Amino Acids (2001) 20: 389–400

Amino
Acids

Impact of separating amino acids between plasma, extracellular and intracellular compartments on estimating protein synthesis in rodents

H. A. Johnson, R. L. Baldwin, K. C. Klasing, and **C. C. Calvert**

Animal Science Department, University of California at Davis, California, U.S.A.

Accepted September 26, 2000

Summary. Three models representing different separations of amino acid sources were used to simulate experimental specific radioactivity data and to predict protein fractional synthesis rate (FSR). Data were from a pulse dose of ^{14}C-U Leu given to a non-growing 20 g mouse and a flooding dose of ^{3}H Phe given to a non-growing 200 g rat. Protein synthesis rates estimated using the combined extracellular and intracellular (Ec + Ic) source pool and extracellular and plasma (Ec + Pls) source pool mouse models were 78 and 120% d^{-1} in liver, 14 and 16% d^{-1} in brain and 15 and 14% d^{-1} in muscle. Predicted protein synthesis rates using the Ec + Ic, Ec + Ic + Tr (combined extracellular, intracellular and aminoacyl tRNA source pool) and Ec + Pls rat models were 57, 3.4 and 57% d^{-1} in gastrocnemius, 58, 71 and 62% d^{-1} in gut, 8.3, 8.4 and 7.9% d^{-1} in heart, 32, 23 and 25% d^{-1} in kidney, 160, 90 and 80% d^{-1} in liver, 57, 5.5 and 57% d^{-1} in soleus and 56, 3.4 and 57% d^{-1} in tibialis. The Ec + Ic + Tr model underestimated protein synthesis rates in mouse tissues (5.0, 27 and 2.5% d^{-1} for brain, liver and muscle) and rat muscles (3.4, 5.5 and 3.4% d^{-1} for gastrocnemius, soleus and tibialis). The Ec + Pls model predicted the mouse pulse dose data best and the Ec + Ic model predicted the rat flooding dose data best. Model predictions of FSR imply that identification and separation of the source specific radioactivity is critical to accurately estimate FSR.

Keywords: Amino acids – Protein turnover model – Protein synthesis rate – Amino acid recycling

Introduction

Amino acids for charging aminoacyl tRNA (protein synthesis) can arise from extracellular (Ec), plasma (Pls), intracellular (Ic) sources or protein degradation through recycling. Experimentally, the amino acid sources sampled may not represent true sources of amino acids used for aminoacyl tRNA

(Tr) formation. Consequently, estimates of protein fractional synthesis rates (FSR) may not reflect true protein synthesis rates. The true precursor pool for protein synthesis is aminoacyl tRNA. However, since separation of aminoacyl tRNA is difficult, other pools are sampled to approximate aminoacyl tRNA specific radioactivity. Based on which pool is used as the precursor pool in the calculation of FSR, estimates of whole body FSR can vary 10–20% (Johnson et al., 1999b). Therefore, the appropriate pool, which best approximates the aminoacyl tRNA specific radioactivity, must be identified to accurately estimate FSR.

Choice of amino acid source specific radioactivity for the calculation of FSR is dependent on which pool approximates the aminoacyl tRNA specific radioactivity. The best source pool should reflect the specific radioactivity of aminoacyl tRNA and may change with the experimental method (continuous infusion, flooding dose, pulse dose, etc.) used to separate free amino acids. The experimental technique (homogenization) used to separate amino acid source pools will also affect the source pools available for use. Amino acids are generally isolated and separated in two different ways; separate Pls, Ec and Ic pools or as a combined pool (Ec, Ic and Tr combined). In Bernier and Calvert (1987), carcasses were homogenized in HCl and the supernatant (Pls, Ec, Ic and Tr combined) was used to estimate Leu specific radioactivity. Obled et al. (1991) separated Pls specific radioactivity from Ec, Ic and Tr specific radioactivity. Smith et al. (1988) measured Pls, Tr and Ec plus Ic specific radioactivities to estimate amino acid recycling and FSR. Hider et al. (1971) estimated extracellular space and used Ic and Ec concentrations to estimate Pls, Ec and Ic specific radioactivities. The consequences of separating free amino acids into combinations of Pls, Ec, Ic and Tr on estimates of FSR, has not been examined quantitatively. In this study, rodent models of protein turnover described previously in Johnson et al. (1999a,b; 2000) and data from Lajtha (1959), Goldspink et al. (1984), Goldspink and Kelly (1984), Kelly et al. (1984) and Lewis et al. (1984) were used to estimate specific radioactivities in Ec + Pls, Ec + Ic + Tr and Ec + Ic. The results were used to determine the source of amino acids for protein synthesis and evaluate the impact of sampling methods of precursor specific radioactivities on protein FSR.

