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Influence of Temperature on QCT: Implications for Mineral Densitometry

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Objective: Inaccuracies in quantitative CT (QCT) for vertebral bone mineral measurements may result from differences between the temperature of the vertebrae and the calibration standards. This study aims to quantify these effects by using scans of marrow-equivalent materials and computer simulation.

Materials and Methods: The CT numbers of fat, water, gelatin suspension, and bone were measured within an anthropomorphic phantom at temperatures between −18 and 38°C. A computer simulation was then performed using these materials to represent marrow fat, soft tissue, and bone in varying proportions over this temperature range. Postprocessing single and dual energy QCT calculations were then performed on the data acquired from the simulation.

Results: A change of 80 HU in the CT number of water on cooling from 38 to −18°C was demonstrated. An increase of 95 HU in the CT number of fat occurred over the same temperature range. Dry cortical bone showed no change in CT number with temperature changes from 24 to −18°C. In the computer simulation, the fat error associated with single energy QCT for trabecular bone mineral densitometry was 20% less for specimens at room temperature than at body temperature. In simulated frozen specimens, varying marrow fat/soft tissue composition had almost no effect on single energy QCT mineral densitometry. Dual energy QCT methods that use a fat-equivalent reference material were significantly influenced by the temperature of the specimen.

Conclusion: The fat error of single energy QCT for mineral densitometry may have been underestimated in previous in vitro studies using vertebral specimens scanned at room temperature. In the simulation, the fat error diminished as the temperature of the specimen was reduced and was negligible when frozen. Fat-equivalent reference materials used for dual energy QCT in vivo should have similar X-ray-attenuating properties at room temperature to those of marrow fat at body temperature.

Index Terms: Computed tomography, quantitative—Attenuation values—Temperature.

The CT number of any material is directly related to its X-ray mass attenuation coefficient and the mass density of the material. The mass densities of all materials vary with temperature, and thus the CT number of any material is temperature dependent (1–3). Detectable changes in the CT number of water occur over relatively small temperature ranges; for example, a rise of 5.6 HU occurs between body and room temperature. This is of importance in the calibration of CT scanners (4,5). Much greater temperature-dependent changes in density (and consequently in CT number) occur in fat over this temperature range and in water when it is frozen (6), with implications for the scanning of tissue specimens (7). The change in attenuation of bone with temperature has not previously been evaluated. In vitro studies of single energy quantitative CT (SEQCT) for mineral densitometry have been performed on bone specimens at room temperature, frozen, or during thawing (8–10). This is

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due to difficulties in handling and storing fresh postmortem specimens and the need to excise and analyze specified volumes of trabecular bone after scanning without loss of marrow content. A correction of SEQCT data of frozen trabecular bone specimens has been used to make results comparable with those of specimens scanned at room temperature (9). The need for this correction was assumed to be due to freezing of the water in the marrow and was not investigated further.

Dual energy QCT (DEQCT) is used to correct QCT mineral densitometry for the influence of fat within trabecular bone on the measurement; increasing marrow fat content causes progressive underestimation of trabecular mineral density (8). Little attention has been paid to the influence of temperature on the results of DEQCT.

The present study was performed to confirm the effect of temperature on the CT numbers of water and fat, to demonstrate the influence of scanner kilovolt peak on this temperature-dependent effect, and to investigate the effect of temperature on the CT number of cortical bone. The rate of change in CT number of thawing specimens was also measured. A computer simulation was then used to demonstrate the implications of temperature changes on QCT of trabecular bone.

