Reprod. Nutr. Dev. 40 (2000) 549–558

Original article

Contribution of erythrocytes and plasma in threonine and lysine transfer across the portal drained viscera and the liver in pigs. Effect of threonine and lysine dietary supply

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(Received 21 June 2000; accepted 22 September 2000)

Abstract — Contributions of erythrocytes and plasma to threonine and lysine transport across the PDV and the liver were determined in growing pigs successively fed a threonine deficient diet and a control well-balanced diet (experiment 1) or a lysine deficient or a well-balanced diet (experiment 2). The animals were surgically prepared for insertion of chronic catheters in the mesenteric vein (MV), the portal vein (PV), a hepatic vein (HV) and the carotid artery (CA). Plasma and whole blood AA concentrations in PV, HV and CA and PV and HV blood flows were determined during 6 hours of para-aminohippuric acid constant infusion. During this period the pigs were continuously fed (1 meal per hour). The contribution of plasma to lysine and threonine transport was higher in pigs fed the well balanced diets. More than 50% of threonine and lysine appearing in the PV and in the HV are transported by the plasma. Our results suggest that erythrocytes are probably little involved in lysine and threonine transfer across the liver and digestive tract of pig continuously fed.

pig / lysine / threonine / blood transport / intestine / liver

Résumé — **Rôle des globules rouges et du plasma dans le transport de la lysine et de la thréonine à travers les tissus drainés par la veine porte et le foie chez le porc. Conséquences des teneurs en thréonine et en lysine des régimes.** Le rôle des globules rouges et du plasma dans le transport de la lysine et de la thréonine à travers les tissus drainés par la veine porte et le foie a été étudié chez des porcs en croissance au cours de deux expériences. Dans l'expérience 1, trois animaux recevaient successivement un aliment déficitaire en thréonine puis un aliment équilibré. Dans l'expérience 2, quatre porcs recevaient successivement un aliment déficitaire en lysine puis équilibré. Les animaux étaient munis de cathéters chroniques dans une veine mésentérique, la veine porte, une veine hépatique et l'artère carotide. Les concentrations sanguines et plasmatiques en acides aminés ont été mesurées dans

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Presented in part at the VIIIth International Symposium on Protein Metabolism and Nutrition [Threonine transfer across the splanchnic area in pigs], September 1–4, 1999, Aberdeen, Scotland.
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la veine porte, l'artère carotide et la veine hépatique. Parallèlement, les débits sanguins dans la veine porte et la veine hépatique ont été déterminés durant 6 heures d'une perfusion continue d'acide para-aminohippurique. Pendant toute la durée des prélèvements, les animaux étaient alimentés en continu. La contribution du plasma au transport de la thréonine et de la lysine est plus importante lorsque l'aliment n'est pas déficitaire en ces acides aminés. Plus de 50 % de la lysine et de la thréonine apparaissant dans la veine porte et dans la veine hépatique sont transportés par le plasma. Nos résultats suggèrent donc que les globules rouges seraient relativement peu impliqués dans les échanges de lysine et de thréonine à travers les tissus digestifs et le foie chez le porc alimenté en continu.

porc / lysine / thréonine / transport / intestin / foie

1. INTRODUCTION

In the animal, amino acids (AA) are transported between tissues in blood. Studies on AA metabolism often consider AA exchanges or transfers only from blood plasma. A major reason for this choice is because plasma is more simple to analyse than whole blood. However, many experimental data have shown that erythrocytes may also contribute to inter-organ amino acid transport [3]. The contributions of plasma and erythrocytes to AA transport vary with both the AA and the blood vessel. For example, in the dog, Elwyn et al. [2] showed that AA are mainly transported from the intestine to the liver in the plasma whereas net transport from the liver to the other tissues occurs presumably in erythrocytes. In pigs, we have suggested in a previous study that the erythrocytes also take part in AA exchanges across the PDV and the liver [13]. In the portal vein (PV), threonine is net transported in both erythrocytes and plasma whereas the other AA appear to be transported mainly in the plasma. Furthermore, the existence of a large concentration gradient across the erythrocyte membrane for lysine implies a compartmentalisation of its metabolism. For this AA, however, the plasma remains the most readily exchangeable pool and is probably the only compartment involved in lysine transfer across the PDV and the liver. In the preliminary study [13] we examined AA fluxes and balance across the PDV and the liver in pigs fed a standard well-balanced diet, but no comparison of different dietary treatments has been made. The aims of the present experiment were: (1) to compare the respective contributions of erythrocytes and plasma to threonine and lysine transport across the PDV and the liver; (2) to determine whether threonine and lysine transport across the splanchnic tissues is modified by dietary AA balance. In this paper, we report the results of two experiments during which we have compared the effects of threonine or lysine dietary levels, from deficient to adequate, on plasma and blood AA balance across the liver and the PDV. The implications of these results on the choice of the blood AA pool to be considered in interorgan or tissue transfer studies is discussed.

