Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens*

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We analysed the respective influences of age and lysine deficiency on skeletal muscle and liver protein turnover. Growing male broilers were fed *ad libitum* on isoenergetic diets containing 200 g crude protein/kg which varied in their lysine content (7·7 or 10·1 g/kg). Fractional rates of protein synthesis (FSR) were measured *in vivo* in the liver and the pectoralis major muscle of 2-, 3- and 4-week-old chickens (flooding dose of L-[4-^{3}H]phenylalanine). Fractional rates of proteolysis (FBR) were estimated for the same tissues as the difference between synthesis and growth. Over the 2-week period liver FSR and FBR were unchanged, whereas muscle FSR decreased with age. This developmental decline was related to the lower capacity for protein synthesis (Cs) without any modifications of the translational efficiency. Whatever the age, lysine deficiency resulted in significant decreases in body weight, tissue protein content and tissue protein deposition, apparently because of reduced amounts of proteins synthesized. We recorded a difference in the response of the two tissues to lysine deficiency, the pectoralis major being more sensitive than the liver. When comparing birds of the same age, liver FSR and FBR were not modified by the diet, whereas muscle FSR, Cs and FBR were higher in chicks fed on a lysine-deficient diet than in the controls. Conversely, when chicks of similar weights were compared, the main effect of the dietary deficiency was an increase in muscle FBR. The results suggest that lysine deficiency not only delayed chick development so that protein turnover was affected, but also induced greater changes in metabolism. Thus, the principal mechanism whereby muscle mass decreased appeared to be a change in FBR.

Lysine: Protein turnover: Chicken

The poultry industry aims to increase the efficiency of the transformation from feed to animal proteins to provide consumers with a product containing more lean and less fat, and to reduce N excretion which is a source of pollution. At present, synthetic amino acids are added to low-protein diets to obtain a well-balanced feed. A deficiency in a single essential amino acid can indeed disrupt growth mechanisms: it decreases chick growth, feed intake and N balance (Akinwande & Bragg, 1985; Okumura *et al.* 1985; Kino & Okumura, 1986*a*, *b*). It caused a reduced protein deposition in the whole body and in the pectoralis major muscle of 3-week-old chickens mainly because lower amounts of protein were synthesized each day (Kino & Okumura, 1987; Tesseraud *et al.* 1992). To our knowledge, all of the studies concerning the effect of an amino acid deficiency have compared chicks at the same chronological age, when chicks fed on a control or an amino acid-deficient diet did not only have different body weights but had, also, different tissue protein masses and different rates of tissue development. To understand better the reduction of growth performance associated with the deficiency of a particular amino acid, we analysed the effect of dietary lysine on *in vivo* protein turnover using 2-, 3- and 4-week-old chickens. We

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therefore determined whether age is associated with variations in protein turnover and response to lysine deficiency. Besides, when discussing protein metabolism in chickens, most authors focus on whole body or on breast (pectoralis major) muscle which is the tissue of greatest interest in growing animals. The liver is of major importance in determining the quantity of amino acids utilized and the proportion of whole-body protein synthesized due to its very high fractional synthesis rate (FSR). It seems vital, therefore, to analyse the mechanisms of regulation of protein turnover in both muscle and non-muscle tissues during growth. Thus we measured tissue-protein synthesis rates in pectoralis major muscle (representative of a completely fast-twitch glycolytic muscle type) and in liver, using the reliable flooding-dose method (Garlick *et al.* 1980, 1994).

