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Relation of Protein Synthesis to Plasma and Cell Amino Acids in Neonates

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ABSTRACT. We hypothesized that more rapidly growing preterm infants would have higher rates of protein synthesis than term infants, and that protein synthesis would be more closely related to intracellular than plasma levels of amino acids. Neutrophils, used as a cell model, were isolated from 1–3 ml blood of 63 infants 27–44 wk postconceptual age. Protein synthesis (^{3}H-leucine incorporation, pmol/h/mg DNA), and 19 amino acids in the leukocytes (nmol/mg DNA) and plasma (nmol/ml) were quantified. Protein synthesis was related inversely to birth weight and gestational age, *i.e.* the smaller and more preterm the infant the higher the rate of protein synthesis. Multiple regression analysis limited to six steps indicated that some plasma amino acids (Val, Ile, Phe, Asp, Ala, Tau) accounted for a significant ($p = 0.03$), but relatively small, proportion, 23%, of the variance in protein synthesis. A greater proportion of the variance in protein synthesis was explained by a set of six intracellular amino acids (Leu, Met, Tyr, Gly, Ala, Tau), with $R^2 = 36\%$, $p = 0.001$. Further, multiple regression identified specific combinations of six plasma amino acids which best explained ("predicted") the levels of each intracellular amino acid predictor of protein synthesis ($R^2 = 0.4$–0.5, $p < 0.001$–0.0001). Activities of some rate-limiting glycolytic enzymes, pyruvate kinase and phosphofructokinase, were correlated with protein synthesis in the leukocytes ($p = 0.036$, and 0.002, respectively). Phosphofructokinase, the major regulating enzyme in glycolysis, also was negatively correlated with birth weight and gestational age. These data indicate that protein synthesis is higher in smaller neonates, and that its level can be predicted from levels of a few intracellular amino acids whose concentrations, in turn, can be predicted from specific sets of different plasma amino acids. (*Pediatr Res* 20: 140–146, 1986)

Abbreviations

LGA, large for gestational age
SGA, small for gestational age
AGA, appropriate for gestational age

The most rapid period of human growth occurs before birth. During the 2nd trimester the fetus increases in weight by about 1000%. After 36 wk gestation the growth rate declines somewhat, but the increment of weight is still about 10% per week. Postnatally, the rate continues to diminish, but by 4–5 months of age birth weight is approximately doubled. This pattern of growth implies that the rate of protein synthesis must be very rapid early in gestation and postnatally to form new cells and to maintain growth of existing cells. Since fetal growth rate reaches it zenith during the 3rd trimester, one may speculate that the rate of protein synthesis also might peak then and decline thereafter. Efforts to quantify rates of protein synthesis and turnover of infants have been made from nitrogen balance measurements and studies of whole body protein turnover using stable isotopes (1–3). These measurements provide an indirect assessment of protein metabolism and requirements. Plasma amino acid patterns also have been used to estimate protein metabolism; however, while they have been shown to reflect recent dietary intake (4, 5), their relation to protein synthesis and to intracellular amino acid pools is largely unexplored. This study was undertaken on the hypothesis that the rate of protein synthesis would not only be greater in small preterm than term infants, but also could be predicted from the intracellular levels of some substrate amino acids.

Classically, either muscle or liver cells have been used as models for protein and energy metabolism; however a biopsy of either tissue is traumatic and not without risk in a small or sick newborn. The circulating polymorphonuclear leukocyte has been used as an alternative, viable cell model. The major metabolic pathways are present in the polymorphonuclear granulocyte and its metabolism is similar to that of other nucleated cells. It derives its energy mainly from glycolysis (6), actively incorporates amino acids for protein synthesis (7) and reflects certain genetic enzyme defects (8) and nutritional biochemical changes (9, 10). It contains many energy-related enzymes, including pyruvate kinase and phosphofructokinase, which are rate-limiting in a major energy pathway of the fetus, glycolysis, and, in the case of pyruvate kinase, generate adenosine triphosphate. Whether the activities of these enzymes are related to the rates of protein synthesis in neonates is not known and also were investigated in this study.

