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Effect of Fixation, Soft-Tissues, and Scan Projection on Bone Mineral Measurements with Dual Energy X-ray Absorptiometry (DXA)

E.-M. Lochmüller,[1] N. Krefting,[1,2] D. Bürklein,[1,2] F. Eckstein[2]

[1]Frauenklinik der Ludwig-Maximilians-Universität München, Innenstadt, Maistr. 11, D-80337 München, Germany
[2]Musculoskeletal Research Group, Institute of Anatomy, Ludwig-Maximilians-Universität München, Pettenkoferstr. 11, D-80336 München, Germany

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Abstract. The objective of this study was to determine the effect of fixation, soft tissues, and scan projection on bone mineral measurements with dual energy X-ray absorptiometry (DXA). In seven fresh cadavers, DXA scans were obtained within 48 hours of death and after 10 months of fixation with 5% formalin/95% ethanol. The measurements showed a high linear relationship ($r^2 > 0.97$; SEE% < 10%), with no significant deviation after fixation (except for total body BMD: −3.1%). In 14 specimens, the precision of femoral and spinal analyses was determined under *in situ* and *ex situ* conditions. There was no significant difference between *ex situ* and *in situ* reproducibility, the coefficient of variation being <3% for the BMC and <2% for the BMD (except at the greater trochanter). The effect of the soft tissues and scan projection was assessed in 83 cadavers aged 80.4 ± 10.3 years. The soft tissues had only a small effect on analyses of the total femur ($r^2 > 0.90$; SEE% <9%), but led to more substantial deviations in regional femoral analyses and in the spine ($r^2 = 0.78$–0.90; SEE% = 8–22%). Comparing lateral with anterior-posterior (AP) spinal scans, the vertebral bodies were found to occupy 40.2 ± 7.2% of the BMC, and 62.0 ± 11.2% of the BMD, the ranges being 26–58%, and 38–91%, respectively. There were large deviations from linearity between *in situ* AP and *ex situ* lateral spinal scans with r^2 values of 0.63 and 0.73 for BMD and BMC (SEE% = 52% and 27% relative to the vertebral body), respectively.

Key words: DXA — Bone densitometry — Fixation — Soft tissue — Reproducibility

Dual energy X-ray absorptiometry (DXA) is the most widely accepted method for quantitative assessment of bone mineral status *in vivo*, and currently serves as an operational definition of osteoporosis by the World Health Organization (WHO) [1–3]. Apart from retrospective and prospective [1, 4, 5] clinical trials of the relationship between bone mineral measurements and fracture risk, a number of experimental investigations have determined the correlation between DXA and mechanical failure loads using biomechanical testing devices [e.g. 6–11]. In view of the high number of novel densitometric techniques and parameters that have been (and will most likely continue to be) developed, for instance, ultrasound of the calcaneus, phalanges, and tibia [4–6, 8–13], peripheral quantitative computed tomography (pQCT) of the upper and lower extremity [14, 15], magnetic resonance imaging of the wrist and heel [16, 17], and others, there will be continuing interest in experimental investigations with large numbers of specimens. Their advantage is that they can be performed in a much shorter time frame than prospective clinical studies, and they do not involve the problem of cumulative X-ray exposure or limited compliance in living volunteers or patients. Biomechanical studies are therefore ideally suited to select the most promising densitometric techniques and parameters, before they are introduced into large scale and more expensive clinical trials.

Since specimens available from pathology dissections include high numbers of patients with severe disease, these samples do not constitute representative samples. It would therefore be advantageous if embalmed specimens from courses of macroscopic dissection could be used, in which the only criteria of inclusion is the testamentary decree several years prior to death. However, if DXA−as a gold standard−is compared to other densitometric techniques, the soft tissue cover should be left intact [9, 10] rather than measurements being performed in excised bones [6–8, 11]. The reason is that relevant measurement errors have been described to occur with *in situ* DXA, which may result from differences in tissue depth [18], soft-tissue inhomogeneity [19–21], and extraskeletal calcification [22].

