Protein Metabolism in Phenylketonuria and Lesch-Nyhan Syndrome

G. N. THOMPSON, P. J. PACY, R. W. E. WATTS, AND D. HALLIDAY

*Nutrition Research Group [G.N.T., P.J.P., D.H.] and Division of Inherited Metabolic Disease [R.W.E.W.],
Clinical Research Centre, Harrow, United Kingdom*

ABSTRACT. Animal and *in vitro* studies have implicated decreased protein synthesis in the pathogenesis of tissue damage in phenylketonuria (PKU) and of growth failure in Lesch-Nyhan syndrome. Protein turnover was measured *in vivo* in ten young adult subjects with classical PKU, two subjects with hyperphenylalaninemia, and three children with Lesch-Nyhan syndrome using techniques based on continuous infusions of [¹³C]leucine and, in Lesch-Nyhan subjects, [²H₅]phenylalanine. The PKU subjects had various degrees of dietary phenylalanine restriction and plasma phenylalanine levels at the time of study ranged from 450–1540 μmol/L (mean 1106). Plasma phenylalanine in the two hyperphenylalaninemic subjects was 533 and 402 μmol/L. Rates of protein synthesis in all PKU subjects (mean 3.71 g/kg/24 h, range 2.68–5.10, [¹³C]leucine as tracer) were in a range similar to or above control values (mean 2.97, range 2.78–3.22, *n* = 6), as were rates of protein catabolism (PKU mean 4.23 g/kg/24 h, range 3.15–5.45; controls 3.64, 3.50–3.91). Protein turnover values in hyperphenylalaninemia were also similar to those in controls. With [¹³C]leucine as tracer, both mean protein synthesis and catabolism values in Lesch-Nyhan subjects (mean 4.80 and 5.64 g/kg/24 h, respectively) were higher than values in control children matched for protein intake (synthesis 4.32 ± 0.74 (SD) and catabolism 4.85 ± 0.57 (g/kg/24 h, *n* = 5). Similar results were obtained in Lesch-Nyhan subjects using [²H₅]phenylalanine as tracer. These results suggest that protein turnover is not decreased in either PKU or Lesch-Nyhan syndrome. This conclusion is inconsistent with the hypothesis that tissue damage in PKU results from impaired protein synthesis. The findings also indicate that growth failure in Lesch-Nyhan syndrome is unlikely to be due to decreased protein synthesis resulting from impaired energy metabolism. (*Pediatr Res* 28: 240–246, 1990)

Abbreviations

PKU, phenylketonuria
HPRT, hypoxanthine-guanine phosphoribosyltransferase
KIC, α-ketoisocaproic acid
BMI, body mass index
GTP, guanosine 5'-triphosphate
APE, atoms percent excess

sis in animal models of PKU (1–4), which has led to wide acceptance of an etiology involving impaired protein synthesis. Such a mechanism has been used in particular to explain the tissue damage that occurs in maternal PKU. Untreated maternal PKU carries a high risk to the fetus of mental retardation, microcephaly, intrauterine growth retardation, congenital heart disease, and other malformations (5, 6). These abnormalities appear related to the control of blood phenylalanine levels throughout pregnancy, but especially at conception (5). Animal models of maternal PKU have shown that maternal hyperphenylalaninemia suppresses active transport of amino acids, especially branched-chain amino acids and methionine, across the placenta (2), resulting in amino acid imbalances in fetal plasma and tissues (2, 7). Free amino acid concentrations have a regulatory role in protein synthesis, with decreased concentrations resulting in decreased polysomal aggregation (8). Inhibition of polysomal aggregation and decreased incorporation of labeled leucine into a wide range of tissues in the animal model of PKU have supported the proposal that decreased protein synthesis is the cause of congenital abnormalities in the offspring of mothers with PKU (2, 3, 9). Imbalances of a number of amino acids other than phenylalanine are present in peripheral tissues as well as brain in both mother and fetus in the animal PKU model (2), suggesting that disturbances in protein metabolism induced by high phenylalanine concentrations would be likely to be manifest throughout the body.

