Day Length Has a Major Effect on the Response of Protein Synthesis Rates to Feeding in Growing Japanese Quail[1]

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ABSTRACT We investigated the effect of day length on mixed protein fractional synthesis rates (K_S) in 14- and 21-d-old Japanese quail (*Coturnix c. japonica*) habituated to either a long day length, 18 h light/6 h dark (LDL), or short day length, 6 h light/18 h dark (SDL), with free access to food during the light period. Rates of protein synthesis were measured by a flooding dose of L-[1-^{13}C]leucine. In both groups, we measured K_S of pectoral muscle, liver and heart after an overnight period of food deprivation and after 2-h food access at dawn. Rates of protein synthesis were also measured in LDL quail starved for 18 h and refed for 2 h. SDL chicks were smaller and had lower tissue weights at 2 wk of age than did LDL chicks ($P < 0.05$). Starvation led to a lower rate of protein synthesis in those animals starved for 18 h. Food availability after starvation for 18 h induced a significant rise in tissue protein synthesis in both SDL and LDL quail ($P < 0.05$). This increase was absent in LDL quail after a 6-h starvation period. There was an increase in K_S to ad hoc changes in food supply. By determining the daily period in which feeding can occur, day length has a major effect on protein synthesis rates. This effect will determine the overall growth chicks are able to achieve that have been subjected to different day lengths. J. Nutr. 131: 268–275, 2001.

KEY WORDS: • *stable isotopes* • *protein synthesis* • *feeding* • *starvation* • *birds*

Growth in juvenile animals is affected by day length (Boon et al. 1997, Schanbacher et al. 1981). This effect is produced by the influence of day length on daily energy intake and energy expenditure (Boon et al. 1997, Charles et al. 1992, Schanbacher et al. 1981). Much of the growth observed in young animals is due to increases in body protein, with skeletal muscle showing the greatest percentage change in body composition as animals grow. Growth is achieved when protein synthesis rates are higher than protein breakdown rates (Waterlow 1984). It therefore seems feasible that day length, via time available for food intake, activity and energy expenditure, will affect protein balance and thus net body growth through changes in protein synthesis and/or breakdown rates.

Changes in timing of food intake have been shown to induce daily variations in protein turnover. In animals experiencing a balanced diurnal periodicity, daytime feeding results in protein gain, and nighttime starvation leads to protein loss (Clugston et al. 1982, Millward et al. 1988 and 1991, Murphy et al. 1990, Pocknee et al. 1978). For net growth of tissues to occur, as in growing and lactating animals, the deposition of protein should exceed protein losses (Garlick et al. 1994). Exposure to short day lengths (SDL),[3] as, for example, occurs seasonally, results in long nocturnal periods of food deprivation and large amounts of food consumed during short light periods (Boon et al. 2000). During these short feeding periods, large amounts of protein amino acids become available to the body. These dietary amino acids have to cater for protein synthesis to cover immediate protein needs (repair, growth and maintenance) and/or as a protein store for use in subsequent periods of food deprivation. Normally, when the supply of amino acids exceeds the protein synthesis capacity of the animal, the excess will be oxidized (Gibson et al. 1996, Millward et al. 1988 and 1991, Waterlow 1984). During starvation, e.g., overnight, protein synthesis rates drop, and during prolonged periods of starvation, rates may even fall below breakdown levels. Starvation over a period of 2–4 d reduces protein synthesis rates considerably in many different tissues and whole body (Cherel et al. 1991, McNurlan et al. 1979 and 1980, Muramatsu et al. 1987). Even short periods of starvation (8–18 h) have been shown to reduce protein synthesis rates in rats and mice (Davis et al. 1993, Garlick et al. 1983, Pocknee et al. 1978, Yoshizawa et al. 1997). Thus, long nocturnal periods of food deprivation may be a limiting factor for growth.

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[3] Abbreviations used: E_A, enrichment of the tissue-free leucine; E_B, enrichment of the tissue protein bound leucine; K_S, percent of protein mass synthesized/h; LDL, long day length (18 h light/6 h dark); MET, Mid-European Time; SDL, short day length (6 h light/18 h dark); t, time of labeling (in h).

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In most studies, starvation and feeding were imposed to investigate acute effects on protein synthesis rates. There is little information concerning the changes in tissue protein turnover rates during periods of starvation and feeding that return daily over a longer time span, as will be experienced by animals exposed to long dark periods and short light periods, such as during the late winter and early spring months. These animals are still able to grow, but to what extent is protein turnover affected? What we aimed to investigate was whether the rates of tissue protein synthesis in animals exposed to such conditions adapted to day length. Are animals that are habituated to short daily feeding periods, and consequently long nocturnal periods of starvation, able to maintain growth at the same rates during the overnight starvation by compensatory stimulation of protein synthesis when food becomes available? We also aimed to investigate whether there were differences between any feeding effects on the rates of protein synthesis in fast turnover (i.e., liver) and slower turnover (i.e., skeletal muscle) tissues.