SUBJECTS AND METHODS

Infants less than 44 wk (<308 days) postconceptual age were eligible for study. Seriously ill infants and those requiring respiratory support were excluded. Patient demographic characteristics are described in Table 1. Gestational age was estimated by the method of Dubowitz *et al.* (11). Expected birth weight, adjusted for sex, parity, and race was estimated from the Brenner nomogram (12). Infants whose actual birth weight was >500 g above or below the expected weight, according to the Brenner scheme, were classified as LGA or SGA, respectively. Those whose birth weights differed from the adjusted, expected weight by ≤500 g were considered AGA. At the time of study, infants

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Table 1. *Patient characteristics*

Classification* (*n*)		SGA (21)		AGA (40)		LGA (2)
Gestational Age (wk)	Range	33–41		27–41		42
	Mean	37.3		36.6		42
		Preterm†	Term†	Preterm	Term	Term
n		13	8	24	16	2
Mean birth wt (g)		1571	2416	1983	3406	4200
Mean wt at study (g)		1692	2406	2196	3295	4300
Mean age at study (days)		10	3	18	3	7
Mean postconceptual age (days)		254	280	253	281	287
Mean gestational age (weeks)		34.9	39.6	33.5	39.7	42

* Classification based on deviation of observed expected weight, adjusted for gestational age, parity, race, and baby's sex (12).

† Preterm = <38 wk gestation; term = ≥38 wk. There were no preterm large for gestational age infants.

ranged in postnatal age from 1 day to 14 wk, with 52 babies studied during the first 2 wk of life. The very low birth weight infants (<1000 g) were the oldest postnatally when studied, but still were <44 wk postconceptual age. Fifty-eight infants were receiving breast milk or commercial formula. Four infants (two term and two preterm, AGA) studied within 48 h of birth were receiving intravenous dextrose solutions. The values for protein synthesis in these infants were consistent with others of the same gestational age. One of the smallest prematures (920 g) was receiving only parenteral amino acid nutritional support. He had the highest value for protein synthesis in our study. Another larger premature SGA infant receiving formula with supplemental parenteral amino acid solution, also had a high value for protein synthesis. The outlying values for both babies were truncated to the upper limit of our normal frequency distribution so that these two values would not exert undue influence on the SDs of the mean protein synthesis value. Blood samples were obtained near the end of the usual between-feed interval. Informed consent was obtained from parents for all babies and the study was approved by the Institutional Review Board.

Our standardized leukocyte isolation technique (9, 13) has been modified to include a Ficoll-Hypaque gradient sedimentation step, to provide a more homogeneous and higher yield of granulocytes (14). One to three ml of heparinized blood is sedimented for 30 minutes in a 10% dextran (Sigma) solution in an ice bath. The leukocyte-rich plasma is further separated using Ficoll-Hypaque (Pharmacia Fine Chemicals). The washed leukocyte pellet is suspended in 0.16 M KCl. Trypan Blue exclusion testing and Wright's staining show >95% polymorphonuclear leukocytes, with >97% viable cells isolated by this technique.

Protein synthesis was determined as incorporation of 4,5^{3}H-leucine at 37° C for 10, 20 and 30 min into a 10% trichloracetic acid insoluble protein fraction of the granulocytes. Incorporation of the isotope remains linear during this interval. The rate is calculated from a least squares regression line fitted to the three points. The assay medium consisted of 100 μl saline containing 1% Eagle's minimum essential medium and 0.4% glucose; 100 μl of 1 mM $MgCl_2$ and 0.05 M tris-HCl (pH 7.9); and 1 μCi of labeled amino acid in 10 μl saline (1 Ci/mmol). The reaction was stopped with cold 10% trichloracetic acid and the precipitate filtered, washed, dried, and solubilized in scintillation fluid [4 g POP and 0.1 g POPOP/liter of "scintanalyzed" toluene (Fisher)] (15). The activity was counted in a Packard Scintillation Counter (52% efficiency) and the uptake of amino acid per hour calculated using the specific activity (1112 dpm/pmol) of ^{3}H-leucine. No correction was made for the intracellular pool of leucine, but the medium concentration was more than five times that of intracellular leucine, thus providing sufficient excess to minimize possible dilution effects (16). Leukocytes have a very short life span in the circulation and minimal or undetectable protein degradation (17); therefore the incorporation of the isotope can be considered to reflect protein synthesis.