Materials and methods

Models

Three models for each tissue were created. All equations are based on mass action kinetics between pools and were published previously in Johnson et al. (2000). The first model (Ec + Pls) partitions free amino acids into Tr, Ic and combines Pls and Ec pools (Fig. 1). Amino acid intake (Leu or Phe in umol min^{-1}) and dose (uCi ^{14}C Leu umol Leu^{-1} or uCi ^{3}H Phe umol Phe^{-1}) enter the Ec + Pls pool and are oxidized from the Ic pool. Flow to aminoacyl tRNA from the Ec + Pls and Ic pools are unidirectional based on the assumption that it would be energetically inefficient and therefore unlikely that aminoacyl tRNA is deacylated once it is formed (Johnson et al., 1999a). Protein synthesis is represented by the flux from Tr to Leu or Phe in protein (Pb) and protein degradation by the flux from Pb to Ic. Recycling is the flux from Pb to Tr. The units for all pools sizes

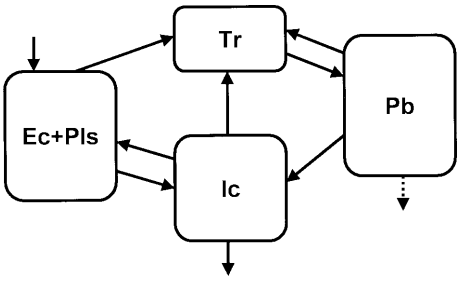

Fig. 1. The Ec + Pls tissue model of liver, gut, muscle, heart, kidney, gastrocnemius, soleus and tibialis for extracellular (*Ec*), intracellular (Ic), aminoacyl tRNA (*Tr*) and protein pools (*Pb*). The dashed arrow represents amino acid (Leu or Phe) in protein exported from the tissue and was only present in the liver and gut tissue models

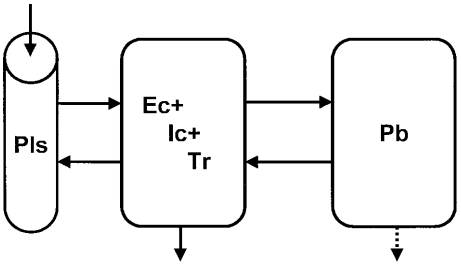

Fig. 2. The Ec + Ic + Tr tissue model of liver, gut, muscle, heart, kidney, gastrocnemius, soleus and tibialis for plasma (*Pls*), free amino acid (*Ec + Ic + Tr*) and protein pools (*Pb*). The dashed arrow represents amino acid (Leu or Phe) in protein exported from the tissue and was only present in the liver and gut tissue models

are umol and all fluxes are umol min^{-1}. Pls and Ec are assumed to rapidly equilibrate. Therefore their specific radioactivities are assumed similar and the pools are combined. Experimentally, the specific radioactivity of Ic is determined by estimating extracellular space and correcting free amino acid (supernatant) specific radioactivity for uCi in Ec. Extracellular space can be estimated using inulin or sorbitol as extracellular markers (Hider et al., 1971).

The second model (Ec + Ic + Tr) partitions free amino acids into Pls and combines Ec, Ic and Tr (Fig. 2). Amino acids (Leu or Phe) in Pb exchange with the combined Ec, Ic and Tr pool representing protein synthesis and protein degradation. There is no representation of recycling or of different sources of amino acids since all free amino acids associated with the cell are represented as one pool. The Ec + Ic + Tr model represents most experiments in which the specific radioactivity of Leucyl tRNA is not determined.

The third model (Ec + Ic) separates free amino acids into Pls, Tr and combines Ec and Ic (Fig. 3). Protein synthesis, protein degradation and recycling are represented by the same fluxes as in Ec + Pls (Fig. 1). However, Ec and Ic are assumed to exchange amino acid rapidly and therefore the specific radioactivities of Ec and Ic are assumed similar and are combined.

