Comparison of estimates of zinc absorption in humans by using 4 stable isotopic tracer methods and compartmental analysis 1–3

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ABSTRACT

Background: Adjustment of gastrointestinal absorption is the primary means of maintaining zinc homeostasis; however, a precise, accurate method for measuring zinc absorption in humans has not been identified.

Objective: The purpose of this study was to compare the estimates of the fraction of dietary zinc absorbed (FZA) by using 4 stable isotopic tracer methods: mass balance (MB), fecal monitoring (FM), deconvolution analysis (DA), and the double isotopic tracer ratio (DITR) method.

Design: All 4 methods were applied to a single data set for each of 6 women. FZA was also determined for each subject by using a detailed compartmental model of zinc metabolism, and that value was used as the reference with which the simpler methods were compared.

Results: The estimates of FZA (± SD) determined by DA (0.27 ± 0.08) and the DITR technique in plasma (0.30 ± 0.10), 24-h urine samples (0.29 ± 0.09), and spot urine samples (0.291 ± 0.089) all compared well with the FZA reference value from the compartmental model (0.30 ± 0.10). The MB and FM methods tended to overestimate FZA compared with the reference value.

Conclusions: The determination of FZA by MB or FM is laborious, is sensitive to subject compliance, and may result in an overestimate. DA, although relatively accurate, has the disadvantage of requiring multiple blood drawings over several days. In contrast, the DITR technique applied to a spot urine specimen obtained ≥3 d after tracer administration provides an accurate measure of FZA and is easy to implement; therefore, it is the recommended method for determination of FZA. Am J Clin Nutr 2000;71:523–9.

KEY WORDS Zinc, stable isotopes, zinc absorption, compartmental modeling, stable isotopic tracer methods, mass balance, fecal monitoring, deconvolution analysis, double isotopic tracer ratio, women

INTRODUCTION

Zinc homeostasis is regulated primarily by changes in zinc absorption and endogenous excretion (1). A reduction in zinc intake from 5.5 to 0.8 mg/d caused a 2-fold increase in the fraction of dietary zinc absorbed (FZA) (2). Also, FZA appears to increase when zinc status is poor (3, 4). Therefore, the ability to measure FZA is essential to studies of zinc metabolism and homeostasis.

Previous studies of zinc absorption were limited by the lack of a precise, accurate measure of intestinal zinc uptake. The mass balance (MB) technique, which measures the difference between dietary zinc intake and fecal output, was used extensively in the past but, because unabsorbed dietary zinc and zinc derived from endogenous sources are both included in the fecal output value, MB measures only net absorption. To measure the homeostatic response to changes in dietary zinc intake, a measure of true absorption, ie, the uptake and transfer of dietary zinc across the mucosal cells, is needed. With the availability of stable isotopic tracers, it became possible to quantify the amount of fecal zinc derived from endogenous sources (5–7). With this information, the MB technique can be corrected to calculate FZA. Alternatively, after an oral stable isotope dose, FZA can be determined from the difference between the amount of isotope administered orally and the excretion of unabsorbed isotope in the stool (5). This approach is called the fecal monitoring (FM) method. However, some of the absorbed tracer is resorbed into the gut and excreted into the feces during the collection period, thereby causing an underestimate of FZA. Methods for correcting the FM technique for the amount of absorbed and resorbed tracer excreted in the feces were suggested by English et al (8) and by Rauscher and Fairweather-Tait (9).

Two other techniques available for determination of FZA are deconvolution analysis (DA) and the double isotopic tracer ratio (DITR) method. Both methods require administration of 2 stable isotopic tracers of zinc, 1 oral and 1 intravenous. With the DA method, both zinc tracers are measured in the plasma over several days; samples are taken frequently during the first 6 h after tracer administration. The fraction of the oral dose absorbed is

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then calculated from the plasma tracer concentrations by decon-
volution (10–12). With the DITR method, FZA is estimated after
simultaneous oral and intravenous administration of 2 different
stable isotopic tracers of zinc. The plasma or urinary ratio of the
oral to the intravenously administered tracer after correction for
differences in dose provides an estimate of FZA (13).