We investigated the effect of day length on the response of protein synthesis rates to starvation and feeding in a rapidly growing strain of Japanese quail (*Coturnix c. japonica*) subjected to either a long day length (18 h light/6 h dark) (LDL) or SDL (6 h light/18 h dark) with free access to food during the light period. We measured the fractional synthesis rate (K_S) of tissue mixed protein after an overnight period of starvation and after 2-h food access at dawn. K_S was measured of two different types of muscle tissue, skeletal and cardiac, and of liver. Skeletal muscle and liver were chosen because both tissues contribute considerably to whole-body protein synthesis. In all organisms where it has been measured, hepatic K_S is high, whereas muscle, with a much lower K_S, contributes by its large size of the protein pool (McNurlan et al. 1980, Waterlow 1984). Cardiac muscle was investigated because of its insensitivity to short-term variation in food availability, as far as function and size are concerned. Japanese quail were used, a species with the highest growth rate of the family Phasianidae (Ricklefs 1973) and therefore likely to be responsive to variations in day length and food availability. In the present study, all measurements were conducted on 2- to 3-wk-old birds, the period of highest rate of body weight gain (10%/d; Boon et al. 2000).

MATERIALS AND METHODS

Animals and housing. Japanese quail (*Coturnix c. Japonica*) neonates, of a strain selected for maximum body mass at the age of 5 wk, were obtained from a commercial quail farm (N.V. Nouwen, Lommel, Belgium). Birds were kept in wooden cages [67 × 39 × 44 cm^3 (l × b × h)] with sawdust bedding at conditions of continuous light and free access to quail starter food and water, as previously shown to ensure maximum possible body mass gain, until 6 d old. A 40-W heating lamp was placed in each cage 20 cm above the bedding to provide a temperature gradient sufficient to allow for selection of the preferred temperature by the chicks. A maximum of 10 chicks were housed in each box. At 6 d of age, the chicks were assigned to either LDL [lights on at 9 h Mid-European Time (MET)] or SDL (lights on at 9 h MET), with free access to food during the light period. At this time, each box housed a maximum of four chicks per box. The 40-W heating lamp was gradually removed to allow the ambient temperature to decrease to room temperature (~21°C). Throughout the experimental period, a pelleted diet (Institute for Animal Science and Health, ID-DLO, Lelystad, the Netherlands; **Table 1**) containing 27.7 g/100 g crude protein and 17 kJ/wet g (gross energy by bomb calorimetry) was fed. Water was always freely available.

The animal care and killing protocol for this experiment were approved by the Animal Experimentation Committee of the University of Groningen, the Netherlands (BG15196).

TABLE 1

Composition of pelleted diet

Ingredient	Amount
	g · kg diet^{-1}
Corn	300
Soybean, extracted	350
Wheat	130
Lucerne flour	70
Fish flour	50
Meat flour	40
Soybean oil	33
Mixture poultry 2%[1]	20
Premix 0.5% without minerals	5
DL-Methionine	1.5
Coccidiostaticum	0.5

[1] The mixture provided the following micronutrients (mg/kg diet): retinol, 12; cholecalciferol, 60; α-tocopherol, 15; menadione, 1.5; thiamin, 1; riboflavin, 5; pantothenic acid, 7.5; niacin, 31; vcyanocobalamin, 0.015; folic acid, 1; pyridoxine, 1; choline, 350; Fe, 52; Cu, 10; Zn, 14; Mn, 59; Na, 1000; Cl, 1500; Ca, 5200; I, 0.8; Se, 0.1; and ethoxyquin, 50.

Experiment 1: Time course of enrichment in plasma, liver and muscle. To establish a protocol for measuring tissue mixed protein fractional synthesis rates by a flooding dose technique (McNurlan et al. 1979), we examined the time course of L-[^{13}C-]Leu enrichment in the free amino acid in plasma and intracellular pools and measured the incorporation of L-[^{13}C-]Leu into mixed tissue protein in pectoral muscle and liver after the administration of a flooding dose of L-[1-^{13}C]Leu. Quail (14 d old, 85 ± 15 g), subjected to an LDL, received a single intramuscular (i.m.) injection in the left pectoral muscle at dawn after an 18-h period of starvation of 1 mL/100 g body mass containing 200 μmol/L leucine, of which 20% was labeled (99 atom% L-[1-^{13}C]Leu; Masstrace Inc., Woburn, MA). After 5 (n = 7), 15 (n = 4) and 30 (n = 4) min, taken as the time after the whole solution was injected, blood was collected from the right wing vein into heparinized tubes, which were then centrifuged (1500 × *g*, 15 min at 4°C) to separate plasma from whole blood. Plasma was stored at −70°C until analysis. Birds were then killed and quickly immersed in liquid nitrogen to cool the body. Whole liver and a sample of the right pectoral muscle were immediately removed and washed in ice-cold saline (9 g sodium chloride/L) to minimize blood contamination. Excess saline was blotted before rapid tissue freezing in liquid nitrogen, weighed (to 0.0001 g) and stored at −70°C until analysis. The tissue harvesting procedure took <2 min, and all tissues were removed in the same order each time. A group of three birds was used for baseline measurements of protein bound and free leucine natural abundance in blood and tissue.

