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Histological Age Prediction from the Femur in a Contemporary Dutch Sample*

The decrease of nonremodeled bone in the anterior cortex

ABSTRACT: This paper presents an uncomplicated and minimally invasive method for age-at-death determination in a contemporary Dutch (West European) population, by modifying the approach of assessment based on the age-related remodeling of bone tissue. In contrast to the usual "osteon count," a "non-remodeled tissue count" is undertaken. To optimize the method, proper zeroing of the polarization filter set of the microscope is essential. Instructions for setting the filters are given. A sample of femoral shaft segments totaling 162 individuals with ages ranging from 15 to 96 years is analyzed. Subperiosteal quantitative assessments are recorded at the most anterior point of the femoral shaft and also at points 25° to the left and to the right of that point. Interobserver agreement in the assessments shows an acceptable degree of correlation. Bone remodeling with age does not progress in a linear, but in a curvilinear manner. Dependence of predicted age on nonremodeled surface counts in the analyzed areas of the anterior cortex of the femur appears to be significant. A set of regression equations is given. Sex can be ignored in age prediction. The small but statistically significant dependence of predicted age on cadaver length corresponds with the present strong secular increase in stature in the Netherlands. A concise catalogue with micrograph examples for every 10-year period in life is available upon request.

KEYWORDS: forensic science, forensic anthropology, age, femur, histology, remodeling, Dutch

Many methods have been developed to estimate the age-at-death from human remains. This field has been dominated by gross anatomical methods as they generally are simple to apply and robust with respect to demands on laboratory equipment (1–5). Although their degree of accuracy has been debated, we conclude from their widespread use that they are considered to have an acceptable degree of exactness for specific purposes (6), for example the assessment of age-at-death and therefore life expectancy in archaeological remains. But an additional objective has also been the identification of single or multiple individuals in forensic circumstances. The results of the latter assessments potentially play a role in court cases and may have serious consequences. In that respect, gross anatomical methods have disadvantages. For instance in the Netherlands they inevitably require the analysis of large skeletal parts that are taken from the body by dissection. For example, accurate age determination may involve recovery of the skull, humerus, femur, plus half of the pelvis, leaving the body seriously mutilated (1,7). In this way collateral damage interferes with possible follow-up work if requested and with a respectful return of human remains to relatives after a successful identification.

With regard to restricting the physical damage to the dead body, the introduction of the so-called "4th rib method" in 1986 is of great help. In this method only the sternal end of the fourth rib is needed (8). In spite of its stated high accuracy (9), there remains a need for an additional but also minimally invasive way of age determination. Such a method would be useful to confirm or to narrow the age range of the diagnosis. It also renders a final diagnosis less dependent on single technique complexity (10). In principle, the histological approach to age determination has been available for a long time but has received surprisingly little utility in the forensic world. Microscopy as a routine technique should have been strongly stimulated by recent technical advances, as preparatory infiltration and embedding of bone specimens in resins for 1 or more weeks and expensive-motorized microtomes are no longer necessary, as the preparation of undecalcified slides is both rapid and cheap (11,12).

Histological Method

After Kerley introduced the principles of histological aging in 1965, a variety of modifications were developed (13,14). In principle, the method quantitatively assesses age-at-death by determining the extent of bone replacement in the cortices of long bones. "Osteon counts" were done to quantify that remodeling status. Over the years, despite various modifications on the method for different bones: mandible, clavicle, humerus, ulna, femur, tibia, fibula, and ribs, the femoral shaft remained the most utilized (13–27).

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In a microscopic transverse section of adult femoral cortical bone, the ongoing replacement process will show as a decreasing amount of surface occupied by the originally deposited circumferential lamellar bone tissue positioned between an increasing amount of surface occupied by newly formed osteons (Haversian systems) and osteon fragments. The latter fragments are leftovers from earlier generation osteons, which, eventually, became incorporated into the ongoing "erosion" and "filling in" process. Therefore, "osteon counts" of osteons and their fragments represent time lapsed. For reasons of simplicity, in this study "non-remodeled tissue counts" are undertaken. The method focuses on the relative decrease in surface area occupied by circumferential lamellar bone tissue with its enclosed non-Haversian canals. It is tested as a parameter for time-lapsed, i.e., age of an individual, as the percentage of decrease should predict age. In order to achieve the best possible accuracy for the Dutch (West European) population, specimens were collected from 162 Dutch individuals of documented identity.

