Insulin, Cortisol and Thyroid Hormones Modulate Maternal Protein Status and Milk Production and Composition in Humans[1,2]

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ABSTRACT The partitioning of dietary and endogenous nutrients during lactation is not well understood. To examine associations between plasma hormone and substrate profiles and indices of either maternal body protein metabolism or lactational performance, we measured plasma insulin, cortisol, prolactin, thyroxine, triiodothyronine, individual amino acid, blood urea nitrogen, and prealbumin concentrations in lactating and nulliparous women in the postabsorptive state. We related these measurements to the subjects' nitrogen balance, urinary 3-methylhistidine excretion, [1-^{13}C]leucine metabolism and milk production. Insulin concentrations showed significant positive relationships with nitrogen balance and prealbumin concentrations; cortisol levels showed a significant negative relationship with nitrogen balance and a significant positive relationship with leucine incorporation into protein. Thyroid hormone concentrations showed significant positive relationships with urinary 3-methylhistidine excretion, leucine incorporation into protein, and milk production. Proline concentrations were associated positively with nitrogen balance and negatively with leucine incorporation into protein, whereas glutamate-glutamine concentrations showed positive associations with leucine oxidation and milk nitrogen concentrations. We propose that insulin and cortisol modulate the channeling of nutrients between anabolic and anti-anabolic aspects of maternal body protein metabolism, whereas thyroid hormones and cortisol modulate nutrient partitioning toward milk production and visceral protein synthesis. We suggest that some nonessential amino acids (proline, glutamate-glutamine) may become limiting during lactation because of their unique contributions to milk protein synthesis. J. Nutr. 124: 1248–1257, 1994.

INDEXING KEY WORDS:

- *lactation* • *insulin* • *cortisol*
- *humans* • *protein metabolism*

Our recent studies have focused on the response of maternal body protein metabolism and milk production to alterations in dietary protein throughout lactation (Motil et al. 1989a, 1989b and 1990). Contrary to expectations, when mothers consumed dietary protein in amounts that approximated recommended dietary allowances (1.0 $g \cdot kg^{-1} \cdot d^{-1}$), they were in significant negative nitrogen balance, despite the fact that the level of intake exceeded considerably the amount of protein secreted in milk. Moreover, the associated reduction in urinary 3-methylhistidine excretion suggested that the level of dietary protein had induced a metabolic adaptation that promoted the conservation of muscle protein stores during lactation.

The homeorhetic (Bauman and Currie 1980) mechanisms that regulate the allocation of dietary protein to milk production or to the maintenance of

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the tissue protein mass of lactating women have not been identified in any detail. Presumably these regulatory aspects are endocrinologic in origin and are associated with alterations in substrate availability (Hart et al. 1978). Although prolactin is thought to play a pivotal role, growth hormone, glucocorticoids, insulin and thyroid hormones also have been implicated in many mammalian species (Hart et al. 1978). Of the hormones studied, prolactin, insulin and cortisol are presumed to be the primary regulators of nutrient partitioning because they show meal- and nutrient-related (Schultz et al. 1987, Carlson 1989) responsiveness.

To improve our understanding of the partitioning of dietary and endogenous nutrients during lactation, we designed the present study to examine the hormone and substrate status of lactating women throughout the postpartum period and to determine whether significant relationships exist between maternal hormonal status and indices of body protein metabolism, milk production and composition, or habitual dietary intake. We made these measurements in women in the postabsorptive state, reasoning that because milk production is maintained during this condition, changes in the hormonal milieu of lactating women would be maximized to facilitate the partitioning of nutrients away from maternal body stores toward the mammary gland for milk production. We hypothesized that insulin and the thyroid hormones would favor anabolic responses with respect to maternal body protein metabolism and milk production, and that the reverse would be true for cortisol. Moreover, we hypothesized that individual amino acids might behave as limiting nutrients and therefore would be inversely associated with indices of body protein metabolism.

METHODS

Subjects. Twelve healthy lactating women and four nulliparous controls were studied. Their average (mean ± SD) ages, heights and weights were 28.0 ± 3.3 vs. 30.7 ± 3.3 y, 166.4 ± 8.1 vs. 164.9 ± 6.8 cm and 63.0 ± 11.6 vs. 60.8 ± 4.1 kg, respectively. The lactating women were divided into three groups of four each, based on postpartum time: 1.5 ± 0.2 mo (L_1), 5.4 ± 0.8 mo (L_5) and 12.2 ± 0.6 mo (L_{12}). Before studies were begun, each individual underwent a review of her medical history, a physical examination, and a routine laboratory evaluation. Informed written consent was obtained from each individual. The study 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.