Experiment 2: The effect of starvation and day length on K_S in fed and starved animals. Having established that flooding was achieved during 15–30 min in all tissues, we used this time period to measure K_S of pectoral muscle, liver and heart in 14-d-old SDL and LDL quail starved overnight or after refeeding for 2 h at dawn. The rate of protein turnover is related to body mass and age (Goldspink et al. 1984, Millward et al. 1981, Waterlow 1984). We therefore repeated the experiment in 21-d-old SDL chicks. The body mass of SDL chicks at that age was closer to that of 14-d-old LDL chicks (**Table 2**). We also measured K_S in 14-d-old LDL chicks starved for 18 h that were refed for 2 h at dawn, similar to the daily starvation duration of SDL chicks. To measure K_S under all conditions, we injected chicks as described in expt. 1 and killed them either 15 or 30 min after injection. Whole liver, a sample of the right pectoral muscle and whole heart were taken and processed as described earlier.

Tissue analysis. A 100- to 200-mg sample of tissue was homogenized in liquid nitrogen using a mortar and pestle. The tissue was

TABLE 2

Characteristics of 14- and 21-d-old Japanese quail exposed to either a long day length (LDL; 18 h light/6 h dark) or short day length (SDL; 6 h light/18 h dark), that were either starved for 6 or 18 h or refed for 2 h at dawn[1]

	14 d				21 d	
	Starved for 6 h	Refed after 6-h starvation	Starved for 18 h[2]	Refed after 18-h starvation[2]	Starved for 18 h	Refed after 18-h starvation
LDL						
n	8	8	8	8	—	—
Body mass, *g*	95.5 ± 10.5	99.1 ± 11.9	94.9 ± 10.9	96.1 ± 14.8	—	—
Muscle, *g*	6.57 ± 1.02	6.58 ± 1.09	7.84 ± 1.15	7.29 ± 1.17	—	—
Liver, *g*	2.83 ± 0.50	2.97 ± 0.47	2.61 ± 0.48	2.78 ± 0.51	—	—
Heart, *g*	0.83 ± 0.14	0.81 ± 0.14	—	0.71 ± 0.15	—	—
SDL						
n	—	—	7	6	6	6
Body mass, *g*	—	—	51.0 ± 7.0*b	65.1 ± 11.0	110 ± 15.8b	126 ± 17.0
Muscle, *g*	—	—	3.04 ± 0.86b	3.90 ± 0.65	8.65 ± 0.64b	8.57 ± 1.82
Liver, *g*	—	—	1.33 ± 0.24a	2.22 ± 0.51	2.41 ± 0.43*b	3.54 ± 0.33
Heart, *g*	—	—	0.48 ± 0.05b	0.60 ± 0.14	0.87 ± 0.10a	0.91 ± 0.27

[1] Values are means ± SD.
[2] Data from expt. 1 for LDL chicks.
* Weight is significantly increased after refeeding compared to the starved state ($P < 0.05$; tissue weights were compared after correction for body mass). Means of 14-d and 21-d-old starved SDL-chicks with a different superscript than 6-h starved 14-d-old LDL quail differ significantly ($P < 0.05$).

mixed with 3 mL of ice-cold 0.2 mol perchloric acid/L, and after centrifugation (1500 × *g*), the supernatant, containing the tissue free amino acids, was removed and neutralized with potassium hydroxide. The amino acids from this solution were purified by ion-exchange chromatography (Dowex H^+ form), dried, and derivatized with 50 μL of pyridine and 50 μL of methylsilyl-*tert*-butylsilyltrifluoroacetamide at 80°C for 1 h. Labeling of the tissue free leucine ^{13}C enrichment, as *tert*-butyl dimethylsilyl (*t*-BDMS) derivative, was then measured by gas chromatography–mass spectrometry (MD 800; Thermoquest, Hemel Hempstead, U.K.) operated in selected ion monitoring mode (*m*/*z* 302 and 303).

