Lipid Composition of the Pectoral Muscles of Chickens with Inherited Muscular Dystrophy.* (29214)

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Asmundson and Julian(1,2) reported the presence of a recessive autosomal gene (am) in New Hampshire chickens which produces in the homozygous bird a condition quite similar to certain forms of muscular dystrophy in man(3). The superficial pectoral muscles of such adult birds contained considerably more fat than those of normal birds (4,5). Elevated fat content has also been found in certain muscles of mice with muscular dystrophy by Shull and Alfin-Slater(6) and in some types of muscular dystrophy in man(3).

This investigation was undertaken to determine the nature of the fat deposited intramuscularly in chickens with inherited muscular dystrophy. An increase in the triglyceride fraction and a decrease in the phospholipid fraction relative to other lipids constitute the major differences in the dystrophic muscle when compared with the normal muscle.

Methods. Seven pairs of 36-week-old normal and muscular dystrophic female New Hampshire birds were used in the study. The animals were sacrificed and the left superficial pectoral muscles were excised, quick-frozen in a dry ice-acetone bath and stored at —20°C. They were thawed and used as needed.

A 0.5-0.8 sample g of wet muscle was placed in a vacuum oven at 70° for 12 hours to determine the moisture content by weight difference. Protein was determined by the Kjeldahl method (N × 6.25) and hydroxyproline was determined by the technique of Neuman and Logan(7). The hydroxyproline content was used as a measure of collagen or connective tissue.

The fat was extracted from the muscle by the $CHCl_3$:CH_3OH:H_2O method of Bligh and Dyer(8). The $CHCl_3$ layer containing the lipid, was evaporated in a 60°C water bath under a stream of N_2. Approximately 70 mg of this fat was chromatographed on a 3.0 g silicic acid-Hiflo Supercell column (70:30 w/w), and eluted by the scheme of Barron and Hanahan(9). Nitrogen was used to maintain a flow rate of 2 ml/min. All the phospholipids were eluted together with 100 ml of methanol:diethyl ether:chloroform (80:21:10 v/v). Each of the other fractions was eluted with 50 ml of an appropriate solvent mixture.

Triglycerides were separated from unesterified fatty acids on an IRA-400 ion exchange column as outlined by Hornstein *et al*(10). The triglycerides were eluted with n-hexane, while the unesterified fatty acids remained on the column.

Aliquots of the triglyceride-unesterified fatty acid fraction from each of the silicic acid columns were saponified by the procedure of Abraham *et al*(11), acidified to pH 1 with 6 N HCl and extracted twice with equal volumes of n-hexane. The hexane was evaporated to dryness in a water bath at 60°C under a stream of N_2. The methyl esters were prepared by the method of Metcalfe and Schmitz(12) and the esters were chromatographed on a 5 ft × ¼ in. stainless steel column packed with 10% diethyleneglycolsuccinate on acid washed chromosorb W (Wilkins Instrument and Research Corp.) at 185°C. Helium was used as a carrier gas (75 ml/min) with a four-filament katharometer (Aerograph A-350-B) as the detector. Identification of the individual esters was made by comparison of retention volumes of the recorded esters with those of known standard fatty acid methyl esters. Esters of fatty acids with a greater retention time than the 18:3 acid were not measured.

Results. The weight of the superficial pectoral muscles of the dystrophic birds was less than that of the normal birds, both in actual weight and percent of body weight (Table I). This indicates that, in agreement with

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TABLE I. Weight and Composition of Superficial Pectoral Muscle.

Constituent	Normal	Dystrophic
Body wt (kg)	2.99 ± .086*	2.27 ± .12
Wt of muscle (g)	120.4 ±4.7	69.7 ±10.5
Idem (% of body wt)	4.0 ± .093	3.0 ± .38
Composition (% of whole muscle)		
Water	72.20 ± .017	49.8 ± 4.2
Fat	2.40 ± .022	37.3 ± 5.6
Protein	24.6 ± .68	13.0 ± 1.3
Hydroxyproline	.12 ± .028	.19 ± .026

* Mean ± standard error of mean.

previous observations(2), the dystrophic muscle in these birds of 36 weeks of age was in an atrophy stage. The reduced body weight in the dystrophic birds beyond that caused by the smaller superficial pectoral muscle may be due to the strain difference.

