Biochem. J. (1981) **194**, 811–819
Printed in Great Britain

The effect of protein depletion and repletion on muscle-protein turnover in the chick

Marnie L. MacDONALD* and Robert W. SWICK†
Department of Nutritional Sciences, University of Wisconsin, Madison, WI 53706, *U.S.A.*

(*Received 18 August 1980/Accepted 11 September 1980*)

Rates of growth and protein turnover in the breast muscle of young chicks were measured in order to assess the roles of protein synthesis and degradation in the regulation of muscle mass. Rates of protein synthesis were measured *in vivo* by injecting a massive dose of L-[1-^{14}C]valine, and rates of protein degradation were estimated as the difference between the synthesis rate and the growth rate of muscle protein. In chicks fed on a control diet for up to 7 weeks of age, the fractional rate of synthesis decreased from 1 to 2 weeks of age and then changed insignificantly from 2 to 7 weeks of age, whereas DNA activity was constant for 1 to 7 weeks. When 4-week-old chicks were fed on a protein-free diet for 17 days, the total amount of breast-muscle protein synthesized and degraded per day and the amount of protein synthesized per unit of DNA decreased. Protein was lost owing to a greater decrease in the rate of protein synthesis, as a result of the loss of RNA and a lowered RNA activity. When depleted chicks were re-fed the control diet, rapid growth was achieved by a doubling of the fractional synthesis rate by 2 days. Initially, this was a result of increased RNA activity; by 5 days, the RNA/DNA ratio also increased. There was no evidence of a decrease in the fractional degradation rate during re-feeding. These results indicate that dietary-protein depletion and repletion cause changes in breast-muscle protein mass primarily through changes in the rate of protein synthesis.

Muscle-protein turnover is an important component of total body protein metabolism during normal growth (Garlick *et al.*, 1976) as well as during adaptation to nutritional deprivation. As in all tissues, the rate of growth of the muscle-protein mass is a function of the relative rates of protein synthesis and degradation. An understanding of the way in which these two processes are regulated would therefore provide insight into the phenomena of development, growth and aging.

The chick is a convenient experimental animal for the study of muscle growth because it grows at a rate almost twice that of the young rat. In studies by Maruyama *et al.* (1978), fractional growth rates of young chicks were manipulated from 0 to 10%/day by imposing partial amino acid or energy restrictions at 1 week of age. Rates of protein synthesis in the breast and leg muscles were determined by the continuous dietary infusion of L-[U-^{14}C]tyrosine. In all studies, the rate of muscle-protein synthesis appeared to be independent of the growth rate, particularly in the breast muscle. In the leg muscle, rapid rates of growth were correlated with slightly higher rates of protein synthesis. In contrast, the fractional degradation rate decreased in both the breast and leg muscles as growth rates increased. These results appeared to indicate that, in the young chick, rapid muscle growth is facilitated by lowered rates of protein degradation.

In studies of the adult fowl, Laurent *et al.* (1978*b*) observed that stretch-induced hypertrophy of the anterior latissimus dorsi muscle was achieved by a marked increase in the fractional rate of protein synthesis. Furthermore, hypertrophy was associated with an increase in the fractional rate of protein breakdown. Similar results have been obtained in studies of muscle-protein turnover in the rat. For example, Millward *et al.* (1976) found that nutritional restrictions that retarded the normal rate of growth were associated with decreased rates of synthesis and of degradation. During rehabilitation on an adequate diet, rapid growth was accompanied by high rates of protein synthesis and of protein degradation (Millward *et al.*, 1975).

* Present address: Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, CA, U.S.A.
† To whom reprint requests should be sent.

0306-3283/81/030811-09$01.50/1 © 1981 The Biochemical Society

The possibility that the fundamental process of muscle growth may be regulated in a different manner in two species, the rat and the chick, is somewhat unexpected. The present study was undertaken to clarify the relative contributions of rates of protein synthesis and degradation to breast-muscle growth in the chick. Dietary treatments were selected to provide a period of atrophy, followed by rapid muscle growth. Rates of protein synthesis were measured with a single massive dose of L-[1-^{14}C]valine by a method similar to that first described by Henshaw *et al.* (1971) and modified by McNurlan *et al.* (1979). This method was chosen so that rates of protein synthesis could be measured in chicks from 1 week to 7 weeks of age. In young chicks, a continuous intravenous infusion (e.g. Garlick *et al.*, 1975) is complicated by clotting of the blood around the cannula, whereas a continuous dietary infusion (Harney *et al.*, 1976; Maruyama *et al.*, 1978) was not feasible in the older birds, owing to size restrictions imposed by the metabolic chambers used to collect expired CO_2.

The massive-dose technique was found to be useful for the measurement of rates of muscle-protein turnover in the chick. Our results demonstrate that protein depletion and repletion cause changes in the breast-muscle protein mass primarily through changes in the rate of protein synthesis. Some of these observations have been reported in a preliminary communication (MacDonald & Swick, 1980).

