MUSCLE PROTEIN SYNTHESIS *IN VITRO* IN CHICKS FED A LOW-PROTEIN DIET

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Summary

Muscle protein synthesis *in vitro* was measured in chicks fed low-protein (10% CP) and control (20% CP) diets. Right leg muscles (*M. gastrocnemius*) were mounted on a support made of stainless steel to stretch in constant tension, whereas left leg muscles were unmounted. Both leg muslces were incubated in Dulbecco's modified Eagle's medium including L-[4-^{3}H] phenylalanine for 60 min to measure *in vitro* protein synthesis. There was no significant difference in fractional synthesis rate (FSR) of muscle protein between both dietary protein levels, whereas FSR with stretch in constant tension was significantly higher than that without constant tension due to an increase in the absolute synthesis rate (ASR) per unit RNA (the efficiency of RNA to synthesize protein). The ASR of muscle protein in chicks fed the control diet was significantly higher than that in the low-protein diet group.

(**Key Words** : Protein Synthesis, Muscle, Low Protein Diet, Chicken, *In Vitro*)

Introduction

The measurement of protein synthesis *in vitro* offers a large number of advantages compared to the *in vivo* study because the condition of *in vitro* incubation can be more easily controlled than the *in vitro* model. Therefore, it has been established that isolated skeletal muscle preparations can be used to examine the influence of various factors affecting the rate of protein synthesis in many species, e.g. rats (Goldspink et al., 1983), rabbits (Palmer et al., 1981) and chickens (Baracos et al., 1989). Palmer et al. (1981) incubated leg muscles isolated from rabbits in the medium including sufficient amounts of nutrients and then estimated the fractional synthesis rate (FSR) of protein *in vitro* from the specific radioactivity of both free and protein-bound labelled amino acids. In avian species, however, little information has been available on FSR *in vitro* of muscle protein.

It has been well known that varying dietary protein levels influence the rate of protein synthesis in the whole-body and tissues of chickens as well as mammals. When chicks were given diets with varying dietary protein levels, whole-body protein synthesis increased with an increment of dietary protein levels up to the required amount recommended by Scott et al. (1982) and National Research Council (1984), and decreased gradually above this level (Muramatsu et al., 1987a; Kita et al., 1989; 1993a). Protein deprivation from diets significantly reduced protein synthesis in the liver of chicks due to a fall in total RNA content (Muramatsu et al., 1983). Furthermore, the feeding of a high-protein diet of twice the requirement also reduced liver protein synthesis as a consequence of the decrease in total RNA content, but did not influence the rate of muscle protein synthesis (Kita and Okumura, 1993). From these results, it is likely that, in the *in vitro* study, dietary protein levels affect the rate of muscle protein synthesis in chickens. In the present study, therefore, we measured FSR of muscle protein *in vitro* and examined the influence of feeding on a low protein diet on muscle protein synthesis *in vitro*.

Materials and Methods

One hundred single-comb White Leghorn male chicks from a local hatchery (Hattori Yokei-en Ltd, Nagoya) were maintained on a commercial chick mash diet (21.5% CP, 12.1 kJ ME/g; Marubeni Siryou Ltd, Tokyo) from hatching until 7 d of age in electrically heated brooders. At this age, 30 birds of uniform body weight were selected and divided evenly into 2 experimental groups of 15 birds each. The birds were placed in individual stainless metabolism cages in a temperature-controlled (29

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±1℃) room. Continuous illumination was provided. Half birds were allowed free access to experimental diets with either 10 or 20% CP. Calculated metabolizable energy of all diets was identically set at 12.6 kJ ME/g. The composition of the diets is shown in table 1.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS (g / kg DIET)

Protein level (%)	10	20
Isolated soyabean protein[1)]	119.5	239.0
L-Methionine	1.5	2.9
L-Threonine	0.6	1.2
Glycine	2.1	4.2
Maize starch	425.6	302.0
Sucrose	200.0	
Cellulose	157.7	
Maize oil	30.0	
Mineral mixture[2)]	58.5	
Vitamin mixture[2)]	2.0	
Choline chloride	1.5	
Inositol	1.0	
Metabolizable energy (kJ/g)	12.6	12.6

[1)] Crude protein content was 84%.
[2)] Kita et al., 1993a.