Materials and Methods

Population

Bone sections were analyzed from the anterior femoral cortex of 162 randomly selected individuals of known age and sex (86 males and 76 females). The specimens were collected from forensic cases at the Netherlands Forensic Institute ($N = 97$) and from dissection hall specimens of the Department of Anatomy, Leiden University Medical Center ($N = 65$). Ages-at-death ranged from 15 to 96 years of age. The ethnicity of the sampled group can be defined as Dutch/West European/Caucasian/White. Only specimens from individuals free of chronic diseases that might have affected bone metabolism were included. Table 1 presents an overview of the age and sex distribution of the sample.

Material and Equipment Preparation

The anterior midshaft of the femur was chosen as the "donor" area as it is most resistant to taphonomic detoriation, even after prolonged interment. Its cortical surface is biomechanically stable and little influenced by traction of muscle attachments (23). Nevertheless and unavoidably, even within the anterior midshaft of the femur, spatial distribution of lamellar bone tissue will to some extent be nonrandomly distributed across a transverse section, as it will reflect some variation in biomechanical loading between individuals (28–30).

A wedged piece of bone was sawn from the anterior femoral midshaft, keeping the continuity of the shaft intact posteriorly. One of the saw cuts of the wedge was always kept perpendicular to the long axis of the femur. From that cut a parallel thick section of circa 2 mm was extracted. Care was taken that the periosteal surface of the bone was not scratched during the sawing. From this thick section a thin ground section was prepared following Maat et al. (11,12). With the help of a marked transparent sheet, the most anterior point of the femur shaft and points 25° to the left and to the right of that point were indicated on the glass cover slip of the prepared slide. To include the overall status of bone replacement in the anterior femoral cortex, the percentage of nonremodeled bone tissue was determined over a surface of $1\,\mathrm{mm}^2$ in the immediate subperiosteal area at each of the three sites. To achieve this, a transparent sheet on an X-ray box with a drawn counting framework of 10×10 squares was projected via a regular prismatic drawing attachment into a regular light microscope. For counting, only the $\times 10$ objective and the $\times 10$ ocular lens were used. The zoom lens of the drawing attachment and a calibrated reference slide enabled the fixation of the magnification of the outer edges of the drawn framework to precisely $1 \times 1\,\mathrm{mm}^2$. As a matter of course, a similar counting framework can be projected into the viewing path by means of calibrated computer software.

A polarization filter was set to show the direction of bone fibers. If filters had not been available, one could have cut them from a sheet of plastic Polaroid filter or from a Polaroid sunglass lens. One filter (the analyzer) was put in the viewing path in the tube of the ocular lens or under the binocular head; the other (the polarizer) was put onto the light source of the microscope. As lamellar bone is arranged in flattened plates of mineralized matrix with a 90° difference in orientation between adjacent plates, transmission with polarized light emphasized the alternating orientation of the lamellar layers (anisotropy). This property of lamellar bone was used to "zero" the analyzer filter in such a way that the non-remodeled surface was enhanced compared with the remodeled surface. To do so, a bone section was positioned on the microscope table in such a way that its subperiosteal circumferential lamellae ran as "horizontal" as possible, parallel to the front of the microscope table (East–West). Subsequently these fibers were enhanced to maximal intensity by turning the polarization filter set to its optimal position. Polar orientation of the filters is kept crossed during the turning (black background). Once this was achieved, the position of the analyzer in the view path (ocular tube) was fixed. For further polarization effects only the polarizer on the light source was turned. The impact of incorrect zeroing on the ability to distinguish between non-remodeled and remodeled cortical surface is demonstrated in Figs. 1 and 2.

Microscopic Analysis

To determine the percentage of nonremodeled surface in the subperiosteal area, the periosteal surface of the bone section opposite to one of the cover slip marks was positioned "horizontally" by hand (East–West). Then the projection of the calibrated framework was positioned with one of its sides against the microscopic image of the periosteal surface in the section (thus opposite the mark on the glass cover slip) (see Figs. 1 and 2). Subsequently, by fine-tuning the polarizer filter on the light source, a one-by-one assessment was made of the degree of remodeling in all 100 squares in the framework. The percentage of nonremodeled surface was equivalent to the number of squares dominated by circumferential lamellar bone with their non-Haversian vascular canals. The same procedure, the "horizontal positioning" of the counting area included, was repeated at all three marked sites: the most anterior point of the femoral shaft and points 25° to the left and to the right of that point. Subsequently,

TABLE 1—*Age and sex distribution of documented specimens.*

Age Interval (Years)	Total Number	Number of Males	Number of Females
10.0–19.9	14	6	8
20.0–29.9	15	8	7
30.0–39.9	21	11	10
40.0–49.9	23	14	9
50.0–59.9	23	14	9
60.0–69.9	15	11	4
70.0–79.9	19	10	9
80.0–89.9	17	8	9
90.0–99.9	15	4	11
Totals	162	86	76