Study design. All lactating and nulliparous women received a constant, controlled diet of measured protein and energy content for 7 d. The subjects were admitted to the Texas Children's Hospital General Clinical Research Center during the last 4 d of the controlled diet. During the admission, milk production was measured for 72 h by the test weighing procedure, and a 24-h aliquot of milk was obtained on d 7 for determination of milk composition. Ninety-six–hour urine and fecal collections were obtained to determine nitrogen balance and rates of urinary creatinine and 3-methylhistidine excretion. [1-^{13}C]Leucine infusion studies were performed with the women in the postabsorptive state on d 7. Plasma hormone and substrate profiles were obtained simultaneously during the infusion studies.

Dietary intakes. All subjects were instructed by the research nutritionist to keep a written record of their food intakes for 3 d including one weekend day. After completion, each subject's food record was reviewed by the nutritionist. Habitual energy intakes were calculated from these records using standard food tables (Gebhardt and Matthews 1986). These estimates formed the basis for the level of dietary energy provided throughout the controlled diets.

The controlled diet consisted of a commercial formula and pudding (Sustacal, Mead Johnson, Evansville, IN) designed to provide protein levels of 1.5 $g \cdot kg^{-1} \cdot d^{-1}$ and energy levels that approximated each individual's habitual intake as determined from the food records (Motil et al. 1990). To promote compliance, fruits and vegetables low in protein were added to the diet in an amount <10% of the total daily energy intake. All subjects consumed four isocaloric, isonitrogenous meals at 0800, 1200, 1700 and 2100 h using standard metabolic techniques (Motil et al. 1990) throughout the 7 d of the experiment. Aliquots of the formula and pudding were stored at –20°C until analysis for nitrogen and energy content. Measured nutrient contents of the formula and pudding were used to calculate actual protein and energy intake during the balance period.

Milk production. Total daily milk production was estimated from the sum of the amount of milk consumed by the infant, as determined by test weighing before and after each feeding (Butte et al. 1984) using an electronic balance (model 3862MP, Sartorius, Göttingen, Germany), and the amount expressed from the breast by mechanical pumping (Egnell, Cary, IL). A 24-h milk sample was prepared from pooled aliquots of milk collected from alternate breasts at sequential feedings (Garza et al. 1986). Milk samples were stored at –20°C until analysis for total nitrogen and energy content.

Nitrogen balance. Ninety-six–hour nitrogen balances were determined from the nitrogen content of food, maternal milk, urine and feces (Motil et al. 1989a). Twenty-four–hour urine samples were collected daily in bottles containing 5 mL of concentrated hydrochloric acid. Fecal samples, demarcated

by a carmine marker administered orally at the beginning and the end of the balance period, were collected continuously. Aliquots of urine and fecal samples were stored at –20°C until analysis for their nitrogen content. Unmeasured nitrogen losses were assumed to be 5 $mg \cdot kg^{-1} \cdot d^{-1}$ (Calloway et al. 1971).

Amino acid kinetic studies. A primed, constant infusion of sterile, pyrogen-free (Pyrogent^R, Mallinckrodt, St. Louis, MO) [1-^{13}C]leucine was administered for 4 h to all subjects in the unfed state (Motil et al. 1989a). At 0800 h, after withholding of food for 12 h, the isotopic solutions were administered intravenously by means of a calibrated infusion pump (model 351, Sage Instruments, Cambridge, MA) as follows: $NaH^{13}CO_3$ and [1-^{13}C]leucine, 2.0 μmol/kg each, as priming doses, followed by [1-^{13}C]leucine (2.2 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) as the infusion dose. Heparinized venous blood samples were obtained at timed intervals before and during the infusion for the determination of the ^{13}C enrichment of plasma free leucine. Breath samples were obtained simultaneously by means of a resuscitation face mask fitted with a two-way nonrebreathing valve (Hans Rudolph, Kansas City, MO) to determine the ^{13}C enrichment of expired CO_2. Four additional breath samples were collected at hourly intervals by means of a respirometer to determine total CO_2 production. The variables of whole-body amino acid metabolism were calculated from conventional tracer dilution expressions and the fractional recovery of CO_2 based on the isotopic enrichments of plasma free leucine and expired CO_2 in breath samples as described previously (Motil et al. 1989a).