Data

Two data sets, which included time course specific radioactivities in different tissues, were used. The first (Lajtha, 1959) was a whole body pulse dose of 0.2 uCi U ^{14}C Leu/

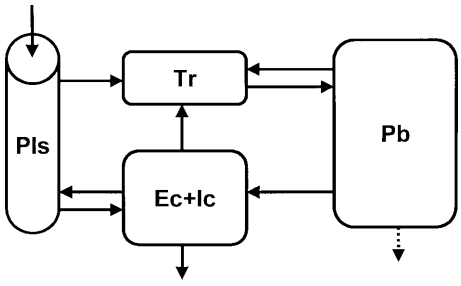

Fig. 3. The Ec + Ic tissue model of liver, gut, muscle, heart, kidney, gastrocnemius, soleus and tibialis for plasma (*Pls*), free amino acid (*Ec + Ic*) and protein pools (*Pb*). The dashed arrow represents amino acid (Leu or Phe) in protein exported from the tissue and was only present in the liver and gut tissue models

0.025 umol Leu given to 20 g (100 d) mice. Six mice were pooled for each time point. Specific radioactivities were measured at 3, 5, 10, 20, 30, 45, 60 and 120 min in plasma, brain, liver and muscle. Organs were frozen and homogenized with 5% trichloroacetic acid (TCA). The extract was the portion containing free Leu (Ec + Ic + Tr) and the precipitate was protein (Pb).

The second data set was from a flooding dose experiment by Goldspink and Kelly (1984), Lewis et al. (1984), Goldspink et al. (1984) and Kelly et al. (1984). A flooding dose of 65 uCi L-4 ^{3}H Phe/150 umol Phe/100 g bodyweight was given to 200 g rats. Data from four rats at each time point were pooled and tissues were collected at 2.5, 5, 10, 15, 20 and 30 min after injection. Tissues were homogenized in 0.3 M $HCLO_4$. The specific radioactivity of free Phe was the supernatant (Ec + Ic + Tr) and the bound Phe (Pb) specific radioactivity was the precipitate of the homogenate. ^{3}H Phe specific radioactivities were determined in plasma, liver, kidney, heart, tibialis anterior, soleus, gastrocnemius and gut.

Pls, Ec + Ic + Tr and Pb Leu or Phe specific radioactivities in tissues were directly from the published data. To determine Ec + Pls and Ec + Ic specific radioactivities, Pls specific radioactivity was assumed to equal Ec specific radioactivity. Then Ic and Ec + Ic specific radioactivities were corrected for Leu or Phe associated with Tr and Ec pool size to determine the specific radioactivity of Ic.

Methods

Data were fit using generalized reduced gradient within ACSL Optimize (Aegis, 2000). ACSL Optimize uses the method of maximum likelihood to estimate parameters based on data input into a model. Model parameters are chosen to maximize the likelihood of fitting the experimental data. Errors are assumed to be normally distributed and statistically independent. For all models, Leu and Phe intake, ^{14}C Leu and ^{3}H Phe dose to the tissue and model predicted FSR (PFSR) were estimated based on fitted specific radioactivities. For the Ec + Pls and Ec + Ic models, the fluxes of Leu or Phe to Tr (from Pls, Ec + Pls, Ic, Ec + Ic and Pb) were also adjusted to fit the data. For the Ec + Ic + Tr models, the exchange of Leu or Phe between the Pls and Ec + Ic + Tr pools were fit to the data. The models also predicted umol Leu or Phe incorporated into Pb min^{-1} (PSR).

Results

The ability of the models to duplicate the specific radioactivities (S) of the experimental data was assessed by low standard deviations on adjusted fluxes

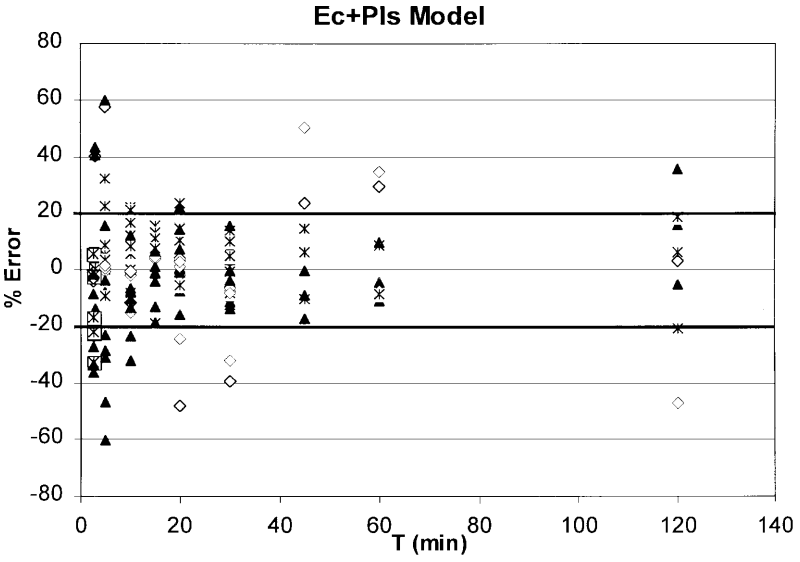

Fig. 4. Percent errors for Ec + Pls model across all data points (species, time points, tissues and pools). Percent error is predicted specific radioactivity by model – observed specific radioactivity. S prefix represents specific radioactivity. Percent errors for specific radioactivity of protein (SPb) = ▲, percent errors for specific radioactivity of intracellular Leu and Phe (SIc) = *, percent errors for specific radioactivity of combined extracellular and plasma Leu and Phe (S(Ec + Pls)) = ◇