Poor growth is characteristic of children with Lesch-Nyhan syndrome (10, 11). Decreased wt percentiles are usually manifest in the first decade, although evidence of subnormal birth wt has recently been documented (11). Although the pronounced dystonic posturing in this condition has made accurate assessment of linear growth difficult, most children are well below the 3rd ht percentile in the first decade (12). Head circumference percentiles also tend to be reduced, though to a lesser degree than those for wt (11). The cause of growth failure in Lesch-Nyhan syndrome is not known, but normal hypothalamic-pituitary function has been demonstrated, including normal thyroid function, growth hormone and prolactin production, and gonadotrophin responses (10, 11). Growth failure appears to precede the onset of renal failure and, although dystonia may cause difficulty in feeding, food intake is generally at a level sufficient for normal growth.

The deficient enzyme in Lesch-Nyhan syndrome, HPRT, is essential for the salvage of the purine bases hypoxanthine and xanthine to the nucleotide inosine monophosphate, the precursor of the adenine and guanine nucleotides (13). Deficiency of the enzyme results in markedly reduced levels of ATP, ADP, and AMP in erythrocytes and platelets (14, 15) and of ATP and GTP in cultured lymphoblasts (16). Protein synthesis is an energy-requiring metabolic process (17, 18) accounting for 15–20% of resting energy expenditure (19). Synthesis of purine nucleotides can proceed either through *de novo* synthesis from phosphoribosylpyrophosphate or through salvage pathways catalyzed primarily by HPRT, but also by adenine phosphoribosyltransferase

The cause of tissue damage in PKU is still a matter of debate. A number of studies have demonstrated reduced protein synthe-

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Correspondence and reprint requests: Dr. G. N. Thompson, The Murdoch Institute, Royal Children's Hospital, Flemington Rd., Parkville, Victoria 3052, Australia.

(13). DNA, RNA, and protein synthesis are impaired in mitotically stimulated lymphocytes, which lack HPRT, when *de novo* purine synthesis is blocked (by azaserine) (20) underlining the dependence of protein synthetic processes on purine nucleotide supply. These findings suggest that purine nucleotide depletion could impair protein synthesis in Lesch-Nyhan syndrome, thus providing a possible explanation for poor growth.

We have examined whole body protein synthesis and catabolism *in vivo* in PKU and Lesch-Nyhan syndrome using stable isotopic techniques to determine whether findings in the animal model and *in vitro* apply in man.

MATERIALS AND METHODS

Subjects. The studies were undertaken with the approval of the Harrow District Ethical Committee. Ten subjects with classical PKU (eight male, two female; mean age 19.3 y, range 14–24), two with hyperphenylalaninemia (one male, one female; ages 22 and 45 y) and six age-matched normal controls (all male, mean age 20.8 y, range 19–23) were studied. Some of these subjects have been studied previously using [$^2\text{H}_5$]phenylalanine techniques to measure phenylalanine hydroxylase activity *in vivo* (21, 22). This technique does not allow meaningful measurement of protein turnover, as the metabolism of phenylalanine is grossly impaired in PKU. Body wt and BMI were within the normal range in all PKU and hyperphenylalanemic subjects (mean wt 63.1 kg $\pm$ 5.5 SD, BMI 23.0 $\pm$ 2.8) and controls (66.2 $\pm$ 9.9 and 22.1 $\pm$ 1.7 kg, respectively). Details of dietary protein and phenylalanine intakes and plasma phenylalanine concentrations in PKU and hyperphenylalanemic subjects are shown in Table 1. Dietary intakes were calculated from a recall history or 3-d diet diary taken at the time of study. It is recognized that these methods do not always give an accurate record of dietary intake, as demonstrated by the incompatibility between stated intake and plasma phenylalanine concentration in some subjects (Table 1). Mean energy intake was 14 360 kJ/24 h (range 6490–17 180). The mean protein and energy intakes in control subjects were estimated to be 1.25 g/kg/24 h (range 0.9–1.5) and 14 900 kJ/24 h (range 7350–16 600), respectively. Plasma phenylalanine concentrations in controls ranged from 47–58 $\mu\text{mol/L}$ (mean 53). Clinical severity in PKU subjects was determined from the dietary tolerance for phenylalanine, defined as the highest intake (mg/kg) consistent with acceptable plasma levels of phenylalanine (200–400 $\mu\text{mol/L}$). Most subjects had discontinued strict dietary control at the time of study, in which case phenylalanine

tolerance was determined retrospectively from an earlier period of good dietary control at age 6–10, or over 17 y. All PKU subjects had a dietary phenylalanine tolerance consistent with the diagnosis of classical PKU (tolerance less than 10 mg/kg/24 h). All subjects were of normal intelligence with the exception of one PKU subject whose IQ was 65. All subjects were studied when clinically well, and there had been no alteration in dietary intake of any subject in the 3 mo preceding the study.