The purpose of this study was to compare the estimates of
FZA by using the MB, FM, DA, and DITR methods. All esti-
mates were made simultaneously in 6 free-living women. The
reference value against which these estimates were compared
was calculated from a compartmental model of zinc metabolism.
This model was derived by using the isotope enrichment data for
plasma, urine, and feces (14). It was assumed that the model pro-
vided the most reliable estimate of FZA because more infor-
mation was used to derive this estimate of FZA than was used for
any of the other methods. Therefore, the overall aim of this study
was to identify the method or methods for measuring FZA that
agree best with the value derived from the detailed compartmen-
tal model of zinc metabolism in vivo.

SUBJECTS AND METHODS

Subjects

Six white women with no acute or chronic health problems
aged 30 ± 11 y (± SD) were recruited for the study [weight:
54.2 ± 8.9 kg; body mass index (in kg/m²): 20.7 ± 2.6]. The usual
dietary zinc intakes of each woman were assessed before the
study by using 3-d weighed-food diet records. Dietary zinc intake
was measured by using a computerized database (NUTRITION-
IST III; N-Squared Computing, Salem, OR), which we updated
by adding any missing information on the zinc content of foods.
The women reported consuming 8.3 ± 3.4 mg Zn/d. The experi-
mental design of this study was approved by the University of
California at Berkeley Committee for the Protection of Human
Subjects. All participants gave written, informed consent.

Experimental design

Details of the experimental design and preparation of stable
isotopic tracers for administration were described elsewhere
(14). In brief, subjects were free living and consumed a con-
stant diet providing 7 mg Zn/d for a 7-d equilibration period
before isotopic tracer administration. Energy was adjusted to
match each subject’s reported intake from the 3-d weighed-
food diet records completed before the start of the study. The
subjects were weighed on days 2, 5, and 7 of the equilibration
period; adjustments were made to the diet if necessary to
maintain a constant body weight. Baseline urine and fecal
samples were collected on day 6. A fecal marker [1 g polyeth-
ylene glycol (PEG) (Sigma Chemical Company, St Louis) in 3
mL distilled water] was administered orally 2 h after the
evening meal on day 7 to mark the beginning of the 6-d meta-
abolic balance period.

On the morning of day 8, the subjects arrived at the metabolic
unit having fasted since 2000 the previous evening. An indwelling
catheter was placed in an arm vein and a fasting blood sample
(8 mL) was drawn into a zinc-free plastic syringe (Monovette;
Sarstedt, Hayward, CA). Fifteen minutes after a breakfast meal
containing 1 mg Zn, each subject consumed 1.3 mg of the oral iso-
topic tracer highly enriched in 67Zn that had equilibrated in 213 g
orange juice overnight. Immediately thereafter, 0.4 mg of another
stable isotopic tracer highly enriched in 70Zn was infused intra-
venously over a period of 1 min into a vein in the arm without the
indwelling catheter. Blood samples (8 mL) were taken via the
catheter 5, 10, 15, 30, 45, 60, 75, 90, and 120 min and 3, 4, 5,
6, and 7 h postinfusion. The samples were placed on ice and the
plasma was separated by centrifugation (13600 × g for 3 min at
room temperature) within 1 h of collection.

Blood samples were taken daily for the next 7 d, during
which the constant diet was consumed, and 24-h urine samples
and total fecal samples were obtained. The urine samples were
collected in 2 portions; the first morning void (spot urine sam-
ple) was collected separately from the rest of the day’s output.
A mock 24-h urine sample was prepared by combining appro-
priate volumes of the spot urine sample and the rest of the out-
put from the previous day, such that the volumes of each com-
bined were in their respective proportion to the total 24-h
volume of urine collected. Urine samples were acidified with 1
mL concentrated HCl (Seastar; Chemicals, Inc, Seattle) per 125 mL
urine before storage.

A second dose of PEG (1 g) was taken 2 h after the evening
meal on day 13 to mark the end of the metabolic balance period.
The subjects consumed a self-selected diet for the remaining 5 d
and continued to collect all fecal output. All plasma, urine, and
fecal samples were stored at −20°C.