The superficial pectoral muscle of the dystrophic hens showed a 15-fold increase in lipid (Table I) over the control birds with a reduction in water and protein. Despite the decrease in protein, the hydroxyproline content (in grams) of the dystrophic muscle is quite comparable to that found in normal muscle, but when expressed as a percent of the whole muscle, it is slightly increased.

With a 15-fold difference between the lipid content of dystrophic and normal muscle, and a 2-fold difference in muscle size, it is difficult to select a baseline for comparing the various lipid constituents. As a consequence, the results are expressed in 3 different ways (Table II); percent of whole muscle, percent of fat, and grams per 16 g of nitrogen.

All of the lipid components were found in greater amounts (% of whole muscle) in dystrophic than in normal muscle. However, the phospholipid fraction increased only about 2-fold, considerably less than the other constituents. When expressed as % of fat, no significant differences were noted in any of the constituents—except in the triglyceride and phospholipid fractions. The triglycerides increased from 54.5% to 85.1% and the phospholipids decreased from 34.8% to 5.13%.

When compared in relation to nitrogen in the muscle, all of the lipid constituents increased in the dystrophic birds. The hydrocarbon-like compounds and the triglycerides increased more in the dystrophic muscle than did other constituents, measuring more than 50 times the normal. The sterol esters, unesterified fatty acids, free sterol and mono- and diglycerides were approximately 25 to 35 times normal while the phospholipids were only 4.1 times the normal level in relation to nitrogen.

The fatty acid composition of the triglyceride and non-esterified fatty acid fraction was very similar in both the normal and dystrophic tissues (Table III). The relative amounts of the various fatty acids were similar to values reported for chicken breast muscle by Marion and Woodroof(13), but were slightly lower in unsaturated fatty acids. This may be due to the inclusion of phospholipid fatty acids which are higher in the unsaturated fatty acids in the analysis by Marion and Woodroof whereas our values are only for the triglyceride and non-esterified fatty acid fraction.

Discussion. Perhaps the most significant observation in this study is that the phospholipid and hydroxyproline concentrations were only slightly different from the normal animal as a percent of the whole muscle. When expressed in absolute terms as grams per muscle, the normal superficial pectoral muscle contained 1.0 g of phospholipid while the dystrophic muscle had 1.2 g. Similarly, the hydroxyproline content of the normal muscle was 0.13 g while the dystrophic one contained 0.14 g. Perhaps this can be considered as evidence that the dystrophic condition does not involve a decrease in number of cells but only an alteration in the composition and possibly in size of the cells. Further, it seems that the control mechanism involving the

TABLE II. Composition of the Lipids of Superficial Pectoral Muscle.

Constituent	% of whole muscle		% of fat		Gram per 16 gram nitrogen		Dystrophic
	Normal	Dystrophic	Normal	Dystrophic	Normal	Dystrophic	Normal
Hydrocarbon-like compounds	.02 ± .007*	.48 ± .16	.96 ± .34	1.10 ± .26	.082 ± .028	4.86 ± 2.26	59.4
Sterol esters	.014 ± .008	.256 ± .13	.50 ± .24	.65 ± .30	.064 ± .037	2.17 ± 1.05	34.0
Unesterified fatty acids	.03 ± .009	.337 ± .12	1.28 ± .42	.90 ± .28	.12 ± .034	3.03 ± 1.22	25.2
Triglycerides	1.30 ± .13	32.3 ± 5.4	54.5 ± 4.3	85.1 ± 1.7	5.34 ± .63	297.9 ± 87	56.0
Free sterols	.10 ± .01	1.28 ± .48	4.34 ± .40	4.03 ± 1.9	.42 ± .047	10.0 ± 3.08	23.8
Mono- & diglycerides	.05 ± .012	.72 ± .20	1.87 ± .31	1.87 ± .37	.203 ± .053	6.53 ± 2.35	32.2
Total phospholipids	.84 ± .14	1.71 ± .22	34.8 ± 4.9	5.13 ± 1.1	3.51 ± 1.02	14.3 ± 2.8	4.1

* Mean ± standard error of mean.

TABLE III. Fatty Acids of Triglycerides and Non-esterified Fatty Acids of Superficial Pectoral Muscle.

