In hypophysectomized rats, there is a marked decrease in the enzyme activity. ACTH only partially corrects this, but insulin *and* ACTH increase the enzyme activity to normal levels. It is suggested that, in the intact rats, insulin and glucagon may exert a stimulatory effect on PNMT activity independent of hypoglycemia or increased glucocorticoid production.

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## Composition of Hypertrophic and Atrophic Muscles in Genetic Muscular Dystrophy of the Chicken* (32677)

D. W. PETERSON, W. H. HAMILTON, AND A. L. LILYBLADE
(Introduced by F. H. Kratzer)
*Department of Poultry Husbandry, University of California, Davis, California 95616*

Hereditary muscular dystrophy in the chicken affects primarily the muscles which normally contain a high proportion of white fibers. The dark or predominantly red muscles are involved to a much lesser degree (1) Similar observations on dystrophic mice have been made by Brust (2) in comparing characteristics of the soleus and gastrocnemius. In dystrophic chickens the white pectoralis muscle has been most studied because of size, accessibility to biopsy, and invariable involvement in dystrophic processes. Hypertrophy of the pectoralis is observed in all dystrophic chickens at an early age. In some birds this is followed by gross atrophy with accompanying fatty infiltration while in others hypertrophy may persist to an advanced age. Asmundson has selected lines of dystrophic chickens which exhibit either early atrophy (line 307) or persistent hypertrophy (lines 304 and 308) (3).

Dystrophic pectoralis muscle when compared with normal pectoralis contains considerably more fat, which may obscure the true relationships of the tissue components if they are merely estimated on a wet-weight basis. It has long been known that in the development of the embryo the water content of the tissues gradually decreases and the nitrogen content gradually increases with age. After birth or hatching this process continues until at the end of the juvenile phase of growth the total body and the muscles reach an ap-

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proximately constant content of water and of nitrogen when these are calculated on the fat-free basis. Since fat is the most variable component of the tissues the constancy of composition of individual organs or tissues is frequently not apparent unless the correction for fat is made (4–7). In the chicken different groups of muscles are found to have different and characteristic values for nitrogen and water content (fat-free) (6).

In this study the fat-free content of several nitrogen fractions and of water of hypertophic and atrophic dystrophic pectoralis muscles was compared with that of normal pertoralis muscles and also with that of chicken muscles which are predominantly red. The latter comparison was made because some investigators have proposed that muscular dystrophy may represent a failure of the muscle to develop beyond the immature state (2,8). Red or slow muscle is considered to be at a more immature or undifferentiated level than white or fast muscle (2,9).

*Methods.* Birds were killed by disarticulation of the cervical vertebrae. Muscles were dissected out, weighed, and immediately frozen on solid carbon dioxide. The entire frozen muscle was homogenized in a Servall omnimixer and samples taken for analysis. The homogenate was weighed and refrozen, and where resampling was necessary the subsequent data was corrected for water loss during storage. Water content was determined by drying for 48 hours at 65°C in a vacuum oven. Total lipid was determined on the dried sample by extraction for 16 hours with a mixture of 70 parts petroleum ether (bp 30–60°C) and 30 parts absolute methanol in a Goldfisch apparatus. Nitrogen fractions were determined as follows: total N by Kjeldahl method, nonprotein N as the nitrogen soluble in 5% trichloracetic acid, noncollagen protein N as the difference between nonprotein N, and N soluble in 0.1% NaOH by the Lowry (10) procedure. Collagen was estimated by the procedure of Lowry *et al.* (10). Total hydroxyproline and collagen hydroxyproline were determined by the method of Bergman and Loxley (11). Free amino acids were estimated as previously described (12).

*Results.* Ratios of atrophic, hypertrophic, and normal pectoralis muscle weights to carcass weights and the composition of these muscles are shown in Table I. The relatively high levels of collagen and lipid are characteristic of an atrophic muscle. When these data are corrected to a fat-free basis (Table II), it is seen that the compositions of atrophic and hypertrophic muscles appear to be identical, except for collagen content. The dystrophic muscles differ significantly from the normal muscle in total nitrogen, nonprotein nitrogen, protein nitrogen, noncollagen protein nitrogen and water. No correction has been made here for collagen nitrogen because of its small overall effect.