Amino acid determinations were made on a Dionex D-300 high pressure Amino Acid Analyzer with automatic program controller and a 25 cm cation exchange column (Pickering Laboratories no. 7050), sodium eluents, post column O-phthalaldehyde derivatization, fluorometric detection, and peak integration by a Spectra-Physics Analyzer System One. Leukocyte suspensions were freeze-thawed twice and the protein precipitated with a final concentration of 0.6% sulfosalycylic acid. Norleucine was added to the supernatant as the internal standard. Plasma was similarly prepared for analysis except the freeze-thaw cycles were omitted and the final concentration of sulfosalicylic acid was 2%. Eluents were mixed in the laboratory from stock solutions supplied by Pickering Laboratories at five pH values ranging from 2.93 to 4.95.

Two column temperatures, 46 and 69° C, were used during a total analysis time of 205 min. A standard amino acid solution was analyzed as a reference between every five samples. Cystine is not measured by this method. The resolution of glutamine, serine, and asparagine is incomplete.

Leukocyte DNA was measured by the modified diphenylamine method described by Giles and Meyers (18). Leukocyte amino acid contents and protein synthesis were referenced to DNA content. To express relative intracellular amino acid concentrations, intracellular water was computed from the DNA content based on simultaneous measurements of intracellular water and DNA made in a separate study of leukocytes isolated from 15 normal neonates by a modification of the method of Baron and Ahmed (19), using ^{14}C inulin to correct for "trapped" water retained between pelleted cells. The intracellular amino acid concentrations of the study babies were computed from the regression of intracellular water on DNA derived from the separate study.

After sonication of an aliquot of cell suspension (Braun Sonic, 199 W, 10 s), the activities of pyruvate kinase and phosphofructokinase were measured spectrophotometrically at 30° C in a Gilford 250 spectrophotometer using a 1 cm light path with microcuvettes and total volume of 215 μl. Determination of pyruvate kinase activity was based on the coupled enzymatic conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase and subsequent reduction of pyruvate to lactate. Phosphofructokinase determination was based on the coupled reactions of fructose-6-phosphate to fructose 1,6 diphosphate by phosphofructokinase, conversion of fructose 1,6 diphosphate to dihydroxyacetone by aldolase and triose phosphate isomerase, and then reduction of dihydroxyacetone to glycerol-1-phosphate. After addition of sample to the appropriate reaction mixture the change in light absorption at 340 μ with oxidation or reduction of NADPH was recorded for 3 min using a strip chart recorder (Gilford 6051). The methods have been slightly modified from those reported from this laboratory (20).

Data were examined initially by a univariate frequency distribution analysis to detect outliers, all of which were checked. Three protein synthesis, four plasma and two cell amino acid measurements were truncated at the upper 2% of the distribution

to bring them closer to the range of the remaining values. All other data were untruncated. Two-tailed t tests, analysis of variance, product: moment correlations and multiple regression analyses were performed using Statisical Analysis Systems programs (21) with an IBM 3801 computer.

RESULTS

Protein synthesis. Complete data for protein synthesis were available in all 63 infants. Figure 1, a best fit curve drawn by computer, shows the inverse, polynomial relationship between birth weight and protein synthesis. The plot is a cubic regression and the equation is given in the legend of Figure 1. When the data were analyzed by type of baby (class = premature, SGA, term), the rate of protein synthesis was significantly higher (p = 0.0097) in preterm and SGA infants in comparison with term AGA and LGA infants (mean ± SD = 4740 ± 2327, n = 24 and 4356 ± 2266, n = 21 *versus* 2717 ± 1255, n = 18, respectively). The birth weights of the SGA and preterm infants were similar (1893 and 1983) and significantly less than the term babies (3496 g). The mean gestational age of the SGA infants was significantly greater than the preterms (36.7 *versus* 33.5 wk) but less than the AGA term babies (40.0 wk). There was a significant negative correlation between birth weight and protein synthesis (r = −0.49, p = 0.0001). Gestational age and weight at study also were inversely correlated with protein synthesis (r = −0.44, p = 0.0001, and r = −0.49, p = 0.0001, respectively). However, when protein synthesis for each type (class) of baby was adjusted for the combined influences of birth weight, gestational age, and weight at time of study, the differences between the groups were not statistically significant, indicating that these covariates exert an important influence on the rate of protein synthesis.

Intracellular water. Cellular water is slightly higher in leukocytes of infants (Table 2) than in adults (22), 68.1 *versus* 66.8% of wet weight corrected for trapped water. Intracellular water is correlated with DNA content: intracellular water (μl) = 0.530 + 0.074 DNA (μg); n = 15, r = 0.85, p = 0.0001.

