Lean body mass of well-nourished women is preserved during lactation[1–5]

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ABSTRACT To determine whether the lean body mass of well-nourished women was mobilized to support milk protein output 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 $g \cdot kg^{-1} \cdot d^{-1}$ were associated with negative nitrogen balance 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 intakes. 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 ± 1.5 wk between 6 and 24 wk postpartum (visits 1–4) and at 52 wk postpartum (visit 5). NP women were studied four times at intervals of 6 ± 1.5 wk (visits 1–4) and again 34 wk later (visit 5), corresponding with 52 wk postpartum for the L and NL women. Body composition was determined at all visits; dietary intakes of all subjects and lactational performance of L subjects were determined at each 6-wk visit. Body composition was measured by clinical anthropometry and whole-body potassium (^{40}K) counting. Dietary intakes were determined from 3-d food records during each 6-wk period by using a nutrient database and were verified for accuracy with 24-h urine collections tested for nitrogen excretion. Milk production was measured during each 6-wk period while the L women were breast-feeding exclusively by the test-weighing procedure; milk composition was determined by chemical analysis.

Anthropometry

Height was measured to the nearest 0.01 m with a wall-mounted stadiometer with a movable headpiece (Holtain Limited, Crymych, United Kingdom). Body weight was measured to the nearest 0.1 kg with an electronic balance (Health-O-Meter, model 451KL; Continental Scale Corp, Bridgeview, IL). Body mass index (kg/m^2) was calculated from height and weight measurements. Prepregnancy weight and maximum pregnancy weight gain were obtained by recall and verified with obstetric records. Midupper arm and thigh circumferences were measured with a metal tape. Measurements of skinfold thickness were taken at five sites—biceps, triceps, subscapular, suprailiac, and thigh—by one individual using calibrated skinfold calipers (Lange, Cambridge Scientific Industries, Inc, Cambridge, MD).

Whole-body potassium

We measured ^{40}K by whole-body counting of natural ^{40}K in the body (7, 8). With the subject in a supine position, the body's natural gamma signal at 1.46 MeV was recorded through use of 30 NaI detectors arranged in two arrays, one above and one below the subject. The gamma signal was directly proportional to the amount of potassium in the body. Lean body mass was calculated from whole-body potassium, assuming a constant relation of 2.355 g K/kg lean body mass (9, 10). Body fat was calculated as the difference between body weight and lean body mass.

Dietary intakes

Each subject was instructed by the research dietitian to record all food and beverage consumption on predesigned forms for 3 d, including 1 weekend day, at each 6-wk visit. The amount of liquids and the size of food portions consumed at each meal during the 3-d period were reported in household measurements by using liquid or dry measuring cups, measuring spoons, and rulers. When a portion of the food or beverage was not consumed, the residual was measured and recorded. All subjects were instructed to continue

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

Characteristic	Group of women		
	Lactating (n = 10)	Nonlactating postpartum (n = 10)	Nulliparous (n = 10)
Race-ethnicity (W:A:H:O)[2]	6:1:2:1	5:4:1:0	7:2:1:0
Age (y)	31.3 ± 3.9[3]	29.8 ± 3.8	29.0 ± 4.9
Height (m)	1.65 ± 0.09	1.62 ± 0.05	1.68 ± 0.06
Weight (kg)	72.2 ± 14.4	67.7 ± 6.5	62.5 ± 9.1
Body mass index (kg/m^2)	26.3 ± 4.0[a]	25.8 ± 2.2[a]	22.2 ± 2.7[b]
Transthyretin (mg/L)	26.8 ± 2.5	28.6 ± 6.2	24.9 ± 5.0
Parity	1.2 ± 0.4[a]	2.2 ± 1.2[b]	—
Prepregnancy weight (kg)	66.1 ± 14.3	61.1 ± 5.9	—
Pregnancy weight gain (kg)	17.7 ± 4.1	17.3 ± 4.5	—

[1] Values in the same row with different superscript letters are significantly different, $P < 0.05$ (ANOVA).
[2] W, white; A, African American; H, Hispanic; O, Asian American.
[3] $\bar{x}$ ± 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 nutrient database (NUTRITIONIST III, version 5.0, N-Squared Computing, Salem, OR) by one individual. The dietary protein and energy intakes of each individual were averaged for each 3-d period from the dietary records by using the nutrient database.