Studies of the quantitative effect of the soft tissues on DXA measurements have been carried out in only small numbers of specimens [19–21], and, although the influence of fixation has been shown to yield no relevant effect on the bone mineral content (BMC) in excised bone specimens [23–27], there have been no prior studies to show whether DXA in fixed bodies deviates from that in fresh cadavers, when being performed with intact (formalin-fixed) soft tissues. Comparing *in situ* with *ex situ* measurements in the spine, we investigated which proportion of the spinal segment is taken up by the vertebral body and by the posterior

Correspondence to: E.-M. Lochmüller

elements, respectively, and whether AP scans are linearly related to those of the vertebral bodies.

The specific questions to be answered by this study were: (1) How are total body, femoral, and spinal *in situ* DXA measurements in fresh cadavers related to those in embalmed bodies after 10 months of fixation in formalin-alcohol solution? (2) What is the precision of the measurements in fixed cadavers compared with that in excised bones? (3) How large are the deviations of *in situ* femoral and spinal DXA (made in cadavers with intact surrounding soft tissues) in relation to *ex situ* measurements of excised bones? (4) What is the contribution of the posterior elements (vertebral arch, costal and spinous process) onto AP measurements of the lumbar spine, and are *in situ* or *ex situ* AP measurements linearly related to those in lateral scan projection, including only the vertebral body?

Materials and Methods

Determination of the Effect of Fixation

In a first step, seven fresh cadavers aged 85.3 ± 4.8 years (two male, five female) were examined within 48 hours of death. Total body, femoral, and AP spinal measurements were performed with a DPX-L scanner (Lunar Corp., Madison, WI), the skin and soft tissues of the cadavers being fully preserved. Lateral spinal measurements could not be taken since it was not possible to bring and hold the cadavers in the required decubitus position on the scanning table. Analysis of the DXA scans was made with Lunar DPX-IQ 4.5 software, determining the bone mineral content (BMC), projectional area, and areal bone mineral "density" (BMD in g/cm^2) of the total body, total femur, femoral neck, greater trochanter, and the lumbar vertebral bodies 2 to 4 (L2–L4).

The cadavers were subsequently embalmed by a 10–15 liter intraarterial injection of a conventional 5% formalin/95% ethanol solution, including 1 kg polyethlylen-glycol 300, 1 kg polyethylen-glycol 1500, 3.2 kg trichlor-acetaldehyd-hydrate, 0.8 kg alkyl-benzyl-dimethyl-ammonium-chloride, 2 l glycerine, and 0.8 l tetrahydro-1,4-oxazin. Intraarterial application was approximately 150 ml/minute. The cadavers were then stored in a 1% formalin/1% phenol solution and the measurements were repeated 10 months later, under identical imaging conditions.

Systematic deviations were evaluated by determining the pairwise differences, and tested for significance with the Wilcoxon signed rank test. The linear relationship was determined by regression analysis, determining the coefficient of determination (r^2) and standard error of the estimate (SEE%).

Measurement Precision in Fixed Cadavers vs. Excised Bones

To compare the reproducibility (precision) of the measurements in fixed cadavers and excised bones, 14 specimens were randomly selected from a course of macroscopic dissection (seven male, seven female, age 75.6 ± 12.0 years). Total body, femoral, and AP spinal (L2–L4) DXA measurements were first taken under *in situ* conditions (with intact skin and soft tissues), repeating the measurements four times on different days (including recalibration of the system and new positioning of the cadavers). In each individual, the coefficient of variation (CV%) was calculated for the various parameters, dividing the mean of the four measurements by their standard deviation. From the individual CV%s we determined the root-mean-square (RMS) average CV% (not the arithmetic mean), the protocol according to the recommendations of Glüer et al. [28].

After the dissection course, the femora and lumbar vertebral columns were excised and dissected free of surrounding soft tissues. The DXA measurements were repeated in a homogeneous environment, and with the bones in a defined position. The femora were placed on a plexiglas tray, 6 cm above the scanning table, within a plexiglas container filled with water to a level of 18 cm (Fig. 1). To simulate AP measurements, the spinal segments were positioned so that the spinous processes were 0.5 cm above the scanning table, the measurements being repeated four times on different days as described above. To simulate lateral measurements, the spinal segments were positioned on a plexiglas tray so that their center was located 12 cm above the scanning table. The paired *t*-test was used to evaluate whether the precision was significantly different under *ex situ* and *in situ* conditions.

