Lean body mass of well-nourished women is preserved during lactation1–5

Kathleen J Motil, Hwai-Ping Sheng, Barbara L Kertz, Corinne M Montandon, and Kenneth J Ellis

ABSTRACT To determine whether the lean body mass of well-nourished women was preserved during lactation, the body composition of 10 lactating and 10 nonlactating women was examined longitudinally at 6-wk intervals between 6 and 24 wk postpartum and at 52 wk postpartum, and that of 10 nulliparous women was examined at equivalent intervals, by using clinical anthropometry and whole-body potassium counting. Milk production was determined at 6-wk intervals during the period of exclusive breast-feeding (6–24 wk postpartum) by the test-weighing procedure. Milk composition was determined by chemical analysis. Dietary intakes were determined at 6-wk intervals between 6 and 24 wk postpartum from 3-d food records with use of a nutrient database. Lean body mass was maintained in women who exclusively breast-fed their infants during the first 6 mo postpartum while consuming dietary protein in amounts that exceeded those of their nonlactating counterparts by 55%. The high protein intakes were sustained throughout lactation despite a progressive reduction by 32% of milk protein output. Lean body mass was preserved throughout lactation in well-nourished women, suggesting that the metabolic needs of milk protein production were met solely by higher protein intakes of the lactating women. Am J Clin Nutr 1998;67:292–300.

KEY WORDS Lactation, protein turnover, lean body mass, human body composition, dietary protein, women, milk protein, breast-feeding

INTRODUCTION Adult women are thought to accrete ≈925 g protein during pregnancy (1). About 60% of the accreted protein is deposited in fetal and placental tissues and ≈40% is deposited in maternal supportive tissues, including the breasts and uterus. Whether additional protein is retained at other sites, such as skeletal muscle, is controversial (2, 3). It has been estimated that after parturition ≈75% of the maternal tissue protein stores accreted during pregnancy can be mobilized to support milk production (4). Because the quantity of protein retained during pregnancy is small compared with that required for lactation, maternal protein stores are rapidly depleted postpartum. Thus, after the first month of lactation, the source of all milk protein must be derived from either diet or prepregnant body protein stores.

With respect to the maternal diet, we previously showed lactating (L) women who consumed the recommended dietary protein allowance of 1.0 g · kg–1 · d–1 were associated with negative nitrogen balances during early, well-established, and prolonged lactation (5). Even when dietary protein consumption increased by 50%, nearly half of the women had a negative nitrogen balance (6). One might infer from these observations that negative nitrogen balances would be associated with losses of lean body mass to support milk production in the presence of a relative inadequacy of dietary protein intake. These observations suggested that the L women supported their milk production at the expense of meeting their own metabolic needs for the maintenance of their nutritional well-being.

We hypothesized, therefore, that mature L women would mobilize body protein stores, and hence, lean body mass, throughout the postpartum period to support milk production, and that the loss of lean body mass would be inversely proportional to the adequacy of their dietary protein intake. The specific aims of this study were 1) to measure lean body mass, body weight, dietary protein intakes, milk production, and milk protein output throughout the first postpartum year in L women; 2) to compare differences in body composition and dietary intakes among L, nonlactating postpartum (NL), and nulliparous (NP) women, and 3) to infer dietary protein adequacy in L women by relating dietary intake and changes in lean body mass with milk production and milk protein output.

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SUBJECTS AND METHODS

Subjects

Thirty healthy women, of whom 10 were L, 10 were NL, and 10 were NP, were enrolled in the study. The enrollment criteria for all women included that they were between the ages of 21 and 38 y and were nonsmoking. The enrollment criteria for the parous women included 1) enrollment in the study before delivery, 2) an uncomplicated pregnancy, 3) a parity of three or less, and 4) delivery of a full-term, appropriate-for-gestational-age infant. The enrollment criteria for the L women included exclusive breast-feeding during the first 6 mo postpartum and gradual weaning of their infants during the second 6 mo postpartum. Each subject underwent a medical history, a physical examination, and routine laboratory tests before study participation to ensure her clinical well-being. The characteristics of the three groups of women, including their racial distribution, ages, anthropometric measurements (height and weight), nutritional status (body mass index and serum prealbumin), and reproductive status (parity, prepregnancy weight, and pregnancy weight gain) are listed in Table 1. Written, informed consent for study participation was obtained from all women. The study was approved by the Institutional Review Board for Human Subjects Research at Baylor College of Medicine and Affiliated Hospitals.

Study design

All L and NL women were admitted for 3 d to the US Department of Agriculture, Agricultural Research Service Children’s Nutrition Research Center or the General Clinical Research Center at Texas Children’s Hospital and were subsequently studied longitudinally at intervals of 6:1:2:1 5:4:1:0 7:2:1:0. Thirty healthy women, of whom 10 were L, 10 were NL, and 10 were NP, were enrolled in the study. The enrollment criteria for all women included that they were between the ages of 21 and 38 y and were nonsmoking. The enrollment criteria for the parous women included 1) enrollment in the study before delivery, 2) an uncomplicated pregnancy, 3) a parity of three or less, and 4) delivery of a full-term, appropriate-for-gestational-age infant. The enrollment criteria for the L women included exclusive breast-feeding during the first 6 mo postpartum and gradual weaning of their infants during the second 6 mo postpartum. Each subject underwent a medical history, a physical examination, and routine laboratory tests before study participation to ensure her clinical well-being. The characteristics of the three groups of women, including their racial distribution, ages, anthropometric measurements (height and weight), nutritional status (body mass index and serum prealbumin), and reproductive status (parity, prepregnancy weight, and pregnancy weight gain) are listed in Table 1. Written, informed consent for study participation was obtained from all women. The study was approved by the Institutional Review Board for Human Subjects Research at Baylor College of Medicine and Affiliated Hospitals.