MATERIALS AND METHODS

Experiment 1

Samples of degassed distilled water, lard (to simulate marrow fat), and 5% gelatin suspension in degassed distilled water (to simulate fat-free marrow components) were placed within 5 cm diameter 200 cm^3 plastic syringes. A dried, defatted human humerus was wrapped in bandage to fit within a syringe. These specimens were scanned in the position of the vertebral body within an oval, water-filled anthropomorphic body phantom (Fig. 1) placed on top of a water and dipotassium hydrogen phosphate mineral densitometry reference phantom. The syringe specimens were scanned after acclimatizing for at least 12 h at (a) body temperature (38°C in a thermostatic water bath); (b) room temperature (24°C); (c) refrigerator temperature 1 (7°C); (d) refrigerator temperature 2 (−3°C); and (e) freezer temperature (−18°C). The bone specimen was scanned at temperatures b and e. The length of the bone (greater tuberosity to lateral epicondyle) was measured to the nearest half millimeter at each temperature. All specimens were scanned within 1 min of removal from the stable temperature environment.

Experiment 2

Specimens of (a) 5% gelatin suspension and (b) lard were scanned immediately after removal from the freezer and left well separated on the bench top in the scan room. Further scans of these specimens were performed at 30, 120, 210, and 340 min after removal from the freezer.

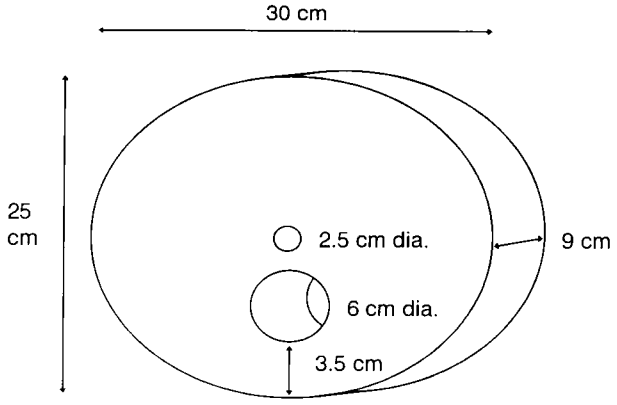

FIG. 1. Anthropomorphic phantom constructed of Perspex filled with water and containing a central air reference channel and a larger specimen channel in the simulated position of a vertebral body.

Scanning was performed on a GE 9800 CT scanner at both 80 kVp, 210 mAs and 140 kVp, 30 mAs for each specimen in experiment 1 and at 80 kVp, 210 mAs in experiment 2. These factors were chosen to give acceptably low standard deviations to the measurements while being similar to those used in vivo. The CT numbers of regions of interest (ROIs) within each syringe specimen, air, and water were measured on each scan. Twelve square centimeter ROIs were used in the water, gelatin, and lard specimens and an irregular ROI containing the 335 highest value pixels was used in the cortical bone at a marked location on the humerus. In addition, 1.66 cm^2 ROIs were used for air, measured within the central reference aperture of the anthropomorphic phantom, and water, measured in the reference phantom. The CT numbers of the specimens were converted to idealized Hounsfield units using the measured air and reference water CT numbers (Eq. 1) to fix the CT number of water at 0 and air at −1,000 (11):

$$HU_{mat} = 1{,}000 \frac{(CT_{mat} - CT_{water})}{CT_{water} - CT_{air}} \quad (1)$$

where HU_{mat} is idealized Hounsfield unit number of the material, CT_{mat} is measured CT number of material, CT_{water} is measured CT number of water, and CT_{air} is measured CT number of air. Repeated scans of the phantoms demonstrated reproducibility of the CT numbers to within ±2.5 (SD) for all materials at both scan energies.

Computer Simulation

The mathematical simulation of QCT was based on the Hounsfield unit numbers of gelatin suspen-

sion, lard, and bone to calculate the expected Hounsfield unit number for theoretical trabecular bone at each temperature and scan energy using Eq. 2:

$$HU_t = HU_b V_b + HU_g V_g + HU_l V_l \quad (2)$$

where HU_t is CT number of trabecular bone with marrow, HU_b, HU_g, and HU_l are CT numbers of bone, gelatin suspension, and lard, respectively, and V_b, V_g, and V_l are volume proportions of bone, gelatin suspension, and lard, respectively.