Fig. 1. *Ex-situ* DXA measurement of an excised femur in a homogeneous environment.

Determination of the Effect of Soft Tissues and Scan Projection

To determine the effect of (formalin-fixed) soft tissues on the DXA measurements, *in situ* measurements were performed in all cadavers from the course of macroscopic dissection (n = 83, 31 male, 52 female; age 80.4 ± 10.3 years, range 46–83 years) with intact skin and soft tissues. Only 77 femoral scans could be obtained because of the presence of hip prostheses. The femoral and spinal scans were repeated in the excised bones. Please note that, for didactic reasons, the spinal canal was opened in 44 specimens during the dissection course to expose the spinal cord. *In situ* AP spinal scans were therefore compared to only 39 *ex situ* AP scans of L2–L4, but lateral scans could be obtained in all 83 specimens under *ex situ* conditions. *Ex situ* AP scans were compared to those in lateral projection, to assess the effect of the posterior elements. *In situ* AP measurements were finally related to lateral measure-

Table 1. Effect of 10-month formalin fixation on *in situ* DXA

		Systematic difference (%)	Level of significance (*P*)	Correlation (r^2)	SEE (%)
Total body	BMC	−2.0%	n.s.	0.98	5.3%
	BMD	−3.1%	<0.05	0.99	1.0%
Total femur	BMC	−0.6%	n.s.	0.97	9.7%
	BMD	+2.2%	n.s.	0.98	3.8%
L2–L4	BMC	+7.0%	n.s.	0.97	7.6%
	BMD	+3.1%	n.s.	0.99	3.3%

SEE = standard error of the estimate relative to the scans in fresh specimens; BMC = bone mineral content; BMD = areal bone mineral "density" (g/cm^2); L = lumbar vertebra; n.s. = nonsignificant

ments in order to assess the aggregate effect of soft tissues and posterior elements onto AP spinal DXA. Systematic deviations were evaluated by computing the mean of the pairwise differences, and tested for significance by the paired *t*-test.

Results

The total body, femoral, and spinal DXA measurements after 10 months of fixation were highly correlated (r^2 > 0.97; SEE < 10%) with those in fresh cadavers (Table 1). There was no significant deviation of the values except for the total body BMD that showed a small decrease of 3.1% on average (Table 1, Fig. 2). The precision of the total body scans was 1.6% for the BMC, and 0.8% for the BMD. With the exception of the femoral neck, the precision was not significantly different under *in situ* and *ex situ* conditions (Table 2), the coefficients of variation ranging from 1.7% (L2–L4) to 6.9% (greater trochanter) for the BMC, and from 0.7% (L2–L4) to 3.0% (greater trochanter) for the BMD.

The measurements attained higher BMC and BMD values *in situ* compared to *ex situ* conditions (range = +0.2%–+12.7% − Table 3). The linear relationship was relatively high for analyses of the total femur (r^2 > 0.92; SEE% < 9% − Table 3), but lower for the femoral neck, greater trochanter, and spine (r^2 = 0.78–0.90; SEE% = 8–22% − Table 3; Figs. 3 a,b).

The vertebral bodies (lateral scans) occupied 40.2 ± 7.2% of the BMC and 62.0 ± 11.2% of the *ex situ* BMD of the spinal segment in the AP scans (Table 4), the range being 26%–58% (BMC) and 38%–91% (BMD). The linear relationship between the values was relatively low, with a coefficient of determination (r^2) of 0.80 and 0.69, respectively, and an SEE% of 41% and 26% (relative to the vertebral body values in the lateral scans). Relating *in situ* AP to *ex situ* lateral spinal scans (Table 4, Fig. 4), there was an increase in the error of estimating vertebral body BMC and BMD, with r^2 values of 0.73 and 0.63, and an SEE% of 52 and 26%.