and percent errors (predicted – observed data) of individual data points of greater than −20% and less than 20%. A 20% percent error was chosen based on previous experience with experimental errors associated with specific radioactivity measurements (Bernier and Calvert, 1987). Figures 4, 5 and 6 show the scatter of percent errors of specific radioactivities vs. time for each model. Solid lines indicate the −20% and 20% percent error levels. For the Ec + Pls model, 5.9% of the total predicted errors fell outside the +/−20% levels. For the Ec + Ic + Tr model, 8.1% and for the Ec + Ic model 6.1% of the total predicted errors were outside the +/−20% level. Predictions were unbiased except for the under prediction of the specific radioactivity of protein (SPb) with the Ec + Ic + Tr model. The largest percent error was with the prediction of SPb with the Ec + Ic model (212%) followed by the prediction of the specific radioactivity of the Ec + Ic + Tr pool (S(Ec + Ic + Tr) at 197%). Table 1 shows the percent errors for each model according to data set (mouse and rat), time point, tissue and pool specific radioactivity. Early (2.5–5 min) and late (45–120 min) time points with 25–56% outliers and the brain and liver data (38 and 27% outliers) were more difficult for all three models to represent. More outliers were associated with the prediction of SPb primarily because the Ec + Ic + Tr model fluxes were difficult to adjust to predict SPb. Overall, the Ec + Pls and Ec + Ic models predictions of the data were approximately the same. The Ec + Pls model predicted the mouse pulse dose Leu data slightly better with 26% of predicted errors outside +/−20% (outliers) and the Ec + Ic model predicted the rat flooding dose Phe data

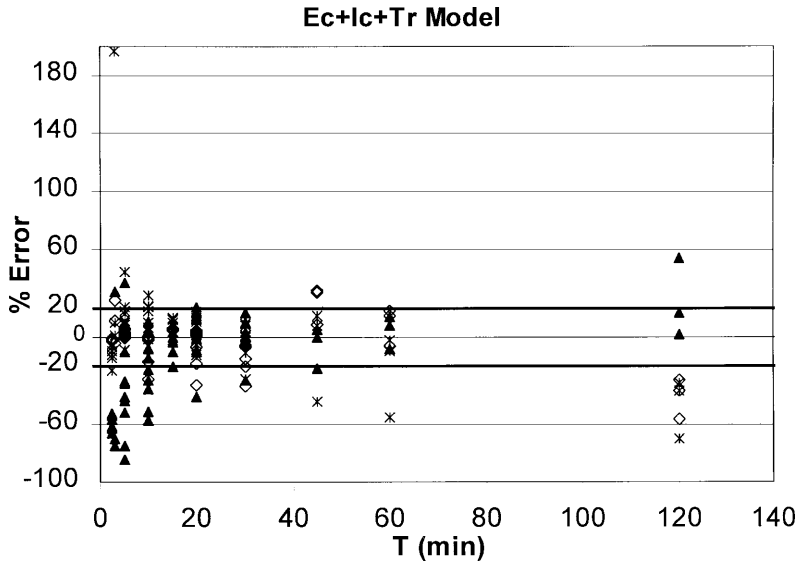

Fig. 5. Percent errors for Ec + Ic + Tr model across all data points (species, time points, tissues and pools). Percent error is predicted specific radioactivity by model – observed specific radioactivity. S prefix represents specific radioactivity. Percent errors for specific radioactivity of protein (SPb) = ▲, percent errors for specific radioactivity of combined extracellular, intracellular and aminoacyl tRNA Leu and Phe (S(Ec + Ic + Tr)) = *, percent errors for specific radioactivity of plasma Leu or Phe (SPls) = ◇