To determine the bound leucine enrichment, the pellet obtained from the treatment of the tissue with perchloric acid underwent acid and alkali treatment to remove RNA and DNA. The protein was then subjected to hydrolysis in 3 mL of 6 mol/L hydrochloric acid at 110°C for 15–18 h. Resulting free amino acids were purified by ion-exchange chromatography, as above, before being vacuum dried. The leucine from this hydrolysis was collected by preparative gas chromatography of the *t*-BDMS derivatives (Smith et al. 1988). Enrichment of the tissue protein leucine was determined by isotope ratio mass spectrometry (Europa Scientific Instruments 2020, Crewe, U.K.) after liberation of the carboxyl carbon as $^{13}CO_2$ by the ninhydrin reaction (Smith et al. 1988).

Calculations and statistical analysis. Mixed protein K_S (%/h) was calculated as

$$K_S = [(E_B/E_F) \times t^{-1}] \times 100$$

where E_B is the enrichment of the tissue protein-bound leucine, E_F is the enrichment of the tissue-free leucine and t is the time of labeling in h (Garlick et al. 1980, McNurlan et al. 1979). The mean enrichments of the free and bound pool over the different time periods (x_1–x_2 min; **Table 3**) after administration of the flooding dose were used. We calculated for each individual quail the difference in enrichment of the free and bound leucine pools over the indicated time periods. Mean values for the free amino acid enrichment over the time course of the experimental period were obtained from the group values.

Data are expressed as means and interindividual SD. Student's *t* test was used to test for differences between group means and to test for the effect of feeding state (starved versus fed) on body mass and tissue K_S within day length (SPSS 1988). We also applied the Student's *t* test to investigate the effect of the duration of starvation within the LDL group on tissue K_S (SPSS 1988). Differences in mean K_S between tissues within day length were subjected to post hoc analysis by Tukey's "honestly significant difference" test, after an effect of tissue on K_S was ascertained by one-way analysis of variance (ANOVA). This test was also applied to test whether the mean K_S for muscle and liver differed over the different time intervals chosen and whether the relative enrichment of muscle and liver free pool enrichment to that of plasma differed over time. ANOVA was used to test for the effect of day length, feeding state and day length × feeding state interaction on tissue K_S. We applied a two-tailed significance level of $P < 0.05$ in all tests.

RESULTS

Experiment 1

The enrichment of the free pools (E_F; atom % excess) increased rapidly after injection (**Fig. 1**). In plasma and liver, the peak enrichment was reached at 5 min and remained

TABLE 3

Mixed protein fractional synthesis rates (K_S) of muscle and liver calculated over different time periods in 14-d-old Japanese quail starved for 18 h after a flooding dose of L-[1-13C]Leu[1,2]

	K_S	
Period, *min*	Muscle	Liver
	%/h	
5–15	0.47 ± 0.18	2.21 ± 0.68a,b
5–30	0.49 ± 0.12	1.46 ± 0.36a
15–30	0.59 ± 0.13	3.61 ± 1.20b

[1] Values are means ± SD, $n = 15$.
[2] Means with a different superscript within tissue differ significantly ($P < 0.05$).

constant over the next 10 min. In muscle, the peak was achieved after 15 min. In the protein bound pool of muscle and liver, we observed a linear increase in leucine enrichment (E_B) over the whole measurement, when the free leucine pool was flooded. The relative enrichment of the tissue free pools to that of plasma is shown in **Table 4**. It is evident from these results that flooding conditions were reached after 5 min for liver. Flooding conditions for muscle were reached only after 15 min and were maintained for the period chosen for protein synthesis measurements (see later; Table 4). Calculated mixed protein K_S for the different time intervals are presented in Table 3. We decided to take the 15- to 30-min interval for the experimental protocol because flooding was achieved and maintained in all tissues and intracellular and plasma free leucine pools were labeled to the same extent. Incorporation of label into protein was linear during this time interval.