Ten days after placement on the experimental diets the birds were used for measuring muscle protein synthesis *in vitro*. After birds were killed by neck dislocation, right and left leg muscles (*M. gastrocnemius*) were removed. Baracos and Goldberg (1986) reported that the tension in order to maintain the normal length of muscle improved protein balance in rat muscles. In the present study, therefore, to maintain muscles at their *in situ* length, right leg muscles were mounted on a support made of stainless steel described by Baracos et al. (1989), whereas left leg muscles were unmounted. All muscles were preincubated for 30 min in a 20 ml flask with 5 ml of Dulbecco's Modified Eagle's (DME) medium (Sigma Chemical Company, St. Louis, MO, U.S.A.) to which was added unlabelled phenylalanine at 1 mM of final concentration. The concentrations on all amino acids in the medium were sufficient to achieve the plasma concentration of amino acids in chickens (Freeman, 1984). The flasks were placed in a temperature-controlled shaking water bath, and temperature was kept at 37℃. After preincubation, both 15 stretched and 15 intact muscles in each dietary treatment were divided evenly into 3 groups of 5 muscles each, and incubated in DME medium including L-[4-^{3}H] phenylalanine (37 kBq/ml, 37 kBq/μmol) for 30, 60 and 120 min, respectively. The medium was equilibrated with 5% CO_2/95% O_2, and all incubations were done under an atmosphere with the same gas. During incubation, the gas was saturated by passing through water to minimize evaporation and continuously bubbled through the medium via polyethylene tubes with needles. At the end of the incubation, muscles were rinsed with physiological saline and plunged into liquid N_2 to freeze deeply. Samples were stored at −20℃ until analyzed.

The specific radioactivity of free and protein-bound phenylalanine in muscles was determined by the method described by Garlick et al. (1980) with modifications (Muramatsu et al., 1993), and FSR was calculated by the method of Rennie et al. (1982). The measurement of RNA content was described previously (Kita and Okumura, 1993).

Statistical analysis of data was performed by ANOVA using the General Linear Model procedures (SAS, 1985). Differences between means were assessed by least significance difference and were considered to be significant at $p < 0.05$.

Results and Discussion

The time course change in the specific radioactivity of both free and protein-bound phenylalanine over 120 min of incubation is represented in figure 1. The specific radioactivity of free phenylalanine increased rapidly within 30 min of incubation and reached the constant level toward 120 min. The specific radioactivity of protein-bound phenylalanine in the low protein diet group elevated from 60 to 120 min of incubation, whereas the specific radioactivity of protein-bound phenylalanine derived from chicks fed a control (20% CP) diet increased from 0 to 60 min of incubation and thereafter was kept constant until 120 min. This result indicated that muscle protein synthesis *in vitro* should be estimated in 60 min, because in the *in vivo* study, the specific radioactivity of protein-bound amino acid continued to increase toward the level of the specific radioactivity of free amino acid. Therefore, in the present study, FSR was estimated from the results of specific radioactivity measured at 60 min of incubation.

Muscle weight, the contents of protein and RNA, fractional and absolute synthesis rates (FSR and ASR, respectively) of muscle protein are shown in table 2. Except for FSR, all parameters obtained from chicks fed the low protein diet were lower than those derived from the control diet group. The feeding on a low protein diet decreased ASR of muscle protein, as was good agreement with whole-body protein synthesis in chicks (Muramatsu