Table 2. *Leukocyte cell water**

	Wt					
	Wet	Dry (mg)	ECW (μg)	ICW (μg)	ICW / DNA (μl/mg)	%ICW
Mean	7.77	2.41	0.09	5.27	77.7	68.1
SD					8.9	5.3

* Fifteen normal neonates. Birth weight = 3378 ± 370 (SD) g. Gestational age = 39.3 ± 1.3 (SD) wk. Post conceptual age at study = 277.5 ± 9.6 (SD) days. ECW, extracellular water = "trapped" water. ICW, intracellular water = cell water, corrected for trapped water. % intracellular water = intracellular water as percentage of wet weight.

Table 3. *Plasma and cell amino acid concentrations*

Amino acids	Plasma		Leukocyte	
	Mean	SE	Mean	SE
	nmol/ml (n = 57)		nmol/mg DNA	
Threonine	122	9	30	4
Valine	103	6	7	1
Methionine	34	3	3	0.5
Isoleucine	37	3	14	3
Leucine	73	4	20	3
Phenylalanine	46	2	6	1
Tryptophane	17	2	0.2	0.1
Lysine	104	7	29	4
Histidine	51	3	21	4
Arginine	49	4	11	2
Aspartic acid	10	1	69	7
GLS*	449	24	145	16
Glutamic acid	54	5	208	24
Citrulline	13	1	0.6	0.2
Glycine	207	10	171	22
Alanine	197	15	57	6
Tyrosine	84	6	7	1
Ornithine	67	6	52	9
Taurine	48	4	1334	93

* Glutamine, asparagine, serine.

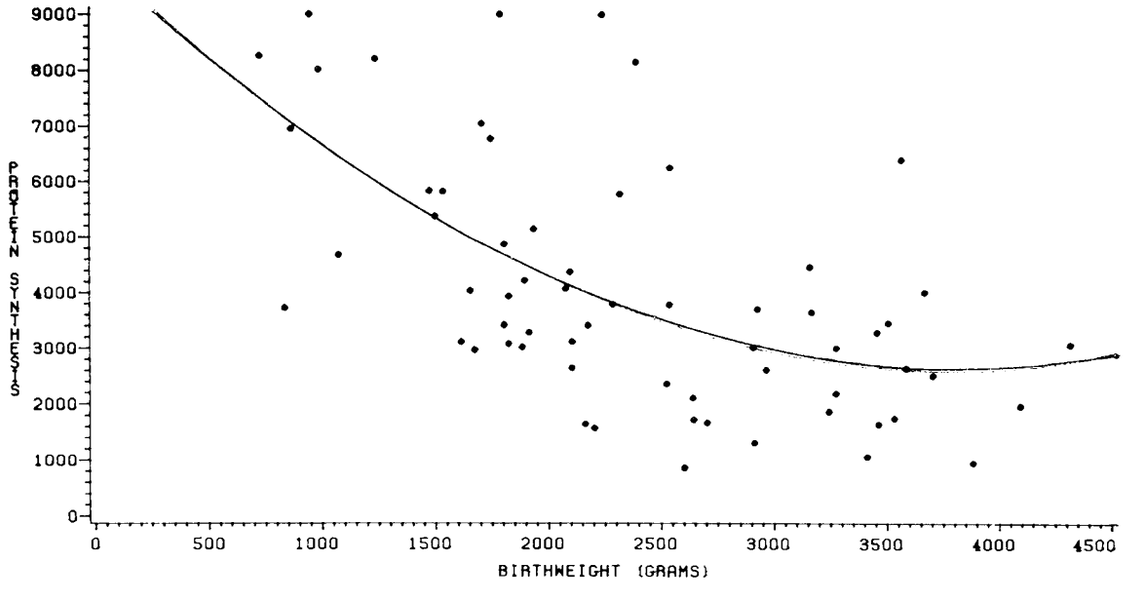

Fig. 1. Protein synthesis *versus* birth weight. Protein synthesis was measured as incorporation of 4,5-^{3}H-leucine, *in vitro* by viable, isolated leukocytes. Each *point* represents one infant. The curve represents a best-fit polynomial regression: protein synthesis = 9978 − 3.78 (birth weight) + 0.001 (birth weight)^2 − 9.58 (birth weight)^3. R^2 = 0.36; p = 0.0001; n = 63. The mean value for protein synthesis was 4067 ± 1763 (SD) pmol/h/mg DNA. The plot illustrates an inverse relationship between protein synthesis and birth weight. The mean birth weight and gestational age were 2385 ± 884 g (SD) and 36.4 ± 3.6 wk, respectively. The mean weight at the time of study (postconceptional age = 266 days) was 2466 ± 805 g.

