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An Isotope Dilution Model for Partitioning Leucine Uptake by the Bovine Mammary Gland

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A model of leucine uptake and partition by the mammary gland (i.e. the udder) of the lactating dairy cow is constructed and solved in the steady state. Model solution permits—if assumptions are made—calculation of leucine uptake from the arterial blood supply, leucine export into the venous drainage, leucine oxidation, and synthesis and degradation of milk protein and constitutive tissue protein within the gland. The experimental measurements required for model solution are blood flow and milk secretion, arterial and venous concentration and plateau enrichment of leucine, and concentration and plateau enrichment of free and protein-bound leucine in milk. The model, at least in theory, is also applicable to other amino acids and provides a means of ranking the nutritional importance of individual amino acids and identifying the most limiting in the gland.

1. Introduction

Traditionally, dairy research has centred on the twin objectives of increasing milk output and improving efficiency of production. With the growing demand from consumers and industry to improve milk protein content, research has now focused on identifying and understanding the factors and mechanisms regulating the partitioning of amino acids towards milk proteins. Such a research programme, directed towards prediction and manipulation of milk composition, is being undertaken jointly between our laboratories.

Much of the knowledge accrued to date on amino acid and protein metabolism in the lactating mammary gland has been derived from the perfused mammary gland (e.g. Roets *et al*., 1979, 1983) and from *in vivo* measurements of tissue protein synthesis (e.g. Champredon *et al*., 1990; Baracos *et al*., 1991) using dairy goats. Such studies, whilst they have provided information on the metabolic pathways of milk synthesis, are generally limited by single measurements. The *in vivo* arterio-venous (A-V) preparation of the mammary gland used for example by Linzell (1971) and Oddy *et al*. (1988) in goats, overcomes this limitation. The preparation, used in conjunction with an infusion of isotopes of specific substrates, allows repeated measurements of the fates of metabolites in the mammary gland to be made *in vivo* throughout lactation. We have established an A-V preparation for the bovine mammary gland and measured leucine exchange plus the enrichment of [1-^{13}C]leucine in blood and secreted milk in order to investigate the influence of diet on partitioning of this amino acid between milk protein output and other metabolic activities.

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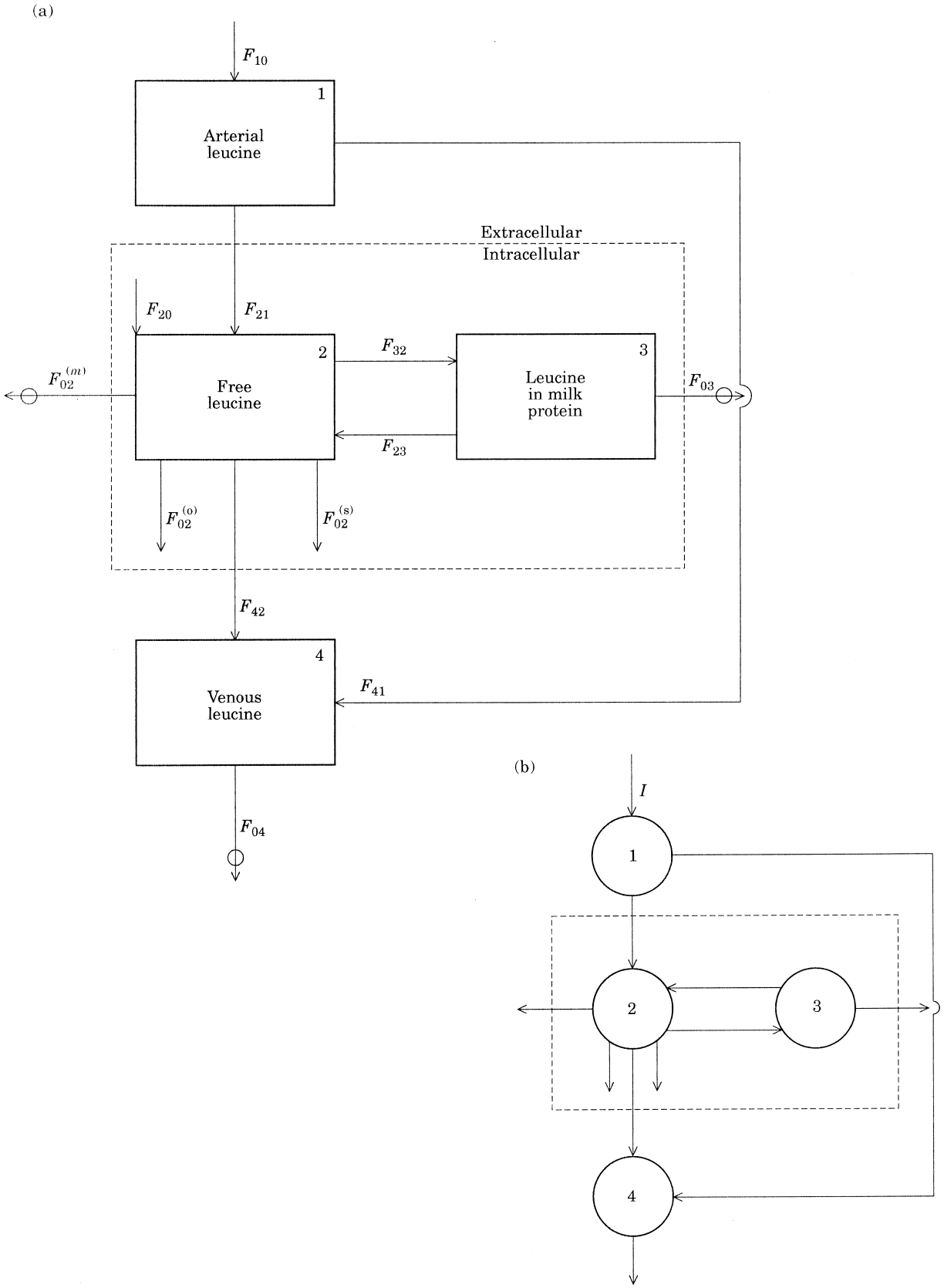