TABLE 1
Characteristics of lactating, nonlactating postpartum, and nulliparous women at visit 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lactating (n = 10)</th>
<th>Nonlactating postpartum (n = 10)</th>
<th>Nulliparous (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>31.3 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.8 ± 3.8</td>
<td>29.0 ± 4.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.09</td>
<td>1.62 ± 0.05</td>
<td>1.68 ± 0.06</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.2 ± 14.4</td>
<td>67.7 ± 6.5</td>
<td>62.5 ± 9.1</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>26.3 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.8 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.2 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transthyretin (mg/L)</td>
<td>26.8 ± 2.5</td>
<td>28.6 ± 6.2</td>
<td>24.9 ± 5.0</td>
</tr>
<tr>
<td>Parity</td>
<td>1.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Prepregnancy weight (kg)</td>
<td>66.1 ± 14.3</td>
<td>61.1 ± 5.9</td>
<td>—</td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>17.7 ± 4.1</td>
<td>17.3 ± 4.5</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values in the same row with different superscript letters are significantly different, P < 0.05 (ANOVA).

<sup>b</sup>W, white; A, African American; H, Hispanic; O, Asian American.

<sup>c</sup>X ± SD.
their typical eating pattern throughout the 3-d interval. During the
period of record keeping, the nutritionist communicated daily with
each subject to monitor compliance and verify the accuracy of each
subject’s record keeping. After completion, all food and beverage
items from each individual’s 3-d record were entered into the nutri-
tive database (NUTRITIONIST III, version 5.0, N-Squared Com-
puting, Salem, OR) by one individual. The dietary protein and
energy intakes of each individual were averaged for each 3-d peri-
d from the dietary records by using the nutrient database.
To assess the accuracy of dietary intake reporting, we mea-
sured urinary nitrogen excretion and dietary energy intake
expressed as a multiple of basal metabolic rate (11). Individual
24-h urine collections were obtained randomly from 5 L and 5
NL women at each visit between 12 and 24 wk postpartum.
Urine samples were collected daily in bottles containing 5 mL
of urine samples were collected at −20 °C until analyzed for total nitrogen concentrations.
Total daily nitrogen excretion was estimated from the amount of
ure produced daily and its nitrogen concentration. The amount of
nitrogen excreted during ad libitum food consumption was
compared with that measured under metabolic conditions of
known dietary protein intake to provide an estimate of the accu-
racy of dietary record keeping (5, 6).
In a similar fashion, dietary energy intake (EI, MJ/d)
expressed as a multiple of basal metabolic rate (BMR, MJ/d) was
calculated as follows:

\[
EI = \frac{(E_d + E_m) - E_n}{BMR}
\]

where \(E_d\) (MJ/d) is the energy available from the diet as reported in
the food records; \(E_m\) (MJ/d) is the energy available from body fat
stores, assuming that all body weight lost or gained by L, NL, and
NP women between the 6- and 24-wk visits was composed of fat,
and that the energy content of body fat is equivalent to ≈38 kJ/g;
\(E_n\) is the energy output from milk; and basal metabolic rate is
equivalent to 75 kJ ⋅ kg\(^{-1}\) ⋅ d\(^{-1}\) in all subjects (12). The ratio,
EI:BMR, was compared with reported values that represent energy
needs at various activity levels to provide an additional estimate of
the accuracy of record keeping (11).

Lactational performance
Milk production was measured for 50 h by the test-weighing
procedure (13). All infants were weighed on an electronic bal-
ance (model 3862MP; Sartorius, Göttingen, Germany) before
and after each feeding for the determination of total daily milk
consumption by the infant. Milk samples collected for storage
were obtained by manual or mechanical pumping (model 40;
Egnell, Cary, IL) and weighed in a similar manner. Total milk
production was calculated from the amount of milk consumed by
the infant and expressed by the mother.
After the test-weighing procedure was completed, a 24-h
pooled milk sample was obtained for compositional analysis
(14). During the milk collection period, the infant’s routine feeding
pattern was monitored such that at each feeding, one breast
was used to feed the infant, while milk was collected from the
other breast with a mechanical pump. This pattern of feeding
was reversed at each subsequent feeding. Two percent of each
milk sample was pooled and stored at −70 °C until subsequently
analyzed for total nitrogen, nonprotein nitrogen, energy, lactose,
and fat content. Protein nitrogen was calculated as the difference
between total nitrogen and nonprotein nitrogen content.

Analytic techniques
Total nitrogen and nonprotein nitrogen concentrations of milk
or urine samples or both were measured by using the micro-Kjel-
dahl method (model 1030; Tecator, Höganas, Sweden). The en-
ergy concentrations of milk samples were measured with use of an
adiabatic bomb calorimeter (model 1241; Parr, Moline, IL). The lactose
concentrations of milk samples were measured by using an
enzymatic method (YSI Lactose Analyzer; Yellow Springs
Instrument Co, Yellow Springs, OH). The fat concentrations
of milk samples were measured after extraction with mixtures of
heptane, diethyl ether, ethanol, and water (1:1:1:1) by the modi-
fied Jeejeebhoy method (15).