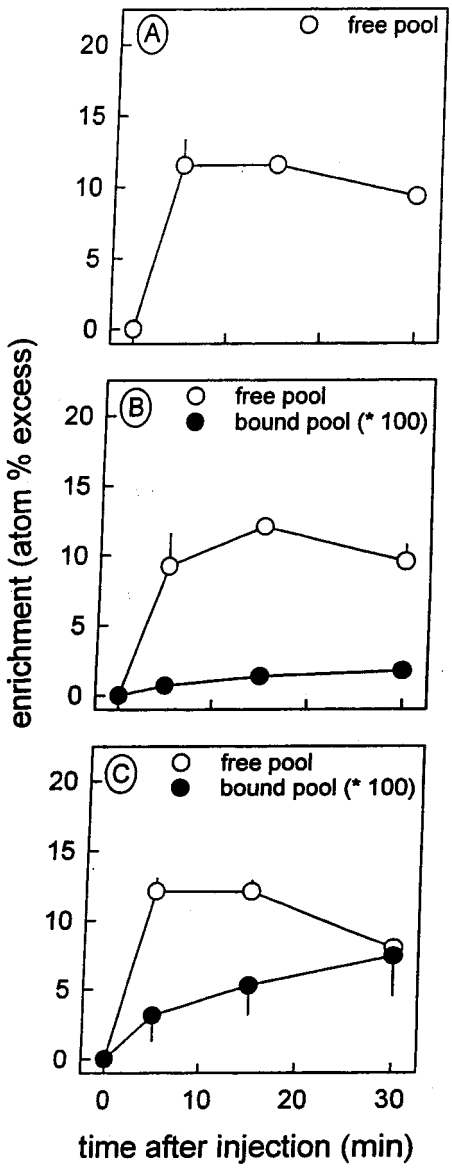

FIGURE 1 Time-related changes in enrichment of L-[1-^{13}C]Leu in the free and bound pool of plasma (*A*), pectoral muscle (*B*), and liver in 14-d-old Japanese quail (*C*) starved for 18 h after a flooding dose of L-[1-^{13}C]Leu. Values are means ± SD, n = 19.

TABLE 4

The ratio of muscle and liver free pool enrichment relative to plasma enrichment over time in 14-d-old Japanese quail starved for 18 h after a flooding dose of L-[1-^{13}C]Leu[1,2]

Time, *min*	Ratio[2]	
	Muscle	Liver
5	0.79 ± 0.11[a]	1.06 ± 0.14
10	0.79 ± 0.08[a]	0.88 ± 0.07
15	1.06 ± 0.04[b]	1.05 ± 0.07
30	1.05 ± 0.15[b]	0.87 ± 0.03

[1] Values are means ± SD, n = 15.
[2] Means with a different superscript within tissue differ significantly ($P < 0.05$).

Experiment 2

Body mass. Body mass varied significantly with day length: SDL chicks weighed 47% less than LDL chicks ($P < 0.001$; Table 2). SDL chicks of 21 d, regardless of feeding state (118 ± 17.6 g), weighed significantly more (18%) than 14-d-old LDL chicks ($P < 0.001$). After correction for body mass, pectoral muscle weighed significantly more and heart less in LDL chicks compared with SDL chicks ($P < 0.05$). A comparison of these LDL chicks with 21-d-old SDL chicks demonstrated that LDL chicks had a significantly lower muscle weight ($P < 0.05$) and higher liver weight ($P < 0.001$), corrected for body mass (Table 2). After correction for body mass, feeding significantly influenced liver weight in only 21-d-old SDL chicks ($P < 0.001$).

***Mixed protein* K_S.** In LDL chicks, there was no significant effect of refeeding on K_S of any of the tissues (**Table 5**) after 6 h of starvation. Starvation for 18 h, however, caused a significant decrease in K_S of pectoral muscle and liver compared with a 6-h starvation period ($P < 0.01$ and $P < 0.05$, respectively; Table 5). Refeeding after an 18-h period of starvation resulted in a marked increase in the rate of synthesis by approximately twofold in both pectoral muscle ($P < 0.01$) and liver ($P < 0.05$; Table 5). In SDL chicks, regardless of age, there was a significant increase in K_S in both pectoral muscle ($P < 0.05$) and liver ($P < 0.01$) after re-feeding. Again, this increase in protein synthesis rate was in the order of twofold to fourfold (Table 5). The magnitude of change in K_S in 18-h starved individuals was the same for the LDL and SDL groups. Interestingly, 14-d-old SDL chicks showed the greatest magnitude of change in protein synthesis rate in pectoral muscle and liver due to refeeding (Table 5). There was no significant alteration in heart K_S on refeeding in either of the SDL groups.

Because none of the tissue K_S values varied with feeding state in the LDL group, we compared mean K_S between the different tissues irrespective of feeding state. In this group, K_S was highest in liver (6.25 ± 1.16%/h), with heart next (2.34 ± 0.42%/h) and pectoral muscle lowest (1.61 ± 0.36%/h). In SDL quail after 18-h starvation, regardless of age, K_S was also highest in liver (5.52 ± 1.03%/h) and lowest in pectoral muscle (0.64 ± 0.23%/h). Heart K_S was intermediate (1.72 ± 0.34%/h; $P < 0.0001$). In the fed state, the difference in K_S between pectoral muscle (1.92 ± 1.02%/h) and heart (2.04 ± 0.68%/h) disappeared in SDL quail, whereas liver K_S (8.61 ± 2.04%/h) remained highest ($P < 0.0001$).

