1077	-1.446	23	.162
	Right Distal Radius	1667	.6370	.1300	4357	.1023	-1.282	23	.213
	Right Proximal Radius	2800	.5416	.1083	5036	0564	-2.585	24	.016*
M-L	Left Distal Tibia	.2174	3.5285	.7358	-1.3085	1.7432	.295	22	.770
	Left Proximal Tibia	6957	1.8200	.3795	-1.4827	.0914	-1.833	22	.080
	Right Distal Tibia	4118	3.8252	.9278	-2.3785	1.5550	444	16	.663
	Right Proximal Tibia	2941	1.6111	.3907	-1.1225	.5342	753	16	.463
	Left Distal Radius	1667	1.3077	.2669	7189	.3855	624	23	.539
	Left Proximal Radius	.0000	.7802	.1593	3294	.3294	.000	23	1.000
	Right Distal Radius	1429	.9493	.2537	6909	.4052	563	13	.583
	Right Proximal Radius	.0667	.7037	.1817	3230	.4564	.367	14	.719

^{*} significant at the p<0.05 level

Table 2: Differences in Harris line raw counts between left and right sides for adults

Paired Differences (Adults) Left - Right

95% Confidence Interval of the

Difference

					Billerenee				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
A-P	Distal Tibia	.197	2.705	.245	288	.682	.803	121	.423
	Proximal Tibia	.244	1.872	.166	085	.573	1.470	126	.144
	Distal Radius	056	.979	.095	244	.132	592	106	.555
	Proximal Radius	.042	.602	.055	067	.151	.761	118	.448
M-L	Distal Tibia	1.000	4.442	.798	629	2.629	1.253	30	.220
	Proximal Tibia	125	2.136	.378	895	.645	331	31	.743
	Distal Radius	115	2.123	.416	973	.742	277	25	.784
	Proximal Radius	.214	1.371	.259	317	.746	.827	27	.415

Table 3: Differences in Harris line raw counts between left and right sides for subadults

Paired Differences (Subadults) Left-Right

95% Confidence Interval of the

Difference

		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
A-P	Distal Tibia	250	2.322	.387	-1.036	.536	646	35	.523
	Proximal Tibia	1.389	3.366	.561	.250	2.528	2.476	35	.018*
	Distal Radius	.455	1.734	.302	160	1.069	1.506	32	.142
	Proximal Radius	.289	1.228	.199	114	.693	1.453	37	.155
M-L	Distal Tibia	783	3.503	.730	-2.297	.732	-1.072	22	.296
	Proximal Tibia	652	3.298	.688	-2.078	.774	949	22	.353
	Distal Radius	.500	2.455	.579	721	1.721	.864	17	.400
	Proximal Radius	.381	1.203	.263	167	.929	1.451	20	.162

^{*} significant at the p<0.05 level

Table 4: Differences in the number of counted Harris lines in A-P versus M-L views for adults

Paired Samples Test									
		Pa							
				95% Confidence the Differe					
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)	
A-P Distal Tibia - M-L Distal Tibia	-4.776	4.552	.393	-5.554	-3.998	-12.147	133	<.001*	
A-P Proximal Tibia - M-L Proximal Tibia	-1.917	2.921	.253	-2.418	-1.416	-7.570	132	<.001*	
A-P Distal Radius - M-L Distal Radius	-1.888	1.671	.144	-2.174	-1.603	-13.080	133	.<.001*	
A-P Proximal Radius - M-L Proximal Radius	366	1.160	.100	564	167	-3.648	133	<.001*	

^{*} significant at the p<0.05 level

Table 5: Frequency of Harris line groupings by orientation for the distal tibia for adults

		None	Low	Moderate	High	Total
A-P Distal Tibia	None	4	8	7	4	23
	Low	1	9	19	13	42
	Moderate	0	2	20	31	53
	High	0	0	2	14	16
Total		5	19	48	62	134

Table 6: Frequency of Harris line groupings by orientation for the proximal tibia for adults

		None	Total			
A-P Proximal Tibia	None	21	18	11	2	52
	Low	7	18	22	5	52
	Moderate	0	3	14	6	23
	High	0	1	1	4	6
Total		28	40	48	17	133