Fatty acid	% of triglyceride and non-esterified fatty acid fraction	
	Normal	Dystrophic
12:0	.1 ± .035*	.1 ± .03
14:0	.8 ± .23	1.0 ± .27
14:1(?)	2.4 ± .47	.7 ± .77
16:0	30.3 ± 2.4	29.0 ± 3.5
16:1	3.7 ± .88	4.4 ± 1.3
18:0	4.6 ± .85	4.7 ± 1.0
18:1	30.9 ± 3.1	31.7 ± 5.3
18:2	12.4 ± 2.3	16.6 ± 1.9
18:3	.6 ± .74	.9 ± .7

* Mean ± standard error of mean.

phospholipids is much more sensitive than those involved with other lipid fractions.

The major increase in the lipid fraction is in the triglyceride fraction. Analysis of the fatty acids in this fraction by gas-liquid chromatography showed that the fatty acids were present in essentially the same relative proportions in both normal and dystrophic animals. It is interesting to speculate that a considerable amount of the amino acids formed in the catabolism of muscle protein may have been metabolized to acetyl-CoA and stored in the form of triglycerides, similar to adipose tissue.

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In vitro Response of Intestinal Segments of Mice with Hereditary Muscular Dystrophy to Various Pharmacologic Agents.* (29215)

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It has been observed in our laboratory that mice with hereditary muscular dystrophy (strain 129/J, genotype dy/dy) have a higher incidence of constipation and fecal impactions than do heterozygous (Dy/dy) or wild-type (Dy/Dy) animals of the same strain. Review of the clinical and experimental literature reveals little information regarding the status of the gastrointestinal tract both in patients with muscular dystrophy and in mice similarly affected. Patterson and Rios (1) reviewed the clinical literature and added a summary of their own observations on a group of patients with pseudohypertrophic dystrophy. In many of the cases in which gastrointestinal disturbances were present, diarrhea and vomiting are the predominant complaints, but constipation and intestinal atony were also frequently reported. To our knowledge, no systematic study of the autonomic nervous function of the gastrointestinal tract has been conducted, either in dystrophy patients or in affected experimental animals. In this communication are presented the results of an *in vitro* study of the responses to various autonomic drugs of selected segments of the gastrointestinal tract of mice with hereditary muscular dystrophy.

Materials and methods. The experimental animals were female mice of 3 genotypes: 1) homozygous dystrophic (dy/dy), 2) heterozygous (Dy/dy) and 3) wild-type (Dy/Dy) controls of the same strain. They had been weaned at 4 weeks of age and were subsequently maintained on Old Guilford Mouse Pellets and tap water *ad libitum*.

Between 8 and 12 animals of each genotype were sacrificed at 4, 12, and 20 weeks of age. At time of sacrifice mice were anesthetized with ether and the intestinal tract was rapidly dissected out and immersed in Tyrode's solution. Three centimeter sections of the proximal portions of the duodenum, jejunum, ileum, and large bowel were removed and were suspended in separate tissue baths containing 9.8 ml of Tyrode's and 0.2 ml of drug solution. Temperature of the baths was maintained at 35 ± 2°C and continual oxygenation was insured by bubbling air through the solution. The tissue was attached by fine sutures to a simple lever and kymograph system with a magnification of 20:1 and a resulting tension on the tissue of approximately 1 g.

All drugs were diluted in distilled water, with the exception of norepinephrine which was prepared in 0.01 N HCl. Drugs were injected into the tissue baths in volumes of 0.2 ml, bringing the total volume of the baths to 10.0 ml. Agents employed to assess the activity of the parasympathetic system included: 1) acetylcholine, in doses of 0.2, 1.0, and 5.0 μg (concentrations of 0.02, 0.10, and 0.50 μg/ml), 2) acetylcholine in the presence of 2.0 mg of hexamethonium, and 3) atropine sulfate in doses of 0.5 and 2.5 μg (concentrations of 0.05 and 0.25 μg/ml). The sympathetic system was tested with: 1) norepinephrine, 0.2 and 2.0 μg (0.02 and 0.20 μg/ml), and 2) guanethidine, 2.0 and 5.0 μg (0.2 and 0.5 μg/ml). In addition, the action of several substances on the muscle itself was determined: 1) barium chloride in doses

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