Amino acids in plasma and cells. Mean values for the intracellular and plasma amino acid levels, obtained from the same blood sample in 57 of the 63 infants, are given in Table 3. Except for methionine, which was lower in SGA than AGA term babies (1.9 *versus* 4.9 nmol/mg DNA, $p = 0.023$), cell amino acid values of the four types of babies were not signficantly different and the data were combined for further analysis.

Figure 2 shows an overlay plot of the intracellular and extracellular (plasma) amino acid patterns. Intracellular concentrations (nmol/ml intracellular water) are higher for most amino acids compared to their plasma concentrations. The cellular concentration of the nonprotein amino acid, taurine (not illustrated), was about 100 times its plasma concentration. The dispensable amino acids glutamine, serine, asparagine, glycine, alanine, glutamic, and aspartic acids also were about three to 100 times their concentrations in plasma. The concentrations of the essential branched-chain amino acids, leucine and isoleucine, were about three times higher in the cells than in plasma. The intracellular concentration of valine was slightly ($p = 0.052$) lower than its plasma level. Of 361 possible simple correlations between levels of amino acids in plasma and cells, there were 34 significant correlations (18 would be expected by chance), but only four were self-correlated on both sides of the cell membrane: histidine, aspartic acid, tyrosine, and ornithine. Plasma alanine was the only extracellular amino acid whose level was correlated significantly (product:moment) with protein synthesis ($r = -0.26$, $p = 0.03$).

Relation of protein synthesis to plasma amino acid pools. A multiple regression analysis, limited to six steps, with protein synthesis as the dependent variable and 19 plasma amino acids as the independent variables showed that a combination of the concentrations of valine, isoleucine, phenylalanine, aspartic acid, alanine, and taurine accounted for a small ($R^2 = 0.23$), but statistically significant ($p = 0.027$), proportion of the variance in protein synthesis (Table 4).

Relation of protein synthesis to intracellular amino acids. The six-step multiple regression analysis with protein synthesis as the dependent variable and the intracellular amino acids as the independent variables selected leucine, methonine, tyrosine, glycine, alanine, and taurine as satisfying the model. The combination ("set") of amino acids accounted for 36% of the variance in protein synthesis ($p = 0.001$). The regression equation for the model is also given in Table 4.

Relation of intracellular to plasma amino acids. To explore the relationships of intracellular to plasma amino acid pools, each of the six intracellular amino acids selected by the model