FIG. 1. Scheme for the uptake and partition of leucine by the mammary gland: (a) total leucine, (b) labelled. The small circles in (a) indicate fluxes that need to be measured experimentally.

TABLE 1
Principal symbols

F_{ij}	Total flux of leucine to pool i from j; F_{i0} denotes an external flux into pool i and F_{0j} a flux from pool j out of the system; a tilde indicates a flux which can be measured experimentally: μmol min^{-1}
I	Constant rate of infusion of labelled leucine into primary pool: μmol min^{-1}
Q_i	Total quantity of leucine in pool i: μmol
q_i	Quantity of labelled leucine in pool i: μmol
e_i	Enrichment of pool i ($=q_i/Q_i$): μmol label/(μmol total leucine)
R	Dilution ratio: dimensionless
t	Time: min

The model described herein was developed to resolve the data generated from this trial. The compartmental modelling presented is of interest in that only a single infusion of isotope is made, unlike in conventional steady-state *in vivo* tracer-kinetic studies where an infusion is made into each pool represented in the scheme in turn, thus enabling the model to be fully resolved by solving a system of N simultaneous equations in N unknowns. A simplified version of the model is also discussed.

2. The Model

The scheme adopted is shown in Fig. 1(a). It contains two intracellular and two extracellular pools. The intracellular pools are free leucine and leucine in milk protein (pools 2 and 3 respectively) while the extracellular are arterial and venous leucine (pools 1 and 4). The fluxes of leucine between pools and into and out of the system are shown as arrowed lines. The intracellular free leucine pool has three influxes, i.e. from the degradation of constitutive mammary gland protein, F_{20}, from the extracellular arterial pool, F_{21}, and from the degradation of milk protein, F_{23}, and five effluxes, i.e. secretion in milk, $F_{02}^{(m)}$, oxidation, $F_{02}^{(o)}$, synthesis of constitutive mammary gland protein, $F_{02}^{(s)}$, incorporation into milk protein, F_{32}, and outflow into the venous pool, F_{42}. The milk protein-bound leucine pool has one influx, from free leucine, F_{32}, and two effluxes, secretion of protein in milk, F_{03}, and degradation, F_{23}. The extracellular arterial leucine pool also has a single influx, entry into the pool, F_{10}, and two effluxes, uptake by the mammary gland, F_{21}, and outflow to the venous pool, F_{41}. The venous leucine pool has two influxes, entry from the arterial pool, F_{41}, and from the mammary gland, F_{42}, and one efflux, F_{04}.

The scheme adopted for movement of label is shown in Fig. 1(b). 1-^{13}C leucine is infused systemically at a constant rate and the enrichment of all four pools monitored. The enrichment of the two intracellular pools cannot be measured directly, therefore the enrichments of the intracellular free-leucine and milk-protein-bound-leucine pools are assumed equivalent to the enrichments of free and protein-bound leucine respectively in secreted milk. The scheme assumes that the only entry of label into the system is into the arterial leucine pool via flux I and that the duration of the infusion is such that the enrichment of constitutive protein can be regarded as negligible.