MATERIALS AND METHODS

Animals and experimental design

One-day-old male broilers (ISA-France) were fed *ad libitum* on either a diet restricted in lysine but well-balanced in other amino acids (L diet, *n* 75) or a control adequate diet (C diet, *n* 75) containing 7·7 or 10·1 g lysine/kg respectively. All the diets were isoenergetic (13·0 MJ metabolizable energy/kg), had the same protein content (200 g/kg) and were in pellet form. The L diet was based on maize and soyabean meal (Table 1) and the C diet was formulated from the L diet by supplementation with crystalline L-lysine HCl (3 g added lysine/kg). During the 4 weeks of the experimental period the birds had free access to water. Relative humidity was maintained at about 45–50% and the ambient temperature was gradually decreased from 32° when the birds were 1-d-old to 26° when they were 3 weeks old. The lighting program, after an initial 48 h of continuous lighting, was reduced to a 16 h light–8 h dark cycle (lights on at 05.00 hours). At 1 week of age (on day 7) all the chickens were weighed; for each treatment, forty-eight of them were selected on a body-weight basis (99·9 (SE 11·1) g in chicks fed on the L diet; 127·5 (SE 13·8) g in the control chicks) and placed in individual cages. Weights were then recorded weekly (on days 14, 21 and 28). At 2, 3 and 4 weeks of age (on days 13–14, 20–22 and 26–28 respectively) we measured growth performances, tissue characteristics and rates of tissue-protein turnover in five to seven chicks from each diet group. The chicks used displayed body weights and growth rates similar to the group mean. Note that fractional protein growth rates (FGR) were evaluated in the same five to seven chicks per age and treatment from the increases in tissue-protein content. For each diet group, regressions were thus established, in which we included additional chicks killed on days 11, 18, 25 and 29 (for details, see p. 856).

Protein synthesis measurements

On the day of the study, feed was removed at 10.30 hours and experiments were carried out between 13.30 and 15.30 hours. Protein FSR were measured *in vivo* according to the flooding-dose method (McNurlan *et al.* 1979; Garlick *et al.* 1980). At 10 min before slaughter, each bird received a single intravenous injection of unlabelled L-phenylalanine (1500 μmol/kg body weight) combined with 18·5 MBq/kg body weight of L-[4-^{3}H]phenylalanine (Amersham, 0·96 TBq/mmol) to flood the precursor pools. This method, previously validated in chickens by Muramatsu & Okumura (1985), has been adapted in our laboratory (Tesseraud *et al.* 1992) and is currently used. In the present study the plasma free [^{3}H]phenylalanine specific activity was at least 84% of the specific activity of the injected phenylalanine in each group. In addition, to determine whether the flooding dose flooded and equilibrated the various precursor pools for protein synthesis, we checked that free phenylalanine specific activities in the liver and in the pectoralis major muscle were close to each other (the liver values were within 91 and 99% of muscle values) and to the

Table 1. *Composition (g/kg) of lysine-restricted diet (L)*

Ingredients	
Ground maize	679
Soyabean meal	160
Maize gluten meal	100
Rapeseed oil	20
Vitamin premix*	5
Salt	3·5
Trace-mineral premix†	1·5
Dicalcium phosphate	16
Calcium carbonate	14
DL-Methionine	1
Calculated composition	
Metabolizable energy (MJ/kg)	13·1
Crude protein	205·3
Methionine + cystine	9·1
Lysine	7·7
Calcium	9·5
Available phosphorus	3·4

* Vitamin content (/kg premix): retinol 0·6 g, cholecalciferol 7·5 mg, α-tocopheryl acetate 3 g, menadione 1 g, nicotinic acid 5 g; thiamin 0·1 g, riboflavin 0·8 g, calcium pantothenate 1·6 g, pyridoxine 0·2 g, cyanocobalamin 1·6 mg, pteroylmonoglutamic acid 200 mg, biotin 40 mg, butylated hydroxytoluene 25 g, choline chloride 100 g, made to 1 kg with milled oat.

† Mineral content (mg/kg premix): Co 335, Cu 8750, I 1225, Se 225, Zn 84000, Fe 44000, Mn 106000.

specific activity in plasma (muscle values were within 87 and 92% of plasma values). There was no age or group effect on these values (variance analysis). Just before slaughter, a general anaesthetic (Tiletamine–Zolazepam, Zoletil; Reading, L'Hay-les-Roses, France) was given in the pectoralis major muscle (right side). The birds were decapitated and exsanguinated, then blood was collected in heparinized tubes which were centrifuged. Plasma was collected and frozen. The livers and the left pectoralis major muscles were quickly excised. The livers were rinsed in cold saline (9 g NaCl/l) to remove blood and wiped. Both tissues were weighed and frozen in liquid N_2 within 3–4 min after exsanguination. This extra time was not taken into account for the calculation of incorporation time. Plasma, liver and muscle samples were then stored at −20° until analysis.