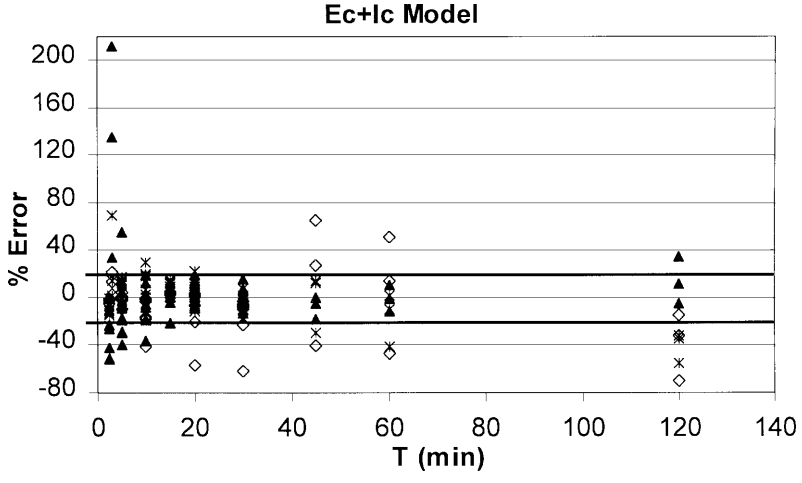

Fig. 6. Percent errors for Ec + Ic model across all data points (species, time points, tissues and pools). Percent error is predicted specific radioactivity by model – observed specific radioactivity. S prefix represents specific radioactivity. Percent errors for specific radioactivity of protein (SPb) = ▲, percent errors for specific radioactivity of combined extracellular and intracellular Leu and Phe (S(Ec + Ic)) = *, percent errors for specific radioactivity of plasma Leu or Phe (SPls) = ◇

Table 1. Ability of the models to predict specific radioactivity data as indicated by number (N) and percent errors[1] that are outliers[2] for species, time, tissue and pool specific radioactivity

	Total N[3]	N Outliers[4]	% Ec + Pls[5]	% Ec + Ic + Tr[6]	% Ec + Ic[7]
Species					
Mouse	215	72	26	42	32
Rat	378	47	34	38	28
Time (min)					
2.5	63	22	36	36	28
3	26	13	24	38	38
5	90	23	39	43	18
10	90	13	31	46	23
15	63	1	0	0	100
20	90	7	42	29	29
30	90	7	29	42	29
45	27	10	20	40	40
60	27	6	33	17	50
120	27	15	13	47	40
Tissue					
Brain	71	27	33	41	26
Gastrocnemius	54	5	40	40	20
Gut	54	3	33	67	0
Heart	54	12	33	33	33
Kidney	54	9	33	33	33
Liver	126	34	24	41	35
Muscle	72	16	26	37	37
Soleus	54	7	42	29	29
Tibialis	54	6	17	66	17
Pool S[8]					
S(Ec + Ic)	66	9	NA[9]	NA	100
S(Ec + Pls)	66	11	100	NA	NA
S(Ec + Ic + Tr)	66	11	NA	100	NA
SIc	65	7	100	NA	NA
SPls	132	21	NA	43	57
SPb	198	60	28	47	25

[1] Percent error is predicted specific radioactivity by model – observed specific radioactivity; [2] Outliers are percent errors (predicted – observed) that are less than −20% or greater than +20%; [3] Total number (N) of data points; [4] Total number (N) of outliers; [5] Percent of total number of outliers for Ec + Pls model; [6] Percent of total number of outliers for Ec + Ic + Tr model; [7] Percent of total number of outliers for Ec + Ic model; [8] S is specific radioactivity of the pool; [9] Not Applicable (NA).

slightly better (28% of total outliers). The Ec + Ic + Tr model had the most outliers for predicting S (40%).

Predictions of FSR by the models (Tables 2 and 3) varied over a wide range but were still within experimental estimates except for the muscle models (gastrocnemius, soleus, tibialis and muscle). Standard deviations on experimental predictions of FSR by the flooding dose, continuous infusion and pulse dose methods are high. Therefore, wide ranges in predicted FSR are still within experimental FSR estimates. Standard deviations (SD) on FSR predicted by the models were very low except for the kidney Ec + Ic + Tr

Table 2. Comparison of predicted FSR (% d^{-1}), by the pulse dose, mouse tissue models, to protein synthesis rates (umol min^{-1}) and to other estimated FSR. Standard deviations are in parentheses following the values