Discussion

In this study we have assessed the effect of fixation, soft tissues, and scan projection on bone mineral measurements with DXA. To our knowledge, this is the first study to evaluate the influence of embalment, including natural soft tissues. The finding of only a negligible effect of fixation is important in the context of experimental, biomechanical studies of the relationship between bone mineral status versus mechanical strength.

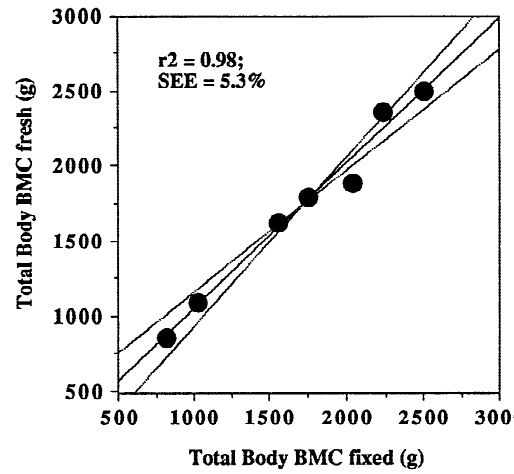

Fig. 2. Regression plot of DXA measurements (total body BMC) before and after 10 months of fixation (n = 7).

Table 2. Precision of DXA under *in situ* vs *ex situ* conditions (n = 14)

		In situ CV%	*Ex situ* CV%	Significance of difference
Total femur	BMC	2.9	2.8	$P = 0.44$
	BMD	1.5	2.2	$P = 0.07$
Neck	BMC	2.2	2.7	$P = 0.06$
	BMD	0.9	2.4	$P < 0.05$
Trochanter	BMC	6.9	5.8	$P = 0.21$
	BMD	3.0	1.8	$P = 0.18$
L2–L4	BMC	1.7	1.3	$P = 0.33$
	BMD	0.7	1.0	$P = 0.16$

BMC = bone mineral content; BMD = areal bone mineral "density" (g/cm^2); L = lumbar vertebra XA under *in situ* vs *ex situ* conditions (n = 14)

Table 3. Effect of soft tissue on femoral (n = 77) and spinal (n = 39) DXA

		Systematic difference (%)	Level of significance (*P*)	Correlation (r^2)	SEE (%)
Total femur	BMC	+5.1	$P < 0.001$	0.92	8.6
	BMD	+0.2	$P < 0.05$	0.94	5.3
Neck	BMC	+11.4	$P < 0.001$	0.88	11.7
	BMD	+4.3	$P < 0.001$	0.87	9.3
Trochanter	BMC	+12.7	$P < 0.001$	0.78	22.0
	BMD	+4.9	$P < 0.001$	0.88	7.7
L2–L4	BMC	+7.7	$P < 0.01$	0.86	15.2
	BMD	+10.0	$P < 0.001$	0.90	9.5

SEE = standard error of the estimate relative to the *ex situ* scans; BMC = bone mineral content; BMD = areal bone mineral "density" (g/cm^2); L = lumbar vertebra able 3. Effect of soft tissue on femoral (n = 77) and spinal (n = 39) DXA