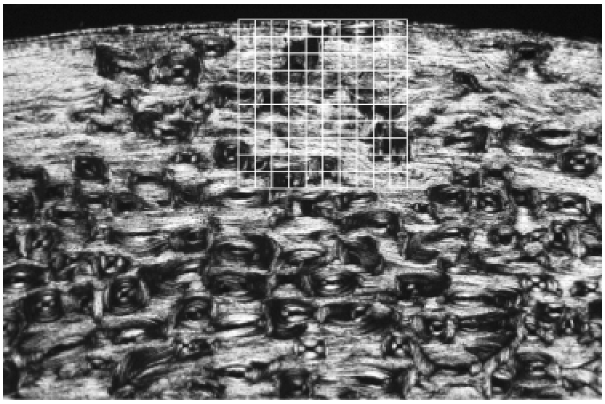

FIG. 1—*Micrograph taken after proper zeroing of the polarization filters (see "Material and Equipment Preparation"). The non-remodeled femoral cortical surface stands out clearly from the remodeled areas and is ready for counting. Counting framework = 1 mm*^{2}*. Percentage of non-remodeled surface is 53%.*

the percentage of nonremodeled surface was calculated for the so-called "entire anterior cortex" (the average of all three areas), for the "most anterior part" alone, and for the "combined antero-lateral parts" (the average of the areas 25° to the left and to the right of the most anterior point).

Interobserver Agreement

The complete sample of slides of all 162 individuals was tested for interobserver agreement with respect to the assessment of the degree of remodeling. After a forensic anthropologist experienced in histological age determination (observer 1, G. M.) had finished all examinations, reexaminations were done by a forensic pathologist trained in tissue microscopy but inexperienced in histological age determination (observer 2, A. M.).

Body Size

To evaluate a possible influence on the rate of bone remodeling from increased weight bearing by the femur because of the increasing body size of the Dutch population over the last century, the body frame of each individual was arbitrarily assessed by visual inspection (classes: slender, medium, robust). In addition, cadaver length (stature) was measured with a measuring tape to the nearest centimeter.

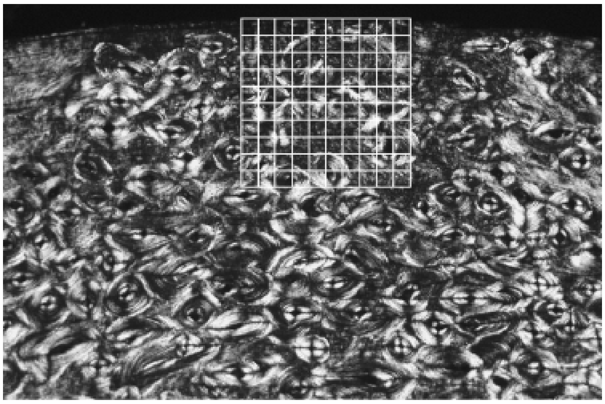

FIG. 2—*Micrograph of the same areas as shown in fig. 1, but now taken after improper zeroing of the polarization filters (see "Material and Equipment Preparation"). The nonremodeled femoral cortical surface does not stand out clearly from the remodeled areas and is not ready for counting. Counting framework = 1 mm*^{2}*.*

Statistical Analysis

Statistical analyses were done using SPSS version 11.0. Interobserver agreement was explored using standard Pearson correlation coefficients. The relationship between age and percentage of nonremodeled bone was analyzed using quadratic (curvilinear, polynomial) regression, with age as the dependent variable and percentage of nonremodeled bone as the independent variable. *p*-values <0.05 were considered significant.

Results

Quantitative Analyses

Interobserver Agreement in the Assessment of the Degree of Bone Remodeling—Agreement in the assessment of the percentages of nonremodeled surface showed high degrees of correlation between both observers. The Pearson correlation between the counts of both observers in the so-called "entire anterior cortex," the "most anterior part," and the "combined antero-lateral parts" of the femoral midshaft was 0.949, 0.911, and 0.944, respectively. For all three situations the two-tailed significance was <0.001. The scatter plot of Fig. 3 shows the high degree of correlation between the counts of both observers for the percentage of nonremodeled bone surface in the "entire anterior cortex." In the range of the lowest percentages observer 2 (inexperienced) tended somewhat to overestimate if compared with observer 1 (experienced).