	FFSR[1]	CIFSR[2]	FSR[3]	PFSR[4]	PSR[5]
Brain	16	12 (8.7)	13		
Ec + Pls				16 (1.6)	0.0014
Ec + Ic + Tr				5.0 (0.49)	0.00043
Ec + Ic				14 (0.00014)	0.0013
Liver	92 (34)	64 (39)	130		
Ec + Pls				120 (6.1)	0.034
Ec + Ic + Tr				27 (0.00045)	0.0083
Ec + Ic				78 (7.0)	0.024
Muscle	14 (5.5)	9.3 (5.6)	9.8		
Ec + Pls				14 (0.78)	0.042
Ec + Ic + Tr				2.5 (0.21)	0.0074
Ec + Ic				15 (0.89)	0.045

[1] FFSR were protein fractional synthesis rates from flooding dose experiments summarized by Johnson et al. (1999a); [2] CIFSR were protein fractional synthesis rates from continuous infusion experiments summarized by Johnson et al. (1999a); [3] FSR were protein fractional synthesis rates from Lajtha (1959); [4] PFSR were protein fractional synthesis rates predicted by fitting the Lajtha (1959) specific radioactivity data to the mouse tissue models; [5] PSR were umol of Phe incorporated into protein per min as predicted by the mouse tissue models.

model (FSR 23% d^{-1}, SD = 19). The Ec + Ic + Tr models under predicted FSR relative to the FSR reported in the papers except with the flooding dose gut and liver models.

The primary source for amino acids for protein synthesis was from the protein pool (Tables 4 and 5). The exception was the flooding dose liver, kidney, heart and gut models. All muscle models were associated with high recycling (90–96%). Model predicted FSR for the flooding dose muscle models were very close (56–57%) and were much higher than experimentally derived FSR (4.5–10%). The sources of amino acids for gut were from the Pls and Ec + Pls pools indicating that Pls was probably the primary source. The Ec + Pls and Ec + Ic pools were the primary sources for kidney protein synthesis indicating that either the Ec was a major contributor or the dose of radiolabeled amino acid needed to be diluted by passing through another pool before being incorporated into protein. Liver (flooding dose) and heart sources of amino acids appeared to be divided approximately equally between pools (Ec, Pls and Ic). Predictions of source of amino acids for protein synthesis are not shown for the Ec + Ic + Tr model since the Ec + Ic + Tr pool is the only source.

Discussion

The implication of these model solutions is that including a separate aminoacyl tRNA pool is critical in tissues that recycle amino acids. Predicting

Table 3. Comparison of predicted FSR (% d^{-1}), by the flooding dose, rat tissue models, to protein synthesis rates (umol min^{-1}) and to other estimated FSR. Standard deviations are in parentheses following the values

	FFSR[1]	CIFSR[2]	FSR[3]	PFSR[4]	PSR[5]
Gastrocnemius	14 (5.5)	9.3 (5.6)	10		
Ec + Pls				57 (0.40)	0.018
Ec + Ic + Tr				3.4 (0.24)	0.0011
Ec + Ic				57 (0.38)	0.018
Gut	120 (28)	77 (36)	61		
Ec + Pls				58 (2.7)	0.059
Ec + Ic + Tr				71 (4.1)	0.071
Ec + Ic				62 (2.8)	0.062
Heart	19 (7.2)	15 (4.9)	10		
Ec + Pls				8.3 (0.46)	0.00063
Ec + Ic + Tr				8.4 (0.21)	0.00064
Ec + Ic				7.9 (0.29)	0.00060
Kidney	80 (14)	29 (8.2)	31		
Ec + Pls				32 (11)	0.0068
Ec + Ic + Tr				23 (19)	0.0050
Ec + Ic				25 (2.1)	0.0054
Liver	92 (34)	64 (39)	43		
Ec + Pls				160 (0.13)	0.18
Ec + Ic + Tr				90 (5.5)	0.10
Ec + Ic				80 (0.88)	0.092
Soleus	14 (5.5)	9.3 (5.6)	9.6		
Ec + Pls				57 (0.012)	0.00072
Ec + Ic + Tr				5.5 (0.34)	0.000070
Ec + Ic				57 (0.27)	0.00072
Tibialis	14 (5.5)	9.3 (5.6)	4.5		
Ec + Pls				56 (0.50)	0.0023
Ec + Ic + Tr				3.4 (0.17)	0.00014
Ec + Ic				57 (0.55)	0.0023

[1] FFSR were protein fractional synthesis rates from flooding dose experiments summarized by Johnson et al. (1999a); [2] CIFSR were protein fractional synthesis rates from continuous infusion experiments summarized by Johnson et al. (1999a); [3] FSR were protein fractional synthesis rates from Goldspink et al. (1984), Goldspink and Kelly (1984), Kelly et al. (1984) and Lewis et al. (1984); [4] PFSR were protein fractional synthesis rates predicted by fitting the Goldspink et al. (1984), Goldspink and Kelly (1984), Kelly et al. (1984) and Lewis et al. (1984) specific radioactivity data to the rat models; [5] PSR were umol of Phe incorporated into protein per min as predicted by the rat models.