The bone content was 5 or 15% by vol. Marrow contained either 15% by vol gelatin suspension with the remainder being lard or 15% by vol lard with the remainder being gelatin. These compositions were chosen to simulate the extremes of marrow fat content encountered in vivo (12). Bone was assumed to contain 1,112 mg/cm^3 of mineral (12). The possible influence of varying beam hardening on the CT numbers of marrow components due to varying mineral contents was not considered. The simulated reference phantom was assumed to be at room temperature for all scans and used bone, gelatin suspension, and lard for calibration. Mineral content was measured using conventional SEQCT calculations at 80 kVp (Eq. 3) and by the DEQCT methods described by Cann et al. (13) (Eq. 4) and by Goodsitt et al. (14) (solution to simultaneous Eqs. 5, 6, and 7 for the unknowns V_b, V_g, and V_l):

SEQCT

$$V_b = \frac{HU_{t_{80}} - HU_{g_{80}}}{HU_{b_{80}} - HU_{g_{80}}} \quad (3)$$

DEQCT of Cann et al. (13)

$$V_b = \frac{(HU_{t_{80}} - HU_{g_{80}}) - (HU_{t_{140}} - HU_{g_{140}})}{(HU_{b_{80}} - HU_{g_{80}}) - (HU_{b_{140}} - HU_{g_{140}})} \quad (4)$$

DEQCT of Goodsitt et al. (14)

$$HU_{t_{80}} = HU_{b_{80}} V_b + HU_{g_{80}} V_g + HU_{l_{80}} V_l \quad (5)$$

$$HU_{t_{140}} = HU_{b_{140}} V_b + HU_{g_{140}} V_g + HU_{l_{140}} V_l \quad (6)$$

$$V_b + V_g + V_l = 1 \quad (7)$$

In the preceding equations, the subscript 80 is the low energy (80 kVp) scan CT number and 140 is the high energy (140 kVp) scan CT number. For each method mineral density (mg/cm^3) = V_b1,112.

RESULTS

The Hounsfield unit values for water and gelatin suspension increased slightly as temperature dropped from 38 to 7°C. On freezing there was a marked decrease to −82 HU for water and −60 HU for gelatin. Further decrease in temperature resulted in a slight increase in Hounsfield unit value. Scan kilovolt peak made no appreciable difference in the Hounsfield unit number of these materials at each temperature (Table 1). Lard showed a more rapid and near linear increase in Hounsfield unit value with decreasing temperature over the entire temperature range (Table 1). At 140 kVp the Hounsfield unit value for lard was between 18 and 24 HU higher than at 80 kVp at all temperatures.

Bone showed a negligible change in Hounsfield unit value with temperature (Table 1), probably within the limits of measurement error. No detectable change in the length of the humerus with temperature was found. The change in Hounsfield unit value with time for the gelatin and lard samples from the freezer during exposure to the ambient room temperature is illustrated (Fig. 2), with both samples taking ~4 h to reach stable states. No visible denaturing of the specimens occurred during the experiments.

Computer Simulation

The mathematical simulation shows that SEQCT with the vertebrae at 38°C underestimates the trabecular mineral density by 15 mg/cm^3 in the presence of 15% lard but by 67 mg/cm^3 with 70% lard. If the vertebrae are at room temperature, the underestimates are only 11 and 56 mg/cm^3, respectively. At a temperature of −18°C, SEQCT underestimates mineral density by 28 mg/cm^3 whatever the lard/gelatin composition of the marrow (Fig. 3).

