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Biochemical correlates of selection for weight-for-age in chickens: twenty-fold higher muscle ornithine decarboxylase levels in modern broilers

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Summary. Little is known about the biochemical correlates of selection for growth in farm or laboratory animals, or the identity of the gene products affected or produced by 'trait-genes'. Modern broiler chickens have about 8-fold greater breast muscle mass than layer chickens at 7 weeks of age and over 2-fold greater breast muscle mass than their 1972 counterparts. This increase in muscle mass is associated with over 20-fold higher levels of ornithine decarboxylase (ODC) in broiler chickens at 1 week of age as compared with layer strain chickens; there is a comparable increase in a relaxed-selection strain of broilers. The increase in ODC levels is larger than the differences in muscle or body weight between broilers and layers at 7 weeks of age, occurs at an age when there is no difference in weights between the strains and precedes the major growth spurt. Increases in ODC levels and hence polyamine synthesis have been associated with, and usually precede, rapid growth and cell proliferation in a wide range of cell types and organisms in response to many different stimuli. Therefore, the correlation of ODC levels with genetic differences in muscle growth make it worth investigating the control of ODC gene expression in these strains.

Key words: Ornithine decarboxylase – Chicken – Muscle – Genetics – Growth differences

Introduction

Most commercially important traits in farm animals exhibit continuous variation, are controlled by many genes and have been improved by the application of quantitative genetic techniques (for review, see Robertson 1980). This approach requires measurements on a large number of animals and enforces the use of simple traits as selection criteria, such as weight-for-age. Commercial broiler chickens demonstrate the overwhelming success in the application of classical animal breeding techniques. Current foundation lines of broilers selected solely for weight-for-age (often referred to as 'male-parent' lines) are now 3½–4-fold the weight of layer strains at 6½ weeks of age and over 2-fold the weight of their 1972 counterparts (Chambers et al. 1981); they differ even more substantially in the amount of breast muscle.

The dissection of these simple selection criteria into their physiological components and the identification of the individual trait-genes and their gene products, are prerequisites not only to a better understanding of the genetic control of commercially important traits (to enable us to develop improved selection protocols; for review, see Bulfield 1980), but also to permit the widespread use of genetic manipulation techniques for improving farm animals (for review, see Bulfield 1985). Although attempts to identify biochemical correlates of selection for increased growth rate have been extensive in both laboratory and farm animals (Bulfield 1980), where differences have been found between selected and control animals when compared on an age basis, these differences have disappeared when the animals were compared on a weight basis (Martin 1974a, b; Bulfield 1980).

It is therefore important that we report that an enzyme associated with DNA synthesis and cell proliferation, ornithine decarboxylase (ODC; EC 4.1.1.17), has over 20-fold higher activity in muscle from a broiler strain of chicken, when compared with a layer strain, at

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Table 1. Ornithine decarboxylase (ODC) activity in 3 tissues of chickens genetically diverging in growth rate

ODC activity[a] in chickens				
Tissue	Week of age	Layer line	Broilers	
			Female line	Male line
Liver	hatch	0.430 ±0.048	0.290 ±0.046	0.381 ±0.043
	3	0.0392±0.0092	0.0280±0.0069	0.0204±0.0031
	7	0.0129±0.0029	0.0240±0.0064	0.0198±0.0030
	10	0.0078±0.0018	0.0328±0.018	0.0275±0.0120
Kidney	hatch	0.0228±0.0014	0.0358±0.0095	0.0246±0.0021
	3	0.0550±0.0074	0.0338±0.0076	0.0042±0.0077
	7	0.0583±0.0059	0.0286±0.0032	0.0656±0.0097
	10	0.0644±0.0046	0.0313±0.0038	0.0630±0.0065
Muscle	hatch	0.0136±0.0033	0.0088±0.0038	0.0098±0.0011
	3	0.0252±0.0030	0.143 ±0.0153	0.112 ±0.033
	7	0.0104±0.0015	0.0376±0.0099	0.0318±0.0069
	10	0.0072±0.0020	0.0117±0.0032	0.0060±0.0023

[a] Enzyme activity is expressed as nmol/min/g wet wt muscle; mean ± SEM of 5 animals. See "Materials and methods" for details of lines of animals

1 week of age. This difference precedes the major growth spurt in these animals and is greater than the difference in body weight or breast muscle weight at 7 weeks of age; the molecular basis of this phenomenon can now be investigated by recombinant DNA techniques.

