Calcium metabolism evaluated by $^{47}$Ca kinetics: estimation of dermal calcium loss

P. CHARLES, F. TAAGEHØJ JENSEN, L. MOSEKILDE AND H. HVID HANSEN

Department of Nuclear Medicine, Aarhus Municipal Hospital and Medical Department III, Aarhus County Hospital, Aarhus, Denmark

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Summary

1. Seventy-seven calcium balance and $^{47}$Ca turnover studies were performed in normal volunteers ($n = 15$) and in patients with osteoporosis ($n = 12$), primary hyperparathyroidism ($n = 8$), osteogenesis imperfecta ($n = 5$), medullary carcinoma of the thyroid ($n = 4$), thyrotoxicosis ($n = 2$) and intestinal bypass for obesity ($n = 11$).

2. After intravenous injection of 20 µCi of $^{47}$Ca two retention curves of $^{47}$Ca were obtained: $R_1(t)$ directly measured on a whole-body counter and $R_2(t)$ calculated from the cumulated daily excretions of $^{47}$Ca in urine and faeces. The data were fitted to a modification of the continuously expanding exchangeable calcium pool model.

3. Dermal calcium loss was estimated from the serum $^{47}$Ca specific radioactivity curve and the constantly increasing difference between the two retention curves. The median dermal calcium loss in 77 studies was 1.50 mmol 24 h$^{-1}$ 1.73 m$^{-2}$ (range 0.13–4.60).

4. The dermal calcium loss might be overestimated by redistribution of tracer or by eventual insufficient collection of urine and faeces. The possible influences of these errors have been evaluated.

5. Patients with primary hyperparathyroidism had a greater ($P < 0.02$) dermal calcium loss (2.64 mmol; range 0.80–4.50) than a control group (1.38 mmol; range 1.25–2.34).

Key words: calcium balance, calcium kinetics, dermal calcium, distribution of $^{47}$Ca.

Introduction

The existence of calcium in sweat was first reported in 1931 [1]. In later studies [2–7] efforts were made to quantify the dermal calcium loss by collecting sweat in arm bags or by washing the clothes of the subjects investigated. Most of these studies were performed during uncommon conditions (i.e. profuse sweating, environmental chambers) and for short periods. The aim of the present study was to describe a method for calculation of dermal calcium loss during physiological conditions, by using the data normally obtained in a combined calcium balance and $^{47}$Ca turnover study.

By this method the dermal calcium loss might be overestimated because of redistribution of the tracer, leading to a reduction in whole-body count rates, or by insufficient collection of urine and faeces. The influence of redistribution of the injected $^{47}$Ca on the estimated dermal calcium loss was evaluated in a phantom experiment, simulating $^{47}$Ca going into deeper parts of the body. Moreover, the effects of early and late redistribution, respectively, were investigated in a normal volunteer and by testing whether the estimated dermal calcium loss was time invariant in normal individuals. The effects of insufficient collection of urine and faeces were evaluated by simulating loss of activity from urine and faeces. Moreover, variation in the daily urine creatinine excretion was carefully analysed.

This work was presented in part at the Symposium on Osteoporosis, Jerusalem, in June 1981.

Materials and methods

Seventy-seven calcium balance and $^{47}$Ca turnover studies [8, 9] were performed in 57 individuals
(42 women and 15 men), 15 normal controls and 42 patients with different calcium metabolic disorders [bypass operation for obesity (n = 11), osteoporosis (n = 12), osteogenesis imperfecta (n = 5), medullary carcinoma of the thyroid (n = 4), primary hyperparathyroidism (n = 8) and hyperthyroidism (n = 2)]. Twenty patients were studied twice. The patients were admitted to a metabolic department, whereas the healthy controls were studied ambulatory. Environmental temperature was cool and temperate. The afternoon temperature outdoors was between -5°C and +20°C and never exceeded 25°C (i.e., profuse sweating never took place).