To assess the accuracy of dietary intake reporting, we measured 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 concentrated hydrochloric acid. Portions of urine samples were stored at $-20\,^{\circ}C$ until analyzed for total nitrogen concentrations. Total daily nitrogen excretion was estimated from the amount of urine 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 accuracy 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:

$$\frac{\text{EI}}{\text{BMR}} = \frac{(E_d + E_{bf}) - E_m}{\text{BMR}}$$

where E_d (MJ/d) is the energy available from the diet as reported in the food records; E_{bf} (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 $\approx$38 kJ/g; E_m is the energy output from milk; and basal metabolic rate is equivalent to 75 $kJ \cdot kg^{-1} \cdot d^{-1}$ 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 balance (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 $-70\,^{\circ}C$ 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-Kjeldahl method (model 1030; Tecator, Höganas, Sweden). The energy 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 modified 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 $\pm$ SDs. Analysis of variance was performed to determine differences ($P < 0.05$) 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 skinfold thicknesses; and intakes of dietary protein, energy, carbohydrate, 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 determine 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 composition, or *4*) body composition and dietary intake. Linear regression was performed to determine relations between the change in body composition (lean body mass and body fat) and reproductive characteristics (prepregnancy 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

TABLE 2
Body weight, lean body mass, and body fat of lactating (L), nonlactating postpartum (NL), and nulliparous (NP) women[1]

Body composition variable	Visit 1	2	3	4	5
Postpartum age (wk)					
L	5.7 ± 0.6	11.9 ± 1.3	17.7 ± 0.7	23.9 ± 0.9	52.0 ± 1.6
NL	5.9 ± 0.6	12.3 ± 0.9	18.8 ± 1.5	24.4 ± 0.8	53.6 ± 1.7
NP	—	—	—	—	—
Body weight (kg)[2,3]					
L	72.2 ± 14.4	71.2 ± 14.1	71.2 ± 14.8	71.0 ± 15.1	69.2 ± 14.9
NL	67.7 ± 6.6	66.5 ± 7.0	65.4 ± 6.5	64.9 ± 7.4	65.8 ± 7.3
NP	62.5 ± 9.1	62.4 ± 9.3	62.4 ± 9.2	62.1 ± 9.9	62.9 ± 9.0
Lean body mass (kg)					
L	45.9 ± 3.7	46.1 ± 4.0	46.4 ± 4.5	46.1 ± 4.8	46.1 ± 4.1
NL	46.0 ± 4.4	46.5 ± 4.1	45.8 ± 4.3	46.4 ± 5.0	47.3 ± 4.5
NP	47.0 ± 3.1	47.2 ± 3.9	47.9 ± 3.4	47.5 ± 3.5	47.5 ± 3.6
Lean body mass (% body wt)[2,4]					
L	65 ± 9[a]	66 ± 9[a]	67 ± 9[a]	67 ± 10[a]	68 ± 10
NL	68 ± 5[a]	70 ± 6[ab]	70 ± 6[a]	72 ± 7[a,b]	72 ± 8
NP	76 ± 8[b]	76 ± 7[b]	78 ± 9[b]	79 ± 10[b]	76 ± 9
Body fat (kg)[2,5]					
L	26.3 ± 11.5[a]	25.1 ± 10.8[a]	24.8 ± 10.9[a]	24.8 ± 11.5	23.1 ± 11.2
NL	21.7 ± 4.6[a,b]	20.0 ± 53[a,b]	196 ± 51[a,b]	185 ± 57	18.5 ± 6.7
NP	15.8 ± 7.3[b]	15.2 ± 6.3[b]	14.6 ± 7.3[b]	14.5 ± 8.4	15.4 ± 7.5
Body fat (% body wt)[2,4]					
L	35 ± 9[a]	34 ± 9[a]	33 ± 9[a]	33 ± 10[a]	32 ± 10
NL	32 ± 5[a]	30 ± 6[a,b]	30 ± 6[a]	28 ± 7[ab]	28 ± 8
NP	24 ± 8[b]	24 ± 7[b]	22 ± 9[b]	22 ± 10[b]	24 ± 9

[1] $\bar{x}$ ± SD; n = 10. Values in the same column with different superscript letters are significantly different, $P < 0.05$ (Fisher's least significant differences test).
[2–5] Repeated-measures ANOVA: [2] P for group effect < 0.001, [3] P for time trend < 0.05, [4] P for time trend < 0.01, [5] P for time trend < 0.001.

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 ± 3.1, −2.8 ± 4.1, and −0.5 ± 2.9 kg in L, NL, and NP women, respectively. The average body weight of L and NL women was 9.7 ± 5.8% and 10.9 ± 5.5% greater than prepregnancy weight, respectively, at 6 wk postpartum ($P <$ 0.01), and 7.7 ± 7.1% and 6.2 ± 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 ± 1.8, −3.2 ± 3.1, and −0.9 ± 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 ± 5.6 and −1.9 ± 3.4 kg in L and NL women, respectively. Nevertheless, body weight of L and NL women was 5.2 ± 9.3% and 7.7 ± 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.