To test for an effect of day length on tissue K_S, we compared for each tissue 14-d-old LDL chicks with 21-d-old SDL

TABLE 5

Mixed protein fractional synthesis rates (K_S) of different tissues of 14- and 21-d-old Japanese quail exposed to either a long day length (LDL; 18 h light/6 h dark) or short day length (SDL; 6 h light/18 h dark) that were either starved for 6 or 18 h or refed for 2 h at dawn[1]

	14 d				21 d	
Tissue	Starved for 6 h	Refed after 6-h starvation	Starved for 18 h[2]	Refed after 18-h starvation[2]	Starved for 18 h	Refed after 18-h starvation
LDL						
n	4	4	4	4		
Muscle, %/*h*	1.57 ± 0.50[a,3]	1.64 ± 0.24	0.59 ± 0.13*[4]	1.39 ± 0.40	—	—
Liver, %/*h*	6.04 ± 1.37[b]	6.46 ± 1.07	3.61 ± 1.19*[4]	9.20 ± 3.71	—	—
Heart, %/*h*	2.33 ± 0.57[a,3]	2.35 ± 0.30	—	2.12 ± 0.84	—	—
SDL						
n			3	3	3	3
Muscle, %/*h*	—	—	0.66 ± 0.22*[a]	2.59 ± 1.06[a]	0.62 ± 0.28*[a]	1.24 ± 0.31[a]
Liver, %/*h*	—	—	6.20 ± 1.00*[b]	10.0 ± 1.93[b]	4.85 ± 0.50*[b]	7.18 ± 0.72[b]
Heart, %/*h*	—	—	1.92 ± 0.37[c]	2.24 ± 1.00[a]	1.52 ± 0.14[c]	1.85 ± 0.23[a]

[1] Values are means ± SD (for *n* see table). K_S was measured by a flooding dose of L-[1-^{13}C]Leu.
[2] Data from expt. 1 for LDL chicks.
[3] Significantly increased compared with 21-d-old starved SDL chicks ($P < 0.01$).
[4] Significantly decreased compared with 6-h starved LDL chicks ($P < 0.01$).
* K_S is significantly decreased in the starved state compared with corresponding values in the fed state ($P < 0.05$). For one combination of feeding state, day length and age, tissue K_S means with a different superscript differ significantly ($P < 0.0001$).

chicks, because of more comparable body mass (Table 2). For this, we entered day length, feeding state and day length × feeding state interaction in one-way ANOVA. Pectoral muscle and heart K_S were significantly higher in the LDL group for both the starved and fed state ($P < 0.01$). Liver K_S was not significantly affected by feeding state or day length.

To test for an effect of day length on pectoral muscle and liver K_S corrected for duration of food deprivation, we compared 14-d-old LDL chicks starved for 18 h with 21-d-old SDL quail (Table 2). To this end, day length, feeding state and day length × feeding state interaction were analyzed using a one-way ANOVA. Pectoral muscle ($P < 0.005$) and liver K_S ($P < 0.01$) varied only significantly with feeding state: K_S of both tissues was greater in the fed compared with the starved state.

DISCUSSION

Day length had a major effect on body mass in rapidly growing quail. Animals subjected to an SDL had a significantly lower average body mass at 2 wk of age compared with birds subjected to an LDL. This was due to the reduction in time available for food intake and consequently protein intake. We showed earlier that growing quail subjected to daily feeding periods of 6 h consumed on average 29% less energy than LDL birds of equal body mass (Boon et al. 2000). Day length reduction would be expected to have a profound influence on body protein turnover (and consequently growth). To measure the likely effects of day length on protein synthesis, we first demonstrated that it is possible to use the flooding dose method to measure tissue protein synthesis in rapidly growing birds. The flooding dose method is a widely adopted technique to measure whole body and tissue protein synthesis rates in small animals (Garlick et al. 1994). It is a convenient method for measuring acute changes in tissue protein synthesis as can occur, for example, after feeding (Davis et al. 1993, Garlick et al. 1983 and 1994). Flooding dose experiments in rats (Garlick et al. 1980, McNurlan et al. 1979, Obled et al. 1989), dogs (Jahoor et al. 1992) and birds (Murphy et al. 1995) show a linear decline in free pool enrichment (E_F) with time shortly after injection of the label. This was not apparent in our study, most likely due to a difference in the route of label administration. In rat studies, the label is usually injected intravenously, leading to a rapid rise of plasma and tissue free pool enrichment (to a maximum level within 2 min), that drops curvilinearly later. In a study with the White-crowned sparrow (*Zonotrichia leucophrys gambelii*), a large dose of ^{3}H-phenylalanine was injected in the pectoral muscle, similar to our study (Murphy et al. 1995). In these birds, free pool enrichment of muscle and liver reached their highest levels within 5 min after injection. The pectoral muscle in quail contains mainly white fast-twitch glycolytic fibers that are not highly vascularized (Hohtola et al. 1998). Because of this poor vascularization, the movement of the injected label from the left pectoral muscle into the bloodstream and then into the muscle free amino acid pools may be attenuated. This may explain the slow rise with time in free pool enrichment of pectoral muscle in our study compared with experimental protocols using intravenous or better vascularized routes. In plasma and liver, we observed the highest E_F levels within 5 min. After 15 min, E_F decreased linearly in all compartments (Fig. 1). We calculated protein K_S using measured and estimated enrichments 15 and 30 min after injection, because we demonstrated clearly that at these time points, flooding conditions were reached in muscle and liver (Table 4). Mixed protein K_S calculated over this time period were comparable to those reported for other bird species (Maeda et al. 1984 and 1986, Murphy et al. 1995).