The balance procedure used has been described in detail elsewhere [9]. It was a 16 day study with 51CrCl3 used as a non-absorbable faecal marker given from 2 days before 47Ca administration. Patients were equilibrated on their 'home diet' with a nearly constant daily content of calcium a week before and during the investigation. On day 8, 20 µCi (0.74 MBq) of 47CaCl2 was given intravenously, after which daily urinary and faecal collections were made for 7 days (9–10 days for faeces). Carmine red was given as a visible marker at the day of 47Ca administration and 7 days later. This procedure secured an individual prolongation of the 51Cr equilibrium period [10] beyond the initial 2 days. The time interval between the appearance of the first and the second carmine red marker in the faeces (mean 6.9 ± SD 0.8 days) was not significantly (P > 0.20) different from the marker interval of 7 days, indicating a constant intestinal transit time during the study. Blood samples were withdrawn after 5, 10, 15, 20, 30, 45, 60, 120, 180 and 360 min and after 1, 2, 3, 4, 5, 6 and 7 days. The 47Ca activity was determined in samples of serum and urine with a scintillation well counter. The 47Ca and 51Cr activity in homogenized faecal samples (< 3.0 g) was determined in a scintillation well counter with the 51Cr count corrected for crossover from 47Ca. The mean recovery of 51Cr and 47Ca in faeces was 100.5 ± 1.3% (n = 10) and 102.9 ± 3.0% (n = 10) respectively. Serum, urine and faecal samples were counted for 47Ca in 30 min or to 10 000 counts. Faecal samples were counted for 51Cr to 10 000 counts. Total se-calcium was determined by atomic absorption spectrophotometry. In addition determination of whole-body 47Ca retention was performed twice a day; in the first 35 studies with a large field of view gamma camera (Ohio Nuclear Sigma 410) coupled to a whole-body scanning facility, later with a medium sensitivity four-crystal stretcher geometry whole-body counter (an array of four scintillation counters with a linear counting geometry) [11]. All measurements were doubled, two from the front and two from the back. Three patients were studied on the whole-body counter as well as with the gamma camera during a full 7 day course of the study, with no differences between the measured or the calculated variables. The 47Ca kinetic data were analysed according to a further development of the continuously expanding exchangeable calcium pool model proposed by Burkinshaw et al. [8] with individual correction for faecal lag time [9].

From the experimental data the following were calculated: retention curve [R1(t)], based on directly measured whole-body retention of 47Ca; retention curve [R2(t)], constructed from urinary and faecal excretions of 47Ca, and serum specific 47Ca activity curve [S*(t)]. The two retention curves differed from each other, R2(t) always being on a higher level than R1(t) with a constantly increasing difference between the curves during the investigation period (Fig. 1). The dermal loss of activity was estimated from the cumulative difference between the curves, and the dermal calcium loss (d) was estimated by a computer from the equation:

\[ d = \frac{R_2(t) - R_1(t)}{\int_0^t S^*(t) \, dt} \]

The reproducibility of the results was evaluated by double determination of all measurable parameters in 13 normal individuals after a single dose of 47Ca. The method error (S) was calculated as:

\[ S^2 = \frac{1}{2n} \sum_{i=1}^{n} d_i^2 \]

where \( n \) is the number of individuals and \( d_i \) the difference between the two calculated dermal losses of calcium in individual i. The coefficient of variation (CV%) was calculated as CV% = 100S/\( \bar{x} \), where \( \bar{x} \) is the mean value.

47Ca going into deeper parts of the body was simulated in a phantom (Remcal, Alderson Research Laboratories Inc., Stanford, CT, U.S.A.). A small core (2 cm x 2 cm x 15 cm) placed centrally in the full size torso of the phantom should illustrate the deeper parts of the body. In four experiments 10, 20, 30 and 40% of the total 47Ca activity respectively was placed in the central core and the rest outside in the body. The 'disappearance factor' (\( \lambda \)) describing the loss of count rates due to altered distribution of 47Ca activity was determined as:

\[ \lambda(x) = \frac{A_{WB}(0) - A_{WB}(x)}{A_{WB}(0)} \]
where $A_{WB(0)}$ is the whole-body $^{47}$Ca activity with all activity outside the central core and $A_{WB(x)}$ the whole-body activity with a certain fraction $(x)$ of the total activity in the central core and the rest outside. All measurements were performed twice with the phantom placed on the whole-body counter, one measurement from the front and one from the back. The mean of these two measurements was used. To evaluate how much the disappearance factor ($\lambda$) found in the phantom experiment might influence the estimated dermal calcium loss, 16 investigations of normals were used. The 15 whole-body count rates [$T(n)$] measured during the 7 days' study in each person were modified [$T_{\text{mod.}}(n)$] according to the equation:

$$T_{\text{mod.}}(n) = 1 + \frac{\lambda(x)n}{15} T(n)$$

$(n = 1, 2, 3, \ldots, 15)$

$T(n)$ is the count rate on the whole-body counter in point number $n$. The 15 modified count rates were used to recalculate the dermal calcium loss for a given $\lambda(x)$.

