Body composition of lactating women determined by anthropometry and deuterium dilution

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1. Body fat, fat-free mass and total body water of ten lactating women were estimated from deuterium-dilution spaces and from skinfold thickness measurements. Deuterium-dilution spaces were calculated from the 6 h (equilibration) and zero-time (extrapolation) deuterium enrichments in saliva, urine, human milk and breath water vapour samples.

2. The deuterium spaces obtained by equilibration were statistically larger than those obtained by extrapolation. Isotope dilution spaces derived from deuterium enrichments in saliva, breath water vapour and human milk did not differ with the exception of the 6 h equilibration value of milk, which was greater than that estimated from saliva. Deuterium-dilution spaces estimated from urine were consistently smaller than those derived from the other biological fluids.

3. No significant differences in body fat, fat-free mass and total body water were observed between anthropometric measurements and deuterium-dilution methods, except for extrapolated values derived from deuterium enrichments in urine.

Studies of body composition in human subjects inherently lack absolute certainty, because methods by necessity must be indirect. Methodological evaluation when applied to human subjects, therefore, has been based on validations in animals and on comparisons between indirect methods. During lactation, dynamic changes occur in body composition as the body reverts to its prepartum state. Questions of relevancy arise when body composition techniques that are validated in normal adults are applied to post-partum, lactating women, because of the changes in fat distribution and metabolism that occur during lactation. In a previous publication, we concluded that equations specific for lactating women were not necessary to predict body density from skinfold thicknesses (Butte et al, 1985).

In the present study, estimations of body fat, fat-free mass (FFM) and total body water (TBW) of lactating women were compared by anthropometric and deuterium-dilution methods. Technical aspects of the deuterium-dilution method and their impact on the prediction of body composition compartments were investigated. Specifically, in vivo isotope fractionation factors of hydrogen in saliva, urine, human milk and respiratory water vapour were measured and incorporated into the calculation of isotope dilution space (IDS); one-point equilibration and multi-point extrapolation modes to calculate IDS were compared, and differences in IDS based on saliva, urine, human milk and respiratory water vapour were evaluated.

SUBJECTS AND METHODS

Subjects

Ten lactating women, mean age 28.4 (SD 4.2) years, and parity equal to 1.2 (SD 0.4), participated in the study. Informed written consent was obtained from each subject and the

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protocol was approved by the Institutional Review Board for Human Research of Baylor College of Medicine and the Clinical Investigations and Publications Committee at Texas Children's Hospital. Mean height and prepregnancy weight were 1.645 (sd 0.032) m and 56.7 (se 4.6) kg respectively. Maternal weight at delivery was 71.2 (sd 6.4) kg, resulting in a weight gain during pregnancy of 14.5 (se 2.9) kg. At the time of study, 3.4 (sd 1.3) months postpartum, the women weighed 61.1 (sd 7.6) kg.

Infants were term and appropriate size for gestational age. All the infants were healthy at the time of the study; five were fed on human milk exclusively and five received food supplements in addition to human milk.

**Experimental design**

Maternal body fat, FFM and TBW were estimated from measurements of skinfold thicknesses and deuterium-dilution spaces. The study was conducted over a 14 d period. For the first 2 d, mothers and infants were admitted to the Clinical Research Center (CRC) at Texas Children's Hospital. Anthropometric measurements were made, and the deuterium dilution procedure begun. Urine, saliva, respiratory water vapour, and milk samples were obtained for the estimation of IDS. Biological specimens were collected at the CRC by the nursing staff or at the home by the mothers after instruction in the CRC.