The fat-free composition of lateral adductor muscle, a red muscle, from juvenile and adult chickens is shown in Table III. It will be apparent that dystrophic pectoralis muscle more nearly resembles the lateral adductor, especially that of the juvenile, in composition (high water content, low nitrogen content) than it does the normal pectoralis muscle (Table II).

The data of Harshaw (13) based on individual analyses of the tissues from 100 normal male chickens has been recalculated in Table IV to a fat-free basis. It will be noted that dystrophic pectoral muscle resembles more nearly in water and nitrogen content the composite of predominantly red leg muscles than it does that of the normal white breast muscle.

Free amino acid content of dystrophic pectoralis muscle in comparison with normal leg muscle and with normal pectoralis is shown in Table V. The dystrophic muscle in this comparison is from line 301, the unselected parent line from which the hypertrophic and atrophic lines previously discussed were selected. No correction for fat has been made on these data since the differences among groups of muscles are so great that such a correction would produce only a small effect. The free amino acid composition of dystrophic pectoralis muscle is intermediate between the characteristic pattern for normal pectoralis muscle and that for the leg muscles. It can be seen that the free amino acids which have been previously reported as peculiarly characteristic of dystrophic pectoralis muscle, such as taurine, serine ethanolamine phosphate and

TABLE I. Wet Weight Composition (Uncorrected) of Normal and Dystrophic Pectoralis Muscles.[a]

| Line | No. of birds | Av. carcass wt.[b] (gm) | Av. muscle wt. (gm) | $\frac{\text{Muscle}}{\text{Carcass}} \times 100$ | (%) $H_2O$ | (%) Total N | NPN (%) | Noncollagen protein N (%) | Collagen (%) | Total lipid (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 200 Control | 5 | 2405 | 114 | 4.76 ± .14[c] | 73.3 | 4.00 | 0.64 | 3.06 | 0.77 | 2.80 ± 0.11[c] |
| 307 Atrophic | 5 | 1783 | 59 | 3.27 ± .28<br>vs. 200 $p$ <.01<br>vs. 308 $p$ <.001 | 69.8 | 2.97 | 0.38 | 2.22 | 1.53 | 12.9 ± 1.1<br>vs. 200 $p$ <.001<br>vs. 308 $p$ <.01 |
| 308 Hypertrophic | 5 | 2000 | 143 | 7.10 ± .27<br>vs. 200 $p$ <.01<br>vs. 307 $p$ <.001 | 76.6 | 3.19 | 0.44 | 2.57 | 0.96 | 4.22 ± 0.44<br>vs. 200 $p$ <.05<br>vs. 307 $p$ <.01 |

[a] All birds 19-week-old males. Muscle weights are for right pectoralis m.
[b] Carcass weight = body weight less pectoral muscles and viscera.
[c] Mean and standard error.

TABLE II. Fat-Free Composition of Pectoralis Muscles.[a]

| Line | No. birds | Total N (%) | NPN (%) | Protein N (%) | NCPN[b] (%) | NPN/NCPN | $H_2O$ (%) | Collagen (%) | Ho proline[c] (%) |
|---|---|---|---|---|---|---|---|---|---|
| 200 Normal | 5 | 4.11 ± .06 | 0.66 ± .01 | 3.45 ± .07 | 3.14 ± .07 | 0.21 ± .006 | 75.4 ± .3 | 0.79 ± .06 | 0.14 ± .004 |
| 307 Atrophic | 5 | 3.41 ± .07 | 0.44 ± .06 | 2.97 ± .06 | 2.55 ± .07 | 0.17 ± .01 | 80.1 ± .3 | 1.77 ± .12 | 0.32 ± .02 |
| $p$ level | vs. normal | .01 | .01 | .01 | .01 | .05 | .001 | .01 | .01 |
| | vs. 308 | NS | NS | NS | NS | NS | NS | .01 | .01 |
| 308 Hypertrophic | 5 | 3.34 ± .03 | 0.45 ± .01 | 2.89 ± .03 | 2.68 ± .02 | 0.17 ± .004 | 79.9 ± .3 | 1.00 ± .04 | 0.20 ± .01 |
| $p$ level | vs. normal | .001 | .01 | .01 | .01 | .01 | .001 | .05 | 0.01 |
| | vs. 307 | NS | NS | NS | NS | NS | NS | .01 | 0.01 |

[a] Mean value ± standard error. NS = not significant. [b] Noncollagen protein nitrogen. [c] Ca. 75% of Ho proline combined in collagen.