Materials and methods

Animals

In the initial experiments (Table 1) animals were all females and were from a light-bodied, egg-laying line, Brown Leghorn J line from IAPGR's flock (formerly the Poultry Research Centre) and unselected for growth or, two broiler lines obtained from D.B. Marshall Ltd (Newbridge, Midlothian, UK). The 'male parent' line broilers had been selected for over 30 generations solely for weight-for-age and were 3-fold heavier than the layer at 7 weeks of age. The 'female parent' line broilers had been selected on an index of weight-for-age and fertility and were 2-fold heavier than the layers at 7 weeks of age.

In the remaining experiments, all animals were female and were obtained as day-olds from Ross Breeders Ltd (Newbridge, Midlothian, UK). These were a commercial White Leghorn-type line selected for egg production and unselected for growth (designated line *L* – layer); a commercial broiler 'male parent' line derived from a White Cornish heavy strain mixed population and continuously selected for weight-for-age and for breast muscle development and conformation since 1957 (33 generations; designated line *S* – selected); and, the same broiler line where selection had been relaxed since 1972 (i.e. selected for 18 generations, relaxed for the last 15; designated line *R* – relaxed). At 7 weeks of age ($n=10$) *L* line females were 469 ± 13.6 g body weight and had 24.8 ± 1.2 g of breast muscle, whereas *S* line were 1864 ± 43.2 g and 197 ± 8.12 g, and *R* line were 1180 ± 41.9 g and 103 ± 4.98 g, respectively. Therefore, *L* and *S* lines differed 3.97-fold in body weight and 7.94-fold in breast muscle weight.

Enzyme assays

The chickens were weighed, killed by cervical dislocation and the total breast muscle was dissected and stored at −70 °C until assayed (storage for up to 6 months had no effect on enzyme activity). Muscle was homogenised in 5 × v/w 0.1 M potassium phosphate buffer pH 7.5 using a Kinematica Polytron homogeniser at 20 s and 12 s bursts. The homogenate was centrifuged for 15 min in an MSE Chilspin refrigerated centrifuge at 2,800 *g*; the supernatant was used for the enzyme assay.

Ornithine decarboxylase (ODC) was assayed by the same procedure developed for histidine decarboxylase (Bulfield and Nahum 1978; Martin et al. 1984) in the following reaction mixture: 1 m*M* potassium phosphate buffer pH 7.5, 1 m*M* L-ornithine hydrochloride (containing approximately 16,000 cpm L-[1-^{14}C] ornithine hydrochloride; Amersham), 0.1 m*M* pyridoxal phosphate and 200 µl muscle homogenate in a final volume of 250 µl, at 30 °C. After 2 h the reaction was stopped with 250 µl of 10% TCA and the $^{14}CO_2$ was entrapped in hyamine hydroxide impregnated filter paper, counted and ODC activity expressed as nmol/min/g tissue wt.

Creatine kinase (CK; EC 2.7.3.2) activity was determined as in Sigma Technical Bulletin 46-UV with the reagents adjusted to 1 ml final volume for the LKB Reaction Rate Analyser (Bulfield and Moore 1974). Glycerol-3-phosphate dehydrogenase NAD^+ (GOPDH; EC 1.1.1.8) and pyruvate kinase (PK; EC 2.7.1.40) activities were determined by semi-automated enzyme assays (Bulfield and Moore 1974). Enzyme activities were expressed as µmol/min/g tissue wt; they were also expressed per mg protein (data not shown), which produced profiles identical to those displayed below.