3-Methylhistidine excretion. Twenty-four–hour urine collections were obtained during the meat-free balance period for the measurement of 3-methylhistidine and creatinine. The fractional rate of muscle protein breakdown was estimated from the output of 3-methylhistidine per unit of creatinine excreted, assuming that the latter is a measure of skeletal muscle mass (Graystone 1968). The rate of skeletal muscle protein breakdown was estimated from 3-methylhistidine excretion, assuming a value of 4.2 μmol of protein-bound 3-methylhistidine per gram of mixed protein in skeletal muscle (Bilmazes et al. 1978). The contribution of skeletal muscle to whole-body protein breakdown was derived from the amino acid kinetic studies, assuming that the content of leucine in body tissues is 3.69 mmol per gram of nitrogen and that 16% of body protein is composed of nitrogen (Motil et al. 1989a).

Hormone and substrate profiles. Heparinized venous blood samples were obtained from subjects in the postabsorptive state during the isotope infusions for the determination of hormone and substrate levels. The specific hormones or substrates and the time of their sampling during the isotopic infusion included the following: glucose, insulin and blood urea nitrogen, 120 and 240 min; prolactin, 90 and 180 min; cortisol and triiodothyronine, 150 and 210 min; thyroxine, 60 and 195 min; prealbumin, 60 min; plasma amino acids, 240 min. Both prolactin samples were obtained at least 2 h after the infant had nursed and 0.5 h after both breasts were emptied by mechanical expression. All samples were placed immediately on ice, then centrifuged (698 × *g*) at 4°C for 20 min. Plasma was separated and stored at –70°C until analysis for individual hormone and substrate concentrations.

Analytic techniques. Nitrogen in the food that was consumed during the experiment and in maternal milk, urine and feces was determined by the micro-Kjeldahl method (model 1030, Tecator, Högänäs, Sweden). Milk and food energy was estimated by adiabatic bomb calorimetry (model 1241, Parr, Moline, IL). Urinary creatinine was measured by enzymatic hydrolysis using standard automated techniques (Ektachem 400, Eastman-Kodak, Rochester, NY). Plasma amino acids and urinary 3-methylhistidine were measured by automated ion-exchange chromatography using ninhydrin detection (model 121-MB, Beckman, Anaheim, CA) after deproteinization with 0.458 mol/L sulfosalicylic acid and centrifugation (2795 × *g*) at 4°C for 10 min. Glucose was measured by the glucose oxidase method (model 27, Yellow Springs Instruments, Yellow Springs, OH). Blood urea nitrogen was measured by the urease method (Sigma Diagnostics, St. Louis, MO). Prealbumin was measured by rocket electrophoresis (Biorad, Richmond, CA). Insulin, thyroxine (Micromedic Systems, Horsham, PA), cortisol, triiodothyronine (ICN Biomedicals, Carson, CA) and prolactin (Hybritech, San Diego, CA) were measured by commercially available radioimmunoassays. The CV of all of these assays was <5%.

The ^{13}C enrichment of plasma free leucine was determined by gas chromatographic-mass spectrometric isotope radiometry (Finnigan MAT 212, San Jose, CA). The $^{13}C/^{12}C$ ratio of respired CO_2 was measured by gas-isotope-ratio mass spectrometry (Nuclide 3″ – 60× SecTorr Nuclide/MAASC, Bellefonte, PA). The CO_2 concentration of breath samples was analyzed by gas solid chromatography (Carle III, Carle Instruments, Anaheim, CA). Total CO_2 production was calculated from CO_2 concentration and respiratory volume after correction for room temperature and barometric pressure.

Statistical analysis. All plasma hormones (prolactin, insulin, cortisol, thyroxine, triiodothyronine) and substrates (glucose, blood urea nitrogen) measured at two different time points in each individual were not significantly different from each other by paired *t* tests and were averaged for subsequent data analyses. All data were analyzed by a standardized computer package for descriptive statistics and are expressed as means ± SD (Minitab,

TABLE 1

Fasting hormone and substrate profiles in lactating women throughout the first postpartum year and in nulliparous women[1]