Conservation of mass principles can be applied to each pool in Fig. 1 to generate differential equations which describe the dynamic behaviour of the system. For total leucine, these differential equations are (mathematical notation is defined in Table 1):

$$dQ_1/dt = F_{10} - F_{21} - F_{41} \quad (1)$$

$$dQ_2/dt = F_{20} + F_{21} + F_{23} - F_{02}^{(m)} - F_{02}^{(o)} - F_{02}^{(s)} - F_{32} - F_{42} \quad (2)$$

$$dQ_3/dt = F_{32} - F_{03} - F_{23} \quad (3)$$

$$dQ_4/dt = F_{41} + F_{42} - F_{04}, \quad (4)$$

and for labelled leucine:

$$dq_1/dt = I - e_1(F_{21} + F_{41}) \quad (5)$$

$$dq_2/dt = e_1F_{21} + e_3F_{23} - e_2(F_{02}^{(m)} + F_{02}^{(o)} + F_{02}^{(s)} + F_{32} + F_{42}) \quad (6)$$

$$dq_3/dt = e_2F_{32} - e_3(F_{03} + F_{23}) \quad (7)$$

$$dq_4/dt = e_1F_{41} + e_2F_{42} - e_4F_{04}. \quad (8)$$

When the system is in steady state with respect to both total and labelled leucine, the derivative terms in eqns (1)–(8) are zero and the equations become:

$$F_{10} - F_{21} - F_{41} = 0 \quad (9)$$

$$F_{20} + F_{21} + F_{23} - F_{02}^{(m)} - F_{02}^{(o)} - F_{02}^{(s)} - F_{32} - F_{42} = 0 \quad (10)$$

$$F_{32} - F_{03} - F_{23} = 0 \quad (11)$$

$$F_{41} + F_{42} - F_{04} = 0 \quad (12)$$

$$I - e_1(F_{21} + F_{41}) = 0 \quad (13)$$

$$e_1F_{21} - e_3(F_{02}^{(m)} + F_{02}^{(o)} + F_{02}^{(s)} + F_{32} - F_{23} + F_{42}) = 0 \quad (14)$$

$$e_1F_{41} + e_3F_{42} - e_4F_{04} = 0. \quad (15)$$

Note that, for the scheme assumed, the enrichment of the milk protein-bound leucine intracellular pool equalizes with that of the free-leucine pool in steady state (i.e. $e_2 = e_3$) otherwise eqns (3) and (7) are inconsistent, so these equations both yield eqn (11) and e_2 can be written as e_3 in eqns (6) and (8) yielding eqns (14) and (15), respectively. The three fluxes, free-leucine and protein secretion in milk and leucine removal from the venous blood pool (i.e. $F_{02}^{(m)}$, F_{03} and F_{04}, respectively), can be measured experimentally (Oddy *et al*., 1988). Algebraic manipulation of eqns (9)–(15) gives:

$$F_{02}^{(o)} + F_{02}^{(s)} = I/e_3 - \tilde{F}_{02}^{(m)} - \tilde{F}_{03} - e_4\tilde{F}_{04}/e_3 \quad (16)$$

$$F_{10} = I/e_1 \quad (17)$$

$$F_{20} = (1/e_3 - 1/e_1)I + (e_3 - e_4)\tilde{F}_{04}/e_3 \quad (18)$$

$$F_{21} = I/e_1 - (e_3 - e_4)\tilde{F}_{04}/(e_3 - e_1) \quad (19)$$

$$F_{32} - F_{23} = \tilde{F}_{03} \quad (20)$$

$$F_{41} = (e_3 - e_4)\tilde{F}_{04}/(e_3 - e_1) \quad (21)$$

$$F_{42} = (e_1 - e_4)\tilde{F}_{04}/(e_1 - e_3), \quad (22)$$

where the tilde denotes an experimentally determined flux. Note that model solution requires the value of e_4 to lie between those of e_1 and e_3. This can be ascertained from inspection of eqns (21) and (22).

Model solution as given by eqns (16)–(22) does not allow separation of $F_{02}^{(o)}$ and $F_{02}^{(s)}$, the leucine oxidation flux and leucine utilization for constitutive mammary gland protein synthesis [see eqn (16)]. However, as the rate of accretion (i.e. synthesis minus degradation) of constitution mammary protein in the lactating ruminant is likely to be small (Knight & Wilde, 1987), an indicator of leucine oxidation can be derived by subtracting eqns (16) and (18) to give:

$$\text{oxidation} + (\text{accretion}) = F_{02}^{(o)} + (F_{02}^{(s)} - F_{20}) \quad (23a)$$

$$= I/e_1 - \tilde{F}_{02}^{(m)} - \tilde{F}_{03} - \tilde{F}_{04}. \quad (23b)$$

Equation (23b) states that the difference between apparent uptake of leucine by the mammary gland and leucine losses in milk serves as an indicator of its oxidation in the gland. Alternatively, oxidation can be determined directly by measuring the trans-organ evolution of isotopically labelled CO_2 arising during a labelled amino acid infusion (e.g. Oddy & Lindsay, 1986; Pell *et al*., 1986; Harris *et al*., 1992).