specific radioactivity changes and FSR was difficult with Ec + Ic + Tr model in tissues with known high rates of recycling (brain, liver (pulse), muscle, gastrocnemius, soleus and tibialis). However, with low recycling tissues such as gut, heart, kidney and liver (flooding dose) the FSR predicted by the Ec + Ic + Tr models was close to FSR predicted by the other models. In tissues with high recycling, S(Ec + Ic + Tr) was too high relative to SPb and so predicted FSR was lower than estimated in the papers (Lajtha, 1959; Goldspink and

Table 4. Percent Leu for aminoacyl tRNA from different sources relative to amount of protein synthesized as predicted from fitting ^{14}C Leu pulse dose specific radioactivity data from Lajtha (1959) to the mouse tissue models. Standard deviations are in parentheses following the values

	% Pls[1]	% Ec + Pls[2]	% Ic[3]	% Ec + Ic[4]	% Pb[5]
Brain					
Ec + Pls		4.8 (2.1)	0		95 (13)
Ec + Ic	27 (0.0010)			0	73 (0.00028)
Liver					
Ec + Pls		7.6 (0.38)	2.0 (0.16)		90 (0.0088)
Ec + Ic	8.8 (1.9)			1.7 (2.6)	90 (6.1)
Muscle					
Ec + Pls		7.3 (1.6)	1.6 (0.011)		91 (9.4)
Ec + Ic	14 (17)			0	86 (0.77)

[1] Leu from plasma which is incorporated into protein (% min^{-1}); [2] Leu from combined extracellular and plasma Leu pool which is incorporated into protein (% min^{-1}); [3] Leu from intracellular Leu pool which is incorporated into protein (% min^{-1}); [4] Leu from combined extracellular and intracellular Leu pool which is incorporated into protein (% min^{-1}); [5] Leu from protein pool which is incorporated back into protein without mixing with the plasma, extracellular or intracellular pools (% min^{-1}).

Kelly, 1984; Lewis et al., 1984; Goldspink et al., 1984 and Kelly et al., 1984) and much lower than FSR predicted by the other models.

With the flooding dose muscle models, however, recycling appeared to increase the predicted FSR. Predicted FSR's among muscle tissues were much closer (57% d^{-1} for gastrocnemius, soleus and tibialis) than FSR estimated in the papers (10 for gastrocnemius, 9.6 for soleus and 4.5% d^{-1} for tibialis). If recycling was not allowed in the model solutions for the Ec + Pls and Ec + Ic flooding dose muscle models, the predicted FSR would be 3.8 (SD = 0.47) and 3.7% d^{-1} (SD = 0.34) respectively, for the gastrocnemius models, 5.02 (SD = 0.55) and 5.01% d^{-1} (SD = 0.45) respectively, for the soleus models and 3.0 (SD = 0.30) and 3.2% d^{-1} (SD = 0.23) respectively, for the tibialis models. Model predictions of specific radioactivities without recycling increased the number of outliers by 1–2 points per model. The predicted FSR without recycling was close to the Ec + Ic + Tr predicted FSR and much lower than estimated FSR. Which FSR and recycling rate is appropriate is unclear. However, the inability of the rat muscle Pls + Ec and Ec + Ic models to distinguish between high and low recycling is probably due to the flooding dose methodology. In Johnson et al. (1999b), a sensitivity analysis of flooding dose Ec + Pls model with high and low rates of recycling indicated that rate of recycling might be difficult to determine. In addition, pulse dose muscle models (Ec + Pls and Ec + Ic) were unable to duplicate the specific radioactivity data without high recycling rates. Therefore if there is high recycling in muscle, then flooding dose FSR model predictions with high recycling may be more accurate.