Statistical methods
All data were analyzed with use of a standardized computer
package for descriptive statistics (MINITAB, version 10.2;
Minitab, Inc, State College, PA) and are expressed as means ±
SDs. Analysis of variance was performed to determine differ-
ces (\(P < 0.05\)) among the L, NL, and NP women in the
descriptive variables (age, height, weight, body mass index,
skinfold thickness, prepregnancy weight, pregnancy weight gain,
and parity) recorded at the first visit to identify those variables
that could potentially confound the main analysis. Analysis of
covariance with repeated measures was performed (BMDP2V,
version 5.1; BMDP Statistical Software, Inc, Los Angeles) to
detect differences among L, NL, and NP women in the following
dependent variables: body weight; lean body mass; body fat;
the proportions of body weight composed of lean body mass and
body fat; triceps, biceps, subscapular, suprailiac, and thigh skin-
fold thicknesses; and intakes of dietary protein, energy, carbohy-
drate, and fat. These analyses were performed while adjusting
for prepregnancy weight (L and NL) or weight at first visit (NP)
as the covariate. When group differences were detected, Fisher’s
least-significant-difference tests were performed to identify
group differences between the L and NL or NP women.
Linear regression was used to derive the coefficients (slopes)
that defined for each L woman the relations among the variables
milk production and milk protein output, dietary intake (protein
and energy), and body composition (lean body mass and body
fat) while adjusting for body weight at each visit. Student’s \(t\)
tests of the mean slopes (against zero) were performed to deter-
mine whether a relation could be detected within the L group
between 1) milk production and dietary intake, 2) milk protein
output and dietary intake, 3) milk production and body composi-
tion, or 4) body composition and dietary intake. Linear regres-
sion was performed to determine relations between the change in
body composition (lean body mass and body fat) and reproduc-
tive characteristics (pregnancy weight, pregnancy weight gain,
age, body mass index, or parity) of L and NL women.

RESULTS
Body weight, lean body mass, and body fat, in both kilograms
and percentage of body weight, of L, NL, and NP women at each
study visit are presented in Table 2. After adjustments were
made for prepregnancy weight, body weight differed among the
groups in that L women tended to be heavier (\(P = 0.14\)) than NL
or NP women at the first four study visits. Body weight of all three groups decreased significantly during the first four study visits and averaged $-1.2 \pm 3.1$, $-2.8 \pm 4.1$, and $-0.5 \pm 2.9$ kg in L, NL, and NP women, respectively. The average body weight of L and NL women was $9.7 \pm 5.8\%$ and $10.9 \pm 5.5\%$ greater than prepregnancy weight, respectively, at 6 wk postpartum ($P < 0.01$), and $7.7 \pm 7.1\%$ and $6.2 \pm 7.4\%$ greater than prepregnancy weight at 24 wk postpartum ($P < 0.01$).

After adjustments were made for prepregnancy weight, the absolute amount of lean body mass did not differ significantly among L, NL, and NP women, and did not change over time in any of these groups during the first four study visits. Lean body mass as a percentage of body weight was significantly lower in L and NL than in NP women at the first four study visits. This percentage increased significantly among all three groups during the first four study visits.

After prepregnancy weight was adjusted for, body fat differed significantly among the groups in that L women had significantly more body fat than NP women at the first three visits, but not the fourth. The fat mass of NL women was intermediate between that of L and NP women, but the differences were not significant. The body fat of all three groups decreased significantly during the first four study visits and averaged $-1.4 \pm 1.8$, $-3.2 \pm 3.1$, and $-0.9 \pm 3.2$ kg in L, NL, and NP women, respectively. Body fat as a percentage of body weight differed significantly among the groups in that it was significantly greater in L and NL than in NP women at the first four study visits. This proportion decreased significantly in all three groups during the first four study visits.