Furthermore, model solution does not allow separation of the milk protein synthesis and degradation fluxes F_{32} and F_{23}, but merely permits calculation of their difference, i.e. net synthesis [eqn (20)]. However, separation can be achieved by assuming that a fixed proportion (≈ 0.1) of the nascent milk protein is cleaved and degraded during the docking and secretory processes (Razooki Hasan *et al*., 1982).

The model can also be used to obtain a dilution ratio for identifying whether or not an amino acid, such as leucine, is limiting protein synthesis in the mammary gland by considering the venous amino acid pool (pool 4). Its steady-state flux equations are eqns (12) and (15), viz.

$$F_{41} + F_{42} = \tilde{F}_{04} \quad (24)$$

$$e_1F_{41} + e_3F_{42} = e_4\tilde{F}_{04}. \quad (25)$$

Eliminating the flux F_{41} by multiplying eqn (24) by e_1 and subtracting (25) yields

$$F_{42} = (e_1 - e_4)\tilde{F}_{04}/(e_1 - e_3) \quad (26a)$$

$$= R\tilde{F}_{04}, \quad (26b)$$

where R, the dilution ratio, is defined by

$$R = (e_1 - e_4)/(e_1 - e_3). \quad (27)$$

R cannot lie outside of the range 0 to 1 as model solution requires e_4 to lie in the range e_1 to e_3. Equations (26a, b) give F_{42}, entry of leucine into the venous pool from the mammary gland. This cannot exceed $\tilde{F}_{04}$ [see eqn (24)]. If leucine is limiting protein synthesis, most likely very little will leave the cell and so the flux F_{42} will be negligible. From an inspection of eqn (26b), it is apparent that F_{42} is negligible if the dilution ratio R or the flux $\tilde{F}_{04}$ tends to zero. $\tilde{F}_{04}$ will be negligible if venous leucine concentration is insignificant. Note that calculation of R requires enrichment in secreted milk to be a reliable proxy for intracellular enrichment. Venous concentration and the index R, used in conjunction with a series of infusions of different amino acids, therefore provide, in theory at

TABLE 2
Experimental measurements

	Cow		
	S_L	T_M	W_L
Milk yield (kg day^{-1})	22.9	22.5	25.6
Plateau enrichment (%):			
e_1	4.55	4.22	4.20
e_3	3.56	3.15	3.78
e_4	4.12	3.76	4.03
Flux (μmol leucine min^{-1}):			
I	35.7	48.8	36.7
$\tilde{F}_{02}^{(m)}$	0	0	0
$\tilde{F}_{03}$	379	379	430
$\tilde{F}_{04}$	460	704	449

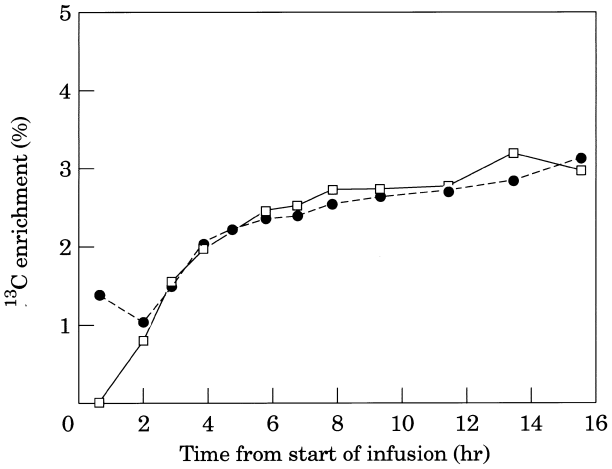

FIG. 2. Time-course of enrichments of free (●) and protein-bound (□) leucine in secreted milk from a dairy cow in late lactation continuously infused with ^{13}C-leucine (Metcalf and co-workers, unpublished data).

least, a means of ranking individual amino acids and identifying the most limiting under particular dietary and physiological conditions.

3. Application

Application of the model is illustrated using data from an experiment conducted at our laboratories with four Holstein–Friesian dairy cows (average weight 670 kg) 24 to 30 weeks into their third lactation. The cows had previously been prepared with catheters into the subcutaneous abdominal vein (which drains the mammary gland), into either the carotid artery or via an intercostal or mesenteric artery into the dorsal aorta, and into the jugular vein.

The cows were fed hourly by autofeeders at two levels of dietary crude protein (CP), based on a grass silage diet [40% dry matter (DM)] with 60% DM provided as low (L) or medium (M) protein concentrate with additional CP provided as protected soya (Sopralin, BP Nutrition). Dietary CP levels were 130 and 155 g kg^{-1} DM for L and M, respectively, and the cows were fed according to milk energy yield. Experimental periods were 3 weeks, with blood and milk samples taken on one day in the final week of treatment. During the sampling period, [1-^{13}C]leucine was infused into the jugular vein and blood samples were taken from the artery and subcutaneous abdominal vein, and milking was performed at 2 hr intervals using oxytocin to ensure complete removal from the gland (Bequette and co-workers, unpublished data).