TABLE 3
Skinfold thickness of lactating (L), nonlactating postpartum (NL), and nulliparous (NP) women[1]

Skinfold thickness (mm)	Visit 1	2	3	4	5
Triceps[2]					
L	25 ± 5[a]	26 ± 6[a]	26 ± 7[a]	27 ± 8[a]	25 ± 6
NL	24 ± 4[a]	23 ± 3[a]	23 ± 4[a]	23 ± 4[a,b]	24 ± 6
NP	17 ± 6[b]	18 ± 7[b]	17 ± 7[b]	18 ± 8[b]	19 ± 12
Biceps[2]					
L	11 ± 4[a]	11 ± 4[a]	12 ± 6[a]	12 ± 7[a]	12 ± 6
NL	10 ± 4[a,b]	9 ± 2[ab]	9 ± 2[ab]	9 ± 2[a,b]	9 ± 3
NP	7 ± 4[b]	6 ± 4[b]	7 ± 4[b]	7 ± 3[b]	8 ± 4
Subscapular[2]					
L	23 ± 6[a]	22 ± 6[a]	23 ± 8	24 ± 9[a]	24 ± 10
NL	20 ± 4[a,b]	19 ± 4[ab]	18 ± 5	20 ± 6[ab]	22 ± 9
NP	15 ± 5[b]	15 ± 6[b]	16 ± 8	16 ± 9[b]	19 ± 14
Suprailiac[2,3,4]					
L	24 ± 6[a]	25 ± 7[a]	23 ± 9[a]	24 ± 8[a]	23 ± 10
NL	20 ± 6[a,b]	17 ± 6[a,b]	16 ± 6[a,b]	15 ± 4[b]	15 ± 5
NP	15 ± 8[b]	15 ± 7[b]	16 ± 9[b]	17 ± 9[a,b]	14 ± 7
Thigh[3,5]					
L	48 ± 11[a]	46 ± 14	47 ± 15	45 ± 13	42 ± 13
NL	47 ± 8[a]	45 ± 8	41 ± 9	40 ± 10	38 ± 9
NP	36 ± 9[b]	36 ± 11	35 ± 12	36 ± 13	33 ± 13

[1] $\bar{x}$ ± SD; n = 10. Values in the same column with different superscript letters are significantly different, $P < 0.05$ (Fisher's least significant differences test).
[2–5] Repeated-measures ANOVA: [2] P for group effect < 0.05, [3] P for time trend < 0.05, [4] P for interaction < 0.05, [5] P for group effect < 0.06.

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 measured by ^{40}K, in free-living women who breast-fed their infants exclusively for the first 6 mo postpartum. We monitored the change in body composition while the women consumed an ad libitum diet because of the ethical constraints associated with the potential long-term consequences for women and their infants of controlled, and possibly inadequate, dietary intakes. We expected to find that there were losses in body weight associated with lactation, that we could measure the contribution of lean body mass and body fat to weight loss, and hence, that we could estimate the potential contribution of maternal body protein stores—as opposed to dietary

TABLE 4
Dietary intakes of lactating (L), nonlactating postpartum (NL), and nulliparous (NP) women[1]

Nutrient	Visit 1	2	3	4
Protein (g/d)[2]				
L	95 ± 28[a]	97 ± 10[a]	100 ± 21[a]	93 ± 22[a]
NL	59 ± 18[a]	54 ± 1 l[b]	58 ± 17[b]	55 ± 22[b]
NP	66 ± 10[b]	63 ± 12[b]	66 ± 15[b]	65 ± 15[b]
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)[2]				
L	1.37 ± 0 53[a]	1.42 ± 0 39[a]	1.45 ± 0.36[a]	1.39 ± 0.45[a]
NL	0.88 ± 0.27[b]	0.85 ± 0.14[b]	0.92 ± 0.23[b]	0.85 ± 0.31[b]
NP	1.09 ± 0.26[a,b]	1.05 ± 0.29[b]	1.07 ± 0.25[b]	1.08 ± 0.32[a,b]
Energy (MJ/d)[2]				
L	8.97 ± 1.43[a]	9.44 ± 1.02[a]	9.75 ± 1.31[a]	8.55 ± 1.19[a]
NL	5.65 ± 2.49[b]	5.16 ± 1.68[b]	5.35 ± 1.73[b]	5.52 ± 1.66[b]
NP	6.88 ± 0.76[b]	6.75 ± 1.12[c]	7.21 ± 1.26[c]	7.25 ± 0.95[c]
Energy ($kJ \cdot kg^{-1} \cdot d^{-1}$)[2]				
L	129 ± 33[a]	138 ± 29[a]	142 ± 29[a]	125 ± 38[a]
NL	88 ± 38[b]	84 ± 21[b]	84 ± 25[b]	88 ± 33[b]
NP	113 ± 33[a,b]	109 ± 25[c]	117 ± 17[c]	117 ± 21[a]
EI:BMR[2,3]				
L_1	1.78 ± 0.46[a]	1.89 ± 0.42[a]	1.96 ± 0.48[a]	1.77 ± 0.59
NL	1.27 ± 053[b]	1.20 ± 039[b]	1.27 ± 0.42[b]	1.33 ± 0.64
NP	1.53 ± 0.41[a,b]	1.50 ± 0.36[b]	1.58 ± 0.34[a,b]	1.62 ± 0.37
EI:BMR[3]				
L_2	1.33 ± 0.37	1.48 ± 0.34	1.52 ± 0.33	1.37 ± 0.43
NL	1.27 ± 0.53	1.20 ± 0.39	1.27 ± 0.42	1.33 ± 0.64
NP	1.53 ± 0.41	1.50 ± 0.36	1.58 ± 0.34	1.62 ± 0.37