Protein turnover was also studied in three children with Lesch-Nyhan syndrome. Each subject had classical clinical features of the disorder and absent HPRT activity in erythrocyte homogenates (23). Detailed descriptions of clinical and biochemical findings have been published previously (11). At the time of study the subjects were receiving dietary protein intakes of 1.66, 1.69, and 1.75 g/kg/d, respectively. These intakes are about $\frac{2}{3}$ the usual intake for age (24), but would be expected to be adequate for normal growth. However, similar percentage reductions in dietary protein intake have been shown to decrease protein turnover (25). Values in Lesch-Nyhan syndrome were therefore compared with those in control children receiving therapeutically reduced protein intakes. This control group consisted of children with tyrosinemia (one subject), methylmalonic aciduria (six subjects) and maple syrup urine disease (two subjects). Some of these subjects have been described previously (22, 26, 27). Details of age, protein and energy intake (assessed by 3-d diet diary), and nutritional status of subjects and controls appear in Table 2. The Lesch-Nyhan subjects were slightly older than controls. However, all subjects were prepubertal, and protein turnover values would not be expected to change significantly between infancy and puberty. All Lesch-Nyhan subjects were wheelchair-bound. They maintained copious voluntary and involuntary physical activity. The severe impairment of speech and motor responses in this condition makes assessment of intellect difficult. None of the three children had major intellectual impairment using the psychologic testing battery described previously (12).

Measurement of protein turnover. Protein turnover was assessed by two independent stable isotope methods based on the continuous infusion of [^{13}C]leucine (28) and [$^2\text{H}_5$]phenylalanine (29), respectively. Abnormal phenylalanine metabolism in PKU precludes the use of [$^2\text{H}_5$]phenylalanine as a tracer for protein metabolism in this condition. PKU and hyperphenylalaninemia subjects and their respective controls were therefore studied using the [^{13}C]leucine technique alone. Although the [^{13}C]leucine method has been widely applied, its use is limited in Lesch-Nyhan syndrome by the need for accurate measurement of expired air CO_2 production. This potential difficulty does not apply to the newly described but less widely tested [$^2\text{H}_5$]phenylalanine method. Both tracers were used in all Lesch-Nyhan subjects, but their respective controls were studied only with tracers that would be expected to be metabolized normally (for example, [^{13}C]leucine was not used in maple syrup urine disease subjects).

Isotopes. L-[1- ^{13}C]leucine (99% ^{13}C), L-[ring- $^2\text{H}_5$]phenylalanine (98% ^2H), L-[ring- $^2\text{H}_4$]tyrosine (98% ^2H) and sodium [^{13}C]

Table 1. Details of PKU and hyperphenylalaninemic subjects*

Subject	Protein intake (g/kg/d)	Phenylalanine intake (mg/kg/d)	Phenylalanine concentration ($\mu\text{mol/L}$)	Treatment
PKU				
1	0.96	48	1410	fd
2	1.05	17	951	d + aa
3	1.00	6	523	d + aa
4	1.03	5	543	d + aa
5	0.77	37	1100	fd
6	1.38	26	912	d + aa
7	0.50	25	1474	d + aa
8	1.64	79	1540	fd
9	0.76	37	1221	fd
10	1.55	77	1382	fd
Mean	1.06	36	1106	
SD	0.34	25	350	
Hyperphenylalaninemia				
1	1.04	52	533	fd
2	1.11	55	402	fd

* fd, free diet; d + aa, diet restricted in natural protein with phenylalanine-free amino acid supplements.

Table 2. Details of Lesch-Nyhan syndrome subjects and controls*

	Lesch-Nyhan syndrome (n = 3)	Controls (n = 9)
Age	9.7 $\pm$ 0.2	7.1 $\pm$ 2.6
Protein intake (g/kg/24 h)	1.70 $\pm$ 0.04	1.63 $\pm$ 0.08
Energy intake (kJ/kg/24 h)	270 $\pm$ 22	315 $\pm$ 31
Wt (kg)	21.2 $\pm$ 1.3	19.8 $\pm$ 4.0
Wt (standard score)†	-3.3 $\pm$ 0.7	-0.3 $\pm$ 0.3
Ht (standard score)†	-2.5 $\pm$ 0.7	-0.3 $\pm$ 0.5

* Values are mean $\pm$ SD.