Sample preparation and analysis

Fecal samples were lyophilized to constant weight and
ground to homogeneity. The total zinc concentration of the
plasma, urine, and fecal samples was measured by atomic
absorption spectrometry (Thermo Jarrell Ash, Franklin, MA)
as described previously (14). Isotopic mass ratios based on a
reference isotope of 66Zn were measured by using inductively
coupled plasma mass spectroscopy and were converted first to
tracer-to-tracee ratios and finally to tracer mass (in mg)
according to equations 1–4, which were reported previously
(14). The latter were then expressed as a percentage of admin-
istered tracer dose/L plasma [assuming plasma volume esti-
mates for each subject as described previously (14)], percent-
age of dose per sample for urine, and cumulative percentage or
fraction of administered tracer dose for feces. A Sciex ELAN
500 ICP mass spectrometer (Perkin-Elmer, Norwalk, CT) was
equipped with a U-5000AT ultrasonic nebulizer (Cetac
Technologies Inc, Omaha) and a 212B autosampler (Gilson
Medical Electronics Inc, Middleton, WI). Mass bias drift was
corrected by using gallium as an isotope ratio internal
standard (15). Plasma and fecal samples were wet ashed in
concentrated nitric acid by microwave digestion (MDS 2000;
CEM Corporation, Matthews, NC) and purified zinc was sepa-
rated by ion exchange chromatography (14). Macrominerals
were removed from the urine samples by using a chelex resin
before separating purified zinc by ion-exchange chromatogra-
phy (14). Fecal PEG content was measured by using a modifi-
cation of the method of Allen et al (16).

Estimation of fraction of dietary zinc absorbed

Mass balance corrected for endogenous zinc excretion

FZA was estimated by using equation 1, where D is the total
dietary zinc intake, F is the cumulative fecal zinc output for the
6-d balance period, and S is the amount of zinc excreted endoge-
nously during that period.
Total dietary zinc intake for the 6-d period after the first PEG dose and up to the second PEG dose was calculated for each subject. Cumulative fecal zinc output during this period, defined by the appearance of the PEG doses in the stool, was measured. Endogenously excreted zinc was estimated by using the following published methods (4, 7).

Isotope dilution

According to the method of Jackson et al (6) modified for the use of the tracer-to-tracee ratio instead of enrichment, the quantity of total fecal zinc derived from gastrointestinal secretion (S) after an intravenous dose of a stable zinc isotopic tracer (highly enriched in $^{70}$Zn in the present study) can be determined by using equation 2:

$$S = F(fp)$$ (2)

where $f$ is the zinc tracer-to-tracee ratio in the pooled feces, and $p$ is the plasma zinc tracer-to-tracee ratio at the midpoint of the balance period. The MB estimate of FZA, corrected by using this method to determine endogenous fecal zinc excretion, will subsequently be referred to as MB-J. Values for endogenous fecal zinc excretion in mg/d ($J$) are determined by dividing $S$ by the number of days in the balance period.

Cumulative isotope excretion

A method for measuring the endogenous fecal excretion of calcium was described by Yerger (7). When this method was applied in the present study, endogenous fecal zinc excretion, $Y$, after intravenous administration of a stable isotopic tracer dose of zinc highly enriched in $^{70}$Zn ($^{70}$Zn$^{\text{tr}}$) can be determined by using the following equation:

$$Y = [(\text{total }^{70}\text{Zn}^\text{tr} \text{in feces})/\text{total Zn}^\text{tr} \text{in urine})]V_q$$ (3)

where $V_q$ is the rate of urinary zinc tracer excretion (in mg/d). The cumulative excretion of $^{70}$Zn$^{\text{tr}}$ in the urine and feces was measured for 6 d after isotopic tracer administration. Endogenous zinc excretion ($Y$) was measured by multiplying $Y$ by the length of the collection period (6 d). MB corrected by using this method to measure endogenous fecal zinc excretion will subsequently be referred to as MB-Y.