<table>
<thead>
<tr>
<th>Body composition variable</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum age (wk)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L</td>
<td>5.7 ± 0.6</td>
<td>11.9 ± 1.3</td>
<td>17.7 ± 0.7</td>
<td>23.9 ± 0.9</td>
<td>52.0 ± 1.6</td>
</tr>
<tr>
<td>NL</td>
<td>5.9 ± 0.6</td>
<td>12.3 ± 0.9</td>
<td>18.8 ± 1.5</td>
<td>24.4 ± 0.8</td>
<td>53.6 ± 1.7</td>
</tr>
<tr>
<td>NP</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>72.2 ± 14.4</td>
<td>71.2 ± 14.1</td>
<td>71.2 ± 14.8</td>
<td>71.0 ± 15.1</td>
<td>69.2 ± 14.9</td>
</tr>
<tr>
<td>NL</td>
<td>67.7 ± 6.6</td>
<td>66.5 ± 7.0</td>
<td>65.4 ± 6.5</td>
<td>64.9 ± 7.4</td>
<td>65.8 ± 7.3</td>
</tr>
<tr>
<td>NP</td>
<td>62.5 ± 9.1</td>
<td>62.4 ± 9.3</td>
<td>62.4 ± 9.2</td>
<td>62.1 ± 9.9</td>
<td>62.9 ± 9.0</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L</td>
<td>45.9 ± 3.7</td>
<td>46.1 ± 4.0</td>
<td>46.4 ± 4.5</td>
<td>46.1 ± 4.8</td>
<td>46.1 ± 4.1</td>
</tr>
<tr>
<td>NL</td>
<td>46.0 ± 4.4</td>
<td>46.5 ± 4.1</td>
<td>45.8 ± 4.3</td>
<td>46.4 ± 5.0</td>
<td>47.3 ± 4.5</td>
</tr>
<tr>
<td>NP</td>
<td>47.0 ± 3.1</td>
<td>47.2 ± 3.9</td>
<td>47.9 ± 3.4</td>
<td>47.5 ± 3.5</td>
<td>47.5 ± 3.6</td>
</tr>
<tr>
<td>Lean body mass (% body wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>65 ± 9a</td>
<td>66 ± 9a</td>
<td>67 ± 9a</td>
<td>67 ± 10a</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>NL</td>
<td>68 ± 5b</td>
<td>70 ± 6b</td>
<td>70 ± 6a</td>
<td>72 ± 7b</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>NP</td>
<td>76 ± 8b</td>
<td>76 ± 7b</td>
<td>78 ± 9a</td>
<td>79 ± 10b</td>
<td>76 ± 9</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>26.3 ± 11.5</td>
<td>25.1 ± 10.8</td>
<td>24.8 ± 10.9</td>
<td>24.8 ± 11.5</td>
<td>23.1 ± 11.2</td>
</tr>
<tr>
<td>NL</td>
<td>21.7 ± 4.6b</td>
<td>20.0 ± 5.3b</td>
<td>196 ± 51b</td>
<td>185 ± 57</td>
<td>185 ± 6.7</td>
</tr>
<tr>
<td>NP</td>
<td>15.8 ± 7.3b</td>
<td>15.2 ± 6.3b</td>
<td>14.6 ± 7.3b</td>
<td>14.5 ± 8.4</td>
<td>15.4 ± 7.5</td>
</tr>
<tr>
<td>Body fat (% body wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>35 ± 9a</td>
<td>34 ± 9a</td>
<td>33 ± 9a</td>
<td>33 ± 10a</td>
<td>32 ± 10</td>
</tr>
<tr>
<td>NL</td>
<td>32 ± 5a</td>
<td>30 ± 6b</td>
<td>30 ± 6a</td>
<td>28 ± 7b</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>NP</td>
<td>24 ± 8a</td>
<td>24 ± 7b</td>
<td>22 ± 9b</td>
<td>22 ± 10b</td>
<td>24 ± 9</td>
</tr>
</tbody>
</table>

or NP women at the first four study visits. Body weight of all three groups decreased significantly during the first four study visits and averaged $-1.2 \pm 3.1$, $-2.8 \pm 4.1$, and $-0.5 \pm 2.9$ kg in L, NL, and NP women, respectively. The average body weight of L and NL women was $9.7 \pm 5.8\%$ and $10.9 \pm 5.5\%$ greater than prepregnancy weight, respectively, at 6 wk postpartum ($P < 0.01$), and $7.7 \pm 7.1\%$ and $6.2 \pm 7.4\%$ greater than prepregnancy weight at 24 wk postpartum ($P < 0.01$).

After adjustments were made for prepregnancy weight, the absolute amount of lean body mass did not differ significantly among L, NL, and NP women, and did not change over time in any of these groups during the first four study visits. Lean body mass as a percentage of body weight was significantly lower in L and NL than in NP women at the first four study visits. This percentage increased significantly among all three groups during the first four study visits.

After prepregnancy weight was adjusted for, body fat differed significantly among the groups in that L women had significantly more body fat than NP women at the first three visits, but not the fourth. The fat mass of NL women was intermediate between that of L and NP women, but the differences were not significant. The body fat of all three groups decreased significantly during the first four study visits and averaged $-1.4 \pm 1.8$, $-3.2 \pm 3.1$, and $-0.9 \pm 3.2$ kg in L, NL, and NP women, respectively. Body fat as a percentage of body weight differed significantly among the groups in that it was significantly greater in L and NL than in NP women at the first four study visits. This proportion decreased significantly in all three groups during the first four study visits.

Body weight, lean body mass, body fat, and the proportions of lean body mass and body fat to body weight did not differ significantly among the three groups of women at visit 5, nor did they change significantly between visits 4 and 5. Total body weight loss between 6 and 52 wk postpartum, consisting entirely of body fat, averaged $-2.9 \pm 5.6$ and $-1.9 \pm 3.4$ kg in L and NL women, respectively. Nevertheless, body weight of L and NL women was $5.2 \pm 9.3\%$ and $7.7 \pm 5.7\%$ greater, respectively, at 52 wk postpartum, than prepregnancy weight ($P < 0.05$). The changes in body weight and body fat were not associated with maternal age, parity, prepregnancy weight, or pregnancy weight gain. Total body weight of NP women showed an insignificant increase of 0.77 kg between the first and fifth study visit.

Triceps, biceps, subscapular, and suprailiac skinfold thicknesses were significantly greater in L than in NP women at the first four study visits (Table 3). Triceps, biceps, subscapular, and suprailiac skinfold thicknesses of NL women were intermediate between those of L and NP women, but these differences were not significant, with the exception of the triceps skinfold thickness. Thigh skinfold thickness was significantly greater in L and NL than in NP women only at the first study visit. None of the skinfold thicknesses changed during the first four study visits except the suprailiac, which decreased significantly in NL but not L and NP women, and the thigh, which decreased significantly in L and NL but not NP women. None of the skinfold thicknesses differed significantly among L, NL, and NP women at the visit 5, nor did they decrease significantly between visits 4 and 5.
Dietary protein and energy intakes differed significantly among the groups of women in that the intakes of these nutrients were significantly greater in L than in NL and NP women (Table 4). Although protein and energy intakes of NL women were lower than those of NP women, these differences were significant only for energy at visits 2, 3, and 4. The protein and energy intakes of the three groups of women did not change significantly during the first four study visits.