Analytical methods

To determine the free and protein-bound phenylalanine specific activities, frozen tissues were finely pulverized in liquid N_2. To 1 g of the powder obtained we added 5 ml of cold 0·2 M-$HClO_4$. The acid-soluble fraction containing free amino acids was separated from the protein precipitate by centrifugation (6000 *g* for 20 min), and $HClO_4$ was neutralized by addition of 0·8–1 ml saturated potassium citrate solution (final pH 6·4) and centrifugation at 2800 *g* for 15 min. The tissue-protein precipitates were washed three more times in cold 0·2 M-$HClO_4$, resuspended in 10 ml 0·3 M-NaOH and incubated at 37° for 1 h. A portion was used to measure tissue-protein content according to the method of Smith *et al.* (1985), by the colorimetric reaction with bicinchoninic acid (Pierce, Rockford, IL 61105, USA). We then reprecipitated the protein from the NaOH solution with 2·5 ml 2 M-$HClO_4$. The supernatant fraction was used to determine the tissue RNA content (Munro & Fleck, 1969). We removed last traces of free phenylalanine from the protein pellet by twice washing the pellet with 10 ml 0·2 M-$HClO_4$ and finally the protein was hydrolysed in 20 ml

6 M-HCl for 16 h at 100–110°. HCl was then removed by evaporation and the amino acids were resuspended in 3·5 ml 0·5 M-sodium citrate, pH 6·3.

Phenylalanine was converted into β-phenylethylamine (Garlick *et al.* 1980), which was assayed by fluorospectrometry (495 nm emission, 390 nm excitation) with ninhydrin and L-leucylalanine (Suzuki & Yagi, 1976).

Calculations

The FSR (% per d) were calculated using the method described by Garlick *et al.* (1980). For better estimating of tissue protein FGR, twenty additional chicks were killed per diet group. The livers and the left pectoralis major muscles were rapidly excised and the same procedures were performed as those used for the tissue samples that had been removed for measuring protein synthesis. We therefore determined tissue-protein mass for thirty-seven or thirty-eight chicks per dietary treatment. Regressions were established for each treatment between total tissue protein mass and empty body weight (r^2 0·97–0·98 for the pectoralis major muscle; r^2 0·91–0·92 for the liver). We thus deduced the amount of tissue protein gained per g body-weight gained. The tissue protein gained per day (mg protein/d) was calculated for each chick used for protein synthesis measurements from its daily growth rate (g/d). FGR was finally derived from the daily change in tissue protein amount divided by the total tissue protein mass present on the day of the experiment. This method, taken from McDonald & Swick (1981), provides a crude estimate of the fractional growth rates. The rates of protein breakdown (FBR) were estimated as the difference between synthesis rates and growth rates in muscle, or (0·7 FSR) and FGR in liver to take account of exported proteins (Goldspink & Kelly, 1984).

Absolute rates of protein turnover, i.e. the total amounts of protein synthesized, gained or degraded each day (ASR, AGR or ABR respectively, g protein/d) in liver or in pectoralis major muscle were calculated by multiplying FSR, FGR or FBR by total tissue protein content present on the day of the experiment. The capacity for protein synthesis, i.e. ribosomal capacity (Cs), was estimated as the RNA:protein ratio (μg RNA/mg protein) because most of the RNA in tissues is ribosomal. We determined the translational efficiency (kRNA) by calculating the amount of protein synthesized per mg RNA and per day (mg protein/(mg RNA per d)).

Statistical methods

Values are given as means with their standard errors. Two-way ANOVA was performed to discriminate between the effects of age and diets and their interaction in the whole population (n 35). Variance analysis and variance–covariance analysis were also performed at each age to study the effect of dietary lysine deficiency in 2-, 3- and 4-week-old chickens.

RESULTS

Effects of age

Growth performances, tissue wet weight, protein and RNA contents increased significantly ($P < 0·001$; Table 2) during the experimental period. Over the 2-week period the gain of tissue wet weight and protein mass corresponded to simultaneous increases in the amounts of proteins synthesized and degraded each day (ASR and ABR, Tables 3 and 4). ASR and ABR increased by approximately 2·4-fold in liver on both diets, about 2·6-fold in the pectoralis major muscle on diet L and by different amounts (2·4- and 4·2-fold respectively) for ASR and ABR on diet C. Since the muscle protein content was increased more than ASR, by 3·9- to 4·3-fold, the proportion of protein synthesized daily (FSR; Table 3) was decreased. In contrast in liver, as ASR and protein content increased by similar amounts, FSR was unchanged.