2. MATERIALS AND METHODS

2.1. Animals and diets

Experiment 1. Three Large White × Landrace pigs (at a starting live weight of 27 ± 2 kg) from the Unité Mixte de Recherches sur le Veau et le Porc herd were used in this experiment. The animals were fitted with chronic catheters in a mesenteric vein (MV), the portal vein (PV), a hepatic vein (HV) and in the carotid artery (CA). Postoperative care of animals and catheters have been previously described [13].

The Control diet (Tab. I) was composed of a mixture of wheat, soybean meal, peanut meal and wheat gluten meal. Free AA

Table I. Composition of the experimental diets ($g \cdot kg^{-1}$).

Ingredients	Exp. 1	Exp. 2
Wheat	725	244
Barley	–	240
Corn[1]	–	232/234
Soyabean meal	20	180
Peanut meal	80	–
Wheat gluten meal	15	50
Cornstarch[1]	80.3/81.7	–
Molasses	30	–
Vegetable oil	–	20
Dicalcium phosphate	20	12
Limestone	12	12
Salt	3.5	3
Potassium carbonate	2.5	–
Trace mineral and vitamins premix	5	5
L-Lysine HCl[1]	4.7	2/–
L-Threonine[1]	1.6/0.2	–
DL-Methionine	0.4	–

[1] For control and deficient diets respectively.

(L-lysine, L-threonine and DL-methionine) were added in order to meet the requirement for essential AA per unit of energy. The threonine content of the threonine deficient diet was reduced by 30% compared with that in the Control diet through decreasing the addition of free L-threonine by 87%. From at least 3 days before the blood flow and AA concentration measurements, pigs were fed hourly with an automatic feeder in order to maintain a near steady state in AA concentrations. The amount of diet offered to the pigs was adjusted to the metabolic weight ($100\ g \cdot kg^{-1}\ BW^{0.75}$). Each pig received successively the two diets during two consecutive periods of one week in a cross-over design. AA fluxes were determined during each period.

Experiment 2. Four female pigs (28 ± 3 kg) were surgically prepared and infused in according to the same protocol as in the experiment 1. Similarly, a Control and a deficient diets (Tab. I) based on a mixture of cereals, soyabean meal and wheat gluten meal were formulated. The deficient diet differed from the Control diet by omission of the 0.2% L-lysine-HCl supplement.

These two experiments were conducted under the guidelines of the French Ministry of Agriculture for animal experiments.

2.2. Infusion protocol and blood sampling

In order to determine portal and liver blood flow, p-aminohippuric acid or PAH (Sigma France, Saint Quentin Fallavier) was infused ($0.2\ mg \cdot kg^{-1}\ BW \cdot min^{-1}$) through the MV at a rate of 60 mL per hour for 7 hours. The PAH solution was prepared as previously described [13]. Blood was sampled in heparinised tubes from the PV, the HV and the CA every 30 min for PAH (2 mL of blood) and, during the four last hours of infusion, every hour for AA concentration measurements (4 mL of blood). Catheters were rinsed with sterile saline after each sampling. At the end of the experiment, pigs were killed with a lethal injection of pentobarbital (Nesdonal) into the carotid catheter and the positions of the catheters were checked in order to validate the accuracy of the surgical procedure.

2.3. Chemical analysis

The PAH concentration in whole blood was determined as described by Ortigues et al. [16] after a deacetylation step through acid hydrolysis. The blood flow rate was calculated according to the indicator-dilution technique [9]. Portal (PF) and hepatic (HVF) blood or plasma flow rates were calculated as $i_{PAH} / ([PAH]_{PV} - [PAH]_A)$ and as $i_{PAH} / ([PAH]_{HV} - [PAH]_A)$ respectively where i_{PAH} was the rate of PAH infusion and $[PAH]_{PV}$, $[PAH]_A$ and $[PAH]_{HV}$ corresponded to PAH blood or plasma concentration in the portal vein, artery and hepatic vein respectively. Hepatic artery blood flow (HAF) was calculated as the difference between HVF and PF. Plasma PAH

concentrations were derived from blood PAH concentrations, corrected for haematocrit (Ht) measurement, in order to calculate plasma flow in the same way: [PAH]plasma = 100 × [PAH]blood / (100 – Ht).