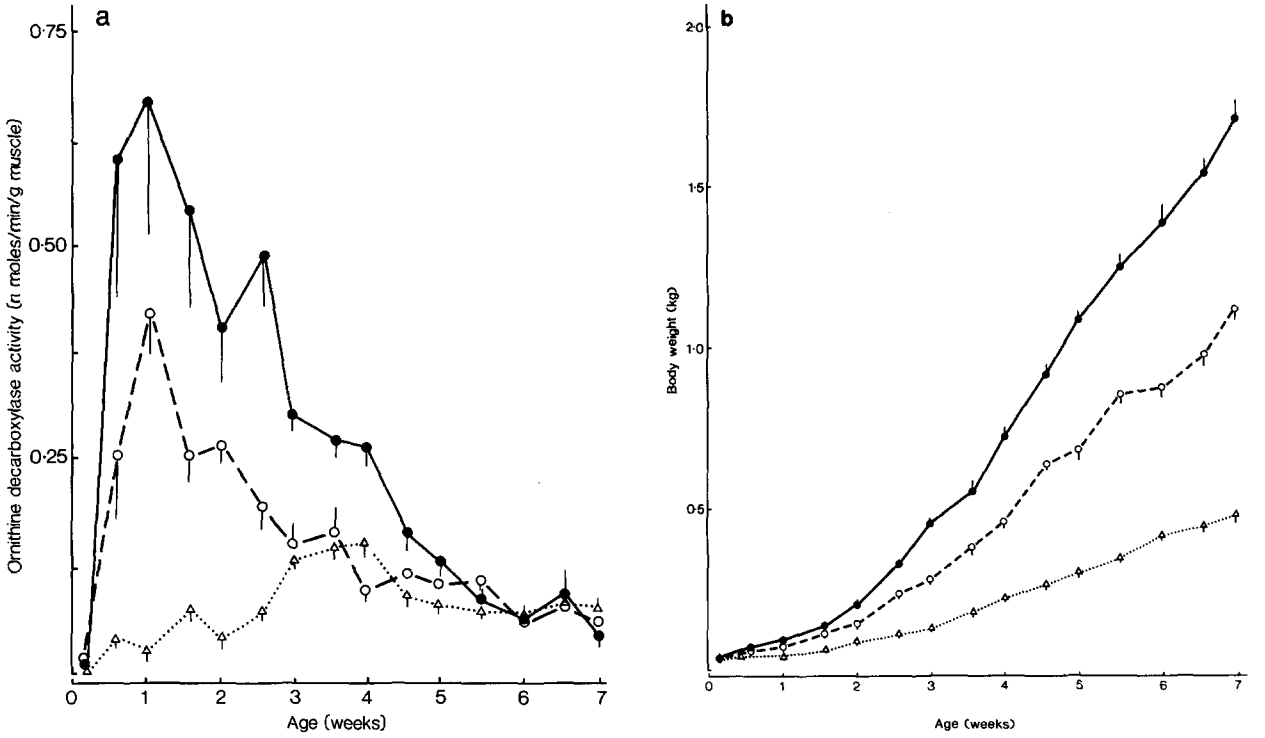

Fig. 1. Muscle ornithine decarboxylase activity (**a**) and body weight from 0–7 weeks of age (**b**), of chicken strains selected and unselected for growth. Each time point represents means ± SEM of 8 females and were from the following strains: (1) a commercial White Leghorn-type line unselected for growth (*line L, dotted lines, open triangles*), (2) a commercial broiler grandparent 'male parent' line (*line S, solid line, filled circles*), (3) the same broiler line where selection has been relaxed (*line R, dashed lines, open circles*)

Results

ODC levels in chicken tissues

The activity of ODC was measured in homogenates of tissues from a broiler line of chickens stringently selected for growth, one less stringently selected and a layer line unselected for growth (Table 1). Liver ODC activities showed a strong developmental profile decreasing 10-fold in the first 3 weeks after hatch; there were, however, no substantial differences between lines. Similarly, there were no line differences in kidney ODC activities; there were, however, large differences between lines in muscle ODC activity. This was particularly noticable at 3 weeks of age where the broiler lines had over 4-fold greater muscle ODC activity. It was therefore decided to construct a more detailed muscle ODC profile up to 7 weeks of age (the age that animals are usually selected for weight), using one selected and two control strains, one unselected for growth (a layer line) and another where selection had been relaxed for several generations.