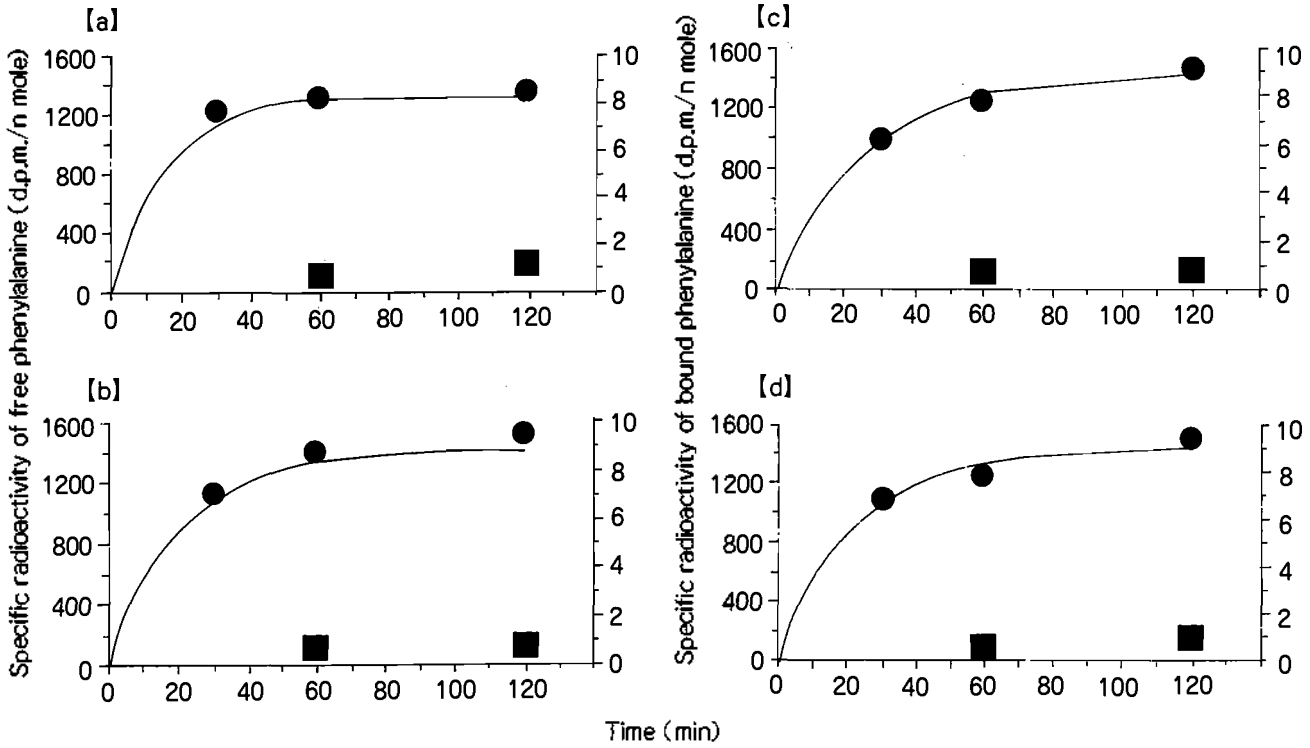

Figure 1. Time course change in specific radioactivity of free (●) and protein-bound (■) phenyalanine in the muscle of chickens.
a): 10%, tension (+), b): 20%, tension (+),
c): 10%, tension (−), d): 20%, tension (−).

et al. 1987b). So far, we have measured muscle FSR *in vivo*, being 9.4%/day, in chicks fed the 20% CP diet (Kita and Okumura, 1993), which was considerably higher than that *in vitro* being 1.60%/day (table 2). Palmer et al. (1981) compared FSR of rabbit muscles *in vivo* and *in vitro* and showed that FSR *in vitro* was nearly one third of that *in vivo*. Similar results were found in FSR of whole-body protein in chicken embryos (Muramatsu et al., 1987a; Kita et al., 1993b) and of rat diaphragm (Preedy et al., 1986). The reason why large difference in FSR was observed between *in vivo* and *in vitro* studies was discussed by Goldspink et al. (1983).

TABLE 2. EFFECT OF DIETARY PROTEIN LEVELS ON THE WEIGHT AND THE CONTENT OF PROTEIN AND MRNA IN THE MUSCLE (*M. gastrocnemius*) OF CHICKS[1)]

Protein level (%)	10	20	Pooled SEM
Muscle weight (g)	0.37[a]	0.59[b]	0.02
Protein (mg/muscle)	64[a]	106[b]	5
RNA (μg/muscle)	549[a]	887[b]	59
FSR[2)] (%/d)	1.43	1.60	0.16
ASR[2)] (mg/d)	0.90[a]	1.68[b]	0.18

[1)] The number of chicks measured the rate of muscle protein synthesis in each treatment was ten.

Means not sharing a common superscript letter within the same row in each experiment are significantly different at $p < 0.05$.

[2)] FSR : fractional synthesis rate, ASR : absolute synthesis rate.

The influence of stretch in constant tension on muscle weight, the contents of protein and RNA, FSR and ASR were also examined in the present study. Although FSR was increased by the stretch in constant tension, no influence of stretch on other parameters was observed (data not shown). When isolated rat skeletal muscles were maintained normal length *in situ*, protein synthesis *in vitro* was similarly improved compared to that with resting length (Goldspink et al., 1983), suggesting that the stimulation of stretch would be one of important factors regulating muscle protein synthesis. Waterlow et al. (1978) proposed two main factors to explain the large variation in FSR; the first was the difference in the RNA concentration (RNA: protein ratio) and the other was the efficiency of RNA to synthesize protein (ASR per unit RNA). From the result shown in table 2, the regression equation for FSR versus ASR per unit RNA was as follows: FSR (%/d) = 616 + 477 × ASR per unit RNA

(μg/g) ($r = 0.76$, $p < 0.001$). However, FSR did not correlate significantly with the ratio of RNA : protein. The high correlation between FSR and ASR per unit RNA suggests that the increase in muscle protein synthesis by stretch in constant tension is regulated, at least in part, by the efficiency of RNA to synthesize protein.

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