Experimental

Animals and diets

White Leghorn cockerels (Sunnyside Hatchery, Oregon, WI, U.S.A.) were fed a control diet beginning at 1 day of age. The diet consisted of 20% (w/w) vitamin-free casein, 8% gelatin, 6% mineral mix (Salts 'N'; Fox & Briggs, 1960), 4% corn oil, 2% cellulose, 0.2% L-methionine, 0.2% choline chloride, and the following vitamins (mg/kg diet): thiamin hydrochloride, 80; *myo*-inositol, 100; nicotinic acid, 100; α-tocopheryl acetate, 25; calcium pantothenate, 20; riboflavin, 8; pyridoxine hydrochloride, 8; menadione, 1; folic acid, 3; biotin, 0.3; vitamin B_{12}, 0.02; cholecalciferol (vitamin D_3), 1000i.u./kg; and retinyl palmitate, 10000i.u./kg. The diet was made up to 100% by adding equal weights of cerelose (glucose monohydrate) and cornstarch. All diet ingredients were purchased from Teklad Test Diets, Madison, WI, U.S.A., and vitamins were purchased separately from ICN Nutritional Biochemicals, Cleveland, OH, U.S.A., or Sigma Chemical Co., St. Louis, MO, U.S.A.

Chicks were housed in a continuously lighted room in wire-bottomed cages heated electrically for the first 2½ weeks of age. At 4 weeks of age, some chicks were switched to a protein-free diet in which the casein and gelatin were replaced by an equal weight of cornstarch and cerelose (1:1). After 17 days, the chicks receiving the protein-free diet were re-fed the control diet. All other chicks remained on the control diet throughout the study.

For each measurement of protein synthesis, six to eight chicks with similar body weights and fractional growth rates (near the group mean) were selected from a group of 50; the remaining chicks were either killed to determine the rate of muscle-protein accumulation or were discarded. Rates of breast-muscle protein synthesis were measured in chicks on the control diet at 1, 2, 4, 6 and 7 weeks of age, in chicks fed the protein-free diet for 7 or 17 days, and in chicks re-fed the control diet for 1, 2 or 5 days.

Injections and tissue analysis

Chicks were kept at 38°C, beginning 45min before the injections and until the time of killing. Injections were given between 11:00 and 14:00h to minimize diurnal variation. L-[1-^{14}C]Valine (37.4mCi/mmol) was purchased from Research Products International, Elk Grove Village, IL, U.S.A., and was combined with unlabelled L-valine to give 444 μmol/ml and 35 μCi/ml, then adjusted to pH7.5. Each chick received an intraperitoneal injection of L-[1-^{14}C]valine (267 μmol/100g body wt.) and groups of three to four chicks were killed by decapitation at 5 or 20min after the injection. The breast muscle was removed quickly, wrapped in aluminium foil and frozen in liquid N_2.

Frozen muscles were weighed and pulverized with a mortar and pestle kept on solid CO_2. A portion of the sample was homogenized in 10% trichloroacetic acid with a Polytron (Brinkmann) homogenizer for the determination of the specific radioactivities of free and protein-bound valine. Another portion was homogenized in 0.2M-$HClO_4$ for the determination of total muscle protein, RNA and DNA.

The homogenate in trichloroacetic acid was treated as described by Harney *et al.* (1976) for the separation of free amino acids and protein, except that the first two trichloroacetic acid washes were saved for the separation of soluble valine, and the third wash, which was discarded, contained 5mM-unlabelled L-valine. The supernatants from animals killed at each time point were pooled, dried, and dissolved in sodium citrate buffer, pH2.2. Norleucine was added as an internal standard. The specific radioactivity of valine was determined in duplicate by separation on Aminex A9 resin (Bio-Rad) with a Beckman 120C amino-acid analyser that had been modified so that a portion of the column effluent (about 40%) was diverted to a fraction collector. Fractions containing valine were counted for radioactivity. The final trichloroacetic acid-precipitated protein was dissolved in formic

acid, and duplicate portions were taken for protein determination by the method of Lowry *et al.* (1951) and determination of radioactivity. Radioactivity was determined by liquid-scintillation counting in a Packard Tri-Carb 3300 instrument, with a xylene/Triton X-100-based scintillation fluid (Fricke, 1975). Correction for quenching was made by automatic external standardization.

A portion of the $HClO_4$ homogenate was pipetted into 1.1M-NaOH for the determination of total protein by the method of Lowry *et al.* (1951). RNA and DNA were solubilized from the remaining homogenate as described by Shibko *et al.* (1967), except that DNA was hydrolysed in 0.8M-$HClO_4$ at 70°C for 45min. RNA was determined by absorption at 260nm after correction for u.v.-absorbing peptides as described by Laurent *et al.* (1978*a*) and DNA was determined by the diphenylamine method as modified by Giles & Myers (1965).

Measurement of rates of protein synthesis

For the measurement of rates of protein synthesis, a massive dose of L-[1-^{14}C]valine (267μmol/100g body wt.) was injected. This dose, which is approx. 10 times the total body pool of free valine, gave a rate of incorporation of radioactivity into mixed breast-muscle proteins that was linear between 5 and 20min (Fig. 1). The specific radioactivity of the trichloroacetic acid-soluble valine ('free' valine) was not constant from 5 to 20min, but increased linearly. The increase in the specific radioactivity of the free amino acid can probably be explained by the slow absorption of the intraperitoneal dose, because a smaller, intravenous dose of L-[1-^{14}C]leucine has been shown to give a linear decrease in liver free leucine specific radioactivity from 2 to 30min after injection in the rat (McNurlan *et al.*, 1979). In the chicks, when a smaller dose was given (133μmol/100g body wt.), the incorporation of radioactivity into protein proceeded with a longer lag (10min) and the specific radioactivity of free valine increased more slowly from 10 to 20min than from 5 to 10min (results not shown).