Fecal monitoring

The FM method for estimating zinc absorption requires the oral administration of a zinc tracer (5), in this case one highly enriched with $^{67}$Zn. The amount of tracer is measured in each stool during the 12-d fecal collection period, and cumulative fecal excretion of the tracer, $f_{\text{oral}}$, expressed as a fraction of the orally administered dose, is calculated. According to this method, FZA is given as

$$FZA = 1 - f_{\text{oral}}$$ (4)

and will be referred to subsequently as FM-N for FM with no correction for resorbed oral tracer.

English et al (8) developed a correction procedure for the resecretion of absorbed oral tracer that contributes to $f_{\text{oral}}$. The cumulative excretion tracer data expressed as a percentage of the administered dose were plotted against time and are shown in Figure 1. The rate of increase of fecal accumulation of tracer, defined as the slope between successive data points, rises rapidly at the beginning of fecal collection because of the passage of unabsorbed tracer directly into the feces. The rate then decreases to a slightly positive slope that is usually <1%/d. It is assumed that this final positive slope is due entirely to the resecretion of absorbed tracer back into the intestinal lumen and its subsequent excretion into the feces. To correct for this endogenous excretion, a line is fitted by linear regression to the data contributing to this slightly positive slope and extrapolated back to the y-axis (Figure 1). The percentage of unabsorbed oral tracer is estimated from the intercept of this line on the y-axis, $y(0)$, where FZA, as a percentage, is then given by

$$FZA = 100 - y(0)$$ (5)

Values for FZA based on equation 5 will subsequently be referred to as FM-E.

Another method for correcting for resecretion of absorbed oral tracer was developed by Rauscher and Fairweather-Tait (9). Apparent absorption (AA) was calculated as in equation 4 but corrected for resecretion of absorbed oral tracer by monitoring the fecal accumulation of a second, intravenously administered tracer at the same time that the oral tracer was measured. AA was converted to the true fractional absorption (TA) by using the fraction of the intravenously administered tracer accumulated in the feces, $f_{\text{oral}}$, such that

$$TA = AA + (f_{\text{oral}})(f_{\text{oral}})$$ (6)

where IV is intravenous. The product $f_{\text{oral}} \times f_{\text{oral}}$ is assumed to correct for resecretion of absorbed oral tracer collected in the feces as part of $f_{\text{oral}}$. Values for TA calculated by using equation 6 will be subsequently referred to as FM-R.

Deconvolution analysis

DA, used previously for estimating calcium absorption (10, 11), was also used to determine zinc absorption (12). With this method, the time course of intestinal uptake of an oral tracer dose can be determined from the tracer concentration responses in the plasma of oral and intravenously administered tracers. The experiment is carried out by administering different oral and intravenous tracers simultaneously so that plasma sampling
is done only once. Under these conditions, the function describing the tracer response in the plasma, $R(t)$ (percentage of dose/L), to the orally administered tracer is given by the convolution integral

$$R(t) = \int_0^t E(\theta) W(t - \theta) d\theta \quad (7)$$

where $E(t)$ is the function describing the rate of first-pass entry (point by point) of the orally administered tracer into the plasma compartment (percentage of dose/h) and $W(t)$ is the function describing the plasma response to an intravenously administered tracer (percentage of dose/L). Because FZA is given by the integral of $E(t)$ from zero to infinity and the integration of equation 7 from zero to infinity yields

$$\int_0^\infty R(t) dt = \int_0^\infty E(t) dt \int_0^\infty W(t) dt \quad (8)$$

FZA can be expressed as

$$\int_0^\infty E(t) dt = \left[ \int_0^\infty R(t) dt \right] / \left[ \int_0^\infty W(t) dt \right] \quad (9)$$

In the present study, FZA could be calculated from the ratio of the integrals from zero to time $t$, assuming that $t$ is great enough for the first-pass absorption process to be completed such that

$$FZA = \left[ \int_0^t E(t) dt \right] / \left[ \int_0^t W(t) dt \right] \quad (10)$$

This value for FZA can be approximated by the ratio of the integrals from zero to time $t$, assuming that $t$ is great enough for the first-pass absorption process to be completed such that

$$FZA \approx \left[ \int_0^t E(t) dt \right] / \left[ \int_0^t W(t) dt \right] \quad (11)$$

In our analysis, the time constant for the absorption process of zinc tracer obtained from our compartmental model averaged 4.7 h (14) and the ratio of plasma integrals for oral to intravenous tracers was performed over a time course of 7 d.