	Group[2]			
	L_1	L_5	L_{12}	NL
Hormone				
Prolactin, μg/L	83 ± 79	21 ± 3	19 ± 8	6 ± 2[a]
Insulin, *pmol/L*	22 ± 9	25 ± 8	22 ± 6	30 ± 6
Cortisol, *nmol/L*	192 ± 43	254 ± 77	232 ± 72	338 ± 221
Thyroxine, *nmol/L*	67 ± 9	81 ± 17	65 ± 9	82 ± 14
Triiodothyronine, *nmol/L*	1.4 ± 0.1	1.7 ± 0.4	1.4 ± 0.1	1.5 ± 0.2
Substrate				
Glucose, *mmol/L*	4.7 ± 0.3	4.4 ± 0.3	4.7 ± 0.2	4.7 ± 0.1
Blood urea nitrogen, *nmol/L*	5.3 ± 1.0	5.0 ± 1.5	4.5 ± 1.3	4.0 ± 0.4[b]
Prealbumin, *mg/L*	214 ± 34	231 ± 20	239 ± 48	210 ± 78
Urinary 3-methylhistidine, *μmol/mmol creatinine*	9.5 ± 1.7	11.1 ± 0.7	9.6 ± 1.3	11.0 ± 2.0

[1]Values are means ± SD, $n = 4$.

[2]L = lactating; subscripts indicate time postpartum: 1.5 ± 0.2 mo (L_1), 5.4 ± 0.8 mo (L_5), 12.2 ± 0.6 mo (L_{12}). NL = nulliparous. [a]$P < 0.01$, L vs. NL. [b]$P < 0.05$, L vs. NL.

version 5.1; Minitab, State College, PA). Linear regression was performed to determine significant ($P < 0.05$) differences among the groups of lactating women over postpartum time for the dependent variables (glucose, blood urea nitrogen, individual amino acids, prealbumin, urinary 3-methylhistidine, prolactin, insulin, cortisol, thyroxine and triiodothyronine). When differences were not detected, values for each of the dependent variables for all lactating women were combined. Two sample t tests were performed to determine significant ($P < 0.05$) differences between lactating and nonlactating women for plasma glucose, blood urea nitrogen, prealbumin, individual amino acids, prolactin, insulin, cortisol, thyroxine, triiodothyronine and urinary 3-methylhistidine. The Mann-Whitney test was used to detect significant ($P < 0.05$) differences between lactating and nonlactating women for plasma prolactin levels (Snedecor and Cochran 1980). Linear or quadratic regressions were performed to determine significant relationships between the independent hormone and substrate variables and the dependent variables associated with measures of body protein metabolism and maternal milk production. Data for maternal milk production and body protein metabolism were published previously (Motil et al. 1989a and 1989b).

RESULTS

Hormone and substrate profiles. Postabsorptive plasma hormone, glucose, urea and prealbumin concentrations and rates of urinary 3-methylhistidine excretion in the lactating and nulliparous women are shown in **Table 1**. Prolactin levels in the lactating women varied across postpartum time; levels were particularly high at 1 mo postpartum. Insulin, cortisol, thyroxine, triiodothyronine, glucose, blood urea nitrogen, prealbumin and urinary 3-methylhistidine excretion showed no significant variations across the stages of lactation. Prolactin and blood urea nitrogen values in the lactating women were significantly higher than those in the nulliparous women. No significant differences in insulin, cortisol, thyroxine, triiodothyronine, glucose, prealbumin or urinary 3-methylhistidine excretion were detected between the groups.

Postabsorptive amino acid profiles in the lactating and nulliparous women are shown in **Table 2**. Plasma valine was the only individual amino acid that showed a statistically significant decline over postpartum time among the groups of lactating women. However, the sum of the nonessential amino acids, although not the sum of the essential or total amino acids, showed a statistically significant decline over postpartum time. Threonine and tryptophan concentrations were significantly lower in the lactating women than in the nulliparous women. Significant differences were not detected in the other individual amino acids between the groups of women. However, the sum of the essential amino acids (threonine, methionine, isoleucine, leucine, valine, phenylalanine, tryptophan, lysine and histidine), but not the sum of the nonessential or total amino acids, was significantly lower in lactating women than in nulliparous women.

Relationships among plasma hormones, substrates and body protein metabolism. Significant relationships between individual postabsorptive plasma hormone concentrations and the variables of body

TABLE 2

Plasma amion acid profiles in lactating women throughout the first postpartum year and in nulliparous women[1]