The relevant experimental measurements are given in Table 2, but pertain to only three of the cows owing to an interruption in the [1-^{13}C]leucine infusion to the fourth. They are based on plasma rather than whole blood values and free rather than total (i.e. free plus bound) plasma leucine. The effective infusion rates I were determined from arterial concentration and enrichment of leucine and plasma flow. Values for $\tilde{F}_{04}$ and I were obtained using plasma flows determined by an indicator-dilution method (Meier & Zierler, 1954; Katz & Bergman, 1969). The flux and enrichment of free leucine in secreted milk were not measured in the experiment, so $\tilde{F}_{02}^{(m)}$ was assigned a value of zero and e_2 was assumed equal to e_3. Justification for the former assumption is provided by Mehaia & Al-Kanhal (1992), who give a value of 3 μM for the concentration of free leucine in cow's milk compared with approximately 15–18 mM for protein bound leucine, and justification for the latter assumption is provided by Fig. 2, which shows data from an earlier pilot study with a cow in a similar stage of lactation as those cows used in the experiment.

The model calculations obtained from these experimental measurements are given in Table 3. Values for

TABLE 3
Model calculations

	Cow		
	S_L	T_M	W_L
Flux (μmol leucine min^{-1}):			
$F_{02}^{(o)}$	0†	73	0†
$F_{02}^{(s)}$	91†	256	62†
F_{10}	785	1156	874
F_{20}	146	256	67
F_{21}	524	755	607
F_{23}	42	42	48
F_{32}	421	421	478
F_{41}	260	401	267
F_{42}	200	303	182
Dilution ratio R	0.43	0.43	0.40

† Model calculation of combined flux was less than that of degradation, so separation of synthesis and oxidation achieved in this case by assuming oxidation to be zero.

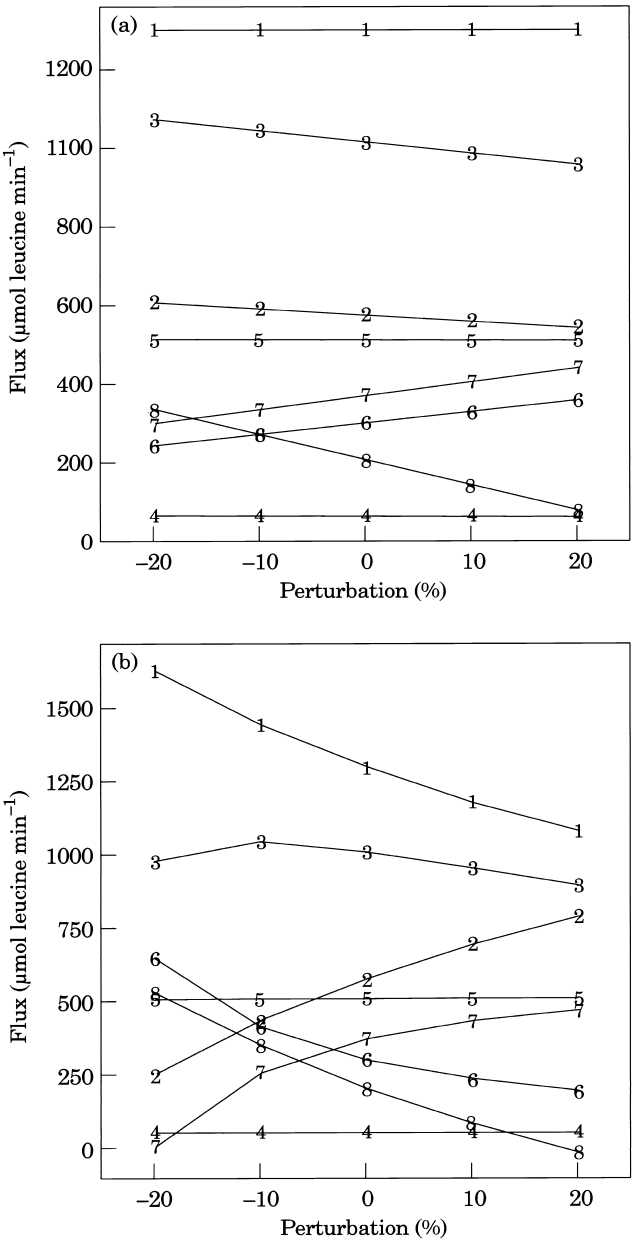

FIG. 3. Model calculation of the fluxes F_{10} (1), F_{20} (2), F_{21} (3), F_{23} (4), F_{32} (5), F_{41} (6), F_{42} (7) and $F_{02}^{(o)}$ (8) when (a) $\tilde{F}_{04}$ and (b) e_1 alone is perturbed.