Table 2. *Effect of lysine deficiency on growth performances and tissue characteristics in 2-, 3- and 4-week-old chickens*†

(Mean values with their standard errors for five to seven chicks per group)

Age (weeks)...	2				3				4				Statistical analysis (*P* values)		
Diet...	C (*n* 6)		L (*n* 6)		C (*n* 6)		L (*n* 7)		C (*n* 5)		L (*n* 5)		Main effects		Interaction
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Age	Diet	Age × Diet
Growth performances															
Body weight (g)	338	6	205*	9	696	26	385*	15	1020	13	593*	20	< 0·001	< 0·001	< 0·001
Growth rate (g/d)	32·1	0·7	15·9*	0·9	49·8	1·6	24·7*	1·8	59·9	4·2	39·1*	1·9	< 0·001	< 0·001	0·10
Pectoralis major muscle															
Wet weight (g)	11·32	0·53	4·22*	0·34	29·55	1·07	9·91*	0·45	45·01	1·14	15·50*	0·85	< 0·001	< 0·001	< 0·001
Protein content (g)	1·47	0·10	0·45*	0·04	3·89	0·16	1·10*	0·06	6·40	0·11	1·74*	0·06	< 0·001	< 0·001	< 0·001
RNA content (mg)	26·60	1·40	13·47*	0·77	52·32	1·94	24·42*	1·09	69·68	2·85	37·65*	2·74	< 0·001	< 0·001	< 0·001
Liver															
Wet weight (g)	13·15	0·67	6·87*	0·58	21·83	1·74	11·57*	0·70	27·79	2·48	17·61*	1·26	< 0·001	< 0·001	0·23
Protein content (g)	1·59	0·07	0·88*	0·08	2·79	0·16	1·54*	0·08	3·55	0·26	2·17*	0·15	< 0·001	< 0·001	< 0·05
RNA content (mg)	96·51	4·63	58·27*	4·91	170·4	9·7	94·52*	5·25	212·0	15·3	138·7*	7·7	< 0·001	< 0·001	< 0·05

C, control; L, lysine-deficient.

* Mean values were significantly different from those for controls of the same age ($P < 0·05$, ANOVA).

† For details of diets and procedures, see Table 1 and pp. 854–856.

Table 3. *Effect of lysine deficiency on pectoralis major muscle protein turnover in 2-, 3- and 4-week-old chickens*†

(Mean values with their standard errors for five to seven birds per group)

Age (weeks)...	2				3				4				Statistical analysis (*P* values)		
Diet...	C (*n* 6)		L (*n* 6)		C (*n* 6)		L (*n* 7)		C (*n* 5)		L (*n* 5)		Main effects		Interaction
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Age	Diet	Age × Diet
Cs (μg RNA/mg protein)	18·2	0·6	30·4*	1·3	13·5	0·3	22·4*	0·9	10·9	0·3	21·6*	0·9	< 0·001	< 0·001	0·13
kRNA (mg protein/mg RNA per d)	12·2	0·6	12·7	0·6	13·6	1·0	13·5	1·0	11·4	0·9	12·2	0·8	0·19	0·86	0·85
FSR (%/d)	22·4	1·4	38·6*	2·5	18·3	1·5	29·7*	1·4	12·5	1·1	26·0*	1·1	< 0·001	< 0·001	0·31
ASR (mg/d)	326	25	172*	14	715	69	326*	22	799	72	451*	19	< 0·001	< 0·001	< 0·05
FGR (%/d)	15·8	1·1	13·8	1·0	10·0	0·6	9·2	1·1	5·9	0·5	8·6*	0·3	< 0·001	0·98	< 0·05
AGR (mg/d)	226	5	61*	4	390	29	103*	15	379	36	150*	7	< 0·001	< 0·001	< 0·05
FBR (%/d)	6·6	1·6	24·8*	2·0	8·3	1·3	20·5*	2·0	6·6	1·2	17·4*	1·1	0·11	< 0·001	0·08
ABR (mg/d)	100	27	111	11	325	55	223	22	421	78	301	18	< 0·001	< 0·05	0·22
Efficiency of protein deposition‡ (%)	71·6	6·0	35·9*	1·9	56·3	4·8	31·7*	4·0	49·0	6·3	33·3*	1·8	< 0·05	< 0·001	0·11

C, control; L, lysine-deficient; Cs, capacity for protein synthesis; kRNA, translational efficiency; FSR, fractional synthesis rate; ASR, absolute synthesis rate; FGR, fractional growth rate; AGR, absolute growth rate; FBR, fractional breakdown rate; ABR, absolute breakdown rate.