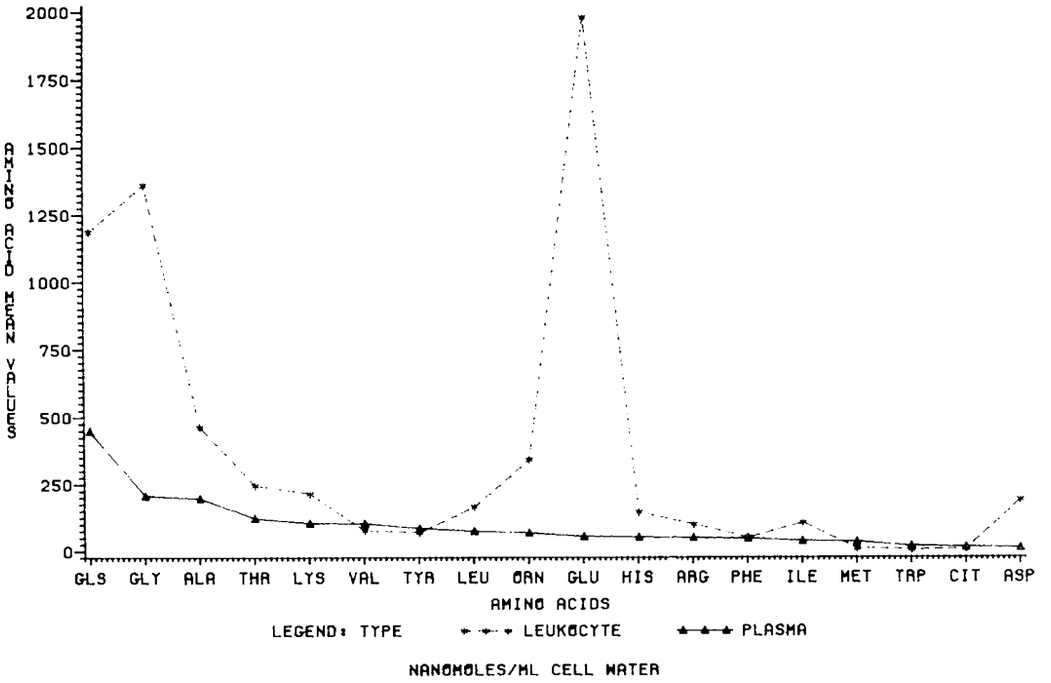

Fig. 2. Intracellular *versus* plasma amino acids. Cell concentrations are expressed as nmol/ml intracellular water. Intracellular water was determined by dry weight corrected for "trapped" water using ^{14}C inulin. Each *point* represents the mean value for paired analysis of each amino acid in the 57 babies. Univariate analysis was used to assess the difference between the cell and plasma amino acid levels. The overlay plot was drawn by computer. All infants were less than 308 days postconceptual age with an average birth weight = 2431 ± 887 (SD) g and gestational age = 36.6 ± 3 (SD) wk. Cell *versus* plasma concentrations of phenylalanine and tyrosine were not significantly different. *p* values for differences: valine = 0.052, citrulline = 0.0006, arginine = 0.0002, all others = 0.0001.

Table 4. *Multiple regression analysis of relation of protein synthesis to plasma and intracellular amino acids**

Relation of protein synthesis to plasma amino acids
Protein synthesis = 4925 − 14 (VAL) + 45 (ILE) + 42 (PHE) − 73 (ASP) − 9 (ALA) − 11 (TAU) $R^2 = 0.23$, $p = 0.027$, $n = 59$
Relation of protein synthesis to intracellular amino acids
Protein synthesis = 3853 + 84 (LEU) − 222 (MET) − 39 (TYR) + 9 (GLY) − 32 (ALA) − 0.5 (TAU) $R^2 = 0.36$, $p = 0.001$, $n = 54$

* Protein synthesis in pmol/h/mg DNA; plasma amino acids in nmol/ml; Leukocyte amino acid concentrations in nmol/mg DNA.

Table 5. *Prediction of intracellular amino acids by plasma amino acid levels**

	Intracellular amino acid predictors of protein synthesis†					
	Leucine	Methionine	Tyrosine	Glycine	Alanine	Taurine
Plasma amino acid predictors of the intracellular amino acid levels	Methionine (−) Phenylalanine (−) Arginine (−) Citrulline Ornithine Taurine	(−) Tryptophane (−) Histidine (−) Arginine (−) Glutamic Acid (−) Citrulline Taurine	Isoleucine Lysine Histidine (−) Arginine (−) Glycine (−) Citrulline	(−) Valine Methionine Lysine (−) Histidine Tryptophane Taurine	Methionine (−) Phenylalanine Lysine Glutamic Acid (−) Citrulline Taurine	Isoleucine Lysine (−) Histidine (−) Arginine (−) Aspartic Acid Glycine
R^2	0.49	0.44	0.45	0.39	0.38	0.45
p	0.0001	0.0002	0.0001	0.0010	0.0011	0.0001

* Stepwise multiple regression analysis with 19 plasma amino acids as independent variables and each of the intracellular amino acid predictors of protein synthesis as a dependent variable. The group of plasma amino acids selected by the model for each intracellular predictor is listed below that intracellular amino acid. The negative (−) sign indicates a negative partial regression coefficient for that amino acid. p = level of significance for prediction of the model (intracellular amino acid) by the set of independent variables (extracellular amino acids).