For DEQCT by the method of Cann et al. (13), the underestimation of mineral content at body temperature is still dependent upon the lard content of the specimens, but the magnitude of the underestimate is approximately one-half that for SEQCT. This method does not calculate marrow fat content. Changing the temperature has no marked effect on the DEQCT mineral density by the Cann et al. method (Fig. 4a and b). With use of simulations of a wide variety of mineral and marrow fat contents at body temperature, multiple linear regression dem-

TABLE 1. *Hounsfield unit values of materials tested*

Temperature (°C)	kVp	Hounsfield unit			
		Water	Gelatin	Lard	Bone
−18	80	−76.9	−57.1	−55.67	1,877.4
	140	−77.2	−57.3	−31	1,330.2
−3	80	−82.2	−60.1	−68.6	
	140	−81.8	−60.3	−43.6	
7	80	5.5	19.8	−103.2	
	140	5.6	19.9	−78.2	
24	80	0	13.4	−121.5	1,882.7
	140	0	13.5	−100.5	1,325.5
38	80	−2.4	10.1	−147.8	
	140	−3.6	8.9	−125.5	

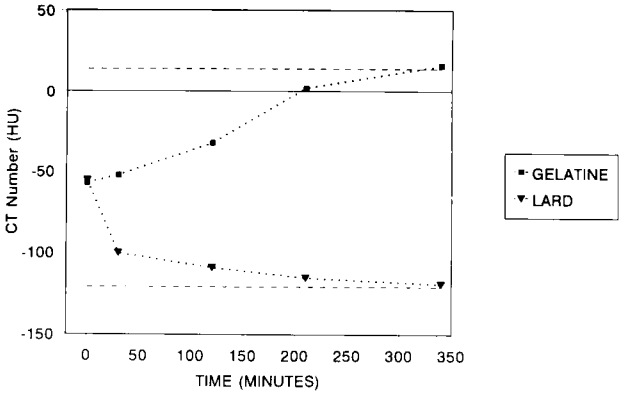

FIG. 2. Change in CT number for gelatin suspension and lard with time after warming from −18°C to ambient temperature (24°C). The dashed lines represent expected values at room temperature.

onstrated a fixed relationship between the true mineral content of the vertebrae and the SEQCT and Cann DEQCT results:

$$BMD_T = 2.025BMD_C - 1.018BMD_S - 7.3 \quad (8)$$

where BMD_T is true mineral density, BMD_C is mineral density by the Cann et al. dual energy method, and BMD_S is mineral density by SEQCT.

The second DEQCT method of Goodsitt et al. (14) gives accurate results for both mineral and marrow fat contents over a wide range of trabecular bone compositions when the vertebrae and reference materials are at the same (room) temperature but shows disparate changes when the temperature is changed, dependent upon the fat content. For the situation relevant to in vivo studies in which the reference phantom is at room temperature and the vertebrae at body temperature, the simulation results again demonstrate that a calibration correction could be applied:

$$BMD_T = 1.0066BMD_G - 0.0335FAT - 7.3 \quad (9)$$

where BMD_G is mineral density by the Goodsitt et al. dual energy method and FAT is fat content by that method.

High fat/low gelatin content causes progressive underestimation of both mineral and fat content with decreasing temperature (Fig. 4b and d). High gelatin/low fat content causes less marked but progressive underestimates of mineral and fat content with decreasing temperature until the temperature is below 0°C. There is then a marked overestimate of both mineral and fat content (Fig. 4a and c).

Although the computer simulation represents an idealized situation where measurement precision is not influencing the results, the measured reproducibility of the original CT numbers can be incorporated to estimate the precision of the QCT measurements: This gave a reproducibility of ±1.6 mg/cm^3 for SEQCT, ±4 mg/cm^3 for DEQCT mineral by the Cann et al. method, ±7.6 mg/cm^3 for DEQCT mineral by the Goodsitt et al. method, and ±9% for DEQCT fat by the latter method.

DISCUSSION

Almost all methods of tissue preservation can influence the CT number of specimens (15). The change in Hounsfield unit number of lard and water at different temperatures confirms previous findings. Although previously documented (6), the magnitude of change in Hounsfield unit of water on freezing is not widely appreciated. The increase in Hounsfield unit of fat is also considerable and is almost linear with decreasing temperature. This feature has not been emphasized in the literature but is important when specimens are scanned even when not frozen. The variation in the Hounsfield unit number of both fat and bone at different scanner energies is recognized when using postprocessing DEQCT (14).