oxidation and constitutive protein synthesis were determined by assuming constitutive protein accretion to be zero (i.e. synthesis equals degradation), and values of milk protein synthesis and degradation by assuming degradation is 0.1 of synthesis. Table 3 shows that uptake of a particular amino acid by the gland is not a reliable indicator of milk protein output. The lowest-yielding cow T_M has the highest leucine uptake ($F_{21} = 755$ μmol min^{-1}), whereas the highest-yielding cow W_L has a significantly lower uptake (607 μmol min^{-1}). However, milk protein synthesis, F_{32}, is 79% of leucine uptake for W_L but only 56% for T_M. The dilution ratio R suggests leucine is not a limiting amino acid in any of the three cases.

The sensitivity of the model to perturbations in the experimental measurements was examined by ascribing values of $I = 65$, $\tilde{F}_{02}^{(m)} = 5$, $\tilde{F}_{03} = 450$, $\tilde{F}_{04} = 650$ (all μmol leucine min^{-1}), $e_1 = 5\%$, $e_2 = 3.2\%$ and $e_3 = 4\%$ to the inputs and solving the model by perturbing each of these inputs in turn by 0, ± 10 and $\pm 20\%$. Model solutions are illustrated in Fig. 3 for the inputs $\tilde{F}_{04}$ and e_1. The average slope of each line so produced was then determined by a five-point linear regression. Scaled values of these slopes are presented in Table 4 which indicates an error of 1% in an input causes, on average, an error of about 2% in a flux sensitive to that input.

The sensitivity of the model to the assumption that milk protein degradation, F_{23}, is a fixed proportion λ ($=0.1$) of milk protein synthesis, F_{32}, can be demonstrated by considering the relative rates $(dF_{23}/d\lambda)/F_{23}$ and $(dF_{32}/d\lambda)/F_{32}$. Separating F_{23} and F_{32} in eqn (20) gives

$$F_{23} = \lambda \tilde{F}_{03}/(1 - \lambda) \tag{28}$$

and

$$F_{32} = \tilde{F}_{03}/(1 - \lambda). \tag{29}$$

Therefore,

$$(dF_{23}/d\lambda)/F_{23} = 1/[\lambda(1 - \lambda)] \tag{30}$$

and

$$(dF_{32}/d\lambda)/F_{32} = 1/(1 - \lambda). \tag{31}$$

For $\lambda = 0.1$, these relative rates become 11.1 for degradation and 1.1 for synthesis, indicating a 1% error in our estimate of λ produces a similar percentage error in synthesis, but an error of about 10% in degradation. F_{23} and F_{32} are the only fluxes in the model affected by the assumption.

4. Simplified Model

Though not generally the case, the enrichments of the extracellular arterial and venous pools can sometimes assume the same plateau value on reaching steady state (Bequette and co-workers, unpublished data). If the steady-state enrichments of extracellular arterial and venous pools are the same, the flux F_{42} describing the export of intracellular leucine into the venous drainage is zero [eqn (22)] and the model can be simplified by merging pools 1 and 4 to produce the three pool scheme shown in Fig. 4, where pool 1 now represents extracellular leucine. The differential equations for the simplified model are as follows. For

TABLE 4
Average slope (%) for each of the fluxes calculated by the model obtained by perturbing each input in turn

	Model calculation							
	$F_{02}^{(o)}$	F_{10}	F_{20}	F_{21}	F_{23}	F_{32}	F_{41}	F_{42}
Input perturbed:								
e_1	−6.9	−1.0	2.4	−0.2	0	0	−3.8	3.0
e_3	0	0	−2.2	0.7	0	0	−2.5	2.0
e_4	0	0	−1.4	−1.4	0	0	5.0	−4.0
I	6.7	1.0	1.3	1.3	0	0	0	0
$\tilde{F}_{02}^{(m)}$	−0.03	0	0	0	0	0	0	0
$\tilde{F}_{03}$	−2.3	0	0	0	0.01	0.01	0	0
$\tilde{F}_{04}$	−3.3	0	−0.3	−0.3	0	0	1.0	1.0

The slope for each flux is expressed relative to the value of the flux obtained when no perturbation is made.