The fractional rate of protein synthesis, in units of day^{-1}, was calculated from the rate of incorporation of radioactivity into protein-bound valine [determined by linear regression of d.p.m./μmol of bound valine against time, from 0.0035 days (5min) to 0.014 days (20min) after injection], divided by the average specific radioactivity of free muscle valine over the time course of incorporation. Because the change in free valine specific radioactivity was linear, the average specific radioactivity was approximated as the arithmetic mean of the values at 5min and at 20min. The rate of protein synthesis per unit of RNA (RNA activity) or per unit of DNA (DNA activity) was determined in a similar manner

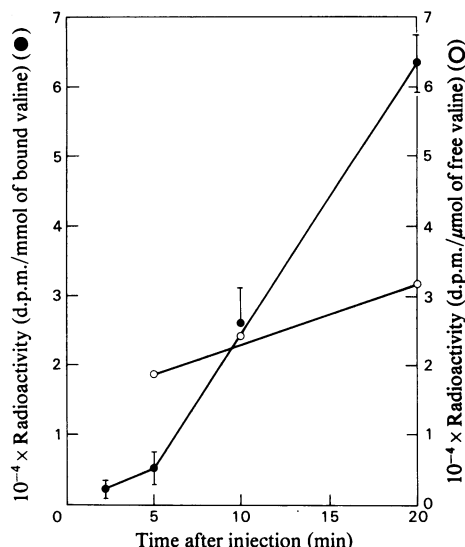

Fig. 1. *Incorporation of* L-[*1-*^{14}*C*]*valine into breast muscle*

Chicks weighing 100g were given intraperitoneal injections of 267μmol of L-[1-^{14}C]valine. Groups of three chicks were killed at 2, 5, 10 and 20min after injection, and the breast muscles were removed and treated as described in the text for the determination of bound valine specific radioactivity in individual birds (d.p.m./mmol ± 1 S.D., ●) and free valine specific radioactivity in pooled supernatants (d.p.m./μmol, ○).

by linear regression of [(d.p.m./μmol of bound valine) × (g of protein/g of RNA)] against time in days, or by linear regression of [(d.p.m./μmol of bound valine) × (g of protein/g of DNA)] against time in days respectively.

The specific radioactivity of protein-bound valine (d.p.m./μmol) was calculated from the specific radioactivity of total protein (d.p.m./mg) and the valine content of mixed breast-muscle proteins, which was determined to be 1.244μmol of valine/mg of protein. This approximation was considered reliable because hydrolysis and amino-acid analysis of labelled breast-muscle proteins from chicks killed 20min after injection showed that more than 96% of the protein-bound radioactivity was present as valine. However, this calculation is subject to the assumption, discussed by Henshaw *et al.* (1971), that there is no significant change in the valine content of mixed muscle proteins due to the treatments. The valine content of proteins may change slightly due to changes in the collagen content of the muscle in chicks fed the protein-free diet, but because collagen is only a small proportion of breast-muscle protein in chicks at 4 weeks of age (Dickerson, 1960), even if no collagen was lost during the protein-free feeding, the valine content of

proteins would be decreased by no more than about 5%. This would lead to a 5% overestimate in the fractional synthesis rate; however, it is not expected that this error would significantly affect the results reported here.

Calculation of rates of protein degradation

The fractional growth rate was determined for each chick as the average fractional growth rate over 48h immediately preceding the synthesis measurement, except for the 1-day-re-fed group, in which the fractional growth rate was determined over 24h only. The fractional rate of muscle-protein growth was determined for each bird from its fractional body growth rate, the amount of breast-muscle protein (mg of protein/g of body wt.) and the amount (mg) of muscle protein gained per g of body weight gained. The latter value was determined by linear regression of total breast-muscle protein (in mg) against body weight (g) of other chicks in the same treatment group.

The fractional rate of protein degradation, in units of day^{-1}, was estimated by subtracting the fractional rate of muscle protein growth from the fractional rate of synthesis. This calculation involves the assumption that the average rate of muscle-protein accumulation over a 48-hour period reflects the rate over the 15-min period of incorporation of radioactivity. In order to obtain an estimate of the fractional degradation rate for each chick, the fractional synthesis rate was re-calculated as follows. The slope of the regression line of (d.p.m./μmol of bound valine) against time in days was extrapolated to zero incorporation, and the rate of incorporation of radioactivity for each chick was determined over the time period from the extrapolated value (from 3 to 4min) to the time of killing. This procedure resulted in nearly identical values for the fractional synthesis rate and S.E.M. as were obtained from linear regression of all values. The fractional synthesis rate and fractional muscle-protein growth rate for each chick was then used to calculate an individual fractional degradation rate as described above.

Results

Massive-dose method

Because some amino acids are toxic (Harper *et al.*, 1970) or are suspected to affect rates of protein synthesis (Buse & Reid, 1975), the effect of a large dose of valine on muscle-protein synthesis was tested by injecting a tracer amount of $NaH^{14}CO_3$ along with a massive dose of unlabelled L-valine. The incorporation of $H^{14}CO_3^-$ into mixed breast-muscle proteins was used as an index of the rate of protein synthesis. This tracer was used instead of a single labelled amino acid because competition between amino acids for transport into the tissue may be reflected in increased or decreased incorporation of label into protein (e.g. McNurlan *et al.*, 1979). Labelled HCO_3^- is fixed into aspartate, glutamate, alanine, glycine and serine in the chick (M. L. MacDonald, unpublished work) and thus its incorporation into protein will reflect the fate of more than one amino acid. As Table 1 shows, a massive dose of L-valine had no significant effect on the incorporation of $H^{14}CO_3^-$-labelled amino acids into breast-muscle protein, which supports the assumption that the valine itself did not affect the rate of muscle-protein synthesis.