The CT number for any material is dependent upon the mass density of the material and the mass attenuation coefficients of the material and water. Mass attenuation coefficients are X-ray energy dependent. Materials having mass attenuation coefficients that decrease less than that of water as scan energy is increased will have a greater CT number at higher energy and a widening difference in CT number between two energies as temperature falls. This would apply to both fat and soft tissues. The latter effect (increasing difference in CT num-

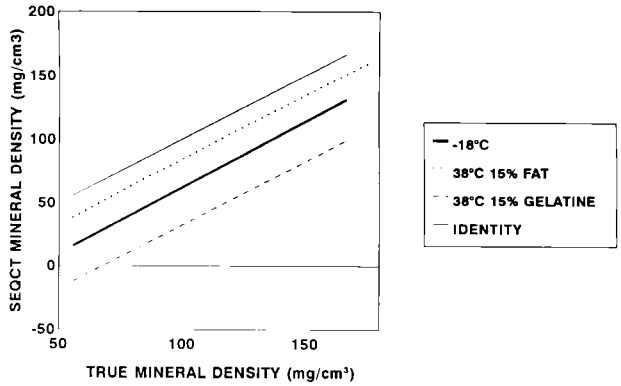

FIG. 3. Computer simulation of the single energy QCT (SEQCT)–measured mineral content of specimens containing between 5 and 15% bone by vol and with varying marrow compositions. The dotted line indicates the results obtained when the marrow contains only 15% fat, the remainder being gelatin; the dashed line indicates the results obtained when the marrow contains only 15% gelatin, the remainder being fat. The thick solid line indicates the results obtained for specimens at −18°C whatever the marrow fat/gelatin ratio. The line of identity indicates the true mineral density without the fat error associated with SEQCT. Marrow compositions between the two extremes of fat and gelatin used in this simulation would give results lying between the dotted and dashed lines.