[1] $\bar{x}$ ± 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.
[3] 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 $kJ \cdot kg^{-1} \cdot d^{-1}$. L_1 represents energy intake before milk energy losses removed; L_2 represents energy intake after milk energy losses removed.

protein intake—to milk production and milk protein output.

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 $g \cdot kg^{-1} \cdot d^{-1}$, 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 ^{40}K 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 5
Milk production and composition of lactating women[1]

Milk constituent	Visit 1	2	3	4	Slope[2]
Amount produced (g/d)	843 ± 160	778 ± 115	758 ± 215	714 ± 207	−7 ± 15
Total nitrogen (mg/g)	2.00 ± 0.18	1.71 ± 0.15	1.66 ± 0.17	1.62 ± 0.17	−0.02 ± 0.01[3]
Protein nitrogen (mg/g)	1.63 ± 0.17	1.38 ± 0.15	1.34 ± 0.16	1.30 ± 0.16	−0.02 ± 0.01[3]
Nonprotein nitrogen (mg/g)	0.38 ± 0.04	0.33 ± 0.03	0.32 ± 0.03	0.31 ± 0.04	0 004 ± 0.002[3]
Protein output (g/d)	8.5 ± 1.4	6.7 ± 1.4	6.3 ± 1.7	5.7 ± 1.4	−0.18 ± 0.13[3]
Lactose (g/L)	62.9 ± 2.7	63.3 ± 3.3	64.1 ± 2.1	65.4 ± 2.5	0.14 ± 0.18[3]
Fat (g/L)	32.3 ± 9.8	33.2 ± 7.1	38.5 ± 8.0	33.7 ± 7.0	0.15 ± 0.41
Energy (kJ/g)	2.8 ± 0.4	2.8 ± 0.3	3.0 ± 0.3	2.8 ± 0.03	0.002 ± 0.018
Energy output (MJ/d)	2.37 ± 0.41	2.13 ± 0.27	2.25 ± 0.59	1.99 ± 0.56	−0.02 ± 0.04

[1] $\bar{x}$ ± SD; n = 10.
[2] Slope represents group mean of the amount each variable changed between 6 and 24 wk postpartum in lactating women.
[3] Significantly different from zero, $P < 0.05$.

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 by the observation that the same energy intake may be appropriate to achieve nitrogen balance for one individual, but not another, depending on factors such as body size, physical activity, and the amount and composition of milk produced. Our study 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 $g \cdot kg^{-1} \cdot d^{-1}$ and 134 $kJ \cdot kg^{-1} \cdot d^{-1}$, 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 intakes of known protein content (5,6). Thus, on the basis of the pattern of urinary nitrogen excretion, we concluded that the dietary records of our L women were reasonable approximations of their actual food consumption. This conclusion was supported further by assessing the availability of energy from the diet, as well as from body fat stores, expressed as a multiple of BMR (11). Using this method, the dietary intakes of our L women, when adjusted for milk energy outputs, were consistent with reports of habitual dietary intakes for sedentary women, although their true dietary intakes may have been underestimated by between 6% and 12%, equivalent to 7–14 $kJ \cdot kg^{-1} \cdot d^{-1}$, as calculated by the Physical Activity Level method (31). The dietary protein and energy intakes of L women were higher throughout the first 6 mo postpartum than those of their NL and NP counterparts and were well above the recommended dietary protein allowance of 1.0 $g \cdot kg^{-1} \cdot d^{-1}$, but not the energy 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 $kJ \cdot kg^{-1} \cdot d^{-1}$, 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

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.

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