**Anthropometry**

Women were weighed on a beam balance (3PY1002; Detecto Medic, New York). Height was measured with an upright extension meter. Skinfold thicknesses were measured to the nearest 0.5 mm using Lange calipers (Cambridge Scientific Industries, Cambridge, MD) at the following sites: triceps, biceps and subscapular (Durnin & Rahaman, 1967). Because of the difficulty in obtaining accurate and reproducible skinfold thickness measurements in the suprailiac regions of post-partum women, the suprailiac skinfold thicknesses were not used in the calculation. Measurements were made by one person (NFB). Body density was predicted from Durnin & Womersley's (1974) regression equation for females of 20–29 years of age: density (g/ml) = 1.1605 – 0.0777 × log (sum of three skinfold thicknesses). Body fat was calculated according to Siri (1956): body fat = (4.95/density – 4.50). FFM was equal to the difference between body-weight and body fat. TBW was estimated from the anthropometrically derived value of FFM and the conversion factor of 0.73 (Forbes, 1987).

**Deuterium-dilution method**

Baseline samples of saliva, urine, human milk and respiratory water vapour were collected from each women before oral administration of the $^{2}$H$_{2}$O tracer (100 mg/kg body-weight). Post-dose samples were collected at approximately 0.5, 1, 1.5, 2, 3, 6, 9 and 12 h and on days 2, 4, 6, 8, 10, 12 and 14 following administration of the $^{2}$H$_{2}$O.

Saliva samples (1 ml) were collected from under the tongue using a plastic syringe and transferred to a crimp-top vial (Wheaton, Millville, NJ) for storage. Urine samples were collected with a specimen cup. A portion of urine (1 ml) was transferred to a crimp-top vial for storage. Human milk was expressed from the left breast using an electric breast pump (Egnell, Inc., Cary, IL). The milk samples were defatted by centrifugation; portions (1 ml) of the defatted milk were transferred to crimp-top vials for storage. For the collection of respiratory water vapour, subjects were instructed to exhale through a polyethylene tube (approximately 750 mm in length) which was partially submerged in a propan-2-ol-dry ice bath at $-60^\circ$ for 2–5 min. Accumulated breath vapour (1 ml) was then thawed and transferred to a crimp-top vial.
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Isotope-ratio measurements

The saliva, urine, human milk and respiratory water vapour samples were reduced to hydrogen gas over zinc-shot at 475° for 30 min (Wong et al. 1987). The $^3\text{H}:^1\text{H}$ ratio was measured by gas-isotope-ratio mass spectrometry (Finnigan Delta-E, Finnigan MAT, San Jose, CA). The results are expressed in delta ($\delta$) per mil ($\permil$) units which are defined as:

$$\delta_{\text{sw}} = \frac{R_{\text{sample}} - 1}{R_{\text{ws}}} \times 10^3,$$

where $R_{\text{sample}}$ and $R_{\text{ws}}$ are the $^3\text{H}:^1\text{H}$ ratios of the sample and working standard respectively. The $\delta_{\text{sw}}$ values were normalized against two international water standards: Vienna-Standard Mean Ocean Water (V-SMOW) and Standard Light Antarctic Precipitation (SLAP) according to Gonfiantini (1984):

$$\delta_{\text{normalized}} = \left[\frac{(\delta_{\text{sample/ws}} - \delta_{\text{V-SMOW/ws}})}{(\delta_{\text{SLAP/ws}} - \delta_{\text{V-SMOW/ws}})}\right] \times \delta_{\text{SLAP/V-SMOW}},$$

where $\delta_{\text{sample/ws}}$, $\delta_{\text{V-SMOW/ws}}$ and $\delta_{\text{SLAP/ws}}$ are the $^3\text{H}$ values of the sample, V-SMOW and SLAP measured against the laboratory working standard respectively. The $\delta_{\text{SLAP/V-SMOW}}$ has a defined value of $-428\permil$ (Gonfiantini, 1984) for $^3\text{H}:^1\text{H}$ isotope ratio measurements. An accuracy of $-4.6\permil$ and a precision of $3.2\permil$ (SD) were obtained on saliva, urine and human milk samples at $580\permil$ enrichment levels of $^3\text{H}$ (Wong et al. 1987).