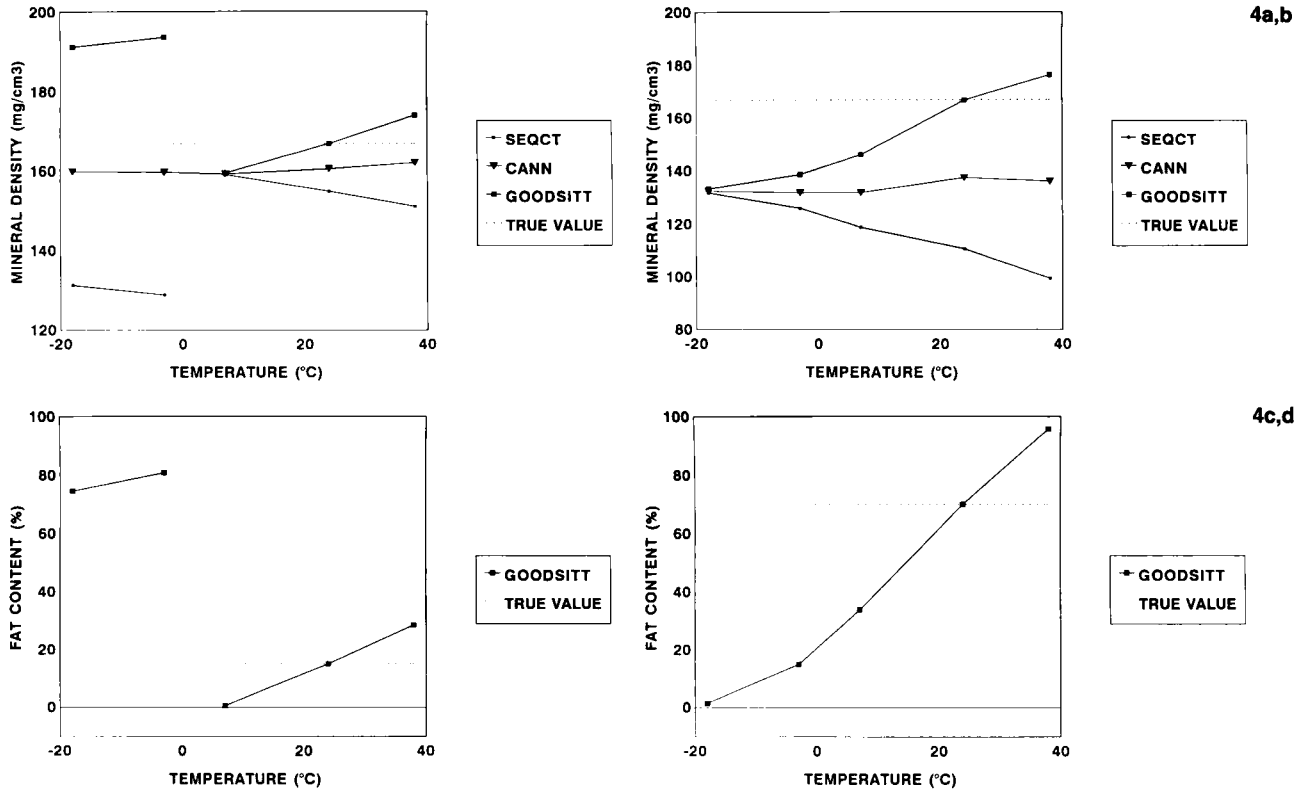

FIG. 4. Computer simulations of the effect of temperature on two specimens of identical mineral content (15% bone by vol, 166.8 mg/cm^3) but varying marrow composition. The dotted lines represent the true mineral value. **a:** Mineral density by single (SEQCT) and dual energy QCT for marrow of 15% fat and 70% gelatin. **b:** Mineral density by single and dual energy QCT for marrow of 70% fat and 15% gelatin. **c:** Fat content by dual energy QCT for marrow of 15% fat and 70% gelatin. **d:** Fat content by dual energy QCT for marrow of 70% fat and 15% gelatin. Discontinuities in the lines for mineral content by single energy QCT and the Goodsitt et al. method (14) on graph (a) and fat content on graph (c) occur due to the effect of freezing when a high water content is present. Cann et al. (13).

ber between high and low kilovolt peak as temperature falls) would be <1 HU for both fat and gelatin over the temperatures used in the present study. The former effect (having a higher CT number at higher scan energy) would be marked for fat (as demonstrated in Table 1), but <1 HU for gelatin.

Bone showed no significant change in either Hounsfield unit number or length on cooling from 24 to −18°C, indicating that the coefficient of thermal expansion for bone is too small to be detected under the conditions of these experiments. The small decrease in the difference between CT numbers at high and low kilovolt peak at −18°C for bone may be due to the converse effect to that described for fat and gelatin above, as the mass attenuation coefficient of bone decreases more rapidly than that for water with increasing kilovolt peak.

The change in CT number of lard on warming to room temperature from −18°C illustrates that even relatively small volumes of fat (~100 cm^3) take >4 h for temperature equilibration. The relationship between CT number and temperature for lard (Table 1) suggests that the lard had warmed to above 0°C within 30 min. Conversely, the gelatin specimen, although also taking >4 h to reach room temperature, had not fully defrosted during the first 3.5 h. The rising CT number of the gelatin sample during this time reflects a reducing proportion of frozen to thawed suspension. Thus, although lard might be expected to self-insulate and warm up slowly, the latent heat of thawing for water keeps the latter sample at 0°C for many hours. These observations emphasize the need for adequate defrosting of frozen specimens before scanning at any assumed temperature.

The computer simulation of vertebral trabecular bone demonstrated results of practical importance: Single energy QCT of specimens at −18°C and 80 kVp provides bone mineral density results that are not affected by variations in the fat/water composition of the marrow. This is because the gelatin suspension and lard have almost identical Hounsfield unit values under such conditions. The systematic underestimation (of ~28 mg/ml in the simulation) could be corrected for by appropriate calibration. The SEQCT of frozen specimens would then retain the high precision of this technique while considerably improving accuracy. These findings are both supported by and explain results obtained when performing QCT on frozen vertebral specimens (16).