AA concentrations were measured in the plasma and whole-blood, after deproteinisation with an equal volume of sulfosalicylic acid solution (solutions at a concentration of 60 $g \cdot L^{-1}$ and 120 $g \cdot L^{-1}$ were used for plasma and whole blood respectively), by ion exchange liquid chromatography and ninhydrin detection (Biotronik LC 5001, Biotronik, Pusheim Banhnof, Germany) using norvaline as an external standard. AA concentrations in erythrocytes were calculated with the following formula: $[AA]_E = ([AA]_{WB} - (100 - Ht) \times [AA]_p)/Ht$.

2.4. Calculations and statistical analysis

AA flux was calculated as the product of blood or plasma flow by the blood or plasma AA concentration. AA net portal appearance was calculated as follows: $([AA]_{PV} - [AA]_A) \times PF$ where PF is the portal blood or plasma flow. AA hepatic vein appearance corresponded to the difference between the hepatic output ($[AA]_{HV} \times HVF$) and the total hepatic input through the portal vein ($[AA]_{PV} \times PF$) and the hepatic artery ($[AA]_A \times HAF$). The positive values for AA net appearance reflect a net addition of AA in the portal or hepatic vein whereas negative values reflect AA uptake by the PDV or by liver.

Statistical analysis was carried out on average values for each animal after controlling the steady state status for amino acid concentrations for each animal. Data were then submitted to variance analyses according to the General Linear Model procedure (GLM) of SAS [22]. The effects of diet, vessels and compartments (plasma, blood and erythrocytes) were tested by the Fischer-Snedecor test and adjusted means were compared by the Student-t test. A difference was declared significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Contribution of plasma and erythrocytes to threonine and lysine transport and transfer across the PDV and the liver

Threonine and lysine concentrations in plasma, whole blood and erythrocyte are presented in the Table II. The difference between intracellular (erythrocyte) and extracellular (plasma) contents was much greater for lysine than for threonine. Whatever the diets and the vessels considered, lysine concentrations were significantly higher in erythrocytes than in plasma. For threonine, the difference between the two compartments was significant only in pigs fed the Control diet in experiment 1 but, in this case, intracellular concentration was significantly lower than plasma concentration. Eventually, in pigs offered the Control diets, only 38–50% of blood lysine but 65% of blood threonine were present in the plasma. A higher lysine concentration in erythrocytes compared with plasma is a common observation in many species, such as dogs [1], pigs [10], calves [8] and sheep [6, 14] although the reason for intracellular lysine accumulation against the concentration gradient is difficult to explain. To our knowledge, there are no experimental data on lysine transport across the membrane of the porcine erythrocyte. Although many differences exist in the AA transport systems of the erythrocytes between different species, the presence of a strong concentrative system for lysine transport of the erythrocytes seems improbable [5, 25]. In rat and sheep, in vitro erythrocyte membrane permeability to L-lysine is not very high and intracellular concentration reaches an equilibrium with extracellular concentration [17, 26]. Felipe et al. [4] described the existence of a rodent transport system that is not sodium dependent and close to the diffusion process. Some authors stated that concentrative system for amino acid transport are present on the membrane of reticulocytes

Table II. Plasma, whole-blood (WB) and erythrocyte (E) threonine and lysine concentrations in carotid artery (CA), portal vein (PV) and hepatic vein (HV). Values are expressed in nmol·mL^{-1}.