Muscle ODC activity profile in divergent lines

A detailed development profile of muscle ODC was determined at half-weekly intervals from hatch to 7 weeks of age. Animals were obtained from a line of White Leghorn type selected for egg production but not selected for growth (line *L*), a broiler line selected for growth rate for 33 generations (line *S*) and the same broiler line where selection was carried out for 18 generations, was then relaxed and no further selection was made for the next 15 generations (line *R*). The developmental profile of ODC activity was very different between the selected lines (both *S* and *R*) and the non-selected line (*L*). The activity of muscle ODC in both *S* and *R* lines peaked at between 4 and 7 days after hatch, but not until 24 days after hatch in *L* animals. A more detailed analysis of the early part of the profile in the selected *S* line animals (data not shown) shows that muscle ODC activity gradually increased from hatch to reach a sharp peak at 5.5 days after hatch, after which it fell (as illustrated in Fig. 1 a). The most significant factor about the profiles of muscle ODC in Fig. 1 a is not their shape but the dramatic differences in ODC activity at 4–7 days after

Table 2. Activity profiles of 3 muscle enzymes in chickens genetically diverging in growth rate

Enzyme activities[a]				
Enzyme	Day of age	Layer line (*L*)	Broiler lines	
			Relaxed line (*R*)	Selected line (*S*)
Creatine kinase	hatch	2.26 ±0.01	1.76 ±0.20	2.64 ±0.03
	4	6.01 ±0.72	1.22 ±0.08	10.6 ±0.46
	11	8.07 ±0.49	1.44 ±0.06	7.56 ±0.35
	21	4.43 ±0.16	4.69 ±0.21	5.21 ±0.20
Pyruvate kinase	hatch	0.443±0.062	0.550±0.034	0.545±0.46
	4	0.859±0.050	1.17 ±0.22	1.52 ±0.094
	11	2.64 ±0.19	5.52 ±0.29	4.40 ±0.39
	21	5.55 ±0.25	5.89 ±0.28	7.12 ±0.33
Glycerolphosphate dehydrogenase	hatch	1.05 ±0.21	2.09 ±0.37	2.24 ±0.33
	4	6.73 ±1.12	15.9 ±1.73	19.1 ±1.32
	11	48.9 ±5.13	67.1 ±5.19	84.7 ±6.86
	21	80.9 ±2.16	95.4 ±2.16	99.3 ±1.70

[a] Enzyme activities expressed as mmol/min/g wet wt muscle for CK and PK and μmol/min/g wet wt for GOPDH; mean ± SEM of 8 animals. See "Materials and methods" for details of animals

hatch. At 7 days of age the muscle ODC activity of the selected *S* line birds is 22-fold higher than in the *L* line birds; the *R* (relaxed selection) line also has considerably higher (13.8-fold) muscle ODC than the *L* line. What is significant is that the differences in muscle ODC activities (and presumably the resulting polyamine levels) precede both the major period of active growth and the period where differences in growth between the lines become apparent (Fig. 1b). There are other enzymes that show a strong developmental profile in growing and differentiating muscle in vivo and in vitro; these include creatine kinase, pyruvate kinase and glycerolphosphate dehydrogenase. A developmental profile of the activity of these enzymes would demonstrate whether the between-line differences in ODC activity are specific to this enzyme or are general to enzymes involved in muscle growth and differentiation.

Developmental profiles of other muscle enzymes

The activities of muscle CK, PK and GOPDH were determined across the growth profile of the two broiler lines (fully-selected *S*, and relaxed selection *R*) and the unselected layer, *L* line (Table 2). Although there were some differences between the *R* and *S* broiler lines and the unselected *L* lines these are around two-fold and are transient; all differences were removed in the cases of PK and GOPDH by the time the animals reached their maximum enzyme activities at 21 days of age. There is, however, a difference in CK activity between the two broiler lines *R* and *S*, which had been separated for 15 generations. Although this is an interesting genetic difference, it is presumably not associated with growth rate as the layer *L* line had activities approaching the *S* line rather than the *R* line (Fig. 1a) and had disappeared by 21 days of age.