TABLE III. Composition of Lateral Abductor Muscles of Normal Chickens.

| Age | Strain | Fat (%) | Fat-free composition | | | | |
|---|---|---|---|---|---|---|---|
| | | | Total N (%) | NPN (%) | Prot N (%) | NCPN (%) | $H_2O$ (%) |
| 5 weeks[a] | Normal New Hampshire | 4.89 | 3.39 | 0.40 | 2.99 | 2.89 | 81.7 |
| 7 weeks[b] | Normal Arbor Acres | 4.14 | 3.25 | 0.38 | 2.88 | 2.84 | 80.9 |
| 1 year[c] | Normal New Hampshire | 4.75 ± 0.69 (3.85–6.11) | 3.56 ± .03 | 0.37 ± .001 | 3.19 ± .03 | 3.07 ± .03 | 77.5 ± 0.3 |

[a] Composite of lateral abductor muscles from five male chickens.
[b] Composite of lateral abductor muscles from three male chickens.
[c] Mean values ± standard errors, individual values for three male birds.

glutathione (12) occur normally at high levels in leg muscle. Those components which are characteristic of normal pectoralis muscle, such as carnosine and anserine, are greatly diminished in dystrophic breast muscle. They are also found in low concentrations in normal leg muscle.

*Discussion.* The distribution of nitrogen fractions and water in dystrophic pectoralis muscle is very similar to that reported by Dickerson (14) for immature pectoralis muscle during early growth of the chick. In Dickerson's data the high percentages of water and the low percentages of protein nitrogen in the immature pectoralis were the resultant of high levels of extra cellular water and low concentrations of fibrillar and sarcoplasmic proteins. Dystrophic pectoralis also contains similarly low levels of these proteins (15, 16).

Progressively decreasing ratios of DNA to total protein occur during growth of juvenile avian muscle (17, 8). Dystrophic pectoralis also is found to have greater than normal or mature levels of DNA (18, 19). It is also similar to juvenile muscle in its greater than normal rate of protein synthesis. (8, 19).

Similarities of dystrophic pectoralis muscle composition to that of red muscle have been pointed out in the data presented above. Resemblances of dystrophic pectoralis to red muscle are also to be found in the data of Cardinet (20). Dystrophic pectoral muscle exhibited low activities of glycolytic enzymes

TABLE IV. Fat-Free Composition of Groups of Chicken Tissues.[a]

| | Mean % and range of fat content | % Total nitrogen | % Water | $p$ Level | | | |
|---|---|---|---|---|---|---|---|
| | | | | vs. leg | | vs. breast | |
| | | | | N | $H_2O$ | N | $H_2O$ |
| Breast muscle | 0.59 (.31–.90) | 3.85 ± .02 | 74.8 ± .1 | .001 | .001 | — | — |
| Leg muscles | 2.89 (1.55–4.16) | 3.40 ± .02 | 77.4 ± .1 | — | — | .001 | .001 |
| Remaining edible[b] | 17.0 (5.9–29.6) | 3.57 ± .04 | 75.8 ± .2 | .01 | .001 | .001 | .001 |

[a] Recalculated to fat-free basis from data of Harshaw (13). Each value is based on eight mean values from individual analyses of 100 birds (male), 10–20 birds in each group sampled at ages of 8, 12, 16, and 20 weeks.
[b] Includes remaining muscle and skin, plus gizzard, heart, and liver.

TABLE V. Free Amino Acids of Normal Leg and Breast Muscles and Dystrophic Breast Muscle[a] (Values Are in mg/100 gm Wet Tissue ± Standard Error—Not Corrected for Fat).