A representative plot of the plasma tracer concentrations expressed as a percentage of the dose per liter after the orally and intravenously administered isotopic tracer doses is shown in Figure 2. The lines through the data points, the functional representations of $^{67}\text{Zn}^u(t)$ and $^{70}\text{Zn}^u(t)$, were obtained by linear interpolation and integrated by using SAAM II (SAAM Institute, Seattle). Values for FZA were determined from the ratio of these integrals from time 0 to 7 d after tracer administration by using equation 11.

**Double isotopic tracer ratio method**

The DITR method, first proposed for measuring FZA by Friel et al (13), is a modification of the well-established double isotopic tracer method for measuring the true absorption of calcium (7, 11). Calcium absorption was estimated from the ratio of oral to intravenous calcium isotopes in a 24-h pooled urine sample after isotope administration. When Friel et al (13) applied the method to zinc, they estimated absorption from any urine sample collected after the slopes of both the oral and intravenous isotopic disappearance curves are declining in a proportional manner by using the following equation:

$$FZA = \left( \frac{\text{enrichment of the sample with the oral tracer/enrichment of the sample with the IV tracer}}{\text{IV dose in mg/oral dose in mg}} \right) \quad (12)$$

In the present study, equation 12 was evaluated by using the zinc tracer-to-tracee ratio instead of enrichment. Values for FZA were then estimated from the isotopic tracer ratios in plasma, 24-h urine samples, and spot urine samples averaged over days 3–7 after tracer administration.

**Reference value of fraction of dietary zinc absorbed**

Our compartmental model of zinc metabolism was developed by using the zinc tracer-to-tracee data in plasma, urine, and feces and total tracee data in urine and feces for the 6 women studied (14). The value of FZA derived from the model for each subject was used as the reference value with which the other tracer methods were compared. FZA was computed for each subject from the ratio of the rate constant describing the fractional transfer of zinc from the intestine to the plasma ($k_{1.5}$) to the sum of the rate constants describing the fractional transfer of zinc from the intestine to the plasma and colon ($k_{6.5}$). In terms of our compartmental model (Figure 1) (14), FZA is given as

$$FZA = k_{1.5}(k_{1.5} + k_{6.5}) \quad (13)$$

**Statistical analyses**

The agreement between each tracer method for measuring FZA and the reference value derived from the compartmental model was determined by using the technique of Bland and Altman (17). This method specifically allows for a pairwise comparison of a single variable (ie, FZA), determined by the 9 dif-

![Figure 2](https://example.com/image2.png)

**FIGURE 2.** Typical example of a plasma data set to which deconvolution analysis was applied. Lines through data (O, intravenous tracer; △, oral tracer) are functional representations fitted to data by linear interpolation. The fraction of dietary zinc absorbed is determined by integration of functions according to equation 11.
TABLE 1
Fraction of dietary zinc absorbed (FZA), estimated by mass balance corrected for endogenous zinc excretion1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dietary zinc intake</th>
<th>Fecal zinc output</th>
<th>Endogenous fecal zinc excretion</th>
<th>FZA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/d</td>
<td>mg/d</td>
<td>mg/d</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.15</td>
<td>7.36</td>
<td>2.30</td>
<td>0.292</td>
</tr>
<tr>
<td>2</td>
<td>7.41</td>
<td>6.12</td>
<td>2.22</td>
<td>0.474</td>
</tr>
<tr>
<td>3</td>
<td>7.02</td>
<td>5.83</td>
<td>2.88</td>
<td>0.580</td>
</tr>
<tr>
<td>4</td>
<td>7.14</td>
<td>5.14</td>
<td>2.33</td>
<td>0.606</td>
</tr>
<tr>
<td>5</td>
<td>7.16</td>
<td>6.96</td>
<td>2.34</td>
<td>0.355</td>
</tr>
<tr>
<td>6</td>
<td>7.15</td>
<td>5.74</td>
<td>1.68</td>
<td>0.432</td>
</tr>
</tbody>
</table>

± SD 7.17 ± 0.13 6.19 ± 0.82 2.29 ± 0.38 1.87 ± 0.68 0.457 ± 0.123 0.398 ± 0.165

1J, isotope dilution method (5); Y, cumulative tracer excretion method (7); MB-J, mass balance corrected for endogenous zinc excretion determined by using the isotope dilution method; MB-Y, mass balance corrected for endogenous fecal zinc determined by using the cumulative tracer excretion method.