With the massive-dose method as with a continuous infusion of a labelled amino acid, it is important to measure the specific radioactivity of the amino acid incorporated into protein. Because the specific radioactivity of aminoacyl-tRNA is difficult to measure, it is conventional to assume that the precursor specific radioactivity is equal to that of the trichloroacetic acid-soluble extract of the tissue of interest. There is now evidence that, in liver, cardiac muscle and skeletal muscle, tRNA may be charged by amino acids from both the extracellular and intracellular pools (Vidrich *et al.*, 1977; Martin *et al.*, 1977; McKee *et al.*, 1978; Chikenji & Elwyn, 1980). In addition, McNurlan *et al.* (1979) have shown that when a large dose of L-[1-^{14}C]leucine is used to measure liver or jejunal-mucosa protein synthesis, the difference in specific radioactivity of the free amino acid in the plasma and in the tissue extract is smaller than with a continuous infusion of [^{14}C]tyrosine. These results indicate that compartmentation of aminoacyl-tRNA may be a less serious problem with the massive-dose technique.

In separate experiments, the massive dose of L-[1-^{14}C]valine gave a fractional synthesis rate of breast-muscle protein similar to that obtained with a continuous dietary infusion of L-[U-^{14}C]tyrosine in chicks at 1 and 2 weeks of age. The fractional synthesis rates in 1- and 2-week-old chicks were 0.42

Table 1. *Effect of a massive dose of valine on the incorporation of $H^{14}CO_3^-$ into mixed proteins of chick breast muscle*

Chicks weighing 148 ± 5g were given intraperitoneal injections of $NaH^{14}CO_3$ (100μCi/100g body wt.) with or without unlabelled L-valine as indicated. Chicks were killed 20min after the injections. Data are expressed as means ± S.E.M. ($n = 4$).

Dose of L-valine (μmol/100g body wt.)	Specific radioactivity of muscle protein (d.p.m./mg)
0	16.2 ± 1.0
133	18.0 ± 2.0
267	18.0 ± 2.4

and 0.20 day^{-1} respectively with the massive-dose method (Fig. 2) and 0.38 and 0.21 day^{-1} respectively with a continuous dietary infusion in NH × SCWL chicks (Maruyama *et al.*, 1978). We have also used a pulse dose of L-[1-^{14}C]glutamate to obtain a direct measurement of the rate of protein degradation in the breast muscle of young chicks fed on a control diet. This tracer yielded an apparent fractional degradation rate of 0.094 day^{-1} and an apparent fractional synthesis rate of 0.18 day^{-1} over a decay period of 1 to 11 days after injection (results not shown). These values are similar to the fractional degradation rate of 0.10 day^{-1} and fractional synthesis rate of 0.20 day^{-1} in 2-week-old chicks with the massive dose of valine (Fig. 2). Because the massive-dose technique gives rates of turnover similar to those obtained by other methods, this technique appears to be useful for the study of protein turnover in muscle.

Changes in muscle-protein turnover with age

Rates of protein synthesis were measured at 1, 2, 4, 6 and 7 weeks of age in chicks fed on the basal diet (Fig. 2). The fractional rates of protein synthesis and degradation in breast muscle were highest in the 1-week-old chicks, and fell by more than 50% by 2 weeks of age. From 2 to 7 weeks of age, there was no significant change in the fractional rates of synthesis, although the fractional degradation rate in the 4-week-old chicks was higher than that of the 2-week-old group.

To assess the relationship of developmental changes in protein turnover to changes in muscle-protein mass, rates of protein synthesis were also expressed per g of DNA, i.e. DNA activity. This term, which has been discussed extensively by Millward and co-workers (Millward & Waterlow, 1978; Millward, 1978; Laurent *et al.*, 1978*a*) relates to the concept that a nucleus 'manages' a certain amount of cytoplasm (Cheek *et al.*, 1971) via its role in transcription. In fact, the capacity for protein synthesis in a given tissue appears to be limited by the total DNA. In rat muscles, the rate of protein synthesis per unit of DNA is relatively constant at all ages, whereas total DNA increases during development (Millward, 1978). In chick breast muscle there was no significant change in DNA activity at any age, whereas the protein/DNA ratio (DNA-unit size) increased more than 2-fold from 1 week to 7 weeks of age (Fig. 3). Because the fractional synthesis rate may be calculated as DNA activity divided by protein/DNA, it is clear that the fall in the fractional synthesis rate from 1 to 2 weeks of age is a result of the rapid rate of accumulation of muscle protein during this time. If the expansion of the DNA-unit size was prevented by nutritional restrictions at 1 week of age, the age-related fall in the fractional rates of turnover would not be seen. These age-related changes in synthesis and degradation complicate the interpretation of experiments in which dietary restrictions were imposed at 1 week of age (Maruyama *et al.*, 1978). In those

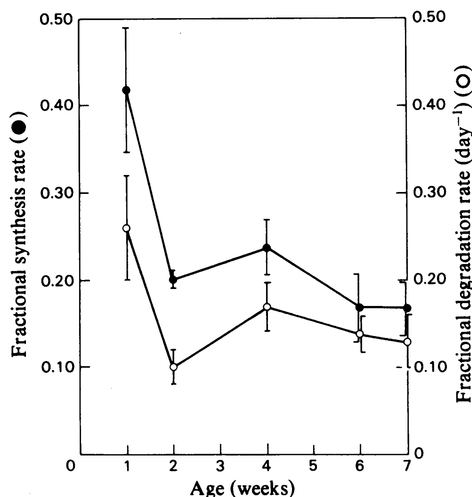

Fig. 2. *Changes with age in the fractional rates of synthesis and degradation of breast-muscle protein*
All values are means ± S.E.M. ($n = 6$). ●, Fractional synthesis rate (day^{-1}); ○, fractional degradation rate (day^{-1}) in chicks fed a control diet beginning at 1 day of age.