† $n = 54$ babies with complete data.

as predictive of protein synthesis, was used as the dependent variable in a similar multiple regression analysis with the 19 plasma amino acids as the independent variables. For each of the six intracellular predictors of protein synthesis, the model selected different groups of six plasma amino acids which accounted for a statistically significant proportion of the variance of that single intracellular amino acid. Results are shown in Table 5. Plasma levels of lysine, histidine, arginine, citrulline, and taurine recurred with the greatest frequency, *e.g.* in four of the six sets of predictors of the intracellular amino acid levels most closely associated with protein synthesis. Thirty-eight to 49% of the variance in the levels of intracellular leucine, methionine, tyrosine, glycine, alanine, and taurine was accounted for by the different combinations of six plasma amino acids. None of the intracellular amino acids was self-represented among the set of six plasma amino acids which together accounted for a significant proportion of its variance.

Leukocyte glycolytic enzymes related to protein synthesis. Sufficient cell suspension was available to determine activities of pyruvate kinase in 38 infants and phosphofructokinase in 36 of the 63 infants. The activities of both enzymes were positively correlated with protein synthesis ($r = 0.34$, $p = 0.036$, and $r = 0.50$, $p = 0.002$, respectively) in these babies. Birth weight and gestational age were correlated negatively ($r = 0.38$, $p = 0.02$ and $r = -0.35$, $p = 0.04$, respectively) with activity of leukocyte phosphofructokinase, which catalyzes the major rate-limiting step in glycolysis.

DISCUSSION

Protein synthesis. Our data show that the lower the birth weight, the higher the rate of protein synthesis, as measured in a cell model, the granulocyte. The relationship between birth weight and protein synthesis is nonlinear. Given the interdependence of birth weight and gestational age, the inverse relationship found between protein synthesis and length of gestation is not unexpected. That protein synthesis is also related inversely to postconceptual age is consistent with data from animal work (23) and stable isotope (^{15}N) studies of whole body protein synthesis in eight preterm infants by Nissim *et al.* (24). Our results also are consistent with those of Pencharz *et al.* (25) who observed increased rates of whole body protein synthesis (^{15}N) in 40 preterm infants, half being SGA. Among the 20 AGA infants, the smaller (birth weight <1500 g) and more immature (gestational age ~28 wk) had somewhat higher (~10%) rates of whole body protein synthesis than the larger (birth weight >1500 g), more mature (gestational age ~33 wk) infants (Table 4 of Ref. 25). However, they did not find a statistically significant correlation between birth weight and whole body protein turnover. Their data indicated that whole body protein synthesis, breakdown, and nitrogen flux were increased in SGA (compared to AGA) infants; however, the difference in nitrogen retention or growth rate between their two groups was not statistically significant ($p > 0.05$). We also noted a significantly higher rate of protein synthesis in preterm AGA infants and in the small group of fetally malnourished, term or near term infants compared to appropriately grown term babies. Covariance analysis indicated that the difference in rate of protein synthesis between the smaller and larger babies was dependent on the combined influences of birth weight, gestational age, and weight at the time of study. The possible difference in our results relating protein synthesis to birth weight and intrauterine nutritional status may be methodologic and reflect the different parameters measured. We measured protein synthesis in an *in vitro* cell model, while Pencharz *et al.* (25) studied total body nitrogen flux *in vivo*. Whether the rate of protein synthesis in granulocytes is similar to protein synthesis rates in major organs, such as liver or muscle, or in the whole body, is uncertain. However 25×10^8 granulocytes are estimated to be produced in the bone marrow/kg/day in adult men. The cell turnover rate is approximately 500% per day compared to 10% for liver. Ten billion (10^9) leukocytes weigh ~0.5 g and contain about 20% protein, hence their incorporation of protein is approximately 17.5 g/day in a 70 kg man or approximately 4 g/day in an infant, an amount roughly equivalent to the daily synthesis of plasma albumin. Because of the rapid turnover, protein degradation appears to be negligible in leukocytes, relative to protein synthesis (17); therefore the rate of incorporation of a labeled amino acid into newly synthesized protein would not be affected significantly by dilution of the pool from protein degradation. Estimations of whole body protein synthesis from distribution and excretion of stable isotopes are dependent upon several assumptions which are difficult to validate and are generally considered to underestimate absolute rates of protein synthesis by 10–20% (17, 26).

