Intestinal Strontium Absorption: From Bioavailability to Validation of a Simple Test Representative for Intestinal Calcium Absorption

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Calcium absorption tests have rarely been validated for being representative for absolute bioavailability (true absorption) or for intraindividual variation. Therefore, we investigated the reproducibility of the absolute bioavailability of strontium chloride, a marker for intestinal calcium absorption, in healthy male volunteers (n = 8) by measuring the area under the plasma strontium concentration–time curve after oral and intravenous administration of strontium. Subsequently, we selected a simple test variable as being representative of absolute bioavailability. The mean absolute bioavailability (±SD) was 25% ± 7%. The best test variable appeared to be the fractional absorption at 240 min (FC240) after oral intake, which demonstrated the highest correlation with absolute bioavailability (r = 0.66). The intraindividual variations of the data for this variable and for the absolute bioavailability are similar to those described for various absorption tests based on the use of calcium isotopes. Thus, the FC240 of strontium offers the potential of a simple clinical test for use as a measure of intestinal calcium absorption and its modulation.

Indexing Terms: pharmacokinetics/metabolism

The importance of information about intestinal calcium absorption in studying the pathophysiology of metabolic bone diseases, e.g., osteoporosis, is evident. However, measurement of intestinal calcium absorption is complicated because the high endogenous concentrations of calcium in plasma, urine, and feces do not allow us to distinguish the transient changes in calcium concentration caused by absorption from background values. Conventional absorption tests are therefore generally based on radioactive and nonradioactive isotopes of calcium. However, neither type of isotope is suitable for application in general clinical practice. Stable strontium, however, is a good alternative marker, being nontoxic, inexpensive, and widely available; moreover, it can be measured with generally applied techniques. Early studies demonstrated that calcium and strontium not only share chemical and physical characteristics, but also are similarly involved in biological processes (1), e.g., intestinal absorption (2–4). Unfortunately, reliable data on the absolute bioavailability (true absorption) are not available, because the data concerning the metabolism of this element are mainly presented as strontium/calcium ratios (I), which masks the individual information from each element.

In developing an absorption test based on stable strontium as a marker, more detailed information about its absolute bioavailability and its intraindividual variation is needed (5). Only with that information can one select a pharmacokinetic variable that, on the one hand, accurately represents intestinal absorption and, on the other hand, allows the design of a simple test. Moreover, for such a test to be applicable in intervention studies, the test variable must be sensitive enough to detect relevant changes in intestinal absorption, i.e., must have good intraindividual reproducibility. Some simple clinical tests based on strontium as a marker have already been introduced (4, 6). Unfortunately, the test variables were not validated for their correlation with absolute bioavailability. Therefore, the aim of our study was to select the pharmacokinetic absorption variable that best represents the absolute bioavailability (true absorption) and also has a low intraindividual variation. Accordingly, we determined the absolute bioavailability of strontium chloride in eight healthy male volunteers.

Materials and Methods
Subjects and Study Design

Eight healthy men [mean age 26.2 years, range 21–31 years; mean Quetelet index (body wt., kg/length2) 22.2, range 19.6–24.7] volunteered for this study, which was approved by the ethics committee of the Free University Hospital in Amsterdam. All procedures followed were in accordance with the Helsinki Declaration. The subjects were in good physical condition, and no subject took any medication during or at least 1 week before this study.

Each subject received an oral dose of strontium chloride at days 0, 14, and 28 (tests 1, 2, and 3, respectively) and an intravenous injection at day 42 (test 4). Thus, the absolute bioavailability of strontium chloride was assessed three times per individual. At the start of each test, blood was sampled for serum calcium, albumin, alkaline phosphatase, γ-glutamyltransferase (GGT), creatinine, parathyroid hormone (PTH), 25(OH) vitamin D, and 1,25(OH)2 vitamin D.

In tests 1–3 the subjects received, after overnight fasting, 200 mL of test solution containing 2.5 mmol of SrCl2 · 6H2O (Merck, Amsterdam, The Netherlands).