Dependence of Predicted Age on Subperiosteal Bone Replacement in the Entire Anterior Cortex of the Femur for Combined Males and Females—A scatter plot of age-at-death on the *y*-axis and the average percentage of nonremodeled bone in the "entire anterior cortex" of the femur for combined males and females on the *x*-axis demonstrated that with increasing age the percentage of nonremodeled bone declined (Fig. 4). As this effect seemed to disappear for percentages over 80%, all percentages of 80% and higher were truncated to 80% for further analyses. Then multiple curvilinear regression analysis was used with both the average of the percentage of nonremodeled bone and its square as

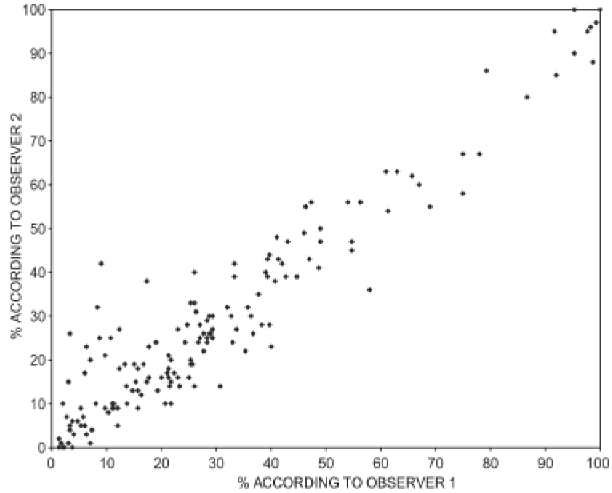

FIG. 3—*Scatter plot for the percentage of non-remodeled bone surface in the so-called "entire anterior cortex" according to observer 1 on the x-axis and observer 2 on the y-axis. The Pearson correlation between the counts of both observers was 0.949.*

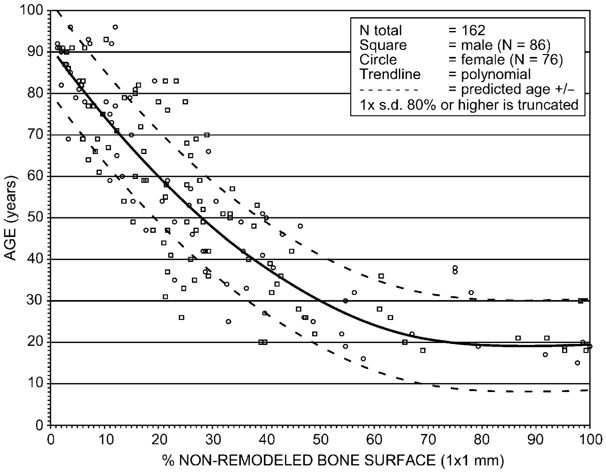

FIG. 4—*Decrease with age of non-remodeled subperiosteal bone surface in the so-called "entire anterior cortex" of the femoral midshaft.*

independent variables. Note that we used "inverse" regression in the sense that we used the outcome of aging, viz. the percentage non-remodeled bone, as a (retrospective) predictor of age. Statistically, the overall dependence was significant ($p < 0.001$) and the standard deviation of the prediction error was ± 11 years (Table 2). The upper and lower limits related to this standard deviation are shown as curved lines parallel to the trend line in Fig. 4. The related regression equation can be found in Table 2.

Inclusion of sex into the regression equation did not significantly improve the R^2 (Table 2). The standard deviation of prediction error was again ± 11 years. Separate equations for males and females are presented in Table 2. They show that for given percentages of non-remodeled bone, the age estimate for females was only 0.876 year higher than for males. The difference was not statistically significant ($p = 0.622$).

Dependence of Predicted Age on Subperiosteal Bone Replacement in the "Most Anterior Part" of the Femur for Combined Males and Females—Regression showed that the overall dependence on the percentage of nonremodeled bone was significant ($p < 0.001$), but the R^2 dropped compared with the "entire anterior cortex" counts (Table 2). This poorer fit was also apparent in the larger standard deviation of the prediction error, which increased to ± 14.9 years. The related regression equation is shown in Table 2.

Dependence of Predicted Age on Bone Replacement in the "Combined Antero-Lateral Parts" of the Femur for Combined Males and Females—As bone sections were turned over many times during their preparation process, their orientation with respect to left or right side was lost. As a consequence the average of the percentages of nonremodeled bone in the areas at 25° to the left and the right, symmetrical to the most anterior point of the femoral shaft, was used. Regression analysis showed that the dependence was significant ($p < 0.001$). The standard deviation of the prediction error was ± 10.6 years. The estimated regression equation is included in Table 2.

Dependence of Predicted Age on Subperiosteal Bone Replacement in the "Most Anterior Part" if Compared with the Replacement in the "Combined Antero-Lateral Parts"—In order to explore whether the "combined antero-lateral parts" and the "most anterior part" yielded independent information for the prediction of age, stepwise linear regression was used. It included all independent variables of the above two analyses as candidates. Both the (truncated) value of the "combined antero-lateral" parts and its square significantly predicted age, but the corresponding values of the "most anterior part" did not.