* Mean values were significantly different from those for controls of the same age ($P < 0·05$, ANOVA).

† For details of diets and procedures, see Table 1 and pp. 854–856.

‡ Efficiency of protein deposition = (100 × FGR)/FSR.

Table 4. *Effect of lysine deficiency on liver protein turnover in 2-, 3- and 4-week-old chickens*†

(Mean values with their standard errors for five to seven birds per group)

Age (weeks)...	2				3				4				Statistical analysis (*P* values)		
Diet...	C (*n* 6)		L (*n* 6)		C (*n* 6)		L (*n* 7)		C (*n* 5)		L (*n* 5)		Main effects		Interaction
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Age	Diet	Age × Diet
Cs (μg RNA/mg protein)	60·7	1·2	66·2*	0·9	61·2	0·9	61·5	1·0	59·8	1·1	64·2*	1·1	0·11	< 0·001	< 0·05
kRNA (mg protein/mg RNA per d)	16·4	1·8	17·1	0·6	18·7	0·4	18·5	1·1	18·0	1·6	17·3	0·3	0·26	0·92	0·84
FSR (%/d)	100·0	11·9	112·8	2·7	114·4	3·1	113·8	7·5	108·1	11·6	111·1	2·0	0·59	0·43	0·65
ASR (mg/d)	1575	158	999*	99	3181	178	1727*	89	3777	317	2398*	126	< 0·001	< 0·001	< 0·05
FGR (%/d)	6·8	0·2	6·0*	0·3	6·6	0·1	5·6	0·5	5·1	0·4	6·0	0·2	0·08	0·37	< 0·05
AGR (mg/d)	107	2	53*	3	185	14	89*	13	179	17	130*	6	< 0·001	< 0·001	0·08
FBR (%/d)	63·3	8·2	73·0	1·9	73·5	2·2	74·0	5·5	70·6	7·7	71·7	1·2	0·55	0·40	0·63
ABR (mg/d)	995	111	647*	67	2042	113	1121*	58	2465	206	1549*	83	< 0·001	< 0·001	< 0·05
Efficiency of protein deposition‡ (%)	10·3	1·2	7·6*	0·4	8·3	0·3	7·3	0·9	6·8	0·3	7·7	0·2	0·08	0·15	0·07

C, control; L, lysine-deficient, Cs, capacity for protein synthesis; kRNA, translational efficiency; FSR, fractional synthesis rate; ASR, absolute synthesis rate; FGR, fractional growth rate; AGR, absolute growth rate; FBR, fractional breakdown rate; ABR, absolute breakdown rate.

* Mean values were significantly different from those for controls of the same age ($P < 0·05$, ANOVA).

† For details of diets and procedures, see Table 1 and pp. 854–856.

‡ Efficiency of protein deposition = (100 × FGR)/(0·7 × FSR).