Urinary nitrogen excretion, normalized for body weight, did not differ significantly between L and NL women, and averaged 11.2 ± 4.1, 11.3 ± 0.9, and 9.9 ± 2.1 mmol·kg⁻¹·d⁻¹ at 12, 18, and 24 wk postpartum, respectively, in L women and 9.2 ± 1.9, 9.2 ± 1.9, and 10.9 ± 3.6 mmol·kg⁻¹·d⁻¹ at 12, 18, and 24 wk postpartum, respectively, in NL women. Control values for L women who consumed known dietary protein intakes of 1.5 and 1.0 g·kg⁻¹·d⁻¹ in a metabolic setting averaged 12.7 and 9.9 mmol·kg⁻¹·d⁻¹, respectively (5, 6), whereas values for NL women who consumed known dietary protein intakes of 1.0 g·kg⁻¹·d⁻¹ averaged 9.3 mmol·kg⁻¹·d⁻¹ (5).

EI:BMR differed significantly among the groups of women in that the ratio was significantly greater in L than in NL women, but not in NP women between visits 1 and 3, and not at visit 4. These differences between the L and NL groups disappeared when energy losses in milk were subtracted from the energy available from the diet and body fat stores. EI:BMR for the three groups of women did not change significantly during the first four study visits. EI:BMR of the L and NP groups approximated 1.35, a cutoff value reported to be compatible with food records reflective of habitual dietary intakes, whereas the ratios of the NL group approximated 1.27, a cutoff value that represents energy intakes associated with minimum survival requirements (11).

The amount of milk produced by L women averaged 773 g/d between 6 and 24 wk postpartum (Table 5). The amount of milk produced did not change significantly as lactation progressed. The total nitrogen, protein nitrogen, and nonprotein nitrogen concentrations of milk showed the expected, significant decrease during the postpartum period. As a result, milk protein output declined significantly as lactation progressed. Milk production, when adjusted for prepregnancy weight, tended to show a positive relation (P = 0.08) with dietary energy intake, but not with protein intake, lean body mass, or body fat (data not shown), between 6 and 24 wk postpartum. Milk protein output, when adjusted for prepregnancy weight, did not show a relation with dietary protein or energy intake (data not shown).

### DISCUSSION

Few studies have examined the impact of the metabolic challenge imposed by lactation on the maintenance of lean body mass in well-nourished women (16–19). As a result, the contribution of the mobilization of lean body mass to milk production and milk protein output has not been documented. In the present study, we quantified the changes in body composition, especially the lean body mass component as opposed to dietary protein or energy intake, but not with protein intake, lean body mass, or body fat (data not shown), between 6 and 24 wk postpartum. Milk protein output, when adjusted for prepregnancy weight, did not show a relation with dietary protein or energy intake (data not shown).

### TABLE 3

<table>
<thead>
<tr>
<th>Skinfold thickness (mm)</th>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triceps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>25 ± 5*</td>
<td>26 ± 6*</td>
<td>26 ± 7*</td>
<td>27 ± 8*</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>NL</td>
<td></td>
<td>24 ± 4*</td>
<td>23 ± 3*</td>
<td>23 ± 4*</td>
<td>23 ± 4b</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>NP</td>
<td></td>
<td>17 ± 6b</td>
<td>18 ± 7b</td>
<td>17 ± 7b</td>
<td>18 ± 8b</td>
<td>19 ± 12</td>
</tr>
<tr>
<td><strong>Biceps</strong></td>
<td></td>
<td>11 ± 4a</td>
<td>11 ± 4a</td>
<td>12 ± 6a</td>
<td>12 ± 7a</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>10 ± 4ab</td>
<td>9 ± 2ab</td>
<td>9 ± 2ab</td>
<td>9 ± 2ab</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>NL</td>
<td></td>
<td>7 ± 4ab</td>
<td>6 ± 4ab</td>
<td>7 ± 4ab</td>
<td>7 ± 3b</td>
<td>8 ± 4</td>
</tr>
<tr>
<td><strong>Subscapular</strong></td>
<td></td>
<td>23 ± 6a</td>
<td>22 ± 6a</td>
<td>23 ± 8</td>
<td>24 ± 9</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>20 ± 4ab</td>
<td>19 ± 4ab</td>
<td>18 ± 5</td>
<td>20 ± 6b</td>
<td>22 ± 9</td>
</tr>
<tr>
<td>NL</td>
<td></td>
<td>15 ± 5b</td>
<td>15 ± 6b</td>
<td>16 ± 8</td>
<td>16 ± 9b</td>
<td>19 ± 14</td>
</tr>
<tr>
<td><strong>Suprailiac</strong></td>
<td></td>
<td>24 ± 6a</td>
<td>25 ± 7a</td>
<td>23 ± 9a</td>
<td>24 ± 8a</td>
<td>23 ± 10</td>
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<td>L</td>
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<td>20 ± 6ab</td>
<td>17 ± 6ab</td>
<td>16 ± 6b</td>
<td>15 ± 4b</td>
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<tr>
<td>NL</td>
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<td>15 ± 8b</td>
<td>15 ± 7b</td>
<td>16 ± 9b</td>
<td>17 ± 9b</td>
<td>14 ± 7</td>
</tr>
<tr>
<td><strong>Thigh</strong></td>
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<td>48 ± 11a</td>
<td>46 ± 14</td>
<td>47 ± 15</td>
<td>45 ± 13</td>
<td>42 ± 13</td>
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<tr>
<td>L</td>
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<td>47 ± 8a</td>
<td>45 ± 8</td>
<td>41 ± 9</td>
<td>40 ± 10</td>
<td>38 ± 9</td>
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<tr>
<td>NL</td>
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<td>36 ± 9b</td>
<td>36 ± 11</td>
<td>35 ± 12</td>
<td>36 ± 13</td>
<td>33 ± 13</td>
</tr>
</tbody>
</table>