	Group[2]			
	L_1	L_5	L_{12}	NL
		μmol/L		
Taurine	28 ± 7	44 ± 28	43 ± 17	35 ± 10
Threonine	135 ± 26	125 ± 9	126 ± 21	149 ± 13[a]
Serine	131 ± 40	118 ± 5	117 ± 35	111 ± 13
Glutamate-glutamine	605 ± 57	532 ± 25	520 ± 75	504 ± 87
Proline	289 ± 93	250 ± 83	212 ± 87	284 ± 24
Glycine	359 ± 157	243 ± 43	243 ± 32	184 ± 63
Alanine	364 ± 85	320 ± 27	300 ± 75	355 ± 43
Citrulline	34 ± 10	31 ± 6	26 ± 4	31 ± 14
Valine	277 ± 65[b]	272 ± 14	218 ± 21	275 ± 17
Methionine	20 ± 3	23 ± 3	22 ± 6	20 ± 5
Isoleucine	67 ± 23	63 ± 5	57 ± 6	69 ± 8
Leucine	136 ± 39	126 ± 10	117 ± 12	129 ± 18
Tyrosine	87 ± 20	84 ± 21	63 ± 19	81 ± 10
Phenylalanine	57 ± 15	62 ± 3	55 ± 7	72 ± 12
Tryptophan	38 ± 2	42 ± 7	40 ± 11	53 ± 8[a]
Ornithine	82 ± 37	67 ± 17	65 ± 19	79 ± 33
Lysine	259 ± 77	242 ± 43	236 ± 65	276 ± 57
Histidine	81 ± 21	86 ± 11	87 ± 27	100 ± 3
Arginine	92 ± 30	104 ± 14	106 ± 27	98 ± 14
Total essential	1071 ± 254	1041 ± 79	961 ± 152	1143 ± 41[a]
Total nonessential	2073 ± 183[b]	1795 ± 109	1695 ± 268	1765 ± 230
Total amino acids	3144 ± 416	2835 ± 181	2657 ± 407	2909 ± 245

[1]Values are means ± SD, $n = 4$.

[2]L = lactating; subscripts indicate time postpartum: 1.5 ± 0.2 mo (L_1), 5.4 ± 0.8 mo (L_5), 12.2 ± 0.6 mo (L_{12}). NL = nulliparous. [a]$P < 0.05$, L vs. NL. [b]$P < 0.05$, concentration vs. postpartum time.

protein metabolism in the lactating women are summarized in **Table 3**. Insulin showed significant positive associations with plasma proline, nitrogen balance and prealbumin concentrations, and cortisol showed significant negative associations with plasma proline concentration and nitrogen balance. The ratio of insulin to cortisol, however, showed a significant curvilinear relationship with nitrogen balance (**Fig. 1**) and a significant linear relationship with prealbumin concentrations (**Fig. 2**). Cortisol and triiodothyronine concentrations showed significant positive associations with rates of whole-body leucine incorporation,

TABLE 3

Relationships between measures of body protein metabolism and plasma hormones in lactating women at 1, 5, and 12 mo postpartum

Dependent variable (*y*)	Independent variable (*x*)	Significance (*P*)	Correlation (*r*)
Plasma amino acid			
Proline	Insulin	<0.01	0.64
Proline	Cortisol	<0.01	–0.69
Glycine	Insulin	<0.05	–0.52
Nitrogen balance	Insulin	<0.01	0.65
	Cortisol	<0.05	–0.51
Leucine incorporation into protein	Cortisol	<0.05	0.51
	Triiodothyronine	<0.05	0.50
Prealbumin	Insulin	<0.01	0.73
3-Methylhistidine excretion	Thyroxine	<0.01	0.73
	Triiodothyronine	<0.05	0.54

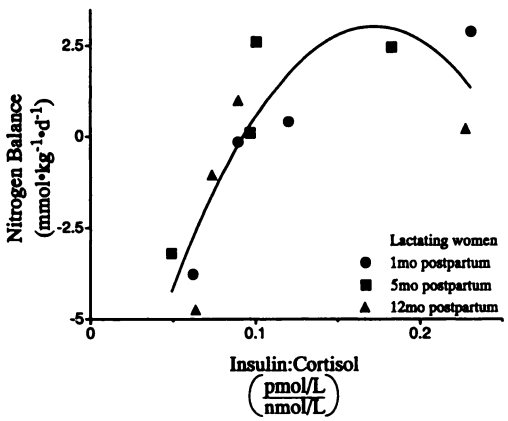

FIGURE 1 Nitrogen balance vs. postabsorptive plasma insulin:cortisol ratio in lactating women at 1, 5, and 12 mo postpartum [$y = -11.1 - 478x^2 + 164x$, $P < 0.01$, $r = 0.81$; units are mmol·kg^{-1}·d^{-1} for y and pmol/nmol for x].

