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Changes in the Phospholipid Composition of Microsomal Membranes of Dystrophic Hamsters (40741)

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Through autosomal recessive inheritance, Syrian hamsters of the BIO 82.62 strain, derived from randomly bred stock, suffer from a myopathy that affects both the skeletal muscle and heart (1, 2). Although these animals generally die from congestive heart failure, there is evidence that suggests that the same defect is responsible for both the heart and skeletal muscle lesions (2).

In previous studies, we reported alterations in lipid metabolism that affect processes occurring in the cytoplasm, the endoplasmic reticulum, and the mitochondria of the dystrophic hamster. Fatty acid synthesis was depressed in the liver and adipose tissue as a result of a decrease in the activities of several enzymes involved in lipogenesis (3). Evidence of alteration in microsomal function included a depression in the rate of incorporation of [^{14}C]glycerol-3-phosphate (G3P) into the glycerides of heart, muscle, and adipose tissue with no change in the rate of incorporation of G3P in the liver (4), as well as changes in the fatty acid composition of the lipids isolated from these tissues (4, 5). More recently, we reported a decrease in the activity of stearoyl-CoA desaturase in the liver and adipose tissue of the diseased animals (6). These differences are apparent in the diseased animals regardless of their age or the severity of the disease.

Evidence of changes in mitochondrial function was derived from studies of fatty acid oxidation by heart and muscle homogenates. We reported (7) a decrease in oleate oxidation by heart homogenates of 180-day-old dystrophic hamsters, a result which was in agreement with the findings of Kako *et al.* (8) and Kelley (9). Oleate oxidation was depressed in muscle of both 35 (unpublished) and 180-day-old hamsters (7), possibly as a result of decreased activity of acyl carnitine transferase in the diseased animals. Additional evidence of alterations in mitochondrial function includes changes in amino acid oxidation (10), oxidative phosphorylation (11), Ca^{2+} transport (12), and electron transport (13).

From these findings, we reasoned that the changes that were observed in the membrane-associated processes may have been caused by alterations in membrane structure. Since phospholipids are a basic constituent of membranes, and because several processes associated with the endoplasmic reticulum of liver and adipose tissue were altered, we elected to investigate possible changes in the lipid component of microsomes. The studies that are reported in this communication include quantification of the phospholipid content and determination of the fatty acid composition of the lipids isolated from the microsomes of liver and adipose tissue of dystrophic and normal hamsters.

Methods. Animals. Both dystrophic (BIO 82.62) and randomly bred female hamsters were obtained from Trenton Experimental Laboratory Animal Company (Bar Harbor, Maine) when they were approximately 25 days old, and were kept in our facilities under identical conditions until used. All animals were allowed free access to water and food. At an average age of 35 days, at least six animals from each group were sacrificed by decapitation and the livers and adipose tissue were excised and suspended in ice-cold 1.15% KCl.

Because of their small size, fat pads from three animals were pooled, but individual livers were used. Livers were homogenized in a motor-driven glass–Teflon Potter–Elvehjem homogenizer in isotonic KCl at 4°C. The motor speed was approximately 1000 rpm and homogenization was complete after five strokes. Adipose tissue was homogenized in a hand-held glass–glass

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homogenizer, in isotonic KCl at 4°C. Homogenization was complete after 10 strokes. The homogenates were centrifuged at 15,000 *g* for 25 min. The supernatant fraction was centrifuged at 102,000 *g* for 90 min and the pellets (microsomal fractions) were retained. The microsomes isolated from livers were resuspended in a volume of isotonic KCl equivalent to the weight of the liver. Adipose tissue microsomes were suspended in a volume of KCl equivalent to one tenth the weight of the pooled fat pads because of low microsomal yields.

Lipids were extracted from microsomes by the method of Folch *et al.* (14). Individual phospholipid classes were separated on thin-layer chromatography plates as described earlier (5). The phospholipid content of the microsomal lipid extract and the individual phospholipid fractions were analyzed according to the method of Bartlett (15). Determination of the fatty acid composition of the lipid extract was performed as previously reported (5).

Results. The phosphorous content of the microsomal lipid extract, sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylethanolamine (PE) from livers and adipose tissue of randomly bred (RB) and dystrophic hamsters (BIO) are shown in Table I. The total phospholipid, PC, and PE concentrations were significantly elevated in the livers of the diseased animals, but no significant changes were observed in adipose tissue.