Using this method, we showed that exposure to SDL (and consequently long overnight periods of food deprivation) resulted in *a*) a pronounced decrease in tissue mixed protein synthesis rates during the overnight starvation and *b*) a steep rise in measured synthesis rates during feeding (Table 5). Shorter periods of starvation, at least as long as

6 h, appeared to have no significant effect on muscle or liver protein synthesis rates. Exposure to an SDL resulted in a pronounced rise in tissue mixed protein synthesis rates during food availability. This increase was absent in birds conditioned to an LDL and hence longer periods of food availability. Being habituated to an SDL did not appear to induce any compensatory higher tissue protein synthesis rates at night and/or increase the deposition of dietary amino acids during feeding. It has been suggested that molting birds, which face high energy demands, may undergo pronounced daily cycling of protein, involving net protein degradation during the night when exposed to long overnight periods of starvation (≥8 h) and net synthesis by day (Murphy et al. 1990). The young birds investigated here were rapidly growing animals, so it is likely that energy demands in these birds will be high. SDL chicks are therefore likely to show a discontinuous 24-h growth curve; net protein gain during the day alternates with net protein loss during the relatively long night. Overall, birds were clearly in a net positive protein balance as they grew, albeit more slowly than the LDL group, over the experimental period. Quail chicks starved overnight for 6 h had prefeeding tissue K_S values comparable to fed rates, suggesting that these birds are able to maintain protein synthesis rates throughout the 24-h period and that the processes involved in regulating protein synthesis are not sensitive to feeding in this condition. A daily starvation period of 6 h also ensured chicks of relatively high tissue K_S values throughout the 24-h period (Table 5). These findings suggest that LDL chicks are able to maintain high rates of protein synthesis, which would contribute to their rapid growth rate. The mechanism for this is not clear at present, but it may involve maintained delivery of food from crop storage over the nightly period.

Because growth in protein is the difference between protein synthesis and protein breakdown (Waterlow 1984), an increase in K_S will result only in increased tissue protein growth when breakdown levels are increased less or even reduced during feeding. However, from evidence gained from other experiments, it is possible to predict that protein breakdown rates have likely fallen either during or immediately after feeding. In mice it has been shown that refeeding for 1 h after an 18-h period of starvation resulted in a decrease in muscle protein breakdown and an increase in muscle protein synthesis (Yoshizawa et al. 1997). The inhibition of protein breakdown rates due to feeding has also been shown in human studies (Gibson et al. 1996, Pacy et al. 1994). It is therefore likely that in our experiment, the increase in K_S, and any decrease in protein breakdown due to feeding, has resulted in an increase in tissue protein growth. Unfortunately, there is no simple method for measuring protein breakdown rates in vivo. However, we can estimate the likely changes in the groups of animals based on their net growth rates. If we assume that net growth over this period is approximately that of protein growth and that the fraction of the tissue weight that is protein has not changed significantly during the experimental period, we can estimate the average rate of protein breakdown in LDL chicks. Muscle growth over this period was 18%/d, and protein synthesis rate was 38%/d. Therefore protein breakdown would average 20%/d. Clearly in this tissue, synthesis, at nearly twice the rate of protein breakdown, would appear to be the most important energy demanding process for growth. In liver, the rate of tissue growth was only 5%/d, and the protein synthesis rate was 145%/d, meaning that protein breakdown rate would be ~140%/d, a much smaller difference in the rates of the processes regulating tissue protein mass. The protein synthesis rates we measured in tissues from this LDL group were not stimulated by feeding, unlike after the longer period of starvation in both SDL groups. It would appear that during the 6 h of starvation, they are receiving a sufficient supply of amino acids from food still being processed in their gut or from other storage forms for protein synthesis to be maintained during the whole day and during the 6-h starvation period. Alternatively, there may be attenuation of the translational process or its signaling pathway, and the process of protein synthesis is not stimulated, in this group, by the postprandial surge in amino acids and hormones that normally takes place. We have shown earlier that feeding during the first 2 h of food access is largely increased in SDL chicks and only minimally in LDL chicks: 11 g versus 4 g, respectively (Boon et al. 2000). This may explain the steep rise in muscle and liver K_S at refeeding in SDL chicks. The fact that no stimulation of protein synthesis occurred in LDL chicks after 2 h food access may also be due to a low food intake level during this period.