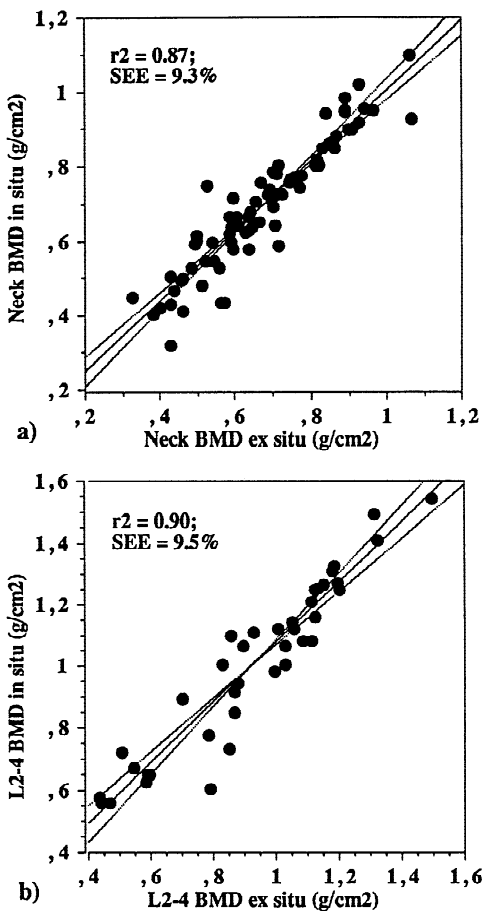

Fig. 3. Regression plot of DXA measurements (BMD) with and without soft tissues: **(a)** Femoral neck BMD (n = 77); **(b)** AP spinal BMD (L2–L4) (n = 39).

Previous investigations in fixed versus fresh bone samples (not including surrounding soft tissues) have also demonstrated that fixation has no significant effect on bone mineral measurements [23–26]. Our results demonstrate that this is also the case for *in situ* measurements with DXA. Since very small increases in BMC and BMD were found in some, and small decreases were observed in other regions of interest, we believe that these changes are random, and not systematic. Edmondston et al. [27] observed that the correlation between BMC and failure loads was not altered by formalin in sheep vertebrae. We have shown previously [29] that the speed of sound of calcaneal ultrasound showed a substantial decrease during fixation, but that the measurements in the embalmed state showed a high linear relationship to those in fresh bones. These results suggest that a comparative evaluation of the capacity of different densitometric techniques to predict mechanical bone strength can be made with specimens from macroscopic dissection courses. This is a substantial advantage because many specimens are available for research purposes and because they constitute a random selection of older people with an average status of underlying disease.

The deviations of the DXA measurements with and without the presence of the surrounding soft tissues can have several sources: Based on the two different energy levels in DXA, only two of the three tissues in the beam path (bone, lean tissue, and fat) can be separated. However, since the composition (fat versus lean) of the soft tissues in front, behind, and within the bone affects measurements of the BMC within the region of interest, their composition must be measured adjacent to the bone. This ratio is then assumed to apply for all the surrounding soft tissue, but if the fat tissue is distributed inhomogeneously throughout these regions, the result is accuracy errors [1, 2, 19, 21]. Other sources of artifacts include variations in tissue depth and extraskeletal calcification, as from the aorta or femoral arteries [1, 2, 18, 22]. Precision errors may contribute to the deviations between *in situ* and *ex situ* measurements at the greater trochanter, but are unlikely to play a significant role at other sites, since the reproducibility was < 3% for the BMC and < 2% for the BMD. Svendsen et al. [21] compared *in situ* DXA with *ex situ* measurements of excised (mechanically cleaned) as well as macerated and defatted bones in a small sample of 14 cadavers. They reported accuracy errors (SEE%) for *in situ* versus excised bones of 7.6/6.7% for femoral BMC/BMD, 7.6/6.2% for the femoral neck, 5.9/3.4% for the greater trochanter, 6.1/5.2% for lumbar vertebrae, and 3.0/2.9% for the forearm. Whereas the accuracy error observed in our study was similar for the

Table 4. Effect of posterior elements on spinal DXA[a]

		Systematic difference (%)	Correlation (r^2)	SEE* (%)	SEE$^+$ (%)
Ex situ (n = 39)	BMC	−59.8	0.80	40.9	16.3
	BMD	−38.0	0.69	26.0	16.1
In situ (n = 83)	BMC	−62.1	0.73	52.1	19.4
	BMD	−43.2	0.63	26.3	15.0

SEE* = standard error of the estimate relative to the lateral scan; SEE+ = standard error of the estimate relative to the AP scan; BMC = bone mineral content; BMD = areal bone mineral "density" (g/cm^2); all systematic difference were highly significant at $P < 0.001$
[a] Comparison between AP scans of L2–L4 (*in situ* and *ex situ*), with lateral scans (*ex situ*)