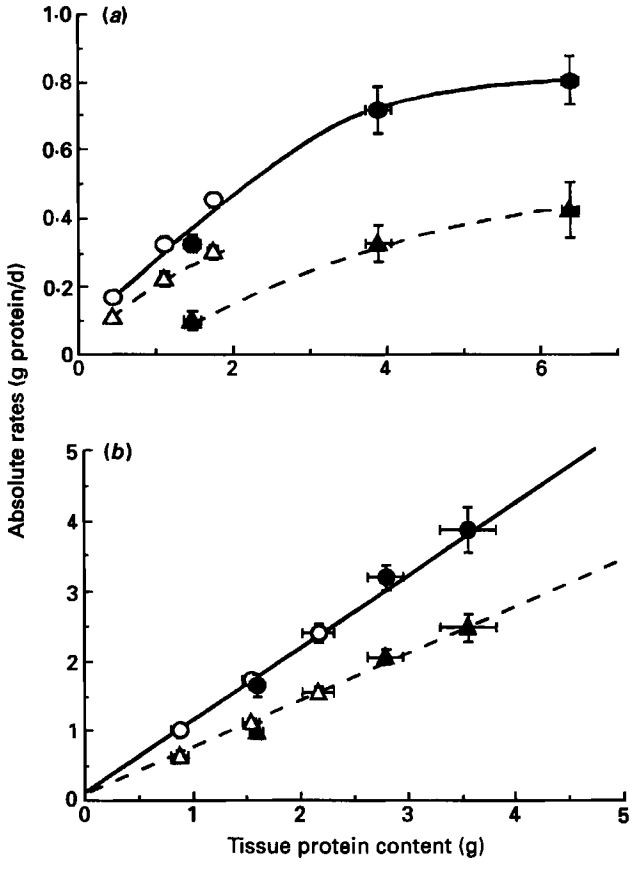

Fig. 1. Protein synthesis (●, ○) and breakdown (▲, △) as a function of tissue protein weight in (a) the pectoralis major muscle and (b) the liver of chicks fed on a lysine-deficient diet (○, △) or a control diet (●, ▲). Values are given as means for five to seven chicks at 2, 3, and 4 weeks of age. Regressions were established between tissue protein weight (PROT) and either protein synthesis (ASR, g/d) or protein breakdown (ABR, g/d). Equations are given in Table 5.

Effects of lysine deficiency

Body weights, tissue wet weights, protein and RNA contents were lower ($P < 0{\cdot}001$) in animals fed on the L diet than in the control group of the same age. Furthermore, wet weight of pectoralis major muscle relative to body weight (kg) was reduced by 40% when a lysine-restricted diet was given. By contrast, liver weight relative to body weight (kg) did not differ significantly between diets.

Two-way ANOVA performed with all animals showed very significant effects of diet on absolute rates of protein turnover expressed in g protein/d (Tables 3 and 4). First, whatever the tissue, protein deposition was decreased by lysine deficiency ($P < 0{\cdot}001$) by 73, 74 and 60% in pectoralis muscle at 2, 3 and 4 weeks of age respectively and by 50, 52 and 27% in liver. Thus, protein synthesis was reduced by an average factor of 1·8. Protein breakdown clearly ($P < 0{\cdot}001$) decreased in liver (−35 to −45% according to age). In the pectoralis major muscle we also recorded a significant effect of diet ($P < 0{\cdot}05$) with slighter changes in ABR (+11% at 2 weeks of age and approximately −30% at 3 and 4 weeks of age). Like the analysis of age effects, the variations in tissue protein mass caused some differences in protein turnover when results were expressed in absolute or fractional terms. Whatever the

Table 5. *Equations of the regressions established between absolute rates of protein turnover (synthesis ASR; breakdown ABR; g/d) and protein weight (PROT, g) in the pectoralis major muscle and in the liver of chicks fed on a lysine-deficient diet (L) or a control diet (C)**

Tissue/diet	*n*	Equation	R^2	RSD
Pectoralis				
L and C	35	ASR = 0·069 + 0·235 (SE 0·035) PROT − 0·019 (SE 0·005) $PROT^2$	0·86	0·092
L	18	ABR = 0·009 + 0·249 (SE 0·101) PROT − 0·047 (SE 0·045) $PROT^2$	0·79	0·043
C	17	ABR = −0·096 + 0·147 (SE 0·079) PROT − 0·010 (SE 0·010) $PROT^2$	0·60	0·124
Liver				
L and C	35	ASR = 0·125 (SE 0·155) + 1·031 (SE 0·070) PROT	0·87	0·376
L and C	35	ABR = 0·075 (SE 0·105) + 0·669 (SE 0·047) PROT	0·86	0·256

RSD, residual standard deviation.
* Corresponding curves are shown in Fig. 1.

age, the reduction of muscle protein mass by about 3·5-fold (Table 2) led to significant increases in FSR and ribosomal capacity Cs (160–210% of the value in the C birds) and particularly in FBR (250–375%; Table 3). Therefore lysine deficiency resulted in lower efficiencies of muscle protein deposition despite increased rates of protein turnover. In contrast to muscle, liver FSR and FBR were not influenced by the diets since the decreases in both absolute rates and protein mass were in the same range, approximately 1·5- to 1·8-fold (Table 4).