Discussion

Growth or weight-for-age can be dramatically altered in both farm and laboratory animals by selective breeding. Many attempts have been made to identify biochemical and physiological correlates of the selection process, but they have generally been unsuccessful (Martin et al. 1974a, b; Bulfield 1980). Similarly, little is known about the 'trait-genes' involved or their products (Bulfield 1985). Commercial layer and broiler chickens provide valuable material for such studies, as they have been selected for many generations for different characteristics, i.e. fertility or growth and conformation. This selection has resulted in modern broilers having about 8-fold greater breast muscle mass at 7 weeks of age compared with layer strain animals, and over 2-fold the amount of muscle as broiler animals unselected since 1972.

During embryonic muscle differentiation, myoblasts fuse to form myotubes which form the myofibres. After birth, the multinucleate myofibres elongate, although the DNA (and hence the nuclear) to cell volume ratio stays the same (for review, see Allen et al. 1979). The myofibre nuclei themselves do not divide; this apparent paradox is explained by the existence of satellite cells that are associated with the muscle fibres (Mauro 1961) and proliferate rapidly after birth in mammals (Cardasis and Cooper 1975) and after hatch in birds

(Campion et al. 1982). Satellite cells resemble undifferentiated myoblast cells in their growth in culture and have the ability to differentiate into myotubes in vitro (Allen et al. 1979). The growth of muscle appears to be limited only by the proliferative ability of the satellite cells and their donation of nuclei to the muscle fibres by fusion (Moss and Leblond 1970). The 8-fold greater breast muscle growth of modern broilers will therefore require extra nuclei and hence increased proliferation of satellite cells. It is important that we have shown (Fig. 1b) that broiler muscle has over 20-fold greater muscle ornithine decarboxylase activity at 1 week of age than muscle from layer strain animals. This large difference in ODC activity is greater than the resulting differences in either muscle mass or body weight of the strains at 7 weeks of age, and occurs at a time when the animals are of nearly identical weights before the major growth spurt, although at the period of maximum relative growth rate (Fig. 1a). In view of the almost complete absence of biologically significant biochemical correlates of growth (Bulfield 1980), these differences in ODC levels need to be evaluated in light of their role in growing tissues.

Ornithine decarboxylase (ODC; EC 4.1.1.17) catalyses the first step in polyamine synthesis and is a truly remarkable enzyme. It has a half-life of only 10–15 min and its synthesis is induced by a wide range of hormones (insulin, thyroxine, growth hormone, nerve and epidermal growth factors, vasopressin, androgens, oestrogens etc.) in a wide variety of target tissues (liver, brain, kidney, uterus, oviduct, etc). Furthermore, ODC synthesis is induced before and during rapid cell division in prokaryotes and eukaryotes and in response to known mitogens. This includes rapid growth of regenerating liver, brain and muscle, chicken oviducts, growing embryos and the uterus and various tumours and transformed cell lines (for reviews, see Russell and Snyder 1968; Morris and Fillingame 1974; Russell and Durie 1978; Cannelakis et al. 1979). A variety of mechanisms have been proposed to explain how increased ODC activity and polyamine levels could cause such widespread effects, including initiation of DNA and RNA synthesis, read-through of termination signals, tRNA availability and binding and fidelity of translation (for review, see Tabor and Tabor 1984). Several mechanisms have been proposed to explain the rapid synthesis and degradation of ODC itself, including an ODC-specific protein kinase, polyamine-induced post-translational modification and the formation of an antizyme complex (Tabor and Tabor 1984; Pegg 1986). Therefore, increased ODC levels and polyamine synthesis have been implicated in rapid cell proliferation in development and in response to hormones.

The dramatic difference in muscle ODC levels between chickens of genetically different growth rates and ODC's putative role in rapidly proliferating tissues makes the phenomenon we have discovered worthy of further detailed study. This can now progress in two ways. First, cDNA for the mouse ODC gene has been cloned (McConlogue et al. 1984; Kontula et al. 1984) and can be used to determine how the expression of the chicken gene is regulated in the different strains. Secondly, as ODC responds to a wide variety of hormones (Morris and Fillingame 1974) including growth hormone and thyroxine, its response to these hormones in muscle in vivo and in satellite cells in culture can be investigated. These approaches will provide, for the first time, a real possibility of unravelling the control of a gene implicated in the regulation of muscle cell proliferation in the response to selection for growth.

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