Dependence of Predicted Age on Body Frame for Combined Males and Females—Body frame, an ordinal variable, was entered into the regression analysis as if it were a "continuous" variable, coded 1 (slender frame), 2 (medium frame), and 3 (robust frame). It did not contribute significantly to the prediction of age ($p = 0.966$).

Dependence of Predicted Age on Cadaver Length for Combined Males and Females—Statistically, the dependence appeared to be significant ($p < 0.001$) and the standard deviation of the prediction error was reduced to ± 9 years. The regression equation obtained for combined males and females is found in Table 2.

Qualitative Analysis

To meet demands for qualitative diagnoses of age at death, a series of characteristic micrographs of human transverse sections through the midshaft of the anterior femur was selected for every period in life of 10 years. The selection was chosen on their closest fit to the trend line of Fig. 4 (decrease of non-remodeled subperiosteal bone surface in the "entire anterior cortex"). The micrographs were produced at two magnifications and compiled into a concise catalog: a general view displaying the complete stretch from periosteal to endosteal surface and a close-up displaying the square millimeter counting framework of 10 × 10 squares. Both bright-light and polarized-light exposures were made (Figs. 5–8). A reprint of the catalogue is available on request (31).

Discussion

To assess age-dependent bone replacement for the prediction of age, only the percentage of non-remodeled surface in transverse sections has been counted. This counted area is equivalent to the area covered by osteons, osteon fragments, and resorption canals (14,19), but it is easier to view and thus less time consuming to count after zeroing of the polarization filters (see "Material and

TABLE 2—*Regression equations expressing the dependence of predicted age on percentage of non-remodeled tissue in the midshaft of the femur.*

Area	Donor	Equation	SD (Years)	R^2
Entire anterior cortex	M+F	$Y = 92.11\ (\underline{2.10}) - 1.86\ (\underline{0.13})\ X + 0.01239\ (\underline{0.002})\ X^2$	11.006	0.783
Entire anterior cortex	M	$Y = 90.68\ (\underline{3.58}) - 1.85\ (\underline{0.14})\ X + 0.01223\ (\underline{0.002})\ X^2 + 0.876$	11.032	0.783
Entire anterior cortex	F	$Y = 90.68\ (\underline{3.58}) - 1.85\ (\underline{0.14})\ X + 0.01223\ (\underline{0.002})\ X^2 + (2 \times 0.876)$	11.032	0.783
Most anterior part	M+F	$Y = 77.15\ (\underline{2.22}) - 0.66\ (\underline{0.11})\ X - 0.001689\ (\underline{0.001})\ X^2$	14.786	0.608
Combined antero-lateral parts	M+F	$Y = 91.49\ (\underline{1.96}) - 1.91\ (\underline{0.13})\ X + 0.01311 (\underline{0.002})\ X^2$	10.602	0.799
Dependence of predicted age on cadaver length				
Entire anterior cortex	M+F	$Y = 164.08\ (\underline{18.91}) - 1.69\ (\underline{0.21})\ X + 0.01181\ (\underline{0.002})\ X^2 - 0.46\ (\underline{0.11})\ Z$	9.162	0.749

M, male; F, female; *Y*, predicted age in years; *X*, percentage of nonremodeled bone, truncated to 80% if higher; *Z*, cadaver length in centimeters; SD, standard deviation of the prediction error.

Underlined values in parentheses = standard deviation of the error. This number is to be neglected in cases of age calculation.