IDS were calculated from the isotopic enrichment of deuterium in saliva, urine, human milk and respiratory water vapour as follows:

$$\text{IDS} = \frac{d}{MW} \times \frac{\text{APE}}{100} \times \frac{18.02}{\text{EOB} \times R_{\text{std}} \times \alpha},$$

where $d$ is the dose (g) of deuterium oxide, $MW$ is the molecular weight of the labelled water (g/mol), APE is the atom percent excess of the isotope, 18.02 is the molecular weight of water (g/mol), EOB is the isotopic enrichment of the isotope over baseline value (per mil), $R_{\text{std}}$ is the $^3\text{H}:^1\text{H}$ ratio of V-SMOW which has a value of 0.00015595 (De Wit et al. 1980) and $\alpha$ is the isotope-fractionation factor of the physiological fluid. The isotope-fractionation factors relative to plasma water were 1.004 for saliva (Wong et al. 1988), 0.993 for urine, 0.942 for breath water vapour and 1.002 for human milk. IDS was calculated from the EOB value at 6 h postdose and from the EOB value obtained by extrapolation to the zero-time intercept using all isotope measurements after 6 h.

TBW was assumed to be equal to IDS. FFM was calculated as TBW/0.73. Body fat was computed as body-weight minus FFM.

Statistics

Repeated-measures analysis of variance was used to detect significant differences between methods (Snedecor & Cochran, 1967). A multiple comparison procedure (Snedecor & Cochran, 1967) was then used to isolate the differences. To assess the agreement between methods, we used the following procedure (Bland & Altman, 1986). First, the between-method differences were plotted against the means of both methods. Regression analysis was then used to test for a relation between the differences and the means. If the slope was not significant, the relative bias (mean difference between methods) and the 95% limits of agreement (mean difference and 2 SD of the differences) were computed. Of all differences 95% were expected to fall within these limits. To determine if the bias (mean difference) was significantly different from zero, a paired $t$ test was used. If the slope relating the between-method differences and means was significant, the 95% limits of agreement were estimated as 2 SE of the estimate around the regression line.
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Table 1. Anthropometric indices of ten lactating women

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Mean</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt (kg)</td>
<td>66.5</td>
<td>62.2</td>
<td>70.4</td>
<td>62.7</td>
<td>51.7</td>
<td>72.3</td>
<td>52.3</td>
<td>56.1</td>
<td>64.1</td>
<td>52.3</td>
<td>61.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.645</td>
<td>1.689</td>
<td>1.676</td>
<td>1.638</td>
<td>1.606</td>
<td>1.626</td>
<td>1.619</td>
<td>1.689</td>
<td>1.594</td>
<td>1.664</td>
<td>1.645</td>
</tr>
<tr>
<td>Biceps (mm)</td>
<td>9.0</td>
<td>4.5</td>
<td>14.0</td>
<td>7.0</td>
<td>5.0</td>
<td>11.5</td>
<td>8.0</td>
<td>8.0</td>
<td>13.0</td>
<td>6.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>23.0</td>
<td>12.5</td>
<td>30.0</td>
<td>23.0</td>
<td>15.5</td>
<td>27.0</td>
<td>19.0</td>
<td>17.5</td>
<td>23.5</td>
<td>20.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Subscapular (mm)</td>
<td>25.5</td>
<td>12.0</td>
<td>24.0</td>
<td>23.5</td>
<td>11.0</td>
<td>20.5</td>
<td>11.0</td>
<td>6.5</td>
<td>19.0</td>
<td>9.8</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Table 2. Body composition compartments derived from anthropometric measurements of ten lactating women