The effect of early redistribution was evaluated in vivo by placing a normal volunteer on the whole-body counter from hour 1 to hour 7 after tracer administration, the count rate being measured hourly. The effect of late redistribution on the dermal calcium loss was evaluated by calculating $d$ after 5.3, 6.3 and 7 days.

The efficiency of urine collection was assessed by measurement of daily urine excretion of creatinine in the last 71 studies. To evaluate the amount of urine and faeces that should be lost to account for, or to double, the calculated dermal calcium loss, a computer simulation was performed on the data from a normal individual (retention curves shown in Fig. 1). The daily dermal calcium loss was calculated after simulation of 5, 10 and 20% loss or increase in the daily amount of faeces and/or urine.

Results

Fig. 2 shows the dermal calcium loss in the different groups of patients and in the controls. The median dermal calcium loss (normalized to standard body surface area ($1.73 \text{ m}^2$) [12] in all 77 studies was 1.50 mmol of Ca/24 h (range 0.12-4.60). The eight patients with primary hyperparathyroidism had a significantly greater dermal loss of calcium (median 2.64 mmol) than the 15 normal controls (median 1.38 mmol) ($P < 0.02$, Mann-Whitney $U$-test). The other patient groups did not differ from the group of normals.

A weak but significant correlation was found between the dermal calcium loss and the total se-calcium in all the subjects investigated ($r_s = 0.29$, $P < 0.01$, Spearman's rank correlation). Excluding the patients with hyperparathyroidism and thyrotoxicosis the correlation was not significant ($r_s = 0.21$, $0.10 > P > 0.05$). For the eight patients with hyperparathyroidism a stronger but less significant correlation was found ($r_s = 0.49$, $P > 0.10$).

The method error for dermal calcium loss in 13 normal volunteers was 0.275 mmol/24 h at a mean value of 1.58 mmol/24 h (coefficient of variation = 17.4%).

Table 1 gives the distribution of the $^{47}$Ca activity in the phantom experiment and the corresponding disappearance factors.

Fig. 3 illustrates the fractional diminution in dermal calcium loss in 16 investigations of normals as a function of the fraction $(x)$ of the total $^{47}$Ca activity suggested to be in a central compartment from the disappearance factors found in the phantom experiment. It is shown that a suggested small compartment containing 20-40% of the $^{47}$Ca activity might explain 10-35% of the estimated dermal calcium loss.
In Table 2 is shown the result of the experiment with the normal volunteer lying on the whole-body counter for 6 h (from hour 1 to hour 7 after tracer administration). No significant 'disappearance' of $^{47}$Ca activity was found.

Table 3 shows the calculated dermal calcium loss after 5.3, 6.3 and 7 days in the 16 controls. No significant difference was found between the values.

Fig. 4 illustrates the efficiency of urine collections. For each person the seven daily urine excretions of creatinine are expressed as percentages of the individual mean value. Excluding the three obvious incomplete collections, the distribution curve is leptokurtic ($\beta_2 = 3.86$) almost symmetrical with a very slight skewness to the right ($\beta_1 = 0.02$) [13]). In the results the amount of dermal calcium loss has been corrected for three obvious incomplete collections. The intra-individual coefficient of variation for all 71 studies was $9.2 \pm 0.4\%$ (mean $\pm$ SEM).

In Fig. 5 is shown a computer simulation on the data from a normal individual. A daily loss of 20% of both faeces and urine was needed to explain the observed difference between the retention curves, with a calculated daily dermal loss of 1.85 mmol of calcium.

A weak but significant correlation was found between the dermal calcium loss and the mineralization rate in all the subjects investigated ($r_s = 0.40$, $P < 0.001$). Excluding the patients with hyperparathyroidism and thyrotoxicosis a weaker but still significant correlation was found ($r_s = 0.26$, $P < 0.05$).

Moreover, a weak but significant correlation was found between the dermal calcium loss and

Fig. 2. Dermal calcium loss in patients with different calcium metabolic disorders and in healthy volunteers. The data from the full 7 day period were used to calculate the dermal calcium loss. The median value for each group is shown. Median value for all 77 investigations was 1.50 mmol of calcium 24 h$^{-1}$ 1.73 m$^{-2}$. 
TABLE 1. Measured whole-body $^{47}$Ca activity ($A_{WB(x)}$) in a phantom during four experiments with various fractions of the total $^{47}$Ca activity in the central core

In each experiment $A_{WB(x)}$ is compared with $A_{WB(0)}$, where all $^{47}$Ca activity is outside the central core. The disappearance factor indicates fractional 'loss' of count rates caused by altered distribution of $^{47}$Ca.