Exp. 1[1]		Thr def			Control				
	Vessel	CA	PV	HV	CA	PV	HV	SEM[2]	P[3]
thr	WB	67.7	84.7	78.7	224.5^{b}	257.4^{b}	248.9	12.1	diet = 0.0001
	Plasma	59.7	80.0	72.9	243.6^{b}	276.6^{b}	267.5		vess = 0.0025
	E	82.7	92.9	89.2	182.4^{a}	213.6^{b}	206.4^{b}		cpt = 0.006
lys	WB	228.1^{b}	260.7^{b}	251.1^{b}	178.6^{b}	214.1^{b}	208.2^{b}	16.4	diet = 0.0005
	Plasma	152.5^{a}	188.4^{a}	177.1^{a}	106.1^{a}	148.3^{a}	135.9^{a}		vess = 0.003
	E	380.6^{c}	402.7^{c}	395.7^{c}	345.6^{c}	366.6^{c}	373.3^{c}		cpt = 0.0001
Exp. 2[1]		Lys def			Control				
	Vessel	CA	PV	HV	CA	PV	HV	SEM[2]	P[3]
thr	WB	197.4	220.9	206.7	155.6	180.4	154.2	14.6	diet = 0.0001
	Plasma	204.3	227.9	213.8	151.3	191.4	160.0		vess = 0.03
	E	182.1	204.7	190.9	163.2	159.1	143.0		cpt = 0.12
lys	WB	205.1^{b}	234.9^{b}	217.8^{b}	266.4^{b}	309.6^{b}	263.2^{b}	12.6	diet = 0.0001
	Plasma	115.3^{a}	146.1^{a}	131.2^{a}	158.7^{a}	221.1^{a}	188.4^{a}		vess = 0.0001
	E	382.5^{c}	406.5^{c}	379.3^{c}	474.1^{c}	477.2^{c}	411.2^{c}		cpt = 0.0001

[1] Values correspond to adjusted means for $n = 3$ in experiment 1 and $n = 4$ in experiment 2. For each amino acid on the same column, different letters in superscript indicate a significant difference between concentration in the different compartments (whole blood, plasma and erythrocyte).
[2] Standard error of the mean.
[3] Levels of probability according to the Fisher-Snedecor test. Diet: comparison of the deficient and the control diets; vess: comparison of the three vessels; cpt: comparison of the three compartments (whole blood, plasma or erythrocyte).

(erythrocyte precursors) and that the maturation of erythrocytes could be the main reason for the loss of the sodium dependent transport system for amino acids [25, 26]. Consequently, because of the relatively short life-span of erythrocytes, we can envisaged that lysine accumulation occurs before the maturation of reticulocyte. In sheep infused with ^{13}C-labelled amino acids, lysine enrichment in the erythrocytes was 40 to 50% lower than that of plasma [14]. These data suggest low exchanges for lysine between extra (highly enriched) and intracellular (poorly enriched) pools but a dilution of labelled lysine by unlabelled intracellular source (peptide hydrolysis or transfer of lysine from an intratissular pool) could be envisaged as well. Unfortunately, in pigs, we do not have similar comparisons for lysine enrichment. A last explanation of the highest lysine concentration in erythrocytes could be the localisation of lysine in the blood cell. Proenza et al. [19] have identified two AA pools in the erythrocyte: one is intracellular and the second one is adsorbed on the erythrocyte membrane. The latter pool has a particularly rapid turnover and probably plays a physiological role during food deprivation [18]. In our experiment, amino acid concentrations in the erythrocytes were calculated from plasma and blood concentration, corrected by the haematocrit,

and it was not possible to separate these two erythrocyte pools.

The Table III represents plasma threonine and lysine fluxes expressed as percent of whole blood fluxes. The contribution of plasma to blood flux was on average 40% for lysine and 65% for threonine. In experiment 1, the contribution of plasma to threonine transport was significantly higher in pigs fed the Control diet than in pigs fed the deficient diet. The same significant difference was noted for lysine in experiment 2. Comparison of the different vessels indicated that for lysine in the both experiments and for threonine in the second one only, the contribution of plasma to lysine and threonine fluxes was higher in the portal and the hepatic vein than in the artery. In spite of the relative low proportion of lysine transported by the plasma in the portal vein (less than 50%), it seems that, when blood go through the intestinal bed, the plasma pool of the portal vein was preferentially enriched by lysine than the whole blood pool. This was confirmed by data presented in Table IV showing that more than 60% of lysine appearing in the portal vein (i.e. net absorption) were transported by the plasma. Our observation was in agreement with previous data which showed that, for lysine, the plasma compartment was probably the most exchangeable pool of blood through the intestine [13] and with the data of Elwyn [3] and Houlier [8] who stated that AA are mainly transported in the plasma from the intestine to the liver. For threonine, the same observation has been done only in one group (control diet in experiment 2). Thus, contrary to lysine, threonine appearing in portal vein was probably transported both by plasma and erythrocytes [13].