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Mean</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density: g/ml</td>
<td>1.024</td>
<td>1.047</td>
<td>1.018</td>
<td>1.026</td>
<td>1.044</td>
<td>1.023</td>
<td>1.038</td>
<td>1.044</td>
<td>1.025</td>
<td>1.039</td>
<td>1.033</td>
</tr>
<tr>
<td>Fat: kg g/kg body-wt</td>
<td>22.3</td>
<td>14.2</td>
<td>25.5</td>
<td>20.3</td>
<td>12.5</td>
<td>24.5</td>
<td>14.1</td>
<td>13.6</td>
<td>21.1</td>
<td>13.7</td>
<td>18.2</td>
</tr>
<tr>
<td>FFM: kg g/kg body-wt</td>
<td>44.2</td>
<td>48.0</td>
<td>44.9</td>
<td>42.4</td>
<td>39.2</td>
<td>47.8</td>
<td>38.2</td>
<td>42.4</td>
<td>43.0</td>
<td>38.6</td>
<td>42.9</td>
</tr>
<tr>
<td>TBW: kg g/kg body-wt</td>
<td>52.3</td>
<td>53.0</td>
<td>52.8</td>
<td>51.0</td>
<td>48.6</td>
<td>53.2</td>
<td>47.8</td>
<td>51.0</td>
<td>47.8</td>
<td>51.0</td>
<td>47.8</td>
</tr>
</tbody>
</table>

Table 3. Body composition compartments derived from deuterium-dilution method for ten lactating women

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Mean</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW*: kg g/kg body-wt</td>
<td>33.6</td>
<td>36.2</td>
<td>32.8</td>
<td>30.2</td>
<td>28.8</td>
<td>36.0</td>
<td>27.4</td>
<td>28.1</td>
<td>34.1</td>
<td>25.9</td>
<td>31.3</td>
</tr>
<tr>
<td>FFM: kg g/kg body-wt</td>
<td>46.0</td>
<td>49.6</td>
<td>45.0</td>
<td>41.4</td>
<td>39.5</td>
<td>49.3</td>
<td>37.6</td>
<td>38.4</td>
<td>46.7</td>
<td>35.4</td>
<td>42.9</td>
</tr>
<tr>
<td>Fat: kg g/kg body-wt</td>
<td>20.5</td>
<td>24.6</td>
<td>25.4</td>
<td>21.3</td>
<td>12.2</td>
<td>23.0</td>
<td>14.7</td>
<td>17.6</td>
<td>17.6</td>
<td>16.9</td>
<td>18.2</td>
</tr>
</tbody>
</table>

RESULTS

The anthropometric indices of the ten lactating women are presented in Table 1. Body composition compartments calculated from anthropometric and deuterium-dilution methods are shown in Tables 2 and 3 respectively.

A comparison of the body composition compartments predicted from anthropometry and deuterium-dilution methods is summarized in Table 4. No significant differences in body fat, FFM or TBW were observed between anthropometry and deuterium-dilution methods (TBW based on 6 h saliva sample). It should be noted that however small the
Table 4. Assessment of the differences observed between anthropometry and deuterium dilution methods for the determination of body fat, fat-free mass (FFM), and total body water (TBW) of ten lactating women
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Anthropometry</th>
<th>Deuterium dilution</th>
<th>Relative bias*</th>
<th>95% Limits of agreement†</th>
<th>Paired t test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>2 SD</td>
<td></td>
</tr>
<tr>
<td>Fat: kg g/kg body-wt</td>
<td>18.2 5.0</td>
<td>18.2 4.4</td>
<td>0.02 2.3</td>
<td>-4.9-4.7</td>
<td>NS</td>
</tr>
<tr>
<td>FFM: kg g/kg body-wt</td>
<td>42.9 3.5</td>
<td>42.9 5.1</td>
<td>-0.02 2.3</td>
<td>-3.6-3.6</td>
<td>†</td>
</tr>
<tr>
<td>TBW: kg g/kg body-wt</td>
<td>516 36</td>
<td>514 35</td>
<td>-0.01 1.7</td>
<td>-56-60</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.
* Mean between-method difference and SD.
† Mean difference and 2 SD of the differences.
‡ Since the slope relating the between-methods differences vs. the mean values was significant, a test of the mean differences was not meaningful.