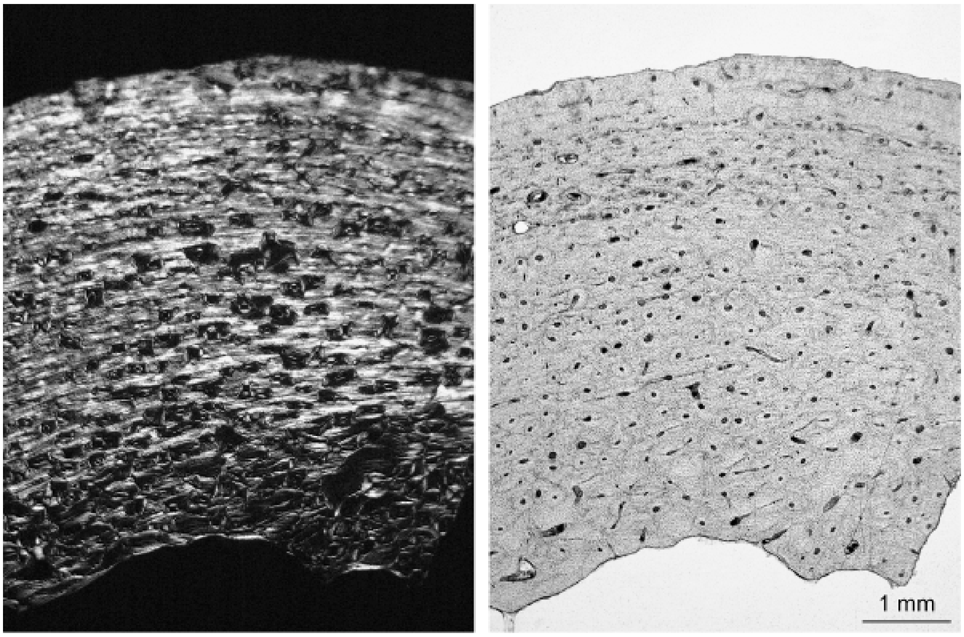

FIG. 5—*Micrograph, covering the periosteal to endosteal surface, of a transverse section through the femoral shaft of a 20-year-old female (polarized and bright light).*

Equipment Preparation"). It also avoids complex decision making on hard to interpret images of various phases of osteon fragmentation (14,19,32).

Quantitative Analysis

The statistically significant correlation for the measurements of the degree of bone remodeling in all three parts of the anterior cortex of the femoral midshaft between both observers was interpreted as a strong indication that interobserver agreement in the assessment of the remodeling process was acceptable (see Fig. 3). The tendency to somewhat overestimate in the counting of the lowest percentages by the inexperienced observer might have been the result of lack of routine to distinguish non-remodeled tissue from remaining osteon fragment tissue of which the lamellae may also run more or less parallel to the periosteal surface. If both are accidentally taken into account, the overall percentage will become too high.

Bone replacement during aging results in a gradual decrease of the percentage of non-remodeled circumferential lamellar bone, but the analysis also shows that this process is not linear (Fig. 4). This was already noted almost half a century ago in the first paper published in this field (13,33). Replacement, by its nature, comes gradually and asymptotically to an end when, because of remodeling, the last remnants of non-remodeled lamellar bone are removed. Probably, linear regressions used in most studies do not reflect an optimal representation of the natural (curved) replacement process (14–19,21,23–27).

Dependence of predicted age on subperiosteal bone replacement, expressed as the percentage of non-remodeled bone in the "entire anterior cortex" of the femur for combined males and females, appears to be significant and has a high R^2 ($R^2 = 0.783$), i.e., 78% of the variance in predicted age is explained by the independent variables. Comparison with the two other studies that also used percentage of non-remodeled bone as a parameter shows that Kerley's dependence came close ($R^2 = 0.756$) (13), but Ericksen's was weaker ($R^2 = 0.518$) (23). The standard deviation of the prediction error of the present study (± 11 years) is close to that of Kerley and Ericksen (13,23). Both were ca. 12 years. Values of the same order of magnitude are also reported from studies based on osteon counts in femora and other bones (10,14,15,18–

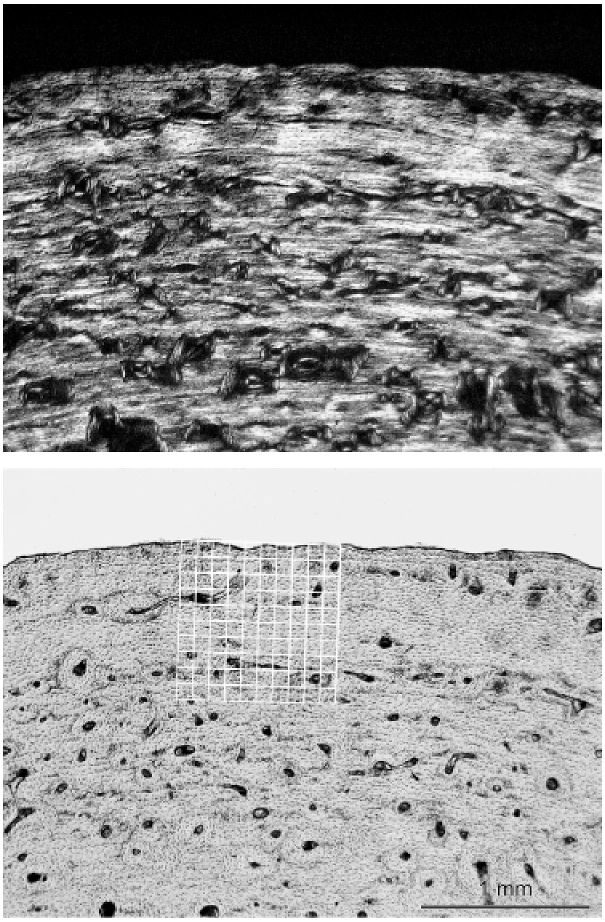

FIG. 6—*Micrograph of the subperiosteal area of a transverse section through the same specimen. Counting framework = 1 mm*^{2} *(polarized and bright light).*