TABLE 2
Fraction of dietary zinc absorbed (FZA) estimated by various methods2

<table>
<thead>
<tr>
<th>Subject</th>
<th>Compartmental model</th>
<th>FM-N</th>
<th>FM-E</th>
<th>FM-R</th>
<th>DA</th>
<th>DI-Pl</th>
<th>DI-24U</th>
<th>DI-spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.181</td>
<td>0.203</td>
<td>0.314</td>
<td>0.298</td>
<td>0.154</td>
<td>0.170</td>
<td>0.189</td>
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<td>2</td>
<td>0.225</td>
<td>0.271</td>
<td>0.289</td>
<td>0.357</td>
<td>0.211</td>
<td>0.218</td>
<td>0.231</td>
<td>0.252</td>
</tr>
<tr>
<td>3</td>
<td>0.392</td>
<td>0.364</td>
<td>0.434</td>
<td>0.444</td>
<td>0.344</td>
<td>0.350</td>
<td>0.395</td>
<td>0.380</td>
</tr>
<tr>
<td>4</td>
<td>0.458</td>
<td>0.477</td>
<td>0.512</td>
<td>0.545</td>
<td>0.417</td>
<td>0.446</td>
<td>0.412</td>
<td>0.417</td>
</tr>
<tr>
<td>5</td>
<td>0.288</td>
<td>0.325</td>
<td>0.365</td>
<td>0.420</td>
<td>0.274</td>
<td>0.315</td>
<td>0.221</td>
<td>0.222</td>
</tr>
<tr>
<td>6</td>
<td>0.262</td>
<td>0.371</td>
<td>NA2</td>
<td>0.409</td>
<td>0.244</td>
<td>0.288</td>
<td>0.269</td>
<td>0.281</td>
</tr>
</tbody>
</table>

± SD 0.301 ± 0.105 0.353 ± 0.0931 0.383 ± 0.0911 0.412 ± 0.0841 0.274 ± 0.0981 0.290 ± 0.0988 0.286 ± 0.0958 0.291 ± 0.0898

1FM-N, fecal monitoring without correction for resecretion of absorbed oral tracer (5; Equation 4); FME, fecal monitoring with correction for endogenous excretion (8; Equation 5); FMR, fecal monitoring with correction for resecretion (9; Equation 6); DA, deconvolution analysis (Equation 11); DI, dual isotope tracer ratio (13; Equation 13); DI-Pl, DI in plasma; DI-24-U, DI in 24-h urine samples; DI-spot, DI in spot urine samples.

2Insufficient stool frequency to allow estimation of FZA by the FM-E method.

3Values with different superscript letters are significantly different, P < 0.005.
The differences between FZA estimated from each tracer method and the model-derived reference value based on the Bland and Altman approach (17) are shown in Figure 3. The 2 corrected MB methods substantially overestimated FZA compared with the reference value by 0.156 ± 0.063 and 0.097 ± 0.084 (x ± SD), respectively. Two of the 3 FM methods provided estimates of FZA closer to the reference values; the average overestimates of FZA by the 3 FM methods were 0.034 ± 0.045, 0.074 ± 0.035, and 0.111 ± 0.035 for FM-N, FM-E, and FM-R, respectively. The DA technique underestimated FZA compared with the reference value in all 6 subjects, but the magnitude of the error was generally smaller than that for FM, averaging −0.027 ± 0.015. The DITR methods produced average values closest to the reference FZA value, with differences of −0.003 ± 0.026, −0.015 ± 0.033, and −0.010 ± 0.037 for plasma, 24-h urine samples, and spot urine samples, respectively. The variabilities of the values produced by the DITR method was slightly larger than that for DA. A comparison of the FZA estimated from each method with that determined from the model using ANOVA showed that MB-J, MB-Y, and FM-R gave estimates of FZA that were significantly different from the reference value (P < 0.001, P < 0.001, and P < 0.01, respectively).