The uptakes of threonine and lysine by the liver did not differ significantly when they were calculated from plasma or blood data (Tab. V). Accordingly, the proportions of plasma and erythrocyte threonine and lysine present in the hepatic vein suggest that the transfer of blood across the liver did

Table III. Plasma threonine and lysine fluxes expressed as percent of whole blood flux[1].

		Vessel[2]				
	Diet	CA	PV	HV	SEM[3]	*P*[4]
Exp. 1						
Thr	Thr def	71.3	70.2	69.8	1.6	diet = 0.003
	Control	59.9	63.5	62.2		vess = 0.7
Lys	Thr def	43.4[a]	48.5[b]	46.2[b]	1.6	diet = 0.9
	Control	44.0	47.6	45.9		vess = 0.04
Exp. 2						
Thr	Lys def	62.9	66.5	65.9	2.2	diet = 0.4
	Control	57.8[a]	67.5[b]	64.9[b]		vess = 0.02
Lys	Lys def	34.4	40.3	38.6	2.1	diet = 0.02
	Control	36.4[a]	46.0[b]	44.1[b]		vess = 0.007

[1] Values correspond to adjusted means for $n = 3$ in experiment 1 and $n = 4$ in experiment 2. Different letters in superscript indicate a significant difference between values on the same line (significant difference between vessels).
[2] Abbreviations used correspond to carotid artery, portal vein and hepatic vein respectively.
[3] Standard error of the mean.
[4] Levels of probability according to the Fisher-Snedecor test. Diet: comparison of the deficient and the control diets; vess: comparison of the three vessels.

not modify the partition of amino acids between the two compartments [23]. However, this last conclusion should be considered very cautiously because of the great variability of hepatic balance data calculated from the difference between three fluxes. Finally, the plasma fraction of blood returned from peripheral tissues in the carotid artery has lower concentrations of amino acids than the venous vessels of the splanchnic tissues. This would suggest that for peripheral tissues as well a great part of

Table IV. Portal vein amino acid appearance (μmol·kg^{-1}·h^{-1}) calculated from plasma and blood amino acid concentrations[1].

Exp. 1		Thr def	Control	SEM[2]	*P*[3]
Thr	Plasma	34.6	82.4	9.2	diet = 0.01
	Blood	47.5	119.4		cpt = 0.02
Lys	Plasma	69.3	102.4	8.7	diet = 0.03
	Blood	100.4	126.4		cpt = 0.009
Exp. 2		**Lys def**	**Control**	**SEM[2]**	***P*[3]**
Thr	Plasma	56.3	95.0	9.6	diet = 0.05
	Blood	86.4	91.7		cpt = 0.2
Lys	Plasma	68.6	139.7	13.9	diet = 0.0001
	Blood	108.2	182.7		cpt = 0.15

[1] Values correspond to adjusted means for $n = 3$ in experiment 1 and $n = 4$ in experiment 2.
[2] Standard error of the mean.
[3] Levels of probability according to the Fisher-Snedecor test. Diet: comparison of the deficient and the control diets; cpt: comparison of plasma and whole blood.

Table V. Hepatic vein amino acid appearance (μmol·kg^{-1}·h^{-1}) calculated from plasma and blood amino acid concentrations[1].

Exp. 1		Thr def	Control	SEM[2]	*P*[3]
Thr	Plasma	–6.8	–11.4	5.7	diet = 0.8
	Blood	–11.4	–9.6		cpt = 0.8
Lys	Plasma	–19.1	–8.7	21.5	diet = 0.3
	Blood	–35.4	10.9		cpt = 0.9
Exp. 2		**Lys def**	**Control**	**SEM[2]**	***P*[3]**
Thr	Plasma	–28.2	–72.3	17.0	diet = 0.05
	Blood	–51.0	–100.0		cpt = 0.2
Lys	Plasma	–25.1	–85.1	29.5	diet = 0.0001
	Blood	–56.2	–174.8		cpt = 0.15

[1] Values corresponds to adjusted means for $n = 3$ in experiment 1 and $n = 4$ in experiment 2.
[2] Standard error of the mean.
[3] Levels of probability according to the Fisher-Snedecor test. Diet: comparison of the deficient and the control diets; cpt: comparison of plasma and whole blood.

amino acid uptake occurred from plasma, as it was already shown in sheep at least for lysine.