* × ± SD; n = 10. Values in the same column with different superscript letters are significantly different, P < 0.05 (Fisher’s least significant differences test).

1, 2, 3, 4 Repeated-measures ANOVA; 1 P for group effect < 0.05, 2 P for time trend < 0.05, 3 P for interaction < 0.05, 4 P for group effect < 0.06.
LEAN BODY MASS OF LACTATING WOMEN

In the present study, lean body mass was preserved in well-nourished L women who breast-fed their infants exclusively between 6 and 24 wk postpartum even though there was a small, progressive loss of body weight throughout the period of lactation. This observation presumably reflects the finding that the L women consumed 55% more protein and 40% more energy than NL women, although other possibilities may include differences in activity pattern between groups or a failure to return to prepregnancy weight during the 1-y period of observation. The higher dietary protein intakes of the L women were sustained even as milk protein output declined by 32% between 6 and 24 wk postpartum. The protein needs for milk production represent quantitatively a small proportion (<3%) of maternal body protein turnover, and hence, of dietary protein needs (20). We assume that the additional protein intakes of L women may be related to the metabolic needs associated with the partitioning of dietary protein into milk proteins or other nonprotein components of human milk.

It is noteworthy that the lean body mass of the L women in the present study was preserved at dietary protein intakes that approximated those in our previous studies in which we documented negative nitrogen balances (5, 6). Although protein intakes of 1.4 g·kg⁻¹·d⁻¹, as recorded in the present study, were generous and exceeded the recommended dietary allowance for L women by 40% (2), we showed previously that mechanisms of adaptation, including lower rates of protein synthesis and degradation, were invoked at these habitual intakes of dietary protein (20), presumably to conserve the lean body mass of L women.

The absence of a significant relation between maternal lean body mass and milk production in the present study further supports the observation that the nutritional status of the mother was maintained during lactation at this intake of dietary protein. Although many studies have documented a general tendency toward body weight loss during lactation (13, 16, 17, 19, 21–28), the contribution of the changes in lean body mass to body weight loss in these women has rarely been reported (16–19). Loss of lean body mass during lactation has been predicted because of the general expectation of maternal protein deposition during pregnancy (3, 29). However, our study showed that the lean body mass of well-nourished L women was preserved throughout 6 mo of exclusive breast-feeding and was not altered during weaning. Nevertheless, the preservation of lean body mass throughout lactation has not been observed uniformly (16, 17). Studies using ⁴₀K counting methods similar to ours suggested that ~67%, or 3.1 kg, of the postpartum weight loss that occurred in L women between 1 wk and 6 mo postpartum was made up of lean body mass (16, 17). Others, using more indirect techniques such as urinary creatinine excretion, did not find losses of lean body mass in L women (19).

### TABLE 4
Dietary intakes of lactating (L), nonlactating postpartum (NL), and nulliparous (NP) women

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Visit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>95 ± 28a</td>
<td>97 ± 10a</td>
<td>100 ± 21a</td>
<td>93 ± 22a</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>59 ± 18a</td>
<td>54 ± 1P</td>
<td>58 ± 17b</td>
<td>55 ± 22b</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>66 ± 10b</td>
<td>63 ± 12b</td>
<td>66 ± 15b</td>
<td>65 ± 15b</td>
<td></td>
</tr>
<tr>
<td>Protein (g·kg⁻¹·d⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1.37 ± 0.53a</td>
<td>1.42 ± 0.39a</td>
<td>1.45 ± 0.36a</td>
<td>1.39 ± 0.45a</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>0.88 ± 0.27b</td>
<td>0.85 ± 0.14b</td>
<td>0.92 ± 0.23b</td>
<td>0.85 ± 0.31b</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>1.09 ± 0.26b</td>
<td>1.05 ± 0.29b</td>
<td>1.07 ± 0.25b</td>
<td>1.08 ± 0.32b</td>
<td></td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>8.97 ± 1.43a</td>
<td>9.44 ± 1.02a</td>
<td>9.75 ± 1.31a</td>
<td>8.55 ± 1.19a</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>5.65 ± 2.49b</td>
<td>5.16 ± 1.68b</td>
<td>5.35 ± 1.73b</td>
<td>5.52 ± 1.66b</td>
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</tr>
<tr>
<td>NP</td>
<td>6.88 ± 0.76b</td>
<td>6.75 ± 1.12c</td>
<td>7.21 ± 1.26c</td>
<td>7.25 ± 0.95c</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ·kg⁻¹·d⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>129 ± 33a</td>
<td>138 ± 29a</td>
<td>142 ± 29a</td>
<td>125 ± 38a</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>88 ± 38b</td>
<td>84 ± 21b</td>
<td>84 ± 25b</td>
<td>88 ± 33b</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>113 ± 33b</td>
<td>109 ± 25c</td>
<td>117 ± 17c</td>
<td>117 ± 21a</td>
<td></td>
</tr>
<tr>
<td>EI:BMR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1.78 ± 0.46a</td>
<td>1.89 ± 0.42a</td>
<td>1.96 ± 0.48a</td>
<td>1.77 ± 0.59</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>1.27 ± 0.53b</td>
<td>1.20 ± 0.39b</td>
<td>1.27 ± 0.42b</td>
<td>1.33 ± 0.64</td>
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</tr>
<tr>
<td>NP</td>
<td>1.53 ± 0.41b</td>
<td>1.50 ± 0.36b</td>
<td>1.58 ± 0.34b</td>
<td>1.62 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>EI:BMR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1.33 ± 0.37</td>
<td>1.48 ± 0.34</td>
<td>1.52 ± 0.33</td>
<td>1.37 ± 0.43</td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>1.27 ± 0.53</td>
<td>1.20 ± 0.39</td>
<td>1.27 ± 0.42</td>
<td>1.33 ± 0.64</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>1.53 ± 0.41</td>
<td>1.50 ± 0.36</td>
<td>1.58 ± 0.34</td>
<td>1.62 ± 0.37</td>
<td></td>
</tr>
</tbody>
</table>