Fig. 1. Comparison of deuterium-dilution spaces derived from (a) urine and saliva, (b) human milk and saliva, and (c) breath water vapour and saliva, of ten lactating women. Individual differences between methods (Δ); relative biases (—); upper and lower 95% limits of agreement between methods (---). The ordinates represent the differences between the deuterium-dilution spaces calculated from the isotopic abundances of deuterium in the urine, human milk and breath water vapour against those derived from saliva.
Fig. 2. Comparison of deuterium-dilution spaces calculated using the 6 h enrichment values and extrapolated zero-intercept enrichment values in (a) saliva, (b) urine, (c) human milk, and (d) breath water vapour of ten lactating women. Individual differences between methods (△); relative biases (---); upper and lower 95% limits of agreement between methods (---). The ordinates represent the differences between the deuterium-dilution spaces derived from the 6 h and the extrapolated enrichment values.

differences between methods for FFM (kg) and TBW (kg), they tended to become smaller as the magnitude of the IDS became larger. Similar method comparisons were made between anthropometry and deuterium dilution using IDS derived from milk, breath water vapour and urine; significant differences in body compartments were demonstrated between anthropometry and deuterium dilution only when the IDS was based on urine samples calculated by extrapolation.

The IDS predicted from the four biological fluids were compared and statistically significant differences were detected (ANOVA, \( P < 0.01 \)). Mean differences in IDS based on saliva and breath water vapour samples were not significantly different from zero, whether they were calculated from the equilibrated (mean difference 0.24 (SD 0.98) kg) or extrapolated enrichment values (mean difference 0.15 (SD 0.73) kg); nor were the mean differences in IDS derived from milk and breath significantly different from zero (mean differences 0.29 (SD 1.2) kg and 0.18 (SD 0.74) kg respectively). The IDS based on isotope enrichment of milk at 6 h was significantly greater than the estimate based on saliva at 6 h (mean difference 0.53 (SD 0.55) kg, \( P < 0.02 \)); no significant differences in IDS were observed for the extrapolated mode of calculation between milk and saliva. The IDS based on urine samples was significantly less than that calculated for saliva, milk and breath, regardless of the mode of calculation (mean differences ranged between 0.8 and 1.3 kg, \( P < 0.01 \)). Comparisons of IDS derived from saliva v. urine, milk or breath samples are illustrated in Fig. 1(a–c).

Deuterium-dilution spaces calculated from equilibrated 6 h and from extrapolated zero-intercept enrichment values are compared in Fig. 2(a–d) for saliva, urine, milk and breath.
Body composition of lactating women

Mean values for body fat, FFM and TBW were essentially identical when estimated by anthropometric or deuterium-dilution methods. The values obtained in the present study were similar to those reported in other studies of well-nourished lactating women (Manning-Dalton & Allen, 1983; Butte et al. 1984). The close agreement between methods indicates that the Durnin & Womersley (1974) and Siri (1956) equations used for the prediction of body fat from skinfold thicknesses are applicable for use in lactating women. The 95% limits of agreement between methods were relatively wide, however, which indicated that the methods could differ substantially when applied to individuals. For example, 95% of the differences between methods for predicting body fat would be within ±8.0%, which represents about 27% of the mean body fat observed in these women.

Although the deuterium-dilution method involves certain assumptions and errors, it is the more direct and precise of the two methods. The anthropometric approach is based on unproven assumptions that the thickness of subcutaneous fat is representative of the total body fat content and that the sites of measurement reflect the average thickness of the entire subcutaneous layer. Changes in fat distribution (Taggart et al. 1967) and differences in the rate of mobilization of fat from diverse adipose-tissue sites (Quandt, 1983) during lactation may account for a significant amount of the observed variability.

When the deuterium-dilution method is applied, major factors that must be considered and resolved are the biological fluid to be sampled, the schedule of sample collection, the validation of IDS and TBW equivalency, the assumed water content of FFM, and isotopic fractionation.