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and 2.5 mmol of CaCl₂·2H₂O (Baker, Deventer, The Netherlands). Blood samples (2 mL) were then withdrawn via an indwelling catheter at 0, 7, 15, 30, 60, and 90 min; 2, 3, 4, 5, 8, 24, and 32 h; and 2, 5, 7, 12, and 14 days. The samples were collected into heparinized tubes and centrifuged at 1500g for 10 min. Plasma was separated and stored at -20 °C until analysis. In test 4, 4 mL of solution containing 1.2 mmol of SrCl₂·6H₂O was injected intravenously in 2 min. The test conditions and frequency of blood sampling were the same as in tests 1–3. Moreover, extra blood samples were withdrawn at 2, 5, 20, and 45 min. Both the test solution and the injection fluid were prepared by the pharmacy of the Free University Hospital. Subjects were allowed to drink and eat ad libitum, beginning 2 h after intake of the test solution.

Analytical Methods

Serum calcium, albumin, alkaline phosphatase, GGT, and creatinine were determined by commercially available methods (Boehringer Mannheim, Almere, The Netherlands). The vitamin D metabolites were separated by HPLC and quantified by a competitive protein-binding assay (7). PTH was analyzed by an IRMA (Medgenix, Brussels, Belgium). All plasma samples were analyzed for strontium by graphite furnace atomic absorption spectrophotometry (8).

Data Analysis

In each subject the absolute bioavailability of orally administered strontium chloride was calculated three times. The absolute bioavailability was calculated from the ratio of the area under the plasma concentration–time curve (AUC₀⁻→ₘ) after an oral dose (oral) and the AUC₀⁻→ₘ of the intravenous dose (i.v.), with both areas normalized for dose as follows:

\[
\text{bioavailability} = \frac{\text{AUC}_\text{oral}}{\text{AUC}_\text{i.v.}} \times \frac{\text{dose}_\text{i.v.}}{\text{dose}_\text{oral}}
\]  

(1)

AUC₀⁻→ₘ was calculated on the basis of the trapezoidal rule by use of the computer program Topfit 2.0 (9).

Absolute bioavailability assessed in this way can be considered the “gold standard” for determining true intestinal absorption, because the calculated ratio from which the absolute bioavailability is determined corrects for all routes of distribution and excretion. The duration of the sampling time of each test was based on 5 T₁/₂ (elimination half-life) (10); i.e., 95% of AUC₀⁻→ₘ is described by the data. Various pharmacokinetic variables were determined for tests 1–3 to select one representative of intestinal absorption. First, we calculated values for the variables described in the literature—i.e., the fractional absorption at 60 min (Fc₆₀) (11) and at 240 min (Fc₂₄₀) (4):

\[
\text{Fc}_t = \frac{[C_t - C_0] \times V_d}{D}
\]  

(2)

where Cₜ = plasma concentration at t minutes, C₀ = plasma concentration at 0 min, Vₜ = volume of distribution represented by 15% of the body weight (BW), and D = dose (2.5 mmol of strontium chloride). Because we expected improvement in precision when using a variable based on more than one concentration, we assessed AUC₀⁻→₆₀ and AUC₀⁻→₂₄₀ (AUC over the interval 0–60 and 0–240 min, respectively). Analogous to Fc, these two latter markers were also corrected for Vₜ (15% of BW) and dose (D), resulting in the quantities AUC₀⁻→₆₀ · 0.15 · BW/D and AUC₀⁻→₂₄₀ · 0.15 · BW/D. Moreover, Fc₅₀, Fc₁₂₀, and Fc₁₈₀ were calculated as potential variables. After testing each one for normal (gaussian) distribution, we assessed the correlation with absolute bioavailability by means of linear regression analysis. Intraindividual variation was calculated by means of one-way ANOVA and expressed as the CV (in %). An indication of reference values for absolute bioavailability and its most representative variable was obtained from the 95% confidence limits based on 24 observations and 7 degrees of freedom.