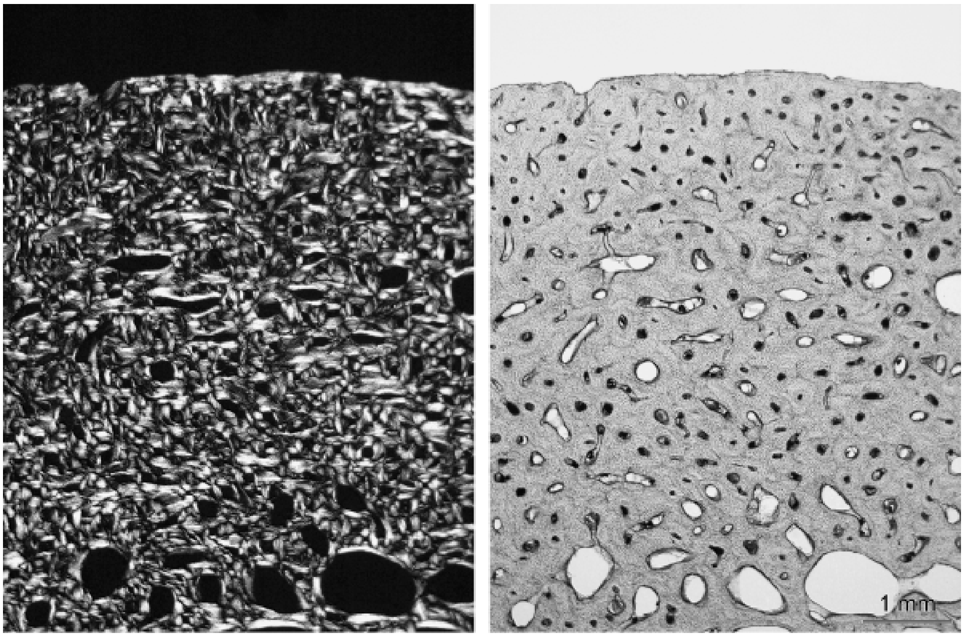

FIG. 7—*Micrograph, covering the periosteal to endosteal surface, of a transverse section through the femoral shaft of a 71-year-old male (polarized and bright light).*

27,34,35). The same holds for age ranges produced by studies based on gross anatomical changes (reviews in (1–5)). In general, they all seem to signal that attempts to become more accurate in age prediction are doomed to fail. The impression is that natural variation in pace of aging between individuals is the limiting factor. Results of analyses may be even further biased by alterations from unnoticed disease. Consequently the only way left to narrow the age range of the diagnoses is to use different "independent" assessment methods and to focus on the common overlap in outcome.

Studies using osteon counts instead of non-remodeled surface counts in femora produced the following R^2: Singh and Gunberg (15), (0.945); Thompson (18), (0.598); Uytterschaut (19), (0.919) and Drusini (21), (0.746). But reported R^2 values higher than 0.8 are a cause for concern, as in view of the impact of known individual variation in speed of aging and the individual effect of differences in mechanical loading on the femur, such results would seem hardly realistic (28–30).

In this study, differences between males and females are negligible and statistically not significant. Except for Ericksen (23), who could only trace a sex difference in the relationship between the numbers of osteons and osteon fragments, lack of difference between sexes seems to be a common finding (13,15,18,35) (confirmed by (35)). For a review see Ericksen (23).

Dependence of predicted age on the subperiosteal bone replacement in the "most anterior part" of the femur for combined males and females shows that, although the dependence is significant, the R^2 drops to 0.634. Thus, only 63% of the variance in predicted age is explained by the remodeling, ca. 37% is not. As the standard deviation of the prediction error also increased substantially to ± 14.3 years, analyzing this region alone should be discouraged.

Dependence of predicted age on the replacement in the "combined antero-lateral parts" of the femur for combined males and females reveals that the dependence is significant, that the R^2 is high, and that the standard deviation of the prediction error is ± 10.6 years. Thus, performance of the "combined antero-lateral parts" alone seems even slightly better than that of the "entire anterior cortex" of the femur (see Table 2). For this reason, and to avoid "needless" extra counting efforts in the "most anterior part" of the femur, one might decide to use the related regression equation. Nevertheless, it is recommend using the "entire