| | Normal leg muscles[b] | Dystrophic pectoralis[c] | Normal pectoralis[d] |
|---|---|---|---|
| Alanine | 19 ± 2 | 19 ± 2 | 8 ± 1 |
| *B*-alanine | 6 ± 1 | 9 ± 2 | 3 ± 1 |
| Anserine | 184 ± 8 | 255 ± 40 | 864 ± 43 |
| Arginine | 11 ± 1 | ND[f] | ND |
| Asparagine | 10 ± 2 | ND | ND |
| Aspartic acid | 14 ± 3 | 2 ± 0.1 | 1 ± 0.2 |
| Carnosine | 30 ± 5 | 140 ± 55 | 348 ± 22 |
| Glutamic acid | 23 ± 2 | 17 ± 2 | 12 ± 2 |
| Glutamine | 74 ± 9 | 21 ± 5 | 10 ± 3 |
| Glutathione | 34 ± 2 | 19 ± 4 | 9 ± 0.4 |
| Glycine | 18 ± 2 | 7 ± 2 | 3 ± 0.4 |
| Histidine | 0 | ND | 0 |
| Hydroxyproline | 7 ± 3 | 1.5 ± .03 | 0.5 ± .05 |
| Leucines | 8 ± 1 | 5 ± .03 | 4 ± .3 |
| Lysine | 7 ± 1 | ND | ND |
| Methionine | 1.4 ± 2 | 0.17 ± .002 | 0.1 ± .004 |
| Proline | 11 ± 1 | 6 ± 1 | 3 ± .2 |
| Serine | 23 ± 2 | 8 ± 1 | 5 ± .6 |
| SEP[e] | 55 ± 7 | 17 ± 7 | trace |
| Taurine | 194 ± 2 | 74 ± 17 | 7 ± 3 |
| Threonine | 13 ± 1 | 6 ± 1 | 7 ± 2 |
| Valine | 5 ± 1 | 4 ± 0 | 3 ± 0.5 |

[a] Adult females from 1 to 3 years of age.
[b] Mean values for four birds (fat not determined).
[c] Mean values for three birds (line 301 unselected dystrophic) mean fat content 10.9%.
[d] Mean values for six birds (mean fat content of five birds was 1.5%).
[e] Serine-ethanolamine phosphate.
[f] ND = not determined.

and high activities of oxidative enzymes in the same manner as normal red (lateral adductor) muscle. Normal pectoral muscle, in contrast, showed high levels of glycolytic activity and low levels of oxidative activity. The low glycolytic activity in dystrophic muscle appeared to be due to a failure of development to the high levels found in the normal muscle.

These data from various sources concerning chemical components of dystrophic muscle provide further evidence in favor of the concept held by some investigators that the physiological properties of dystrophic muscle are like those of red or slow muscle which represents a more immature state of development than white or fast muscle (2, 9).

In Tables I and II it is seen that components representing largely extracellular material, namely collagen and fat, occur at higher levels in dystrophic than in normal pectoral muscle. Again, a resemblance to the juvenile phase of growth is suggested since during early growth of normal pectoralis collagen develops more rapidly than the sarcoplasmic and fibrillar proteins (14, 21). This may be the situation occurring in hypertrophic muscle. The atrophic muscle, which contains even higher percentage levels of collagen than the hypertrophic muscle may be, in addition, undergoing catabolism of the intracellular proteins at a greater rate than of the connective tissue which would account as well for its higher level of collagen as the hypothesis of fibrosis. Total collagen of the atrophic muscle as a ratio to carcass weight is lower than this ratio in the hypertrophic muscle.

Fat content of hypertrophic muscles is similar to that of normal red muscle (Tables I and III) but in the atrophic muscle fat levels are much higher. The composition of the increased fat is similar to that of adipose tissue, the principal component being triglyceride (22). Infiltration of muscle tissue by fat is common in atrophic processes (23, 24) and suggests replacement of lost muscle tissue by fat, but the mechanism for such a process has not been explained.

In agreement with the suggestion of Weinstock (8) much data thus far accumulated indicates that it may be profitable to consider the primary process in dystrophic muscle as a state of arrested development at a juvenile stage. Gross muscle atrophy, at least in the chicken, is a process subsequent to hypertrophy of this juvenile type muscle and is quite variable chronologically, since hypertrophy may persist to advanced ages (1).

*Summary.* Analysis of hypertrophic and atrophic pectoral muscles of chickens with hereditary muscular dystrophy for total nitrogen, nonprotein nitrogen, noncollagen protein nitrogen, and collagen indicates that when considered on the fat-free basis the two types of muscle do not differ in composition, except for collagen content. Dystrophic muscle differs in all these components from normal pectoral muscle. The composition of dystrophic muscle suggests that it is a juvenile type of muscle which fails to develop to the normal mature state.

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