Results

The biochemical analytes concerning calcium and bone metabolism were in all individuals within the normal reference ranges (Table 1). As an example, Fig. 1 demonstrates three plasma concentration–time curves of strontium in one individual after oral administration of 2.5 mmol of strontium chloride. The shapes of these curves were almost identical. For each individual the concentrations measured after administration of strontium were adjusted for the endogenous strontium concentration at t = 0 min of test 1 (0.15 ± 0.05 μmol/L). Because the plasma concentration of strontium did not return to the value for endogenous strontium at the start of each test, the plasma concentrations of tests 2–4 were corrected for the resting values of the preceding tests. In this way a maximum plasma concentration (Cₘₚₖ) of 25.7 ± 6.7 mmol/L was obtained, with a corresponding tₘₚₖ of 174 ± 44 min. Within one individual, Cₘₚₖ and tₘₚₖ varied by as much as 25% and 26%, respectively. The mean absolute bioavailability of strontium chloride was 25% ± 7% (±SD), with 95% confidence limits between 20% and 32%. The values obtained for each test are given in

| Table 1. Biochemical variables involved in calcium and bone metabolism of eight healthy male volunteers. |
|-------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Serum                                           | Mean (SD)        |                  |
| Calcium, mmol/L                                 | 2.20 (0.10)      |                  |
| Albumin, mmol/L                                 | 43 (2)           |                  |
| Creatinine, μmol/L                              | 84 (10)          |                  |
| GGT, U/L                                        | 14 (4)           |                  |
| Alkaline phosphatase, U/L                       | 48 (12)          |                  |
| Plasma                                          |                  |                  |
| PTH, pmol/L                                     | 3.2 (0.5)        |                  |
| 25(OH)D, nmol/L                                 | 53 (19)          |                  |
| 1,25(OH)₂ surfaces, pmol/L                      | 115 (17)         |                  |

* Calcium concentration corrected for the serum albumin concentration.
Table 2 to illustrate the intraindividual differences for absolute bioavailability and Fc240.

The correlation (\( r \)) between the absorption variables and absolute bioavailability ranged from 0.45 and 0.66 (Table 3). Fc240 demonstrated the highest correlation (Fig. 2). Intraindividual variation varied from 20% (Fc90) to 29% (Fc190). We calculated this variation for \( n = 23 \) tests, omitting the results for test 2 for one individual because of poor compliance to the pretest conditions. Table 3 demonstrates that, in this case, the variation for several variables (e.g., absolute bioavailability, Fc240) was decreased by >5%. The reference range for Fc240, based on 24 observations and 7 degrees of freedom, was 0.074—0.118.

Discussion

The results of this study demonstrate that within one individual the absolute bioavailability, i.e., the true absorption of strontium, fluctuates considerably. Considering the close correlation between the pharmacokinetics of calcium and strontium (1), it is reasonable to expect a comparable variation for intestinal calcium absorption.

Knowledge about the absolute bioavailability of a substance is essential to the development of an absorption test for that substance, because the test variable or analyte should represent intestinal absorption. Assessment of absolute bioavailability by the ratio of the areas under the plasma concentration–time curves (extrapolated to infinity) after an oral and an intravenous dose is a generally accepted method in clinical pharmacology for determining the absorption of orally administered drugs (12). The plasma concentration–time curve after an oral load reflects the results of absorption, distribution, and excretion, whereas the curve after an intravenous dose represents only distribution and excretion. Thus, by comparing the area under these curves, we can determine true absorption, i.e., absolute bioavailability. In this study the mean value for the absolute bioavailability of strontium chloride was 25% ± 7%, which is in accordance with results reported earlier for studies in humans (13).

In the past the absolute bioavailability of calcium, and to a much smaller extent of strontium, has been studied in various ways [e.g., metabolic balance studies (14)]. However, the pharmacokinetic approach as described in this study has not been reported before. Nevertheless, a variety of variables for calcium as well as strontium [Fc90 (15), Fc120 (16), Fc240 (4), and Fc300 (17)] have been introduced over the years. One of the rare studies on the relationship between absolute bioavailability and the test analyte was carried out by Marshall and Nordin (15), who compared their single-tracer method (Fc90) with net calcium absorption as determined by balance measurements (15). For that study some reservations must be kept in mind, because net absorption does not account for endogenous secretion into the intestine (18). Because the plasma concentrations after oral and intravenous administration will be affected to almost the same extent by intestinal secretion, the absolute bioavailability of strontium, calculated from the ratio of the AUC values, will not be affected by this process.