We analysed the effect of lysine deficiency by comparing chicks at the same chronological age, when chicks fed on L or C diets had different body weights, tissue weights and protein contents. To take this problem into account we plotted absolute rates of protein turnover against tissue protein weight (Fig. 1). For the pectoralis major muscle, polynomial regressions were established (Table 5). As for protein synthesis (ASR), no difference between diets was observed. In contrast, chicks fed on the L diet seemed to degrade higher amounts of muscle proteins than the control group. This is confirmed by the ABR: protein weight ratios, which reached 0·248, 0·203 and 0·173 with the L diet, and 0·068, 0·084 and 0·065 with the C diet in 2-, 3- and 4-week-old chicks respectively. For the liver, linear regressions were established (Table 5) in which chicks fed on C or L diets could not be discriminated. Indeed, whatever the component of protein turnover (ASR or ABR), covariance analysis using the liver protein weight as a covariate indicated that this covariate had a significant effect ($P < 0·001$) and that there was no difference between diets ($P > 0·80$).

DISCUSSION

We compared tissue-protein turnover in young growing chicks aged 2, 3 or 4 weeks and fed on either a control diet (C) (adequate diet according to the requirements of growing chicks) or a lysine-restricted diet (L) which contained 76% of the lysine in the C diet. The results reported here show that tissue protein turnover was greatly affected by age and lysine deficiency and its responses varied in liver and skeletal muscle. The higher reduction of protein mass in muscle compared with liver resulted from increases in muscle fractional rates (FSR and especially FBR), whereas liver FSR and FBR were unchanged. Finally, we observed that when birds of the same weight were compared the conclusions were different from those drawn from an age comparison. Thus, the reduced rates of muscle protein accretion as a result of amino acid imbalance appear to be due mainly to changes in the rate of protein degradation.

Effect of lysine deficiency

Our results demonstrated that whatever the tissue or the age, lysine deficiency reduced the amount of protein gained each day (AGR, g/d). Protein turnover is extremely sensitive to food intake (Arnal *et al.* 1987; McNurlan & Garlick, 1989; Muramatsu, 1990) and the protein deposition is influenced by the nature or the quality of proteins (amino acid balance) as well as by the energy intake and the amount of dietary protein. In the present experiment we could not determine whether the diet-induced variations of protein turnover were due to the decrease in protein quality *per se* or to the consequent alterations in protein or non-protein energy intake since feed intake was reduced approximately 1·5-fold in the L groups compared with the controls. Whatever the age, neither the feed intake:body-weight gain ratios nor the feed intake:body weight ratios were lower in chicks fed on the L diet (results not shown). Thus we may speculate that decreased protein deposition in L groups did not result primarily from a deficiency in protein and/or energy.

Tissue-specific changes

In avian species, most studies have been carried out on the regulation of whole-body rather than tissue-protein turnover. However, a change (or lack of change) in whole-body protein turnover does not necessarily mean that all body components respond equally or in the same direction. In the present experiment, therefore, we measured fractional rates of protein synthesis and breakdown in skeletal muscle as well as in liver. The tissue FSR observed here in 2-week-old chicks are similar to the few previous values obtained in chicks of the same age, e.g. 20–25% per d in the biceps muscle and 95–110% per d in liver (Nieto *et al.* 1994).

The pectoralis major muscle seemed more sensitive than the liver to lysine deficiency: whatever the age, we recorded a higher decrease in tissue weight (-65 *v.* -40 to -50% in pectoralis major and liver respectively) and in protein deposition (at 2 and 3 weeks of age: -75 *v.* -50% in pectoralis major and liver respectively; at 4 weeks of age: -60 *v.* -25%). Moreover, we showed that the efficiency of protein deposition declined considerably in the pectoralis major muscle, whereas we observed only a slight change in the liver. The difference of tissue response could be due to the fact that the liver may be protected from diet-induced atrophy on account of its vital properties, unlike the breast muscle which is a product of genetic selection, has little functional utility and could become a major source of amino acids in deficiency states. A higher loss of skeletal muscle mass compared with liver mass and differential changes in protein turnover occurring in each tissue are consistent with results obtained in various catabolic states such as ageing (Mosoni *et al.* 1995) or sepsis (Vary & Kimball, 1992*a*, *b*). In addition, it could be speculated that liver is less sensitive since it is supplied with nutrients before muscle.