total leucine [Fig. 4(a)]:

$$dQ_1/dt = F_{10} - F_{01} - F_{21} \tag{32}$$

$$dQ_2/dt = F_{20} + F_{21} + F_{23} - F_{02}^{(m)} - F_{02}^{(o)} - F_{02}^{(s)} - F_{32} \tag{33}$$

$$dQ_3/dt = F_{32} - F_{03} - F_{23}, \tag{34}$$

and for labelled leucine [Fig. 4(b)]:

$$dq_1/dt = I - e_1(F_{01} + F_{21}) \tag{35}$$

$$dq_2/dt = e_1F_{21} + e_3F_{23} - e_2(F_{02}^{(m)} + F_{02}^{(o)} + F_{02}^{(s)} + F_{32}) \tag{36}$$

$$dq_3/dt = e_2F_{32} - e_3(F_{03} + F_{23}). \tag{37}$$

In steady state, the derivative terms are zero and eqns (32)–(37) become:

$$F_{10} - F_{01} - F_{21} = 0 \tag{38}$$

$$F_{20} + F_{21} + F_{23} - F_{02}^{(m)} - F_{02}^{(o)} - F_{02}^{(s)} - F_{32} = 0 \tag{39}$$

$$F_{32} - F_{03} - F_{23} = 0 \tag{40}$$

$$I - e_1(F_{01} + F_{21}) = 0 \tag{41}$$

$$e_1F_{21} - e_3(F_{02}^{(m)} + F_{02}^{(o)} + F_{02}^{(s)} + F_{32} - F_{23}) = 0. \tag{42}$$

For this scheme, the enrichment of the milk protein-bound leucine intracellular pool equalizes with that of the free-leucine pool in steady state (i.e. $e_2 = e_3$), otherwise eqns (34) and (37) are inconsistent, so these equations both yield eqn (40) and e_2 can be written as e_3, in eqn (36) yielding eqn (42). Algebraic manipulation of eqns (38)–(42) gives:

$$F_{02}^{(o)} + F_{02}^{(s)} = I/e_3 - e_1\tilde{F}_{01}/e_3 - \tilde{F}_{02}^{(m)} - \tilde{F}_{03} \tag{43}$$

$$F_{10} = I/e_1 \tag{44}$$

$$F_{20} = (1/e_3 - 1/e_1)I + (e_3 - e_1)\tilde{F}_{01}/e_3 \tag{45}$$

$$F_{21} = I/e_1 - \tilde{F}_{01} \tag{46}$$

$$F_{32} - F_{23} = \tilde{F}_{03}, \tag{47}$$

where the tilde again denotes an experimentally determined flux. These equations can also be applied when all three enrichments attain the same plateau (i.e. $e_1 = e_2 = e_3$), in which case the degradation of mammary gland protein flux F_{20} is zero. As the export of intracellular leucine into the venous drainage is zero, a dilution ratio cannot be derived for this simplified scheme.

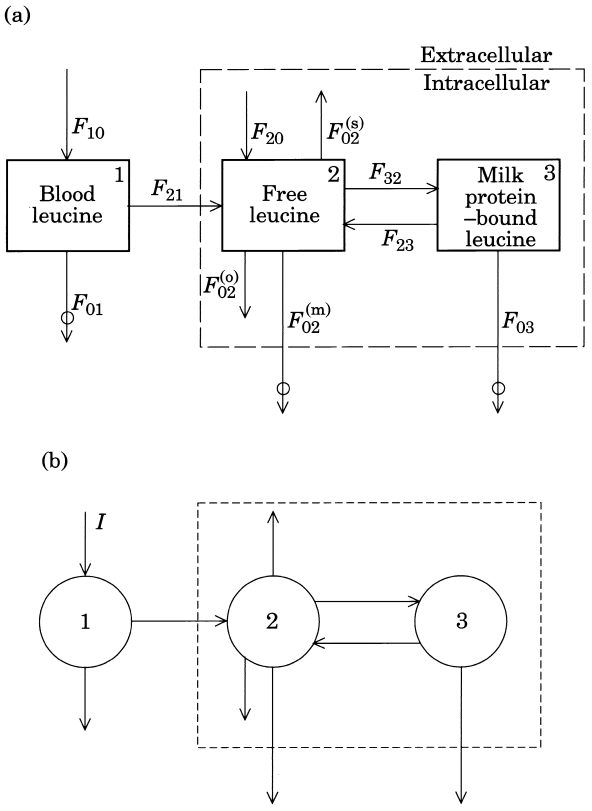

FIG. 4. Simplified scheme for the uptake and partition of leucine by the mammary gland: (a) total leucine, (b) labelled. The small circles in (a) indicate fluxes that need to be measured experimentally.

5. Discussion

The compartmental model, described here, of leucine metabolism in the lactating mammary gland, was constructed to interpret isotope dilution data from *in vivo* trans-organ studies with dairy cows undertaken at our laboratories. Model representation was primarily based on information from published investigations by Verbeke *et al*. (1959), Davis & Mepham (1976), Roets *et al*. (1979, 1983), Oddy & Lindsay (1986), Oddy *et al*. (1988), Champredon *et al*. (1990) and Baracos *et al*. (1991), and from investigations currently being conducted at our own laboratories into the precursors of milk protein synthesis.