In selecting a good test variable we first formulated some conditions regarding its nature. First, the variable should well represent the absolute bioavailability. Second, the intraindividual variation should be as low as possible. Third, the variable should allow the design of a simple clinical test.

To obtain a representative variable, we calculated fractional absorptions for \( t = 240 \) min; after this time, absorption would no longer be predominant, and the test would be too time-consuming. The correlation between Fc240 and absolute bioavailability appeared to

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**Table 2. Absolute bioavailability (\( f \)) and fractional absorption at 240 min (Fc240) in eight healthy adult men.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Fc240</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>13</td>
<td>33</td>
<td>0.132</td>
<td>0.057</td>
<td>0.173</td>
<td></td>
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<tr>
<td>2</td>
<td>41</td>
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<td>38</td>
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<td>0.085</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>28</td>
<td>24</td>
<td>0.085</td>
<td>0.096</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>31</td>
<td>20</td>
<td>0.101</td>
<td>0.139</td>
<td>0.085</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>23</td>
<td>25</td>
<td>0.087</td>
<td>0.085</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>28</td>
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</tr>
<tr>
<td>7</td>
<td>15</td>
<td>34</td>
<td>20</td>
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<td>0.127</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>22</td>
<td>19</td>
<td>0.080</td>
<td>0.089</td>
<td>0.079</td>
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</tr>
</tbody>
</table>

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increase with time (Table 3), with FC240 being the most representative variable. Improvement of the correlation was not achieved by calculating correlations for a marker on the basis of more than one concentration (AUC0–t), whether corrected for BW (AUC0–t ⋅ 0.15 ⋅ BW/D) or not. However, we note that our test population was homogenous with regard to body weight. In the case of underweight or overweight patients, such a correction might improve the correlation with absolute bioavailability. Nevertheless, this observation has important consequences for the test procedure, by indicating that blood samples may be collected by venipuncture instead of by an indwelling cannula. This will make the test more practical for wider application. The results of an earlier study of 63 patients with various disorders of the bone and calcium metabolism demonstrated that the FC240 of strontium correlated very well with the FC240 of calcium \((r = 0.66)\) \((19)\). Thus, FC240 not only is the most representative variable for intestinal absorption of strontium but also is easy to assess and corresponds well with calcium absorption.

The second condition for a good test variable, a low intraindividual variation, is of special importance in intervention studies. However, for neither strontium nor calcium has much research been done on this topic. Moreover, the data available are difficult to interpret because of poorly described study designs and differences in expression of the variation (e.g., variance, SD) \((20–22)\). In our study the intraindividual variation ranged from 4% to 46% for absolute bioavailability and from 5% to 48% for FC240. The variation for FC240 is comparable with variance values of FC240 reported earlier \((4, 23–27)\). The relatively high intraindividual variation is highly unlikely to be attributable to the use of strontium instead of calcium isotopes. In the literature on calcium isotope tests, this variation is often misleadingly interpreted as low (10% or less) because of insufficient or incorrect statistical analysis of the data. For example, recalculating the data of deGrazia et al. \((28)\), who performed two balance studies in seven patients, we obtained an intraindividual variation of 24% (by one-way ANOVA) instead of the 8.4% reported. In 1988 Heaney et al. \((20)\) reported a mean variation of 12% for FC300 of calcium in a comparable group of volunteers \((n = 6, three tests per person)\). Various reasons may explain this much lower variation. First, as shown in Fig. 1, plasma concentrations vary less at later times on the plasma C–t curve, resulting in a lower variation for FC300 than for FC240. However, at \(t = 300\) min, distribution and excretion dominate the decreasing contribution of intestinal absorption; thus FC300 values are less representative of intestinal absorption than the earlier values. Second, the modality of the oral calcium administration, i.e., with a test meal, may have a modulating effect on the transit time through the stomach and the intestine. We preferred to standardize our test on the fasting state, because many of the breakfasts described in the literature contain various factors that influence intestinal calcium and strontium absorption; e.g., marmalade and fruit juice may contain considerable amounts of citrate, which enhance intestinal absorption of calcium \((29)\). The fact that the citrate content of a test meal is subject to (seasonal) variation may also be a source of variability of the test variable in the long term. No uniformity in the composition of the meals applied in various tests is reported in the literature; each test seems to use a meal containing different stimulating and inhibiting factors, which makes it very difficult to compare the reference values for the various tests. On the other hand, one could also argue that the function of the pylorus is regulated by a meal, and that the fasting state introduces an artifact of either immediate passage into the duodenum or pylorospasmus in some individuals. Third, as demonstrated in Table 3, one outlier value has a strong effect on the calculation of intraindividual variation in a small group.