LDL chicks starved for 18 h had comparable tissue protein synthesis rates at dawn as birds habituated to repeated 18-h periods of starvation (Table 5). We had thus no support for our first hypothesis that birds habituated to 6-h feeding periods develop adaptive mechanisms by which they can maintain high protein synthesis rates during daily periods of prolonged starvation. We cannot, however, exclude that the drop in K_S during the overnight starvation was delayed in SDL chicks compared with 18-h starved LDL chicks, resulting in less protein loss during the night. We have shown that growing quail habituated to a long daily overnight period of starvation were able to offset (partially) the effects of starvation by exploiting their crop as a temporary place for food storage and slow delivery for digestion and absorption (Boon et al. 2000). In this way, food is present in the gastrointestinal tract during a large part of the night, as was evident from a high RQ. LDL chicks starved for 18 h may not have developed this strategy of using their crop as a food store, or their crop may be less accommodating to large amounts of storage. There may be a role of adaptations in crop volume, emptying regulation (neural or endocrine) or hypertrophy that requires further investigation. The loss of protein during the night may, therefore, be less in SDL chicks than LDL chicks starved for 18 h. LDL chicks that were refed for 2 h after an 18-h period of starvation showed a comparable stimulation of muscle and liver K_S as 21-d-old SDL chicks during feeding (Table 5). We hypothesized that SDL chicks, conditioned to repeated long daily overnight periods of starvation, would show a higher increase in tissue K_S during feeding than LDL chicks. Because feeding is restricted to only 6 h of the 24-h period, a large amount of amino acids become available to the body in a relatively short period. For efficient growth to occur, these dietary amino acids must be rapidly and efficiently deposited as body protein with minimal oxidative losses. Adopting this strategy, chicks could enlarge their amino acid store for periods during which no feeding can occur. Muscle K_S in particular may be responsive. Muscle is known to contain the largest reserve of mobilizable protein in the body (Waterlow 1984) and has been suggested to perform a storage function in meeting amino acid demands in the postabsorptive state (Murphy et al. 1995). However, we were unable to find evidence for this hypothesis (Table

5). Results indicate that protein synthesis rates are highly flexible and respond quickly to ad hoc changes in food supply.

Feeding had a significant effect on liver and pectoral muscle K_S after an 18-h period of starvation. This has been demonstrated in other studies after prolonged overnight starvation (Cherel et al. 1991, Davis et al. 1993, Garlick et al. 1983, Pocknee et al. 1978, Rennie et al. 1982, Yoshizawa et al. 1995 and 1997). Feeding, however, did not influence the protein synthesis rate of cardiac muscle. Heart is a rhythmically active organ that supplies tissues with nutrients and perfuses organs that remove waste products from the body. It is vital for life and growth, and its function cannot be compromised due to the short-term absence of dietary nutrients. Other studies have shown that in rats, cardiac muscle is sensitive to hormones and amino acids after prolonged periods of starvation (Preedy et al. 1984 and 1995). This relative insensitivity of heart muscle in quail to periods of starvation and feeding could be due to the time periods used in this study.

Whole body absolute protein turnover rates in mature animals of different species are positively related to metabolic mass ($g^{0.75}$; Goldspink et al. 1984, Millward et al. 1981, Reeds et al. 1980, Waterlow 1984, Waterlow et al. 1978). Protein turnover rates per unit mass on the other hand are negatively related to metabolic mass ($g^{-0.25}$). In our study, there was a significant difference in body mass between 14-d-old LDL and 21-d-old SDL chicks (96 g versus 118 g). However, based on the relationship between protein turnover rate per unit mass and metabolic mass, we calculated that the difference in body mass between the two groups could only account for ~5% of the change in mass corrected protein turnover rates. This is a marginal effect considering the scale of differences observed after feeding and the variation within the groups. It is therefore unlikely that this difference in body mass has affected the comparison between tissue K_S values. This relationship also explains the higher K_S values in the 14-d-old SDL group that had a significantly lower body mass than equal-aged LDL chicks (Tables 2 and 5).

In conclusion, the results showed that day length affects protein synthesis rates by determining the daily duration of food access in diurnal animals. In this way, day length will determine the level of overall growth juvenile animals are able to achieve when exposed to different day lengths.

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