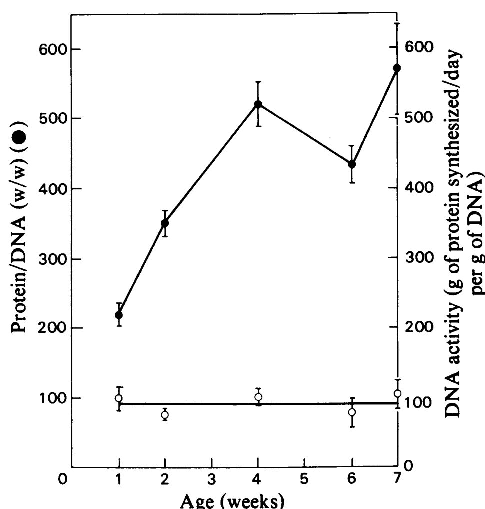

Fig. 3. *Changes with age in DNA activity and DNA-unit size*
DNA activity (g of protein synthesized/day per g of DNA, ○) was calculated as described in the legend to Table 3. ●, DNA-unit size (protein/DNA, w/w). All values are means ± S.E.M. ($n = 6$).

experiments, different conclusion could be made if age controls or weight controls were compared with chicks fed on deficient diets. For this reason, we have used somewhat older chicks (4 weeks of age) to examine changes in muscle-protein turnover on a protein-free diet followed by rehabilitation on a good diet.

Effect of a protein-free diet

A group of 4-week-old chicks previously fed the basal diet was given a protein-free diet for 17 days. By 7 days, the chicks were losing weight at a rate of 1.5% day^{-1} and muscle protein at a rate of 2.2% day^{-1} (Table 2). This rate of loss remained constant up to 17 days on the deficient diet, by which time the chicks had lost an average of 30% of their initial body weight and 66% of the initial muscle protein. The extensive loss of breast-muscle protein may be unique to this muscle, because only 30% of the combined leg-muscle protein is lost in rats fed a protein-free diet for 30 days (Garlick *et al.*, 1975). It may be that leg muscles are 'protected' from atrophy on account of their continual activity (Wechsler, 1966), unlike the breast muscles of the domestic fowl, which are a product of genetic selection and have little functional utility.

The fractional synthesis rate decreased by almost 50% after 1 week on the protein-free diet, whereas there was no apparent change in the fractional degradation rate. The amount of protein synthesized

Table 2. *Rates of growth and protein turnover in breast muscle during protein depletion and re-feeding*

Chicks (4 weeks old) were fed a protein-free diet for 17 days, then re-fed the control diet. The fractional rate of protein synthesis was measured from 5 to 20 min after the injection of a massive dose of L-[1-^{14}C]valine. The fractional degradation rate was calculated for each chick by subtracting the fractional rate of muscle-protein growth from the fractional synthesis rate. All values are means ± S.E.M., $n = 8$ for the 5-day-re-fed group; $n = 6$ for other groups.

	Body weight (g)	Fractional growth rate (day^{-1})	Fractional muscle-protein growth rate (day^{-1})	Fractional synthesis rate (day^{-1})	Fractional degradation rate (day^{-1})
Control (4 weeks old)	257 ± 4	0.045 ± 0.009	0.063 ± 0.010	0.24 ± 0.03	0.18 ± 0.03
Protein-free diet for 7 days	222 ± 3	−0.015 ± 0.003	−0.022 ± 0.002	0.13 ± 0.03	0.15 ± 0.03
Protein-free diet for 17 days	176 ± 3	−0.016 ± 0.004	−0.023 ± 0.003	0.21 ± 0.03	0.23 ± 0.03
+1 day re-fed	187 ± 3	0.056 ± 0.012	0.106 ± 0.010	0.32 ± 0.12	0.21 ± 0.05
+2 days re-fed	223 ± 2	0.103 ± 0.015	0.172 ± 0.020	0.41 ± 0.05	0.24 ± 0.04
+5 days re-fed	283 ± 2	0.064 ± 0.010	0.096 ± 0.020	0.45 ± 0.03	0.36 ± 0.05
Control (6 weeks old) (age control for 17-day protein-free)	444 ± 4	0.027 ± 0.004	0.031 ± 0.003	0.17 ± 0.04	0.14 ± 0.02
Control (7 weeks old) (age control for 5-day-re-fed)	530 ± 6	0.036 ± 0.011	0.037 ± 0.005	0.17 ± 0.03	0.13 ± 0.03

Table 3. *Rates of protein synthesis per unit of DNA (DNA activity) and per unit of RNA (RNA activity) in breast muscles of chicks during protein depletion and re-feeding*

DNA activity and RNA activity were calculated by linear regression of [(d.p.m./μmol of bound valine) × (protein/DNA)] against time or [(d.p.m./μmol of bound valine) × (protein/RNA)] against time from 5 to 20 min after the injection of a massive dose of L-[1-^{14}C]valine. The slope of these regression lines was divided by the average specific radioactivity of free valine over the period of incorporation. All values are means ± standard error of the slope, or means ± S.E.M. ($n = 6$–8).