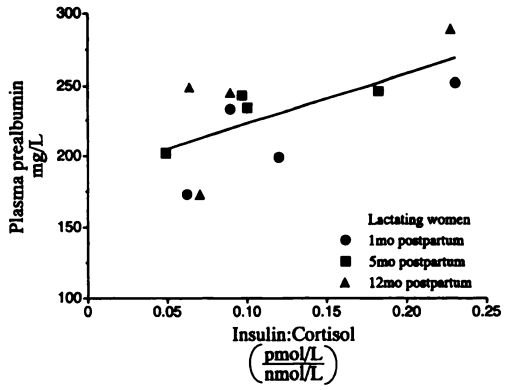

FIGURE 2 Plasma prealbumin vs. postabsorptive plasma insulin:cortisol ratio in lactating women at 1, 5, and 12 mo postpartum [$y = 187 + 357x$, $P < 0.02$, $r = 0.61$; units are mg/L for y and pmol/nmol for x].

and thyroxine and triiodothyronine showed significant positive relationships with urinary 3-methylhistidine (Table 3). None of the other hormones measured in this study showed significant relationships with these variables of body protein metabolism in the lactating women.

Significant relationships between postabsorptive plasma concentrations of substrates and body protein metabolism in the lactating women are summarized in **Table 4**. Plasma proline concentrations showed a significant positive association with nitrogen balance and significant negative associations with leucine flux and incorporation into protein. Blood urea nitrogen and plasma citrulline concentrations also showed significant negative associations with leucine incorporation into protein. Plasma glutamine-glutamate concentration showed a significant positive relationship with leucine oxidation. Plasma glycine concentration showed significant negative associations with plasma prealbumin (Table 4) and insulin concentrations (Table 3). None of the other substrates showed significant relationships with the variables of body protein metabolism in the lactating women.

Relationships among plasma hormones, substrates, and milk production and composition. Significant relationships between plasma hormones or substrates and milk production or composition in the lactating women are summarized in **Table 5**. Thyroxine, triiodothyronine, plasma alanine concentrations and urinary 3-methylhistidine excretion showed significant positive associations with the amount of milk produced. Plasma glutamine-glutamate and glycine concentrations showed significant positive associations with milk nitrogen concentration. None of the other hormones or substrates showed significant relationships with milk production or composition in the lactating women.

Relationships among diet and plasma hormones and substrates. Dietary energy intakes showed negative associations with blood urea nitrogen ($P < 0.05$, $r = -0.61$) and plasma citrulline ($P < 0.05$, $r =$

TABLE 4

Relationships between measures of body protein metabolism and plasma substrates in lactating women at 1, 5, and 12 mo postpartum

Dependent variable (y)	Independent variable (x)	Significance (P)	Correlation (r)
Nitrogen balance	Proline	<0.05	0.58
Whole-body protein metabolism			
Flux (leucine)	Proline	<0.01	–0.73
Incorporation (leucine)	Proline	<0.05	–0.56
	Citrulline	<0.05	–0.57
	Blood urea nitrogen	<0.01	–0.68
Oxidation (leucine)	Glutamine-glutamate	<0.05	0.52
Prealbumin	Glycine	<0.05	–0.53

TABLE 5

Relationships between milk production and composition and plasma hormones and substrates in lactating women at 1, 5, and 12 mo postpartum

Dependent variable (*y*)	Independent variable (*x*)	Significance (*P*)	Correlation (*r*)
Amount produced (*g/d*)	Thyroxine	<0.05	0.57
	Triiodothyronine	<0.05	0.61
	3-Methylhistidine	<0.05	0.62
	Alanine	<0.05	0.54
Nitrogen (*μmol/g*)	Glutamine-glutamate	<0.05	0.55
	Glycine	<0.05	0.63

–0.41) concentrations and showed a positive relationship with plasma alanine concentration ($P < 0.05$, $r = 0.61$) in the lactating women. No significant relationships were detected between dietary energy or protein intakes and other substrate or hormone concentrations.

DISCUSSION

The initiation and maintenance of lactation pose a metabolic dilemma for the lactating woman insofar as her nutrient needs must be balanced against those required for milk production. Although the nutrients that support lactation are derived either from the diet or from maternal body stores, the endocrinologic mechanisms that regulate the relative contribution of these two sources to milk in well-nourished lactating women are unknown. Although the importance of these endocrinologic factors may be readily apparent for some ruminant and nonruminant species, there is no assurance that these factors necessarily apply to the regulation of the partitioning of nutrients between maternal body stores and milk production in humans.