1 ± SD; n = 10. Values in the same column with different superscript letters are significantly different, P < 0.05 (Fisher's least significant differences test).

2 Repeated-measures ANOVA: P for group effect < 0.001.

EI:BMR represents the ratio of the sum of the energy intake (EI, MJ/d) available from the diet and from endogenous body fat stores lost between 6 and 24 wk postpartum and of basal metabolic rate (BMR, kJ/d) estimated to be 75 kJ·kg⁻¹·d⁻¹. L 1 represents energy intake before milk energy losses removed; L 2 represents energy intake after milk energy losses removed.
Nevertheless, our study is limited insofar as the amount of dietary protein needed to achieve nitrogen balance in L women is related, in part, to their dietary energy intake, and hence, energy balance, a factor that we have not measured in the present study. The effect of energy intake on nitrogen balance is complicated further by the fact that the range of ad libitum dietary protein intakes in the L group was not broad enough over a prolonged period to detect the potential consequences of dietary protein deprivation or excess on lean body mass in L women. Thus, we were unable to prove our hypothesis that the loss of lean body mass in L women would be inversely proportional to their dietary protein intakes, but only can infer that the consumption of protein and energy intakes of 1.4 g·kg⁻¹·d⁻¹ and 134 kJ·kg⁻¹·d⁻¹, respectively, was sufficient to maintain lean body mass in L women.

The use of food records to estimate dietary intakes has been criticized on the grounds that this technique may not accurately represent actual food consumption. However, the 24-h urinary nitrogen excretion data, a measure of the nutritional adequacy of dietary protein intake (30), showed nitrogen losses in the present study that paralleled values measured concomitantly with dietary protein intake (30), showed nitrogen losses in the present study that paralleled values measured concomitantly with dietary protein intake (30). The absence of a difference between L and NL women in the absence of measurable changes in lean body mass, body fat losses explained entirely the changes in body weight of L women. Nevertheless, although the L women consumed less energy than the current recommended dietary allowance of 11.3 MJ/d for lactation (2), the observation that the multiples of energy intake and BMR, after adjustment for milk energy output, of L women approximated those of the NP group suggests that the energy cost of milk production in our L women was limited to the energy secreted in milk. Thus, dietary energy needs of L women parallel their milk energy output. In contrast, the low dietary protein and energy intakes observed frequently in NL women are thought to represent underreporting or active dieting (21), as evidenced by the low multiples of energy intake and basal metabolic rate that fell within the range of minimum “survival requirement” (11). Thus, the dietary intakes of the NL women in the present study may be underestimated by 20–25% using the Physical Activity Level method, equivalent to 24–32 kJ·kg⁻¹·d⁻¹, and at best, should approximate the intakes of the NP women.

Although the focus of the present study was on maternal body protein stores, in the absence of measurable changes in lean body mass, body fat losses explained entirely the changes in body weight of L women. The amount of weight lost by L women in the present study, 0.27 kg/mo, was considerably less than that reported in some (13, 21–23, 27, 28), but not all (17, 24), studies of L women during the first 6 mo of breast-feeding. In the present study, weight and compositional measurements were excluded during the first 6 wk postpartum because this period is associated with rapid weight loss (17, 19, 22, 25), presumably in conjunction with mobilization of body water. Once lactation is well established, further weight loss associated with tissue mobilization shows a direct relation with dietary energy intakes (17, 19, 21, 24, 28), as was the case in our L women. Nevertheless, although the L women consumed less energy than the current recommended dietary allowance of 11.3 MJ/d, they were still heavier 1 y after pregnancy than before they became pregnant. The weight loss of L women in the present study paralleled that of their NL counterparts, presumably because milk energy output accounted for the differences in dietary energy consumption between the two groups. Others have suggested that weight loss is quantitatively similar, although the pattern of early weight mobilization may differ, between L and NL women (21, 22, 27). The absence of a difference between L and NL women in the relation between body fat and dietary energy intake, after accounting for milk energy output, supports our expectation that the postpartum changes in body weight of L and NL women represent the