	Total muscle DNA (mg)	Protein/DNA (g/g)	DNA activity (g of protein synthesized/day per g of DNA)	RNA/DNA	RNA activity (g of protein synthesized/day per g of RNA)
Control (4 weeks old)	4.69 ± 0.12	517 ± 35	101 ± 10	4.25 ± 0.69	28.5 ± 6.8
Protein-free diet for 7 days	4.85 ± 0.31	328 ± 37	43 ± 4	2.39 ± 0.56	18.8 ± 5.2
Protein-free diet for 17 days	3.56 ± 0.25	217 ± 19	43 ± 3	2.30 ± 0.54	19.1 ± 2.8
+1 day re-fed	3.56 ± 0.24	217 ± 5	69 ± 26	2.23 ± 0.27	31.2 ± 3.8
+2 days re-fed	3.81 ± 0.24	283 ± 18	125 ± 26	2.65 ± 0.31	45.8 ± 4.5
+5 days re-fed	4.90 ± 0.23	338 ± 40	152 ± 40	4.21 ± 0.97	33.0 ± 3.8
Control (6 weeks old)	7.55 ± 0.57	427 ± 36	74 ± 20	4.27 ± 0.52	17.2 ± 3.9
Control (7 weeks old)	9.85 ± 1.2	568 ± 65	100 ± 20	4.53 ± 1.38	20.3 ± 2.9

per unit of DNA decreased by 57%, whereas total muscle DNA remained unchanged (Table 3). DNA activity was lower because of a decrease in the RNA/DNA ratio and a decrease in the amount of protein synthesized per unit of RNA (RNA activity) (Table 3). RNA activity is primarily a measure of the activity of the ribosomes in protein synthesis (Millward *et al.*, 1973; Laurent *et al.*, 1978*a*), because the majority of muscle RNA is ribosomal (see Young, 1970).

By 17 days on the protein-free diet, both the fractional synthesis and degradation rates were higher than those of the 7-day group, and did not appear to be different from the values in the 4-week-old (weight control) birds. However, the total amounts of breast-muscle protein synthesized and degraded per day were less in the 17-day group than in the 7-day group (Table 4). Furthermore, RNA activity and DNA activity were equally decreased in chicks fed on the deficient diet for either 7 days or 17 days (Table 3). The fractional synthesis rate was thus higher after 17 days than after 7 days on the protein-free diet because of a decrease in the size of the protein pool, and not because of an increase in the total amount of protein synthesized. On the basis of DNA activity and total amounts of protein turned over, it is clear that the extensive loss of muscle protein on the protein-free diet was achieved by a decrease in the rate of protein synthesis, and was not accompanied by an increase in the rate of muscle-protein breakdown. In this situation, in which two-thirds of the breast-muscle protein was lost by 17 days on the deficient diet, basing conclusions solely on fractional rates of synthesis is misleading.

This discrepancy, which has been encountered by others (e.g. Garlick *et al.*, 1975) arises because protein synthesis is expressed as a fractional rate, whereas it is, in all likelihood, a zero-order (constant amount) process with respect to the protein pool. Protein degradation may indeed be a constant-fraction process, but when it is calculated from the fractional rates of protein synthesis and protein accumulation, as in the experiments reported here, its meaning will again depend on the fractional synthesis rate. This does not imply that fractional rates of protein synthesis are meaningless, only that they must be used with caution. The fractional rate of synthesis reflects the intensity of protein synthesis, which provides a useful comparison between species and between organs within a species. Furthermore, the fractional synthesis rate is calculated directly from measurements of specific radioactivities. Nevertheless, in the non-steady state, it may be advisable to report rates of protein synthesis per unit of DNA or as total amounts of protein synthesized in addition to fractional rates. The methods of expressing turnover rates, and the kinetics of protein turnover, have been discussed in detail by Waterlow *et al.* (1978).

Table 4. *Total amounts of breast-muscle protein synthesized or degraded each day during protein depletion and re-feeding*

Total protein synthesis and breakdown were calculated by multiplying the fractional synthesis rate or fractional degradation rate (Table 2) by the protein content of the breast muscles at the time of death.

	Total protein synthesis (mg/day)	Total protein breakdown (mg/day)
Control (4 weeks old)	538	394
Protein-free diet for 7 days	184	214
Protein-free diet for 17 days	158	176
+1 day re-fed	275	192
+2 days re-fed	437	253
+5 days re-fed	704	539
Control (6 weeks old)	546	434
Control (7 weeks old)	704	512

Effect of re-feeding

On re-feeding the chicks on the basal diet, the fractional rate of muscle-protein growth increased from -2.3% day^{-1} to 17% day^{-1} after 2 days (Table 2). The rapid growth was accompanied by a doubling of the fractional synthesis rate (Table 2) and the total amount of protein synthesized (Table 4) by 2 days after re-feeding. DNA activity increased by 350% and total muscle DNA, which had fallen by 25% by 17 days on the protein-free diet, was restored to pre-depletion values by 5 days of re-feeding (Table 3). The protein/DNA ratio, however, was still lower after 5 days of re-feeding than in the 4-week-old controls. The marked increase in DNA activity was accomplished by an increase in RNA activity after 1 day of re-feeding, followed by an increase in the RNA/DNA ratio after 5 days of re-feeding. Catch-up growth occurred despite an apparent 60% increase in the fractional rate of protein degradation (Table 2) and a 3-fold increase in the total amount of protein degraded (Table 4). There was no apparent decrease in the rate of degradation at any of the three time points after re-feeding.