In the present study we examined the protein nutritional status of lactating women from several perspectives. We measured nitrogen balance (Waterlow et al. 1978) and blood urea nitrogen concentrations (Jackson et al. 1984) to assess the overall adequacy of the diet in the support of maternal body protein needs and milk production. We assumed that plasma prealbumin concentration (Socolow et al. 1965) and urinary 3-methylhistidine excretion (Bilmazes et al. 1978) reflected the anabolic activity of the liver and the rate of catabolism of myofibrillar proteins, respectively, in response to alterations in the adequacy of the maternal diet. Furthermore, we assumed that under conditions of relative dietary protein insufficiency, depressed plasma amino acid concentrations would indicate their potentially limiting status for protein synthesis (Yoshida et al. 1966). These indices of body protein metabolism were measured in the context of the hormonal milieu of the individual in the postabsorptive state. We assumed that the postabsorptive state reflected a period of metabolic "stress" that would exaggerate the adaptive metabolic responses that support body protein metabolism and milk production in the mother.

The results of our study demonstrated that, on a group basis, few differences existed in postabsorptive plasma hormone and substrate profiles between lactating and nulliparous women. The exceptions were the significantly higher plasma prolactin and blood urea nitrogen concentrations and the significantly lower total plasma essential amino acid concentration in the lactating group. A more detailed analysis of the data from the lactating women, however, showed statistically significant relationships among plasma hormone and substrate profiles, indices of body protein status, and milk production and composition, irrespective of habitual dietary intake. More specifically, plasma insulin and cortisol concentrations or their ratios showed positive relationships with nitrogen balance, plasma prealbumin concentrations, or rates of leucine incorporation into protein. Thyroid hormone concentrations, on the other hand, were related positively to myofibrillar protein catabolic activity, rate of leucine incorporation into protein, and milk production. Selected amino acids, including proline, glutamate-glutamine, glycine and alanine, were associated individually with insulin or cortisol concentration, nitrogen balance, indices of body protein metabolism, plasma prealbumin concentration, or milk production and composition. Thus, these observations underscore the interactive nature of the endocrine and nutrition status of the mother during lactation.

Hormonal modulation of lactation. Prolactin is essential for the initiation of lactation, but a regulatory role, with respect to the partitioning of nutrients into the mammary gland for milk production, is less likely (Mena et al. 1981). In the present study, prolactin

concentration was significantly higher in lactating women than in nulliparous women and tended to be higher during early, as opposed to later, well-established lactation. However, there was substantial interindividual variation in prolactin concentration, especially during early lactation, even though food was withheld from all women for 12 h and all milk samples were obtained at least 2 h after nursing. Nevertheless, the absence of long-term interrelationships between prolactin and either milk yield or composition suggested that once lactation has been initiated, prolactin is not active in the partitioning of nutrients towards milk synthesis in well-nourished women. Others, however, have suggested that prolactin may have a regulatory influence with respect to milk production and the disposition of dietary nutrients in poorly nourished mothers (Lunn et al. 1980).

In the present study, plasma insulin, cortisol and thyroxine concentrations, but not triiodothyronine concentration, tended to be lower in lactating women than in nulliparous women, although significance was not achieved. Furthermore, insulin and thyroid hormone concentrations did not change with postpartum time, whereas cortisol concentration tended to be lower in early, rather than later, stages of lactation. Similar patterns for insulin (Illingworth et al. 1986) cortisol (Sartin et al. 1988) and the thyroid hormones (Hart et al. 1978, Iwatani et al. 1987) have been documented in adult women and other mammalian species. These findings are consistent with the expectation that, during lactation, depressed plasma hormone concentrations in the mother permit the partitioning of nutrients into milk rather than maternal body stores (Jones et al. 1984).

In the present study, plasma thyroxine and triiodothyronine were the only hormones among those measured that showed positive relationships with milk production. These findings are in contrast with those in cattle in which plasma thyroxine concentrations were inversely related to milk yield (Hart et al. 1978). Although the administration of thyroid-releasing hormone has been associated with increased milk production in women, this response resulted primarily from augmented prolactin concentration rather than thyroxine or triiodothyronine release (Peters et al. 1991). Conversely, the thyroid hormone concentrations in the present study were not associated with milk nutrient concentrations.

We detected no relationships in the present study between insulin or cortisol and milk production or composition. Nonetheless, others have shown that insulin (Girard et al. 1987) and cortisol (Thatcher and Tucker 1970) enhance milk production in rats by increasing mammary gland cell number and metabolic activity, and by enabling the preferential partitioning of glucose (Jones et al. 1984) and individual amino acids (Viña and Williamson 1981) to the mammary gland while limiting the utilization of these nutrients by peripheral (e.g., liver, adipose) tissues. Whether the same would apply to humans remains a question unanswered by the present results.