† Number of SD from expected mean for age.

Table 3. Leucine flux and oxidation and protein synthesis, catabolism, and net loss in PKU, hyperphenylalaninemia, and controls

Subject	Leucine kinetics ($\mu\text{mol/kg/h}$)		Protein turnover (g/kg/d)		
	Flux	Oxidation	Synthesis	Catabolism	Net loss
PKU					
1	96.9	10.8	3.28	3.70	0.42
2	138.0	13.8	4.74	5.27	0.53
3	142.9	9.1	5.10	5.45	0.35
4	106.9	9.8	3.71	4.08	0.37
5	115.7	14.3	3.87	4.42	0.55
6	139.8	16.2	4.71	5.34	0.63
7	84.1	13.5	2.69	3.21	0.52
8	116.0	24.8	3.48	4.43	0.95
9	82.6	12.3	2.68	3.15	0.47
10	86.5	12.5	2.82	3.30	0.48
Mean	110.9	13.8	3.71	4.23	0.52
SD	22.4	4.2	0.84	0.85	0.16
Hyperphenylalaninemia					
1	91.4	16.0	2.88	3.49	0.61
2	109.9	16.5	3.56	4.19	0.63
Controls					
Mean	95.4	17.5	2.97	3.64	0.67
SD	4.7	2.2	0.18	0.18	0.08
Range	91.6–102.6	15.1–21.3	2.78–3.22	3.50–3.91	0.58–0.82

Table 4. Leucine kinetics and protein turnover in Lesch-Nyhan syndrome and controls using [^{13}C]leucine as tracer

	Infusion rate ($\mu\text{mol/kg/h}$)	CO_2 production (mL/min)	KIC APE	CO_2 APE	Leucine kinetics ($\mu\text{mol/kg/h}$)			Protein turnover (g/kg/24 h)		
					Flux	Oxidation	Disposal to protein	Synthesis	Catabolism	Net loss
Lesch-Nyhan syndrome subjects										
1	7.42	118	4.6	0.002	151.2	8.9	142.4	5.43	5.77	0.34
2	6.97	124	5.0	0.006	131.8	22.4	109.3	4.17	5.03	0.85
3	7.04	120	4.2	0.007	160.9	28.3	132.7	5.06	6.14	1.08
Mean	7.14	121	4.6	0.005	147.8	19.9	128.1	4.80	5.64	0.76
Controls ($n = 5$)										
Mean	6.89	124	5.1	0.004	126.9	14.4	112.5	4.32	4.85	0.53
SD					14.9	5.5	19.4	0.74	0.57	0.21

Table 5. Phenylalanine kinetics and protein turnover in Lesch-Nyhan syndrome and controls using [$^2\text{H}_5$]phenylalanine as tracer

	Infusion rate ($\mu\text{mol/kg/h}$)	Phenylalanine MPE*	Tyrosine MPE*	Phenylalanine kinetics ($\mu\text{mol/kg/h}$)			Protein turnover (g/kg/24 h)		
				Flux	Hydroxylation	Disposal to protein	Synthesis	Catabolism	Net loss
Lesch-Nyhan syndrome subjects									
1	2.92	6.3	0.41	48.6	3.0	45.6	3.87	4.17	0.29
2	3.16	6.5	1.44	50.5	15.3	37.2	3.20	4.33	1.13
3	4.33	7.1	0.84	63.0	7.5	55.5	4.77	5.40	0.64
Mean	3.47	6.6	0.90	54.0	8.6	46.1	3.95	4.63	0.69
Controls ($n = 7$)									
Mean	2.87	4.8	0.51	49.9	4.1	45.9	3.93	4.28	0.35
SD				6.1	2.0	5.8	0.50	0.52	0.17

* MPE, mol percent excess.

bicarbonate (99% ^{13}C) were obtained from Cambridge Isotopes Laboratories (Woburn, MA). The isotopic and isomeric purities of the amino acid tracers were verified by gas chromatography/mass spectrometry and by chiral column gas chromatography. Isotopes were infused in a solution of normal saline that was shown to be sterile and pyrogen-free.