Various authors have shown that the IDS derived from saliva and plasma are comparable (Schoeller et al. 1982; Wong et al. 1988). In the present study we found that the IDS based on saliva, human milk and breath, calculated in the same manner, i.e. equilibration or extrapolation, were not significantly different from one another, with the exception of the saliva–milk comparison at 6 h. IDS derived from 6 h milk values was higher than that based on saliva, indicating lower enrichment values in milk; the isotope may have been diluted by pre-existing milk in the gland. IDS based on urine samples was consistently less than the IDS based on the other fluids; slight biological fractionation in the re-absorption of water from the renal tubules may account for this difference. Mean differences observed between urine and the other fluids were generally less than 1.0 kg and the 95% limits of agreement averaged ±0.65 kg; therefore, the practicality of obtaining urine samples in some protocols may outweigh the slight loss in accuracy.

IDS may be calculated from a single enrichment value taken at equilibrium or from the zero-time intercept obtained from extrapolation of several post-dose enrichment values. In normal adults, isotope equilibrium in plasma, urine, saliva and respiratory water vapour is reached by 3 h post dose (Wong et al. 1988). IDS calculated from the deuterium enrichment in the 3, 4, 5 and 6 h post-dose samples were within 3% of each other. The isotopic loss

The mean differences between these two approaches (6 h equilibrium minus the value derived from the multi-point extrapolation for calculating IDS) were 0.33 (SD 0.43) kg for saliva, 0.53 (SD 0.32) kg for urine, 0.83 (SD 0.41) kg for human milk and 0.72 (SD 0.62) kg for breath water vapour. No significant relations were seen between the differences and the mean IDS by regression analysis. The mean differences observed for the two modes of calculation were significantly different from zero in the four fluids (P < 0.01–0.05). The mean differences between 6 h and extrapolated values for saliva, urine, milk and respiratory water vapour were equal to 1.1, 1.8, 2.6, and 2.3% of their respective group mean IDS.

DISCUSSION

The mean differences between these two approaches (6 h equilibrium minus the value derived from the multi-point extrapolation for calculating IDS) were 0.33 (SD 0.43) kg for saliva, 0.53 (SD 0.32) kg for urine, 0.83 (SD 0.41) kg for human milk and 0.72 (SD 0.62) kg for breath water vapour. No significant relations were seen between the differences and the mean IDS by regression analysis. The mean differences observed for the two modes of calculation were significantly different from zero in the four fluids (P < 0.01–0.05). The mean differences between 6 h and extrapolated values for saliva, urine, milk and respiratory water vapour were equal to 1.1, 1.8, 2.6, and 2.3% of their respective group mean IDS.
during equilibration is assumed to be negligible. The extrapolation approach accounts for isotopic loss before equilibration, but assumes that the rate of loss is mono-exponential. In our study, IDS calculated from the 6 h equilibrium enrichment were consistently higher than IDS predicted by extrapolation, and the differences between modes of calculation ranged from 1.1 to 2.6% of their respective mean IDS. The time-course of deuterium enrichment in the four fluids demonstrated that 100% equilibration had not been achieved by 6 h in all women. Pooling of water in the bladder and mammary gland may contribute to unequal distribution of isotope at 6 h post-dose. If this is the case, calculation of IDS by extrapolation may be theoretically more accurate; however, differences in IDS that range from 1.1 to 2.6% approach our analytical precision in measurements of $^2\text{H}:^1\text{H}$.

The deuterium-dilution technique overestimates TBW to the degree that deuterium exchanges with non-aqueous hydrogens. Estimates of TBW by tritium have been reported to differ from those obtained by desiccation by 4–15%, depending on age and animal species (Sheng & Huggins, 1979). Error may be introduced by loss of tracer through urine, breath and skin during the equilibration period. Forbes (1987) estimated that 1.4% of the dose could be lost in the initial 5 h post-dose in a human adult. Calculation of FFM from TBW using the conversion factor, 0.73, introduces an uncertain degree of error. The authors wish to thank the women who participated in the study, C. J. Heinz for field work, L. L. Clarke for isotope ratio measurements, M. E. Lewis for manuscript preparation and Y. Garza and E. R. Klein for editorial review. This work is a publication of the USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, TX. This project has been funded in part with federal funds from the U.S. Department of Agriculture, Agricultural Research Service, under Cooperative Agreement no. 58-7MN1-6-100. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES


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