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Fraction of activity in central core</th>
<th>Measured whole-body $^{47}$Ca activity* (c.p.m.)</th>
<th>Disappearance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$x$</td>
<td>0.10</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.10</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.20</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.30</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.40</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Count rate measured for 5.5 min and corrected for physical decay.

TABLE 2. Whole-body $^{47}$Ca activity ($A_{WB}$) and serum specific $^{47}$Ca activity ($S^*$) in a normal subject 1-6 h after intravenous injection of $^{47}$Ca

No renal or faecal loss of $^{47}$Ca activity took place during the experimental period.

<table>
<thead>
<tr>
<th>Time after administration of tracer (min)</th>
<th>67</th>
<th>121</th>
<th>174</th>
<th>228</th>
<th>297</th>
<th>352</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{WB}$ (fraction of initial count rate)</td>
<td>1.00</td>
<td>1.01</td>
<td>1.02</td>
<td>1.02</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>$S^*$ (fraction of initial value)</td>
<td>0.456</td>
<td>0.370</td>
<td>0.339</td>
<td>0.317</td>
<td>0.284</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Dermal calcium loss ($d$) estimated as a function of time ($t$) in 16 investigations of normal subjects

<table>
<thead>
<tr>
<th>$t$ (days)</th>
<th>$n$</th>
<th>$d$ (mmol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>5.3</td>
<td>15</td>
<td>1.63</td>
</tr>
<tr>
<td>6.3</td>
<td>16</td>
<td>1.63</td>
</tr>
<tr>
<td>7.0</td>
<td>16</td>
<td>1.64</td>
</tr>
</tbody>
</table>

the estimated body surface area ($r_b = 0.34$, $P < 0.01$) in all the individuals excluding patients with hyperparathyroidism and thyrotoxicosis.

Discussion

The accuracy of the present method is unknown. However, the result obtained is not incompatible with those of other investigators who have found lower [2, 5], higher [3, 7] or identical mean values [4] with different techniques. The range for normal adults during physiological conditions varied in these investigations from undetectable to 9.13 mmol of calcium/24 h. Except for the

FIG. 3. Fractional diminution of dermal calcium loss in 16 investigations of normals as a function of the fraction ($x$) of the total $^{47}$Ca activity suggested to be in a central compartment from the disappearance factor found in the phantom experiment. Means ± SEM are shown. The curve was obtained by least squares fitting. For calculation procedure see the text.
FIG. 4. Distribution curve for 497 24 h urine creatinine excretions in 71 studies. The seven 24 h urine excretions of creatinine obtained for each individual are expressed as percentages of the individual mean value.

FIG. 5. Computer simulation showing the calculated changes in initially estimated daily dermal calcium loss (1.85 mmol of Ca/day) in a normal individual after simulation of 5, 10 or 20% loss or increase in the daily excretion of urine (○), faeces (△) and urine and faeces (●); ■, measured dermal calcium loss.

Present method, dermal calcium estimation has always in some form or other included collection of sweat for chemical analysis. This collection procedure may change the normal production of sweat and thereby introduce an error of the estimate. Moreover, the collection has often been performed from certain parts of the body, and disagreements exist whether or not the sweat from one part of the body is representative for the whole-body sweat in its chemical composition [13, 14]. In the present study the dermal calcium loss was calculated for the whole body, during physiological conditions and without any change in sweat production, apart from the influence of admission to a metabolic department with a perhaps slightly reduced physical activity. Moreover the individual value was calculated as the average daily loss in a 7 day period, whereas most of the previous investigators for practical reasons collected sweat in shorter periods. Isaksson et al. [7] estimated the dermal calcium loss indirectly from the dermal loss of potassium and the Ca/K ratio in sweat samples. The dermal potassium loss was determined from an extended conventional potassium balance combined with an isotope dilution technique. In this study the average dermal calcium loss in 13 patients was 3.0 mmol of calcium/24 h.
The dermal calcium loss was calculated from the difference between the two retention curves \( R_1(t) \), directly measured on the whole-body counter, and \( R_2(t) \), calculated from urinary and faecal \( {^{47}}\text{Ca} \) excretion. The difference between these two retention curves may be caused by (1) redistribution phenomena, which change \( R_1(t) \), (2) insufficient collections of urine and faeces, which changes \( R_2(t) \) and (3) dermal calcium loss.