3.2. Effect of threonine and lysine dietary balance on their utilization by tissues and their transfer across the PDV and the liver

In pigs fed the Control diets, free lysine and free threonine concentrations in the plasma, whole-blood and erythrocytes were significantly higher than in pigs fed the lysine or the threonine deficient diets (Tab. II). This accumulation is a common feature of the metabolic response of animals to the addition of any essential AA to a diet when the supplies exceed the requirement [7, 11, 21, 24]. On the other hand, in pigs fed the lysine and threonine supplemented diets (control diets), plasma and blood concentrations of several other essential but non limiting amino acids (only threonine and lysine data are shown) were lower than in pigs fed the deficient diet. This was probably a consequence of the enhanced AA utilization for tissue protein deposition and confirmed that lysine and threonine supplies were limiting performance in the deficient diets.

Appearance of lysine and threonine in the PV increased with their additional intake (Tab. IV). One more time, additional dietary lysine appeared in the portal vein preferentially in plasma whereas the increase in dietary threonine did not modify the contribution of plasma to threonine transfer across the intestine. However, the concomitant increases in lysine and threonine PV appearance with threonine and lysine addition respectively were unexpected since threonine or lysine intakes were not significantly different between the 2 groups of pigs when these amino acids were not limiting (Tab. VI). Moreover, this observation has been done for the other essential amino acids as well (data not shown). An effect of threonine and lysine dietary content on the absorption of non-limiting AA is unlikely, unless the uptake of non-limiting AA by the gut tissue is less when the diet is well-balanced. In other words, amino acid deficiency may alter the ability of intestine to synthesis protein. The consequence is that the decrease in protein turnover in the intestine may have lowered losses of endogenous protein and then re-absorption of their constitutive amino acids.

Table VI. Threonine and lysine intake in g per day.

Exp 1	Thr def	Control
Thr	5.4[a]	7.3[b]
Lys	9.8	10.6
Exp 2	**Lys def**	**Control**
Thr	9.3	8.9
Lys	11.7[a]	14.9[b]

On the same line, values with different superscript are significantly different.

In the experiment 2 of this work, the uptake of plasma and blood lysine by the liver significantly increased with additional lysine dietary supply (Tab. V) but this is not true for threonine (experiment 1). In the case of lysine, the increase in liver uptake helps to limit lysine accumulation in the systemic plasma by favoring lysine catabolism in the liver. In pigs, threonine seem to be less efficiently extracted into the liver than the other essential AA [20]. In a previous study using labelled tracer, we have shown that threonine entry in the liver was low and fairly constant for low and adequat dietary supplies, which corresponds to the experimental situations of experiment 1, but increased sharply with excess supply [12]. This might have spared threonine for protein synthesis in the peripheral tissues when dietary threonine supplies did not exceed the requirement. The increase in liver threonine uptake observed with lysine addition in experiment 2 follows the parallel increase in threonine portal vein appearance with

lysine addition and contribute to balance net intestine output for peripheral tissue supply.

The present results indicate that in fed pigs both plasma and erythrocytes take part in the transport of threonine and lysine in the portal and hepatic veins and in the arterial blood. However, erythrocytes seem to be little involved in the exchanges of amino acids between blood and splanchnic tissues (intestine and liver) especially for lysine. Moreover, plasma amino acids seem to be preferentially taken up by peripheral tissues. Similarly, in calves, during the fasted state the erythrocyte appeared to be more involved in the transport of amino acids than in the fed state [15] probably because of the rapid depletion of the plasma compartment. These results imply slow exchanges between the plasma and erythrocyte AA. The question is whether or not erythrocyte AA can be really mobilised and used directly by tissues when dietary supply is low. Nevertheless, since AA concentration in the erythrocytes can be high, especially for lysine, and because, from the present data, it is not possible to exclude direct exchanges between tissues and erythrocytes, there is no doubt that blood data should be used if we want to calculate total AA balance across a given tissue. However, in the case of studies using tracers, because of slow exchanges of threonine and lysine between plasma and erythrocytes, it is perhaps necessary to consider the erythrocytes as a particular compartment during interorgan AA exchanges.

ACKNOWLEDGEMENTS

The authors would like to thank Y. Lebreton and F. Legouëvec for surgical procedure and animal care and Y. Colleaux for amino acid analyses.

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