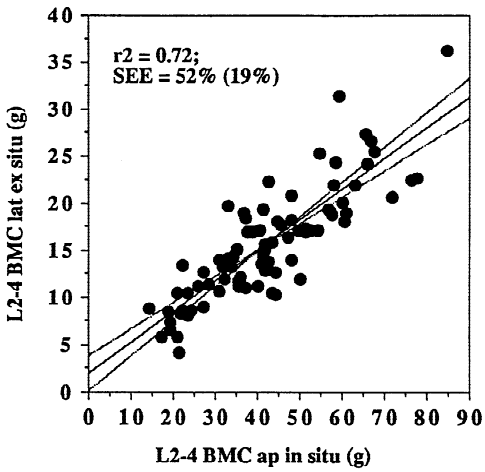

Fig. 4. Regression plot of spinal DXA measurements (BMC of L2–L4) obtained in AP projection *in situ* versus *ex situ* in lateral projection (n = 83).

total femur, it was higher for the femoral neck, trochanter, and the lumbar spine. This discrepancy may have the following sources: Svendsen et al. [21] made two measurements *in situ* and two *ex situ*, respectively, and used positioning devices to ideally match the positions relative to the scan table. In this way, precision errors were reduced, whereas we have determined the aggregate error (accuracy and precision) occurring when a single measurement is taken. The second reason may be the different study samples. The 14 individuals investigated by Svendsen et al. [21] had an average age of 61 years, whereas our sample was much larger and included older individuals with a mean age of 80 years. In these individuals, the relative fat tissue content varied widely (from 4.2% to 52.2% body weight), and this larger variation may have also caused the fat tissue distribution to be more inhomogeneous throughout the body, thus increasing the accuracy errors involved in DXA, particularly in the spine.

When comparing AP and lateral scans in the lumbar spine, we found only a moderate correlation between both types of measurements, this being in agreement with previous clinical studies [30–33] that have compared both scan projections. Duboeuf et al. [32] found a correlation coefficient of 0.64 for lateral versus AP BMD of L2–L4 in 101 young healthy women. In 22 patients with spinal osteoporosis, they reported a lateral BMD of 58% of the AP BMD. Grampp et al. [33] studied 47 premenopausal women (33 ± 7 years) and 41 postmenopausal women (64 ± 9 years) without, and 36 postmenopausal women (70 ± 6 years) with vertebral fractures, and reported a correlation of 0.74 for lateral versus AP spinal BMD. The lateral BMD values amounted to 80% of AP measurements before menopause and 72% after menopause [33]. We find a relatively low correlation between both measurements also under *ex situ* conditions (r = 0.69). This demonstrates that the deviations are not caused by accuracy errors involved in an inhomogeneous fat distribution but from the variability in bone mineral status of the predominantly trabecular vertebral body and the mainly cortical posterior elements. On average, the vertebral body contained 40% and the posterior elements 60% of the total AP BMC in our study, the relationship varying between 26% and 58% between individuals. Both the low values of the vertebral body and the highly variable relationship with the posterior elements will most likely be due to the older age of individuals in our study. Some of them had severe trabecular bone loss in the vertebral body, some degenerative changes in the spine (with increased calcification of the vertebral discs and apophyseal joints), and some both. This result demonstrates that in the elderly it is problematic to make accurate estimates of trabecular bone loss in the vertebral body from AP scans. This may, however, be less problematic in immediate postmenopause, where degenerative changes are less frequent.

We conclude that for comparative densitometric/biomechanical studies, accurate DXA measurements can be made in cadavers that have been fixed over substantial periods of time. The soft tissues cause a slight overestimation of the DXA measurements (up to 13%), with accuracy errors (SEE%) of less than 9% in the total femur, but errors of 8–22% for regional analyses of the femur and lumbar spine. Relatively large deviations from linearity are observed between AP measurements of vertebral segments and lateral measurements of the vertebral bodies under both *in situ* and *ex situ* conditions. These deviations are attributed to a high variability of trabecular bone loss in the vertebral body as well as the presence of degenerative changes in the posterior elements in elderly individuals.

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