Model solutions to predict the specific radioactivity changes in the data implied that the separation of Pls amino acid from Ec + Ic + Tr would lead

Table 5. Percent Phe for aminoacyl tRNA from different sources relative to amount of protein synthesized as predicted by fitting ^{3}H Phe flooding dose specific radioactivity data from Goldspink et al. (1984), Goldspink and Kelly (1984), Kelly et al. (1984) and Lewis et al. (1984) to the rat tissue models. Standard deviations are in parentheses following the values

	% Pls[1]	% Ec + Pls[2]	% Ic[3]	% Ec + Ic[4]	% Pb[5]
Gastrocnemius					
Ec + Pls		5.5 (12)	0		95 (0.42)
Ec + Ic	5.5 (14)			0	95 (0.038)
Gut					
Ec + Pls		100 (11)	0		0
Ec + Ic	94 (12)			0	5.8 (3.7)
Heart					
Ec + Pls		45 (5.5)	55 (9.0)		0
Ec + Ic	37 (8.2)			63 (16)	0
Kidney					
Ec + Pls		70 (47)	0		30 (5.3)
Ec + Ic	17 (1.6)			75 (0.068)	8.9 (0.41)
Liver					
Ec + Pls		45 (0.00065)	54 (0.0013)		1.9 (0.12)
Ec + Ic	34 (0.66)			66 (0.042)	0
Soleus					
Ec + Pls		7.4 (8.5)	0		93 (11)
Ec + Ic	7.4 (10)			0	93 (0.20)
Tibialis					
Ec + Pls		5.1 (6.0)	0		95 (0.39)
Ec + Ic	5.1 (7.8)			0	95 (0.30)

[1] Phe from plasma which is incorporated into protein (% min^{-1}); [2] Phe from combined extracellular and plasma Phe pool which is incorporated into protein (% min^{-1}); [3] Phe from intracellular Phe pool which is incorporated into protein (% min^{-1}); [4] Phe from combined extracellular and intracellular Phe pool which is incorporated into protein (% min^{-1}); [5] Phe from protein pool which is incorporated back into protein without mixing with the plasma, extracellular or intracellular pools (% min^{-1}).

to underestimation of FSR with the pulse dose or flooding dose methods. The separation of Pls + Ec from Ic amino acids appeared to be better for the pulse dose method and the separation of Pls from Ec + Ic was better for simulations data from the flooding dose method. If recycling was occurring, the Tr pool specific radioactivity needed to be separated. In order to confirm that recycling was occurring in the flooding dose muscle models, the specific radioactivity of the aminoacyl tRNA should be measured.

References

Aegis Technologies (2000) Optimize, ACSL: Advanced continuous simulation language. Aegis Technologies, Huntsville, Alabama

Bernier JF, Calvert CC (1987) Effect of a major gene for growth on protein synthesis in mice. J Anim Sci 65: 982–995

Goldspink DF, Kelly FJ (1984) Protein turnover and growth in the whole body, liver and kidney of the rat from foetus to senility. Biochem J 217: 507–516

Goldspink DF, Lewis SEM, Kelly FJ (1984) Protein synthesis during the developmental growth of the small and large intestine of the rat. Biochem J 217: 527–534

Hider RC, Fern EB, London DR (1971) Identification in skeletal muscle of a distinct extracellular pool of amino acids, and its role in protein synthesis. Biochem J 121: 817–827

Johnson HA, Bladwin RL, France J, Calvert CC (1999a) Development and evaluation of a model of whole body protein turnover based on leucine kinetics in rodents. J Nutrition 129: 728–739

Johnson HA, Baldwin RL, France J, Calvert CC (1999b) Recycling, channeling and heterogeneous protein turnover based on leucine kinetics in rodents. J Nutrition 129: 740–750

Johnson HA, Baldwin RL, Klasing KC, France J, Calvert CC (2000) A rodent model of protein turnover used to design an experiment for measuring the rates of channeling, recycling and protein synthesis. J Nutrition (accepted)

Kelly FJ, Lewis SEM, Anderson P, Goldspink DF (1984) Pre and postnatal growth and protein turnover in four muscles of the rat. Muscle Nerve 7: 235–242

Lajtha A (1959) Amino acid and protein metabolism of the brain – V. turnover of leucine in mouse tissues. J Neurochem 3: 358–365

Lewis SEM, Kelly FJ, Goldspink DF (1984) Pre and postnatal growth and protein turnover in smooth muscle, heart and slow and fast twitch skeletal muscles of the rat. Biochem J 217: 517–526

Obled C, Barre F, Arnal M (1991) Flooding-dose of various amino acids for measurement of whole-body protein synthesis in the rat. Amino Acids 1: 17–27

Smith BC, Deibler GE, Schmidt K, Sokoloff L (1988) Measurement of local cerebral protein synthesis in vivo: Influence of recycling of amino acids derived from protein degradation. Proc Natl Acad Sci USA 85: 9341–9345

Authors' address: Heidi A. Johnson, Animal Science Department, University of California at Davis, Davis, CA 95616 U.S.A.,
Fax: 530 752-0175, E-mail: HAJohnson@UCDavis.edu

Received June 11, 2000