Opposite responses of muscle absolute synthesis rate and fractional synthesis rate

Because of the large reduction in muscle mass the FSR (% per d) and ASR (g/d) responded in opposite directions irrespective of age. On the one hand, lysine deficiency resulted in decreases in ASR, in agreement with the results obtained in growing chickens (Tesseraud *et al.* 1992), in growing pigs (Salter *et al.* 1990) and in humans (Conway *et al.* 1980; Meredith *et al.* 1986). On the other hand, it led to increases in FSR, in keeping with our previous results (Tesseraud *et al.* 1992). This observation apparently conflicts with the results of Kino & Okumura (1987), who recorded a reduced FSR in whole body in chicks fed on S amino acid- or histidine-devoid diets (diets completely lacking in an amino acid). Differences between the results could be attributed to the following reasons. The amino acid in question may be important since Kino & Okumura (1987) recorded lower FSR with

a methionine + cystine-free diet than with a histidine-devoid one. Other possibilities are the severity of amino acid deficiency, as suggested by McDonald & Swick (1981) for protein restriction, and the genotypes used (more or less fast-growing chicks; see review by Griffin & Goddard, 1994); finally, the results might vary in accordance with the measurement of protein turnover performed in the whole body or in particular tissues and organs. The present investigation provides at least the evidence that lysine deficiency does not change FSR and FBR in liver, in contrast to muscle.

Influence of diet-induced delay in chick development

The first apparent effect of lysine deficiency was to reduce the body weight so that a 3-week-old chick fed on diet L was similar in weight and development to a 2-week-old control. Therefore the modifications of protein metabolism could simply be related to a delay in development. Indeed, between 2 and 4 weeks of age we obtained a developmental decline of FSR in the pectoralis major muscle, which is in good agreement with the results found in whole body or skeletal muscle of chickens (McDonald & Swick, 1981; Muramatsu & Okumura, 1985; Tesseraud *et al.* 1994) as well as in whole chick embryos (Muramatsu *et al.* 1987). Note that age-related decreases in FSR were related to decreased capacities for protein synthesis, whereas the translational efficiency was not significantly modified, as previously reported in mammals (Attaix *et al.* 1988; Lobley, 1993). Consequently this hypothesis could explain the relatively similar muscle FSR when comparing 2-week-old chicks on diet C with 3- or 4-week-old chicks on diet L. The fact that muscle FBR was always higher in chicks fed on a lysine-deficient diet suggests, however, that the dietary treatment results not only in delayed development but also induces great changes in muscle protein metabolism.

The results presented also suggest that the effect of lysine deficiency on either protein synthesis or degradation depends on whether one compares for equal weights or ages. First, with comparisons at the same age the reduced protein deposition seems to be due mainly to decreases in protein synthesis. This is in keeping with the results obtained with lysine-deficient diets either in growing chicks (Tesseraud *et al.* 1992) or pigs (Salter *et al.* 1990). Only Fuller *et al.* (1987) failed to agree since they found a decrease of protein deposition in growing pigs without any significant changes in protein synthesis or breakdown. Second, a different conclusion is reached when comparing chicks of similar weight or muscle protein content: thus, muscle protein degradation was always higher in the L group, whereas synthesis was unchanged (Fig. 1 and Table 5). Similarly, if one compares 2-week-old birds on diet C with 3- or 4-week-old birds on diet L, which present similar protein contents at 1·47, 1·10 and 1·74 g respectively, the striking effect of lysine deficiency is on the FBR. Indeed FBR is more than double, in the lysine-deficient 3- or 4-week-old chicks, that in the controls aged 2 weeks, whereas ribosomal capacity and FSR are similar, and the translational efficiency is identical. Compared in this way, prolonged lysine deficiency may affect muscle mass by changing protein degradation rates, as suggested by other experiments in chicks with mild dietary treatments, e.g. slight protein restriction (Maruyama *et al.* 1978), amino acid imbalance or feed restriction (Nieto *et al.* 1994). According to Nieto *et al.* (1994), only complete fasting reduced muscle FSR. These findings contradict the idea that FBR is less sensitive to dietary treatment than FSR or more generally that degradation rates are primarily regulated by genetic or intrinsic mechanisms, whereas protein synthesis rates are more responsive to nutritional and environmental determinants (see discussion by Tomas *et al.* 1991). In order to deepen understanding the regulation of tissue protein deposition by dietary factors and particularly dietary protein quality will require further investigations of protein turnover including physiological and hormonal factors.

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