Discussion

One purpose of the present study was to investigate whether muscle-protein turnover in the young chick changes during development, nutritional restriction or re-feeding in a manner that is unique to this species. As shown in Fig. 3, the DNA activity in breast muscle varied from 70 to 100 in chicks

ranging from 1 week to 7 weeks of age. These values are similar to the values of 61 to 77 in the anterior and posterior latissimus dorsi muscles of fully-grown cockerels (Laurent *et al.*, 1978*b*), which indicates that DNA activity in skeletal muscle may be relatively constant throughout development of the chick. These results are similar to those obtained in a fast-growing (CFY) strain of rats in which DNA activity did not change markedly with age (Millward & Waterlow, 1978). In this respect, the pattern of growth is similar in young rats and chicks.

Dietary treatments were selected to provide a period of muscle atrophy followed by rapid growth. On the protein-free diet, atrophy was achieved by a marked decrease in the rate of protein synthesis, which was accompanied by a smaller decrease in the rate of protein degradation (Tables 2 and 4). The observation that a decreased rate of protein synthesis is responsible for the loss of breast-muscle protein on a protein-free diet differs from previous studies in which milder dietary restrictions were imposed on chicks at 1 week of age (Maruyama *et al.*, 1978). In those studies, it appeared that a low rate of protein degradation played an important role in the achievement of rapid rates of growth of the breast muscle, and that synthesis was not changed by the dietary treatments. There are three possible explanations for the differences between results in the latter study and the present work. (1) The age-related fall in the fractional rates of synthesis and degradation (Fig. 2) may complicate the interpretation of experiments in which dietary restrictions are imposed at 1 week of age. Because the fall in the fractional synthesis rate from 1 week to 2 weeks of age is a consequence of the expansion of the muscle-protein pool, dietary restrictions that retard the normal rate of growth would also retard this age-related change. In this case, it may be appropriate to compare restricted birds with their weight controls instead of age controls. When this was done, the fractional rates of synthesis of breast-muscle proteins were lower in the chicks on various dietary restrictions than in the controls, and there was little or no apparent change in the fractional rates of degradation (Maruyama *et al.*, 1978). (2) Rates of protein turnover may change in a different manner in response to a more severe restriction (protein-free) than when a milder restriction is given (low-lysine, low energy or low protein/energy). However, it is unclear why a protein-free diet, which is a more severe restriction, would lead to decreased rates of muscle-protein breakdown, whereas the milder restrictions appeared to increase rates of breakdown. In rats, the fractional rate of breakdown of muscle protein is increased only after 4 days of starvation, whereas other dietary restrictions (including a protein-free diet for 30 days) caused a decreased rate of breakdown (Millward *et al.*, 1976; Millward & Waterlow, 1978). However, it may be that the lack of dietary protein in the chick induces a different response than when some protein is present. (3) Rates of muscle-protein turnover in the younger chicks (1 to 2 weeks of age) may respond differently to a nutritional shift than in somewhat older birds (4 to 6 weeks of age). Although this possibility cannot be discounted, it is the least likely because the breast muscles of newly-hatched chicks are fully differentiated and are not morphologically unique (George & Berger, 1966). Nevertheless, more information is needed about the extent of changes in rates of protein synthesis and degradation due to nutritional deprivation in chicks at various stages of development.

To our knowledge this is the first report of changes in muscle-protein turnover during nutritional rehabilitation of chicks, despite the economic implications of compensatory growth in the poultry industry (Johnson & Sell, 1976). Our results indicate that rapid muscle growth was achieved by an immediate increase in the rate of protein synthesis. There was no evidence of a lowered rate of protein degradation during regrowth of the muscle; instead, the fractional degradation rate increased by 60% by 5 days of re-feeding. These results are similar to those obtained in rats when rates of protein synthesis were measured by a continuous infusion of [^{14}C]tyrosine (Millward *et al.*, 1975).

In contrast, Young *et al.* (1971) reported that during the re-feeding of rats after prolonged protein depletion, muscle growth was facilitated by the cessation of protein breakdown. These conclusions were based on the disappearance of radioactivity from muscle proteins labelled *in vivo* by [4-^{14}C]aspartate. However, these results could be explained by increased retention of label in muscle after re-feeding. The likelihood of reutilization is supported by the observation that total muscle radioactivity increased for 7 days after the injection of [^{14}C]aspartate. In addition, if the fractional rate of protein synthesis (k_s) is calculated from their data from the change in specific radioactivity of muscle protein, the average k_s from 4 to 6 days after re-feeding is not higher in the re-fed than in the depleted rats. The apparent k_s in the muscle of the re-fed rats is almost certainly lower than the 'true' k_s owing to reutilization of label, because direct measurements of rates of protein synthesis showed a 3.5-fold increase in the fractional rate of synthesis by 3 days of re-feeding (Millward *et al.*, 1975).