Redistribution was simulated in a phantom in order to evaluate whether it in fact could influence the measured count rates. The experiment showed a reduction of 1–2% of the total count rates, assuming that 20–40% of the total activity, after redistribution, was concentrated in a relatively small central compartment. During redistribution \( {^{47}}\text{Ca} \) is concentrated in the skeleton, which partly is located in the central part of the body (lumbar spine and long bones); however, much of the skeleton is superficially located (the skull and the thoracic and pelvic bones).

It is uncertain what the actual redistribution of \( {^{47}}\text{Ca} \) activity is \textit{in vivo}, but we feel that a distribution with as much as 40% of the total activity in the central part of the body must illustrate the 'worst case'. Accepting this theoretical worst case distribution the directly calculated dermal calcium loss should be reduced by 35%.

The early redistribution (i.e. the first hours) accounts for \( {^{47}}\text{Ca} \) going from the vascular phase to the extracellular space of the body. Here the spatial distribution is expected to be unchanged, in accordance with the finding that a normal person placed on the whole-body counter for 6 h showed no fall in count rates, corrected for physical decay.

The late redistribution due to \( {^{47}}\text{Ca} \) going into bones was assessed by using the mathematical model to calculate the dermal calcium loss after 5.3, 6.3 and 7 days. The dermal calcium loss was time-invariant, showing that the late redistribution did not influence the calculated dermal calcium loss \textit{in vivo}.

The experiments \textit{in vivo} disclosed no effects of redistribution on dermal calcium loss, neither in the first 6 h, where redistribution is expected to be greatest, nor between days 5.3 and 7. The interval between these periods was, however, not investigated and redistribution therefore may still explain some of the difference between the two retention curves. Based on the results \textit{in vivo}, we think, however, that the reduction in dermal calcium loss due to redistribution is much less than the 35% found in the 'worst case' phantom experiment.

It cannot be excluded that a small amount of activity could be lost during collection of urine and faeces. These collections were, however, strictly controlled and according to precise instructions. An analysis of the daily urine creatinine excretion only showed three collections (out of 497) with obvious losses of urine. Correction for these losses has been made in the results. The almost symmetrical distribution of the curve for daily creatinine excretion (excluding the three obvious insufficient collections) with a very slight skewness to the right excludes that frequent minor losses of urine took place. Compared with loss of urine, faecal losses had only to a minor degree an influence on the calculated dermal calcium loss. As shown in the computer simulation, a daily loss of 20% of faeces resulted in only a 25% increase in the dermal calcium loss. The simulation also showed that as much as 20% of both urine and faeces should be lost every day to explain all the difference between the two retention curves. Moreover the steadily increasing difference between the two retention curves in each individual demonstrated that the 'unexplained' loss of activity was a continuous process. It was not intermittent, as should be expected if it was caused by single major insufficient collections.

In conclusion redistribution and insufficient collection of urine and faeces might explain some of the difference between the two retention curves, and thereby result in an overestimation of the dermal calcium loss. We find, however, that an appreciable amount of calcium is lost through the skin every day. An estimate of calcium balance which does not take this into account will involve a systematic error in a positive direction.

The significant correlation found between the dermal calcium loss and serum calcium and the enhanced dermal calcium loss in the hyperparathyroid group, could suggest some relationship between the dermal calcium loss and the serum concentration of calcium. Furthermore, the weak correlation found between the dermal calcium loss and the body surface area in all the individuals with normal serum calcium levels, and without known increased bone turnover, could suggest that the magnitude of the dermal calcium loss in part depends on the body surface area.

The estimated dermal calcium loss could theoretically be influenced by an enhanced redistribution effect in patients with an enhanced bone turnover. This theory was supported by the significant positive correlation found between the dermal calcium loss and the mineralization rate among all the individuals investigated.

The described calculation of dermal calcium loss can easily be performed as a part of a combined balance and turnover study, if the retention curves are calculated both from whole-body
counting measurements and from urinary and faecal excretion data. Determination of mineralization rates from serum specific radioactivity curves and whole-body retention curves constructed from urinary and faecal excretion data alone [15], will involve an overestimation, as the activity lost in the sweat will be included in the estimated activity lost from the serum pool by mineralization and other irreversible losses. Mineralization rates should therefore be estimated from directly measured whole-body retention curves.

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