Procedure. After an overnight fast, an i.v. cannula was inserted into a hand vein for blood collection. The hand was warmed for 5 min in a heated box (air temperature 60°C) before collection of all blood samples in adult subjects to obtain "arterialized"

specimens (30). Isotopes were infused into a vein in a separate limb. After collection of baseline blood and expired air samples, priming bolus doses of L-[^{13}C]leucine (adults 0.5 mg/kg, children 0.7 mg/kg) and sodium [^{13}C]bicarbonate (0.08 mg/kg) ([^{13}C]leucine model) and/or L-[$^2\text{H}_5$]phenylalanine (0.5 mg/kg) and [$^2\text{H}_4$]tyrosine (0.08 mg/kg) ([$^2\text{H}_5$]phenylalanine model) were given. A continuous infusion of L-[^{13}C]leucine (adults 0.5 mg/kg/h, children 0.7 mg/kg/h) and/or [$^2\text{H}_5$]phenylalanine (0.5 mg/kg/h) was then given over the next 4 h (6 h in PKU and hyperphenylalaninemia subjects). The infusion time was ex-

Fig. 1. Protein synthesis in PKU and hyperphenylalaninemic subjects in relation to plasma phenylalanine concentration.

tended in the latter subjects beyond that usually used in this technique because impaired amino acid transport in PKU could have prolonged the time required for isotopic equilibrium to be achieved. Blood and expired air samples were collected at 15- to 20-min intervals in the final 2 h of each infusion and at ½-h intervals from 2–4 h in PKU subjects. Where the [^{13}C]leucine method was applied, measurement of expired air total CO_2 production rate was accomplished by indirect calorimetry during the final 2 h of each infusion using a ventilated hood system as described previously (31).

Analyses. Blood samples were centrifuged at 4°C immediately after collection and the plasma stored in -70°C until analyzed. Plasma KIC concentration and ^{13}C -enrichment were measured using the quinoxalinol-trimethylsilyl derivative and a Finnigan 4500 gas chromatograph/mass spectrometer (Finnigan MAT, San Jose, CA) as previously described (32). Plasma phenylalanine and tyrosine were derivatized to their tertiary butyl dimethyl silyl derivatives and their concentrations and deuterium enrichments were determined by electron impact ionization gas chromatography/mass spectrometry using selected ion monitoring and β -methyl-phenylalanine or α -methyl-tyrosine as internal standards in a manner similar to that described previously (33). Plasma leucine concentration was determined by the same method using norleucine as internal standard. ^{13}C -enrichment of expired air CO_2 was determined by isotope ratio mass spectrometry. Plasma amino acids were assayed in the sample collected immediately

before the isotope infusion by amino acid analyzer (LKB, Milton Keynes, UK).

Calculation of whole body protein turnover. Protein turnover was determined as described previously (28, 29, 34). In the fasting, steady state the amino acid flux [Q $\mu\text{mol/kg/h}$] was calculated by isotope dilution:

$$Q = i \cdot \left[\frac{E_i}{E_p} - 1 \right] \quad (1)$$

where i is the rate of infusion of tracer ($\mu\text{mol/kg/h}$) and E_i and E_p are the respective enrichments of the labeled amino acid in the infusate and of [$^2\text{H}_5$]phenylalanine or [^{13}C]KIC at plateau in plasma. Leucine turnover was calculated from [^{13}C]KIC enrichment, as this is presumed to more accurately reflect intracellular leucine enrichment (34). Plasma [$^2\text{H}_5$]phenylalanine enrichments were corrected to expected intracellular levels by multiplying by 0.8 as described previously (29). The rate of conversion of phenylalanine to tyrosine [Q_{pt} ($\mu\text{mol/kg/h}$)] was calculated from the plasma enrichments of [$^2\text{H}_5$]phenylalanine and [$^2\text{H}_4$]tyrosine using principles similar to those applied in equation 1 (29):

$$Q_{pt} = Q_t \cdot \frac{E_t}{E_p} \cdot \frac{Q_p}{[ip + Q_p]} \quad (2)$$

where Q_t is the tyrosine flux ($\mu\text{mol/kg/h}$) calculated from phenylalanine flux by assuming constancy of the ratio of phenylalanine:tyrosine entering the free pool from protein breakdown [discussed previously (29)], Q_p is the phenylalanine flux ($\mu\text{mol/kg/h}$) estimated independently by the primed constant infusion of [$^2\text{H}_5$]phenylalanine, E_p and E_t the respective enrichments of [$^2\text{H}_5$]phenylalanine and [$^2\text{H}_4$]tyrosine in plasma, and ip the rate of infusion of [$^2\text{H}_5$]phenylalanine ($\mu\text{mol/kg/h}$). The oxidation of leucine (O) was determined from the rate of appearance of $^{13}\text{CO}_2$ in expired air (28, 34).