### Table 5

#### Milk production and composition of lactating women

<table>
<thead>
<tr>
<th>Milk constituent</th>
<th>Visit</th>
<th>Amount produced (g/d)</th>
<th>Total nitrogen (mg/g)</th>
<th>Protein nitrogen (mg/g)</th>
<th>Nonprotein nitrogen (mg/g)</th>
<th>Protein output (g/d)</th>
<th>Lactose (g/L)</th>
<th>Fat (g/L)</th>
<th>Energy (kJ/g)</th>
<th>Energy output (MJ/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>Slope&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Amount produced (g/d)</td>
<td>843 ± 160</td>
<td>778 ± 115</td>
<td>758 ± 215</td>
<td>714 ± 207</td>
<td>−7 ± 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen (mg/g)</td>
<td>2.00 ± 0.18</td>
<td>1.71 ± 0.15</td>
<td>1.66 ± 0.17</td>
<td>1.62 ± 0.17</td>
<td>−0.02 ± 0.01&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein nitrogen (mg/g)</td>
<td>1.63 ± 0.17</td>
<td>1.38 ± 0.15</td>
<td>1.34 ± 0.16</td>
<td>1.30 ± 0.16</td>
<td>−0.02 ± 0.01&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonprotein nitrogen (mg/g)</td>
<td>0.38 ± 0.04</td>
<td>0.33 ± 0.03</td>
<td>0.32 ± 0.03</td>
<td>0.31 ± 0.04</td>
<td>0.004 ± 0.002&lt;sup&gt;2&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein output (g/d)</td>
<td>8.5 ± 1.4</td>
<td>6.7 ± 1.4</td>
<td>6.3 ± 1.7</td>
<td>5.7 ± 1.4</td>
<td>−0.18 ± 0.13&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose (g/L)</td>
<td>62.9 ± 2.7</td>
<td>63.3 ± 3.3</td>
<td>64.1 ± 2.1</td>
<td>65.4 ± 2.5</td>
<td>0.14 ± 0.18&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (g/L)</td>
<td>32.3 ± 9.8</td>
<td>33.2 ± 7.1</td>
<td>38.5 ± 8.0</td>
<td>33.7 ± 7.0</td>
<td>0.15 ± 0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/g)</td>
<td>2.8 ± 0.4</td>
<td>2.8 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>2.8 ± 0.03</td>
<td>0.002 ± 0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy output (MJ/d)</td>
<td>2.37 ± 0.41</td>
<td>2.13 ± 0.27</td>
<td>2.25 ± 0.59</td>
<td>1.99 ± 0.56</td>
<td>−0.02 ± 0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> T ± SD; n = 10.
<sup>2</sup> Slope represents group mean of the amount each variable changed between 6 and 24 wk postpartum in lactating women.
<sup>3</sup> Significantly different from zero, P < 0.05.
overall metabolic response in energy balance, regardless of the reproductive status of the mother.

Regional differences in body fat loss among postpartum women have been documented; body fat is mobilized most readily in the lower body (13, 16, 21, 22). The thigh (22, 32) and suprailiac (13, 21) have been cited as the areas of greatest fat loss in L and NL women. In the present study, the thigh was the major site of fat mobilization in not only L, but also NL, women. Fat mobilization is thought to occur most readily in the thigh region because lipoprotein lipase activity is reduced in the peripheral adipose tissues of postpartum women, thereby permitting increased rates of lipolysis at this site (33).

Prepregnancy weight in relation to body mass index and pregnancy weight gain are considered to be two of the major determinants of weight loss during the postpartum period (25, 27). In the present study, L and NL women had normal body mass indexes (34). Both groups gained similar amounts of weight during pregnancy and subsequently lost similar amounts of body weight during the postpartum period. In general, each pregnancy is thought to contribute ≈1 kg of retained weight in well-nourished women (35–37). As a group, L and NL women in the present study weighed ≈3.1 and 4.7 kg, respectively, more than their prepregnancy weight at 1 y postpartum. Only two L and two NL women reached their prepregnancy weight within this time period. The aging process itself increases the body mass index by ≈0.15 units/y (38). The increase in the body mass index of NP women in the present study by 0.14 in 1 y, equivalent to a weight gain of 0.4 kg/y, represents only 10% of the actual weight retained by L and NL women. Thus, these observations support the concern that reproduction is an antecedent to maternal obesity in later life (35–37).

In summary, the preservation of lean body mass throughout lactation in well-nourished women suggests that the metabolic needs of milk protein production can be met solely by the maternal diet. However, the consumption of dietary protein in amounts that exceed by 40% the amount needed for milk protein output raises questions about the partitioning of dietary protein during lactation. The discrepancy between the maternal diet and milk protein output may reflect the unique metabolic needs associated with the partitioning of dietary protein into the components of human milk.

We thank the nursing and dietary staff of the Metabolic Research Unit, the US Department of Agriculture, Agricultural Research Service Children’s Nutrition Research Center, and the General Clinical Research Center, Texas Children’s Hospital, for study support; R Shypailo and J Posada for technical support; S Vaidya and M Thotathuchery for laboratory analyses; and K Fraley and EO Smith for statistical support.

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30. Motil KJ, Opekun AR, Montandon CM, Berthold HK, Davis TA, Klein PD, Reeds PJ. Leucine oxidation changes rapidly after dietary protein intake is altered in adult women but lysine flux is unchanged as is lysine incorporation into VLDL-apolipoprotein B-100. J Nutr 1994;124:41–51.