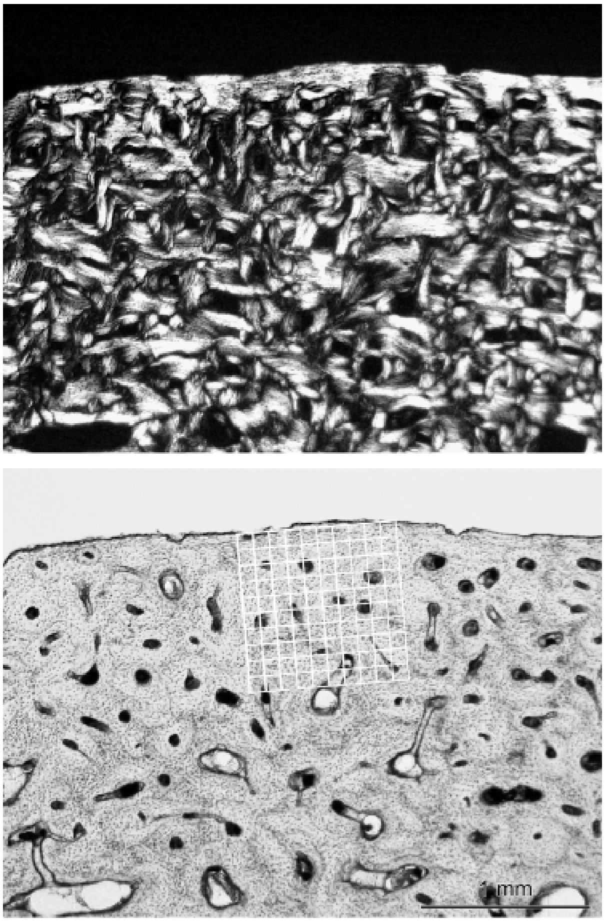

FIG. 8—*Micrograph of the subperiosteal area of a transverse section through the same specimen. Counting framework = 1 mm^2 (polarized and bright light).*

anterior cortex" and its corresponding regression equation. It avoids the risk of misjudgments because of unnoticed isolated atypical fields of bone tissue relatively void of remodeling activity.

During the last century and a half, the Dutch population has been subject to a substantial increase in body size. Adult Dutch males increased 17 cm in stature from 1865 to 1997 AD (36). In general, that shift in body size will have enlarged the average loading on femoral shafts and thus may have caused a change in the remodeling rate of cortical tissue of the young compared with older individuals of our sample group. If so, present regression formulas for age assessment may become outdated in the future. This study shows that dependence of predicted age on body frame for combined males and females is virtually absent. Thus, body frame can be ignored in the prediction of age. Nevertheless, dependence of predicted age on cadaver length (stature) for combined males and females proved to be statistically significant. The regression formula (Table 2) expresses that every centimeter increase in stature comes with a 0.46-year decrease in predicted age. In other words, increase in stature results in a slightly increased remodeling activity. Although this effect is small, the result does fit in with the present strong secular trend in growth in the Netherlands.

Catalogue for Qualitative Analysis

In a qualitative approach, age is assessed by the researcher's experience to interpret the overall degree of bone replacement in the microscopic section (expert opinion). In daily practice it has shown to be of great assistance to have a reference series of micrographs typical of the status of bone replacement for every period of 10 years from young to old age. Such a catalogue with micrograph depictions showing in polarized and bright light is useful in cases where bone specimens to be diagnosed are incomplete, damaged, or of poor quality (too thick, cremated, etc.). But also in case of the availability of complete sections of fine histological quality they are probably useful, as in literature there was a strong indication that qualitative seriating of specimens estimates age with greater accuracy and less bias than (quantitative) regression techniques (37).

Conclusions

For the Dutch population, a quantitative histological method of age estimation was tested on a collection of documented specimens by analyzing the relative decrease in surface area occupied by non-remodeled circumferential lamellar bone together with its enclosed non-Haversian canals in the anterior femoral cortex. In microscopic transverse sections, quantitative assessments were taken at the most anterior point of the femur shaft and at points 25° to the left and to the right. The method performed well.

Interobserver agreement in the assessment of bone remodeling in the anterior femoral midshaft between an experienced and inexperienced examiner was of an acceptable degree.

Dependence of predicted age on non-remodeled tissue surface in the "entire anterior cortex" of the femur for combined males and females appeared to be curvilinear and statistically significant. With increasing age the percentage of non-remodeled bone declined. Taking into account that this effect disappeared for percentages over 80%, a set of useful regression equations was given. After proper zeroing of the polarization filter set, application of the proposed method was simple to execute (non-remodeled tissue counts), produced sound age assessments (although not substantially more accurate than the traditional osteon counts), and was more in line with the natural curvilinear progress of remodeling. Sex could be ignored in age prediction.

Although dependence of predicted age on cadaver length for combined males and females was statistically significant, it added little to the accuracy of age prediction. In practice, as cadaver length is often missing, we recommend such data be ignored.

To meet demands for qualitative age assessments, a concise catalogue from the authors is recommended, which consists of a series of characteristic micrographs for every age period of 10 years.

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