Previously, one other isotope dilution model of amino acid and protein metabolism in the lactating mammary gland has been published, that by Oddy *et al*. (1988). This too focuses on leucine metabolism, but in the dairy goat. The Oddy model, which follows earlier work by Nissen & Haymond (1981), was constructed for the simultaneous determination of individual rates of protein synthesis and degradation in mammary tissue and in milk. The scheme contains five pools: mammary tissue protein, milk protein, free leucine, 4-methyl-2-oxopentanoic acid (MOP) and CO_2, and is based upon the metabolism and interconversion of [1-^{14}C]leucine and its transamination product [^{3}H]MOP across the isolated mammary gland. Direct measurements from each pool (with the exception of mammary tissue protein), including their transient specific radio activities, are required for model solution and the flux calculations rely upon accurate determinations of mammary blood flow and A-V difference of concentration of metabolites. The present model, which does not consider the keto-acid transamination and oxidation pathways directly and contains one less pool, requires fewer measurements with these taken only under steady-state conditions.

Any determination of protein synthesis is dependent upon the choice of precursor pool (France *et al*., 1988; Champredon *et al*., 1990; Baracos *et al*., 1991). In the Oddy model, the activity of the precursor pool for milk protein synthesis is represented by the specific radioactivity of free leucine in arterial blood. In the present model, isotopic activity of the precursor pool is represented by the enrichment of free leucine in secreted milk. In applying both models, it was assumed that the extracellular plasma pool is the only pool participating in the exchange of labelled and unlabelled leucine with the tissues of the mammary gland. This assumption is not strictly correct. The role of erythrocytes in the transport of amino acids to and possibly from tissues is recognized (Felig, 1975), and consideration has been given to the choice of whole blood or plasma as the extracellular pool exchanging amino acid with the tissues (Heitmann & Bergman, 1980; Barrett *et al*., 1987). These latter workers have observed differences in sheep and dogs between blood and plasma measurements of the net and isotopic exchange of amino acids with several body tissues, with differences occurring both between tissues and between amino acids. However, information for the mammary gland is limited and more equivocal, though Hanigan *et al*. (1991) concluded that net uptake of most amino acids by the bovine mammary gland derived from plasma measurements does not adequately represent amino acid uptake from the whole blood. Thus, using plasma measurements in solving both the Oddy and present models might not be wholly appropriate.

An assumption inherent in most analyses of tissue protein metabolism is that only blood- or plasma-free amino acid exchanges with the tissues. However, evidence is now emerging for ruminants that a significant proportion of the total amino acid in circulating blood and plasma is bound in peptides and proteins, and that there appears to be a removal of these by the mammary gland (Hanigan *et al*., 1991). In applying the present model using free rather than total circulating leucine, any utilization of leucine arising from the intracellular hydrolysis of extracellulary derived peptides and proteins is, to some extent, accounted for, but subsumed in the estimate of the constitutive protein degradation flux. Also, the model does not explicitly represent the use of amino acid carbon for the syntheses of non-essential amino acids and fatty acids, which is known to occur in the mammary gland of the lactating ruminant. Less than 1% of total leucine utilization in the mammary gland is thought, however, to be used for these syntheses (Verbeke *et al*., 1959; Oddy *et al*., 1988).

Secreted milk contains a heterogenous mixture of proteins. In the dairy cow, casein proteins comprise approximately 80% of total milk protein whilst the remainder is made up of various whey proteins (Miller *et al*., 1990). All casein proteins and some 70% of whey proteins are synthesized in the mammary gland. The remaining whey proteins are synthesized in the liver, transported to the mammary gland and then secreted in milk (Larson, 1979). The present model fails to embody this influx to the mammary gland of preformed whey proteins, which comprise about 6% of total milk protein output, and assumes that all protein secreted in milk is synthesized in the mammary gland.

Following the synthesis of most if not all milk proteins, a signal sequence on the newly synthesized protein is recognized by a specific recognition protein

(SRP)—an event which is required for the transport of the protein through the endoplasmic reticulum for eventual secretion (Meyer, 1982). The signal sequence and the SRP are cleaved during this process and presumably degraded intracellularly, since they do not appear in secreted milk. As stated earlier, a value of 0.1 of total protein synthesis is ascribed in the present model calculations to allow for this retention and re-entry process. Also, there is now also evidence to suggest that nascent milk proteins are degraded intracellularly as well (Razooki Hasan *et al*., 1982; Oddy *et al*., 1988), though there is no consensus as to its extent and therefore it has not been taken account of in the calculations presented here.

Despite these limitations, the model described provides a useful vehicle for obtaining information on the uptake and partitioning of leucine by the bovine mammary gland, indicating aspects of regulation that could be manipulated to direct more of the amino acid towards milk protein synthesis. Because of the level of representation adopted, the model is also applicable to other amino acids and provides, in theory at least, a means of ranking individual amino acids and identifying the most limiting in the gland.

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