In the steady, fasted state:

$$Q = S + ID = C \quad (3)$$

where S is the loss of tracee from the free amino acid pool to protein synthesis ($\mu\text{mol/kg/h}$), ID is the irreversible disposal of tracee by oxidation (O , $\mu\text{mol/kg/h}$) or hydroxylation (Q_{pt} , $\mu\text{mol/kg/h}$), and C is the rate of entry of tracee into the free amino acid pool from protein catabolism ($\mu\text{mol/kg/h}$). Rates of protein synthesis and catabolism (g/kg/h) were calculated by assuming the phenylalanine and leucine contents of protein to be 280 and 629 $\mu\text{mol/g}$ protein, respectively (35).

Table 6. Plasma amino acid concentrations in PKU and hyperphenylalaninemic subjects*

	PKU		Hyperphenylalaninemia	Normal range
	On diet	Off diet		
Taurine	51 (29–66)	44 (29–51)	40 (32–49)	53 (33–73)
Threonine	109 (85–141)	87 (56–124)	71 (70–73)	122 (80–164)
Serine	121 (80–140)	107 (77–154)	109 (108–110)	110 (72–148)
Proline	159 (99–212)	107 (62–166)	84 (79–90)	161 (91–231)
Glycine	250 (163–292)	217 (183–254)	211 (109–314)	220 (154–286)
Alanine	289 (156–472)	292 (149–401)	209 (155–263)	309 (163–455)
Valine	168 (119–221)	131 (102–146)	159 (116–202)	184 (116–252)
Methionine	12 (8–23)	19 (12–31)	20 (14–26)	18 (6–30)
Isoleucine	51 (35–72)	45 (30–59)	40 (30–51)	53 (31–75)
Leucine	110 (83–130)	75 (60–111)	98 (74–123)	103 (73–133)
Tyrosine	51 (25–80)	35 (24–48)	34 (30–38)	46 (30–62)
Ornithine	44 (32–57)	42 (34–51)	27 (26–29)	50 (34–66)
Lysine	158 (130–199)	145 (119–201)	105 (86–124)	168 (128–208)
Histidine	91 (85–101)	78 (59–107)	46 (46–46)	90 (58–122)
Arginine	89 (59–111)	78 (53–99)	75 (70–81)	83 (55–111)

* All values are mean (range), $\mu\text{mol/L}$. On diet, dietary restriction of natural protein with phenylalanine-free amino acid supplements; Off diet, free diet.

Steady state of measured species (plasma KIC, phenylalanine and tyrosine enrichment and concentration, leucine concentration, and $^{13}\text{CO}_2$ enrichment in expired air) was achieved in all studies as defined by visual inspection of the plateau and by plateau coefficient of variation of less than 10%. In PKU subjects, plateau enrichments were identical between 2–4 h and 4–6 h of infusion time, suggesting that the amino acid transport inhibition that has been documented elsewhere in PKU (36) did not impair the access of labeled leucine to the intracellular space in these subjects. Statistical comparisons were made using unpaired *t* test.

RESULTS

Individual and meaned leucine and phenylalanine kinetic data are shown in Tables 3–5. In PKU subjects, the mean leucine, KIC, and CO_2 ^{13}C -enrichments above baseline were $5.19 \text{ APE} \pm 1.54 \text{ SD}$, $4.20 \text{ APE} \pm 1.25$, and $0.0057 \text{ APE} \pm 0.002$, respectively; mean CO_2 production rate was $183.3 \text{ mL/min} \pm 14.0 \text{ SD}$, mean [^{13}C]leucine infusion rate was $4.86 \mu\text{mol/kg/h} \pm 0.36$, and mean calculated rate of leucine entering protein was $97.1 \mu\text{mol/kg/h} \pm 22.0$.

Protein turnover values derived from the [^{13}C]leucine and [$^2\text{H}_5$]phenylalanine models are shown in Tables 3–5. Rates of protein synthesis in all PKU subjects were in a range similar to or above control values, as were rates of protein catabolism (Table 3). Any trend toward increased synthesis in individual subjects was compensated by an increase in whole body protein catabolism, so that net protein loss during fasting tended to be lower in PKU subjects than in controls (not statistically different). Protein turnover values in the two hyperphenylalaninemic subjects were similar to those in PKU subjects and controls. Protein synthesis did not change significantly with plasma phenylalanine concentration (Fig. 1), and there was no apparent relationship between energy intake and protein synthesis.