Hormonal modulation of maternal body protein metabolism. In contrast to the relationships between maternal hormonal profiles and milk production, much less is known about the role of hormones in partitioning nutrients to maternal body stores during lactation. Among the hormones measured in the present study, only insulin and cortisol showed a relationship to all indices of body protein status in lactating women, even in the presence of relatively depressed plasma concentrations, and irrespective of dietary intakes. The positive relationship between insulin and nitrogen balance, as well as plasma prealbumin concentrations, supports an anabolic role for this hormone in the maintenance of maternal body protein stores, whereas the negative relationship between cortisol and nitrogen balance supports an antianabolic role. Others also have shown that during short-term fasting, insulin, albeit in pharmacologic doses, reduces muscle protein degradation in humans (Fukagawa et al. 1985) and enhances muscle protein synthesis in rodents (Garlick et al. 1983), whereas pharmacologic doses of cortisol depress muscle protein synthesis (Southorn et al. 1990). Furthermore, the strong correlation between the ratio of insulin and cortisol and nitrogen balance, as well as plasma prealbumin concentrations, further advances the idea of an integrated, highly regulated metabolic process with respect to the maintenance of body protein stores (Reeds et al. 1987).

Although a link has been suggested between glucocorticoids and wasting of peripheral tissue protein stores, we suggest that cortisol had an anabolic effect, presumably on hepatic protein synthesis, in the lactating women. The direct relationship between plasma cortisol level and rate of leucine incorporation into protein, rather than urinary 3-methylhistidine excretion, argued in favor of an anabolic, rather than catabolic, function for this hormone in women in the postabsorptive state. Others also have documented enhanced rates of hepatic protein synthesis during food deprivation in rodents (Southorn et al. 1990) when pharmacologic dosages of glucocorticoids have been administered. In the present study, there also were positive associations among thyroxine, triiodothyronine, rate of leucine incorporation into protein, and urinary 3-methylhistidine excretion, which, when taken together, suggested that these hormones similarly may have modulated both muscle protein degradation and hepatic protein synthesis during fasting in lactating women.

Amino acids and lactation. During lactation, plasma essential and nonessential amino acids reflect the balance between supply and demand for maternal

body protein metabolism and milk production. In this context, we do not propose that plasma amino acid concentrations limited protein synthesis. Rather, we regard lower concentrations as an indication that demand has exceeded the available supply. In other words, the plasma amino acid concentration reflected a proportional limitation.

In the present study, the generally lower concentrations of plasma essential amino acids in lactating women, compared with those in nulliparous women, were compatible with their increased utilization for the synthesis of milk proteins. On the other hand, although essential amino acids are partitioned into milk production throughout lactation, the nitrogen component of nonessential amino acids may become a limiting nutrient as lactation progresses, as reflected in their significant decline over postpartum time. Two "nonessential" amino acids, glutamate-glutamine and proline, constitute approximately 19% and 17%, respectively, of the amino acid content of human milk caseins (Richardson et al. 1979). We presume that the availability of nonessential nitrogen is important to milk production because of the strong positive associations among plasma glutamate-glutamine concentration, milk nitrogen concentration, and rates of amino acid oxidation noted in the present study. In addition, we found significant associations between plasma proline concentration and insulin and cortisol concentrations, nitrogen balance, and amino acid incorporation into protein, which, when taken together, suggested that proline may be a limiting nutrient for overall maternal body protein synthesis. The importance of this speculation is that in women in the postabsorptive state, milk proteins relatively rich in proline are synthesized from other endogenous protein sources that are relatively poor in proline. Under these circumstances of enhanced need, the ability of adults to synthesize proline may be limited (Jaksic et al. 1991) and may lead to a relatively depressed plasma concentration. Similar observations have been made in dairy cows during early lactation when intense muscle protein breakdown is associated with low plasma proline concentration (Motyl and Barej 1986).

In summary, we propose that the hormonal status of the lactating women was the primary determinant of the allocation of endogenous substrates to meet the needs both of the mother and of milk production. Our findings suggest that, in women in the postabsorptive state, insulin and cortisol modulate the mobilization of endogenous nutrient stores, whereas thyroxine and triiodothyronine modulate the diversion of these nutrient stores to milk protein synthesis. We also contend that, under these conditions, individual nonessential amino acids may become limiting nutrients for protein synthesis.

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