Dual energy QCT, introduced to correct for the inaccuracies of SEQCT caused by variations in the fat/water ratio of the marrow, has remained an experimental technique. This is because it is both less precise than SEQCT and involves a higher radiation dose. Several methods of DEQCT have been described. The earlier (preprocessing) techniques usually involved additional hardware and software modifications to the scanner (17), although some scanners are designed to perform such techniques (18). Preprocessing methods are not widely available and most centers performing DEQCT use postprocessing methods in which separate scans are performed through the same region of tissue at two different energies. Energy-dependent changes in the Hounsfield unit value of the different components of trabecular bone can then be used to estimate the relative proportions of those components in the ROI. The simplest method of Cann et al. (13) was described for identifying calcification in pulmonary nodules but is also used in vertebral trabecular bone mineral densitometry. This method examines only the change in CT number of the ROI in trabecular bone at different scan energies and assumes that this change is due to the change in CT number of bone. All other components within the ROI are assumed to have no change in CT number with scan energy. Thus, for example, bone shows a reduction in CT number of 557 HU between scans of 80 and 140 kVp (Table 1). If an ROI in trabecular bone showed a reduction of 55.7 HU between two scans at these energies, then it would contain 10% bone. This result would be obtained whether the rest of the sample consisted of water with a CT number of 0 HU at both energies or ice with a CT number of −82 HU at both energies. The assumption that the CT number of all nonbone materials does not change with scan energy is true only for water and gelatin suspension in the computer simulation but not for fat. An error is therefore introduced that is proportional to the fat content and the difference in CT number of fat at high and low scan energies. Thus, at body temperature this method makes a correction of ~50% to the fat error in SEQCT, though knowledge of both the SEQCT and the DEQCT result may allow a recalibration to give a more accurate result (Eq. 8). The difference in CT number of fat between high and low scan energy is only minimally affected by temperature (Table 1). The results of the Cann et al. method for mineral densitometry are therefore relatively insensitive to changes in temperature of the vertebrae, as demonstrated in the simulation (Fig. 4).

Goodsitt et al. (14) described two methods of DEQCT. Under the conditions of the simulation, both methods would give identical results. These methods incorporate the change in Hounsfield unit number of fat with scan energy by scanning and measuring a fat substitute within the reference phantom. It is the difference between the Hounsfield unit value of fat in the calibration reference phantom (at room temperature) and fat in the specimen that causes the temperature-dependent errors in DEQCT (Fig. 4d). At temperatures below freezing, fat content determined by DEQCT is particularly misleading.

The experimental model uses the Hounsfield unit values of gelatin suspension, lard, and bone at 24°C as the reference phantom equivalents. It therefore gives correct mineral and fat results for DEQCT by the method of Goodsitt et al. (14) for vertebrae at this temperature. The errors in fat and mineral estimation at 38°C (Fig. 4) can be corrected by appropriate calibration in the simulation (Eq. 9) but would in practice depend upon the reference materials present in the calibration phantom.

There are several estimates of the influence of marrow fat on SEQCT vertebral trabecular bone mineral densitometry in the literature (8,19–22). All these estimates were derived from CT measurements made in postmortem bones that were stored and scanned at various temperatures. None were performed above room temperature. The data from our experiments suggest that the error in SEQCT mineral densitometry caused by marrow fat in vivo would be underestimated by 20% if postmortem bones were scanned at room temperature and by 50% if they were scanned at 1–4°C.

Dual energy QCT methods that require a fat-equivalent reference material may be significantly affected by the difference in temperature between the patient and the reference phantom. Solid plastic reference materials are now established but should have properties over the range of acceptable room temperatures that mimic those of the appropriate marrow components at body temperature.

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