The effect of nutritional rehabilitation in rats has also been studied with a non-radioisotopic technique in which the urinary excretion of 3-methylhistidine is used as an index of the rate of breakdown of myofibrillar proteins. With this method, Funabiki *et al.* (1976) observed an immediate increase in the fractional breakdown rate of myofibrillar proteins

when rats were re-fed an adequate diet after 2 weeks on a protein-free diet. However, Haverberg *et al.* (1975) obtained different results in rats fed either a low-protein or a low-protein/low-energy diet for 2 weeks before repletion. In rats previously fed the low-protein diet, re-feeding caused an increase in 3-methylhistidine excretion, suggesting an increased rate of breakdown of actomyosin. In contrast, the protein/energy-depleted rats exhibited decreased excretion of 3-methylhistidine by 4 days of re-feeding.

At this time, no known radioisotopic technique gives satisfactory rates of degradation in muscle under all conditions (MacDonald *et al.*, 1979). In addition to reutilization of labelled amino acids during decay measurements, the turnover of myofibrillar proteins may be non-random. If so, the interpretation of decay curves may be complicated when different treatments are being compared (Millward *et al.*, 1975). On the basis of direct measurements of rates of protein synthesis and indirect estimates of rates of protein breakdown, it seems likely that, in both the rat and the chick, rates of muscle-protein synthesis and degradation are decreased on a protein-free diet and are increased during re-feeding; thus the rate of protein breakdown changes in a way that limits the rate of muscle-protein accumulation or loss. As discussed by Millward *et al.* (1975), the increased rates of breakdown during muscle growth may be unique to this tissue and may result from the way in which myofibrils proliferate.

This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and U.S. Public Health Service grant no. AM 14704. We thank Miss Elizabeth Johnson for excellent technical assistance, Mr. James Sommercorn for helpful discussions, and Dr. Richard A. Freedland of the University of California (Davis), in whose laboratory some of the sample analyses were performed.

References

Buse, M. G. & Reid, S. S. (1975) *J. Clin. Invest.* **56**, 1250–1261

Cheek, D. B., Holt, A. B., Hill, D. E. & Talbert, J. C. (1971) *Pediatr. Res.* **5**, 312–338

Chikenji, T. & Elwyn, D. H. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 349

Dickerson, J. W. T. (1960) *Biochem. J.* **75**, 33–37

Fox, M. R. S. & Briggs, G. M. (1960) *J. Nutr.* **72**, 243–249

Fricke, U. (1975) *Anal. Biochem.* **63**, 555–558

Funabiki, R., Watanabe, Y., Nishizawa, N. & Hareyama, S. (1976) *Biochim. Biophys. Acta* **451**, 143–150

Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975) *Biochim. Biophys. Acta* **414**, 71–84

Garlick, P. J., Burk, T. L. & Swick, R. W. (1976) *Am. J. Physiol.* **230**, 1108–1112

George, J. C. & Berger, A. J. (1966) *Avian Myology*, Academic Press, New York

Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93

Harney, M. E., Swick, R. W. & Benevenga, N. J. (1976) *Am. J. Physiol.* **231**, 1018–1023

Harper, A. E., Benevenga, N. J. & Wohlheuter, R. M. (1970) *Physiol. Rev.* **50**, 428–558

Haverberg, L. N., Deckelbaum, L., Bilmazes, C., Munro, H. N. & Young, V. R. (1975) *Biochem. J.* **152**, 503–510

Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) *J. Biol. Chem.* **246**, 436–446

Johnson, R. L. & Sell, J. L. (1976) *Anim. Nutr. Health* (Nov./Dec.), 13–15

Laurent, G. J., Sparrow, M. P., Bates, P. C. & Millward, D. J. (1978*a*) *Biochem. J.* **176**, 393–405

Laurent, G. J., Sparrow, M. P. & Millward, D. J. (1978*b*) *Biochem. J.* **176**, 407–417

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

MacDonald, M. L. & Swick, R. W. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 349

MacDonald, M. L., Augustine, S. L., Burk, T. L. & Swick, R. W. (1979) *Biochem. J.* **184**, 473–476

Martin, A. F., Rabinowitz, M., Blough, R., Prior, G. & Zak, R. (1977) *J. Biol. Chem.* **252**, 3422–3429

Maruyama, K., Sunde, M. L. & Swick, R. W. (1978) *Biochem. J.* **176**, 573–582

McKee, E. E., Cheung, J. Y., Rannels, D. E. & Morgan, H. E. (1978) *J. Biol. Chem.* **253**, 1030–1040

McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979) *Biochem. J.* **178**, 373–379

Millward, D. J. (1978) *Biochem. Soc. Trans.* **6**, 494–499

Millward, D. J. & Waterlow, J. C. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2283–2290

Millward, D. J., Garlick, P. J., James, W. P. T., Nnanyelugo, D. O. & Ryatt, J. S. (1973) *Nature (London)* **241**, 204–205

Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975) *Biochem. J.* **150**, 235–243

Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188

Shibko, S., Koivistoinen, P., Tratnyek, C. A., Newhall, A. R. & Friedman, L. (1967) *Anal. Biochem.* **19**, 514–528

Vidrich, A., Airhart, J., Bruno, M. K. & Khairallah, E. A. (1977) *Biochem. J.* **162**, 257–266

Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, pp. 179–223, North-Holland, Amsterdam

Wechsler, W. (1966) *Methods Achiev. Exp. Pathol.* **1**, 431–438

Young, V. R. (1970) in *Mammalian Protein Metabolism* (Munro, H. N., ed.), vol. 4, pp. 586–674, Academic Press, New York

Young, V. R., Stothers, S. C. & Vilaire, G. (1971) *J. Nutr.* **101**, 1379–1390