Amino acids and protein synthesis. Our leukocyte results indicate that the rate of protein synthesis was more closely related to a set of intracellular amino acid levels than to a set of plasma amino acid levels. Why this particular set of intracellular amino acids (Table 4) was selected by the program to satisfy the model is not evident at this time. However, each of the amino acids comprising the set is known to have some physiological importance. For example, leucine, a branched-chain α-keto amino acid, is considered to be an important regulator of protein synthesis. Studies by Goldberg and Tischler (27), Adibi (28), and

Buse and Reid have shown that leucine stimulates protein synthesis in various rat tissues, possibly by facilitating translation. Tyrosine, another component of the set of intracellular amino acid predictors of protein synthesis, is considered a conditionally essential amino acid for human neonates because of the slow maturation of hepatic phenylalanine hydroxylase which converts phenylalanine to tyrosine. Alanine is considered a major gluconeogenic amino acid because of its transamination to form pyruvate and subseqeunt entry into the Krebs cycle via acetyl CoA. Glycine, another of the intracellular predictors of protein synthesis, occasionally may be limiting because endogenous glycine production in preterm infants on a limited glycine intake may be insufficient to meet demands (30). Infants fed supplemental glycine were reported to have improved growth (31). The biologic significance of taurine, also selected by the program, is not yet clear. Although not a constituent of proteins, it is present in very high concentrations in liver, brain, and muscle, and may have an important biologic function to protect cell membranes against oxidants and reduced oxygen molecules (32). It may be a conditionally essential amino acid for preterm infants, who may develop abnormal electroretinograms after prolonged treatment with total parenteral nutrition solutions or formulae lacking taurine. This retinal-response defect can be reversed by administration of taurine (33).

Winkler *et al.* (7) have shown that incorporation of a labeled amino acid into protein in human granulocytes can be altered by the addition of a mixture of amino acids into the incubation medium, probably by competitive membrane transport. That the levels of the six intracellular amino acid predictors of protein synthesis in our infants could, in turn, be predicted by specific, apparently unrelated, groups of plasma amino acids suggests that the extracellular amino acids might affect the intracellular concentrations of these amino acids indirectly by altering their transport into, or metabolism by, the cell. It is conceivable that *in vivo* excesses or imbalances of certain nutrients (*e.g.* amino acids) in plasma could, through an effect on membrane transport, result in relative intracellular substrate excesses or limitations of other amino acids. The fact that none of the predictor intracellular amino acids was represented among the plasma amino acids which largely accounted for its variance indicates that there is not a simple direct relation between the intracellular and plasma levels of an amino acid.

Our results demonstrate that measurements of plasma amino acid concentrations alone, are not likely to reflect either levels of intracellular amino acids or of protein synthesis; however, combinations of intracellular amino acids appear to account for a significant proportion of the variance in protein synthesis in neonates, as well as in adults, although a different set of cell amino acids comprise the predictors for adults (22). Combinations of certain plasma amino acid levels may prove useful, however, for predicting the intracellular levels of those amino acids which are statistically most closely associated with protein synthesis.

Glycolytic enzymes. The cytosolic glycolytic enzymes, pyruvate kinase and phosphofructokinase, were correlated directly with protein synthesis, and inversely with birth weight and gestational age. Pyruvate kinase and phosphofructokinase catalyze two of the three rate-limiting steps of glycolysis, the major energy-producing pathway in the leukocyte. Both plasma and intracellular alanine was found to be negatively correlated with protein synthesis in these infants, and alanine is known to inhibit to both pyruvate kinase and phosphofructokinase activity (34). For example, the maximal enzyme velocity for pyruvate kinase in leukocytes of pregnant women is inhibited by alanine (35). Fructose diphosphate normally stimulates the enzyme and can overcome the inhibition by alanine; however, this response is less effective in the leukocyte enzyme from mothers carrying fetally malnourished babies, which suggests an alteration in the protein structure of the enzyme (35).

SUMMARY

With polymorphonuclear leukocytes as the model, the rate of protein synthesis in neonates is inversely related to birth weight, gestational age, and weight at time of study. The activities of the glycolytic enzymes pyruvate kinase and phosphofructokinase also are inversely related to protein synthesis. Phosphofructokinase activity is correlated negatively with both birth weight and gestational age. A significant proportion of the variance in protein synthesis can be predicted from a combination of the levels of six intracellular amino acids (leucine, methionine, tyrosine, glycine, alanine, and taurine). Specific, but different, sets of plasma amino acid levels account for a significant proportion of the variance of each of these predictor intracellular amino acid concentrations.

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