Plasma amino acid concentrations in PKU and hyperphenylalaninemic subjects are shown in Table 6. Mean concentrations of many amino acids were in the lower normal range in PKU subjects on normal diets without phenylalanine-free amino acid supplements, whereas in subjects treated with protein-restricted diets and phenylalanine-free amino acid supplements, the mean concentrations of all amino acids other than phenylalanine were similar to those in controls.

Whole body protein synthesis and catabolism values in Lesch-Nyhan syndrome were either similar to or higher than those in control subjects. Values for net gain varied substantially, with the variation (SD) being greater in controls when the [^{13}C]leucine model was used. This almost certainly resulted from the difficulties encountered in measuring expired air CO_2 production rate in children; these difficulties are particularly prominent in Lesch-Nyhan syndrome. An error in CO_2 production rate measurement will manifest in calculation of synthesis, but not catabolism. Similar SD for both synthesis and catabolism values when the [$^2\text{H}_5$]phenylalanine model was used appear to reflect the absence of error introduced by CO_2 production estimations with the [^{13}C]leucine model.

DISCUSSION

Inhibition of protein synthesis in neural tissue by increased phenylalanine levels has long been regarded as a likely mechanism of neurologic damage in PKU (1, 3, 4, 9, 37). More recently, this mechanism has also been suggested to account for congenital defects such as low birth wt and heart malformations in the offspring of PKU mothers (2). The decrease in protein synthesis is presumed to result from the well-documented reduction in blood and brain of concentrations of amino acids other than phenylalanine in PKU (38, 39). The conclusion that decreased protein synthesis results from inadequate amino acid supply would, however, seem incompatible with the increased levels of aminoacyl-tRNA in the brain of hyperphenylalaninemic animals

(4). These increased concentrations were found in the presence of grossly depleted intracellular free amino acid pools, suggesting that aminoacyl-tRNA protein synthetic precursors can still be formed in adequate quantities in the presence of severely limited amino acid supply. In the current study, depletion of a number of plasma amino acids was noted in the five PKU subjects who were not receiving phenylalanine-free amino acid supplements, yet these subjects still sustained levels of protein synthesis similar to those in controls. This suggests that selected amino acid deficiency in PKU is insufficient to impair protein synthesis at the whole body level. Our data also do not support a direct relationship between protein turnover changes and the process of neurologic damage in that protein turnover appeared unrelated to the plasma phenylalanine concentration (Fig. 1), despite the close relationship between phenylalanine concentration and both prenatal and postnatal neurologic damage in PKU (5, 6, 40). The possibility of an alternative explanation to amino acid imbalance for congenital abnormalities in maternal PKU is further indicated by the dissimilarity between the pathologic findings in children of PKU mothers compared with those in pregnancies complicated by severe protein malnutrition (41, 42). Finally, methionine adenosyltransferase deficiency, a disorder accompanied by massive increases in plasma methionine, is not usually associated with neurologic abnormality (43). Methionine shares a common transport system with phenylalanine (44) and increased concentrations might be expected to have similar effects on amino acid transport to increased phenylalanine concentrations.

Despite the lack of inhibition of whole body protein synthesis in PKU subjects in our study, other mechanisms secondary to alterations in nonphenylalanine amino acid concentrations may be etiologically important. Indeed, Lou *et al.* (45) have suggested a link between decreased neurotransmitter levels and amino acid imbalance in the causation of neurologic symptoms after ceasing dietary therapy in older PKU patients. These authors proposed that the mechanism involves impaired transport at the neuronal cell membrane level, or inhibition of tyrosine hydroxylase activity, rather than decreased transport of amino acids into the cerebrospinal fluid. The techniques used in our study would not be expected to detect an isolated change in protein synthesis in a single organ such as the brain. It remains possible that increased phenylalanine concentration may have a tissue-specific effect on CNS protein metabolism. Other mechanisms of damage unrelated to protein metabolism have also been proposed, the most plausible being a direct toxic effect of phenylalanine and/or its metabolites on neuronal function (46–48).