**DISCUSSION**

Stable isotopic tracers are useful for measuring mineral absorption. In this article, we used 4 previously published stable isotopic tracer techniques that yielded 9 different estimates of FZA. The same data set from a group of 6 women who were fed a standard zinc diet was used for all comparisons. Estimated values for FZA based on these relatively simple measures of the data were compared with a reference value for FZA obtained from a detailed, physiologically based compartmental model fitted to all of the tracer and tracee data for each subject.

Estimates of FZA from the compartmental model averaged 0.301 (reference value) in these 6 women consuming a standard diet containing 7 mg Zn/d (Table 2). Both the DA technique, applied to plasma tracer data over the first 7 d after the isotope dose, and the DITR technique, whether estimated from plasma, 24-h urine samples, or spot urine samples averaged over days 3–7, provided reliable and comparable approximations of the reference value of FZA (Table 2).

The 2 corrected MB methods significantly overestimated FZA compared with the reference value. Endogenous zinc excretion estimated by using both the Jackson et al (6) and Yergey (7) methods agree well with values predicted by using the compartmental model, published previously, which averaged 2.01 ± 0.34 mg/d (14). Possible sources of error in the MB techniques include inaccurate measurements of dietary zinc intake and fecal zinc output.

All of the FM methods overestimated FZA compared with the reference value (Figure 3). Wastney and Henkin (19) showed that, in theory, the FM-N technique overestimates FZA for short fecal-collection periods and underestimates it for longer collection periods. The uncorrected technique gives an accurate value only over a narrow band of days. Because the collection period that gives accurate values differs among subjects (as a result of variance in rates of fractional absorption, fractional secretion, and gastrointestinal transit time) and because the best collection period is unknown, Wastney and Henkin (19) concluded that the FM technique should not be used to estimate zinc absorption.

A critical problem with the FM technique is that previously absorbed and resecreted oral tracer is excreted along with the unabsorbed tracer in the feces and there is no way to differentiate between the 2 sources of tracer in the feces. Several investigators attempted to provide a solution to this problem. One solution (FM-E), detailed in a review by Krebs et al (20), is based on the assumption that the rate of excretion of resecreted absorbed tracer
is constant and can be determined from the slope of the fecal accumulation of the tracer plotted as a function of time after all of the unabsorbed oral tracer has passed through the gastrointestinal tract. On the basis of this assumption, extrapolation of this slope back to zero time should correct for all of the resecreted oral tracer. When applied to our data over a 12-d collection period, this correction leads to a larger overestimate of FZA than does no correction at all (Figure 3). Another potential solution to this problem (FM-R) was proposed by Rauscher and Fairweather-Tait (9), who attempted to correct for resecretion by monitoring the accumulation of an intravenous zinc tracer in the feces and used this information to correct the fecal secretion of orally administered tracer according to equation 6. When applied to our data, this correction leads to an even greater overestimate of FZA (Figure 3) compared with our reference estimate.

In summary, we compared 4 different techniques for estimating FZA—MB, FM, DA, and DITR—with a reference value derived from a compartmental model. The MB method substantially overestimates FZA. The FM technique, even when corrected for resecretion of absorbed oral tracer, also overestimates FZA. The DA and DITR techniques provide estimates of FZA that are close to the model derived value. The DA technique requires multiple blood draws over several days to define the response of the orally and intravenously administered tracers in plasma. In contrast, the DITR technique requires only a single plasma sample, a 24-h urine sample, or a spot urine sample obtained ≥2 d after tracer administration. The spot urine sample requires the least subject involvement of all the procedures. We therefore recommend the DITR technique with use of a spot urine sample collected ≥2 d after tracer administration (or the average of several spot urine samples) as the method of choice for estimation of FZA when detailed compartmental modeling and the extensive sampling it requires cannot be performed.

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