The intraindividual variation was only slightly improved by calculating a variable based on more than

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### Table 3. Characteristics of measures of pharmacokinetic absorption of strontium.

<table>
<thead>
<tr>
<th>Correlation with f (t)</th>
<th>Intraindividual CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 24)</td>
</tr>
<tr>
<td>f</td>
<td></td>
</tr>
<tr>
<td>FC240</td>
<td>0.45</td>
</tr>
<tr>
<td>FC90</td>
<td>0.35</td>
</tr>
<tr>
<td>FC120</td>
<td>0.49</td>
</tr>
<tr>
<td>FC180</td>
<td>0.60</td>
</tr>
<tr>
<td>FC240</td>
<td>0.66</td>
</tr>
<tr>
<td>AUC0–40</td>
<td>0.34</td>
</tr>
<tr>
<td>AUC0–240</td>
<td>0.61</td>
</tr>
<tr>
<td>AUC0–240 ⋅ 0.15BW/D</td>
<td>0.39</td>
</tr>
<tr>
<td>AUC0–240 ⋅ 0.15BW/D</td>
<td>0.63</td>
</tr>
</tbody>
</table>

\(f\), absolute bioavailability; FC\(_t\) = fractional absorption at \(t\) minutes after oral intake of strontium chloride; AUC\(_{0–t}\) = area under the plasma concentration–time curve over the interval 0 – \(t\) minutes (in mmol/L); BW = body weight; D = dose (2.5 mmol of SrCl\(_2\) ⋅ 6H\(_2\)O).

\(^\ast\) Results computed after omitting results of one test for one subject.

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![Fig. 2. Three plasma C–t curves of one individual after oral administration of 2.5 mmol SrCl\(_2\).](image-url)
one concentration (AUC\textsuperscript{0-240}). The remaining variation indicates that, for the test conditions described here, the test is more suitable for discriminating alterations in the level of absorption (i.e., low, intermediate, and high) within one subject than for discriminating small, clinically less relevant, changes.

Overall, we considered FC\textsubscript{240} as the best test variable. Although AUC\textsuperscript{0-240} has a lower intraindividual variation than does FC\textsubscript{240}, the latter is preferred because of its markedly better correlation with absolute bioavailability. Moreover, the test procedure for FC\textsubscript{240} causes less discomfort to the patient than does the test procedure for AUC\textsuperscript{0-240}. Although the application of FC\textsubscript{240} as a test variable has been described before (\textsection 4), we emphasize that the results we obtained are only valid for the test conditions described in this article. If the fasting state is replaced by a test meal, which is common in most absorption tests, the optimum variable probably will be found at another time point on the plasma C-t curve because of altered pharmacokinetics. Also, the amount of strontium and the strontium/calcium ratio administered may affect the absolute values of FC\textsubscript{240}; given that earlier ex vivo experiments (2, 30) have demonstrated that strontium may use the same absorption mechanisms as calcium.

On the basis of the results of this study we advise the following test procedure: overnight fasting, blood sample (C\textsubscript{0}), oral intake of 200 mL of test solution (t\textsubscript{0}), and a blood sample at t = 240 min (C\textsubscript{240}).

In conclusion, the results of this study make clear that FC\textsubscript{240}, measured by only two plasma concentrations, provides reliable information about the absolute bioavailability of strontium and thus indirectly also of calcium. Although the applicability of this variable in patients still needs further investigation, it clearly has the potential to be a simple test that gives information about intestinal calcium absorption and its modulation.

We thank the volunteers for their enthusiastic participation in this study, Geert Jan Blok and Piet van der Wal for their clinical assistance, and the Department of Pharmacology for preparation of the test solutions. We also thank Joris Briefles for the accurate analysis of the numerous samples.

References