Findings in animal models of PKU contrast with those of our study in suggesting that raised phenylalanine concentrations impair protein synthesis (1, 2, 4). These studies have, in general, used bolus doses of a labeled amino acid and measured incorporation of that label into protein as an indicator of protein synthesis. As pointed out by Appel (1), this technique will not only detect changes in the protein synthetic rate, but also changes in the transport of the amino acid into the cell. Transport disturbances in PKU appear to decrease both the uptake and efflux of amino acids across the cell membrane (36). Thus, immediate resynthesis back into protein of amino acids released into the intracellular space from protein catabolism would not be measured by bolus techniques. It is possible that the continuous infusion method used in our study could also underestimate this immediate reuse of amino acids (49), but a smaller error may be predicted. Further, the maintenance of isotopic plateau of KIC over a prolonged infusion period suggests that transport disturbances did not impair isotopic equilibration in these subjects.

The Lesch-Nyhan syndrome children in our study had severe growth impairment. Despite the major abnormalities in purine nucleotide production that result from HPRT deficiency in Lesch-Nyhan syndrome, whole body protein synthesis was able to proceed at a normal or increased rate in the subjects studied.

This finding has two important implications. First, protein synthesis appeared to be preserved in these subjects in the face of reduced energy supply and, second, growth failure in Lesch-Nyhan syndrome probably arises through a mechanism other than reduced protein synthesis resulting from ATP/GTP depletion (16). The slightly higher protein turnover values in Lesch-Nyhan subjects compared with controls are most likely attributable to changes in body composition that may have altered the metabolically active body mass in terms of protein turnover.

The devastating effects of Lesch-Nyhan syndrome on the brain and the more clinically subtle testicular atrophy that accompanies this disorders (11) have been correlated with high activities of HPRT in both brain and testes of normal subjects (50). Testicular manifestations appear at puberty, whereas those in the brain appear in infancy, suggesting that damage to these tissues is most likely to occur during periods of maximal cell proliferation when the demand for purine salvage through HPRT is greatest. In contrast, there is no suggestion that growth failure is age-related; percentile charts show a steady departure from normal percentiles throughout childhood, a process that probably begins prenatally (11). This lack of temporal relationship between impaired growth and periods of expected maximal growth velocity further supports the conclusion that growth failure is not directly related to impaired energy supply in Lesch-Nyhan syndrome.

If the pathogenesis of growth failure in Lesch-Nyhan syndrome does not primarily involve decreased availability of GTP and ATP, then it must be proposed either that the growth failure results from another secondary effect of energy depletion, or that the enzyme deficiency has other consequences that affect growth. Although the subjects in our study had low food intakes, the intakes were well above those that allow other children with inborn metabolic errors to grow perfectly well. The static nature and prenatal onset of growth failure (11) would seem to favor intrauterine damage to a growth control mechanism. However, such a mechanism has yet to be identified; the major growth-related hormones have been shown to be normally produced in Lesch-Nyhan syndrome (10, 11).

The [^{13}C]leucine and [$^2\text{H}_5$]phenylalanine models used simultaneously in Lesch-Nyhan subjects resulted in some differences in absolute protein turnover values. The differences are consistent with those seen in comparison of the models in normal subjects and reflect the different assumptions made in each model (29). However, qualitative comparisons between the values obtained with either model in the Lesch-Nyhan children and controls were similar. The slight differences between the two models in control values were contributed to by the different patient composition between control groups studied with [$^2\text{H}_5$]phenylalanine and those studied with [^{13}C]leucine, and by the use of KIC enrichment to calculate protein turnover for the [^{13}C]leucine model as compared with [$^2\text{H}_5$]phenylalanine enrichment calculated using the intracellular enrichment correction factor (29) for the [$^2\text{H}_5$]phenylalanine model. As with all studies of whole body protein turnover using isotopic methods, a number of model-related limitations must apply to interpretation of results of this study. These have been discussed in detail previously (51). Limitations specific to our study include the possible effects of minor differences in the quality or quantity of protein intake and in body composition between control and PKU/Lesch-Nyhan subjects. If any effect were to be noted from the decreases in protein intake in PKU subjects, the response to more substantive decreases in protein intake previously reported in normal subjects (25) would predict lower protein synthetic values. Such changes were not seen in our study. Despite the decreases in protein intake, growth and BMI measurements in PKU subjects reported here were similar to those in controls, a finding consistent with the normal growth curves previously reported in PKU (52).

In summary, *in vivo* techniques of measuring protein turnover in PKU and Lesch-Nyhan syndrome subjects used in this study have failed to support substantial animal model and *in vitro*

evidence that has previously implicated decreased protein synthesis in the pathogenesis of tissue damage in PKU and of growth failure in Lesch-Nyhan syndrome.

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