Analysis of the fatty acid composition of liver microsomal lipid extract (Table II) shows a significant decrease in the relative concentrations of palmitic (16:0), palmitoleic (16:1) and oleic (18:1) acid and a concomitant increase in stearic (18:0), linoleic (18:2), linolenic (18:3), arachidonic (20:4), and docosahexanoic (22:6) acid concentrations. Although a similar pattern of change was seen in the fatty acid composition of adipose tissue microsomal extract (Table II), significant changes were only observed in two fatty acids. The concentrations of palmitoleic and oleic acid were decreased in the microsomes of the diseased animals.

In Table III are shown the ratios of saturated/monounsaturated fatty acids and the ratios of saturated/unsaturated fatty acids of the microsomal extract of liver and adipose tissue. The ratio of saturated/monounsaturated fatty acids of both liver and adipose tissue microsomes of the diseased animals was significantly higher than that of the normal controls. However, the ratio of saturated to unsaturated fatty acids of microsomal lipids was significantly lower in liver of the dystrophic hamsters, but was not different from controls in adipose tissue.

Discussion. The endoplasmic reticulum is the site of several major processes of lipid metabolism. Alterations in the structural composition of these membranes may affect their proper function and cause aberrations in lipid metabolism in the whole organism. In previous studies, we observed that several processes associated with the

TABLE I. THE PHOSPHOROUS CONTENT OF LIVER AND ADIPOSE TISSUE MICROSOMAL LIPID EXTRACT (TOTAL PL), SPHINGOMYELIN (SM), PHOSPHATIDYLCHOLINE (PC), PHOSPHATIDYLSERINE (PS), AND PHOSPHATIDYLETHANOLAMINE (PE) OF RANDOMLY BRED (RB) AND DYSTROPHIC HAMSTERS (BIO)

	μg P/g tissue				
	Total PL	SM	PC	PS	PE
Liver					
RB	239 ± 8.0[a]	17.9 ± 2.6	89.1 ± 3.9	28.7 ± 1.6	40.5 ± 3.6
BIO	292 ± 15*	16.6 ± 1.6	122 ± 7.5*	36.5 ± 3.3	52.2 ± 2.9*
Adipose					
RB	121 ± 6.2[b]	14.7 ± 2.9	48.9 ± 0.3	14.4 ± 0.3	30.7 ± 8.5
BIO	132 ± 5.9	23.5 ± 9.1	47.3 ± 7.2	18.6 ± 3.6	27.4 ± 5.9

[a] Values are of $\bar{X}$ ± SEM of at least six samples.
[b] Values are of $\bar{X}$ ± SEM of three samples. Each sample is composed of microsomes isolated from fat pads of three animals.
*Denotes statistically significant differences, $P < 0.05$.

TABLE II. RELATIVE PERCENTAGE COMPOSITION OF MAJOR FATTY ACIDS OF LIVER AND ADIPOSE TISSUE MICROSOMES OF RANDOMLY BRED (RB) AND BIO 82.62 DYSTROPHIC (BIO) HAMSTERS

	16:0	16:1	18:0	18:1	18:2	18:3	20:4	22:6
Liver								
RB	23.4 ± .3″	3.8 ± .2	9.8 ± .4	17.1 ± .6	16.4 ± .4	1.1 ± .05	8.8 ± .3	9.1 ± .3
BIO	18.7 ± .3*	2.4 ± .1*	11.1 ± .3*	10.6 ± .3*	22.2 ± .4*	1.3 ± .03*	10.8 ± .3*	13.0 ± .2*
Adipose tissue								
RB	20.3 ± .4″	5.6 ± .5	8.0 ± .6	24.2 ± 1.1	18.7 ± .9	1.9 ± .1	4.6 ± .6	1.6 ± .05
BIO	19.7 ± .7	4.0 ± .4*	9.0 ± .5	19.5 ± .9*	20.6 ± .9	1.9 ± .1	5.6 ± .6	1.6 ± .04

″ Values are $\bar{X} \pm$ SEM of at least eight samples.
′ Values are $\bar{X} \pm$ SEM of three samples. Each sample is composed of microsomes isolated from fat pads of three animals.
* Denotes statistically significant differences, $P < 0.05$.

endoplasmic reticulum (microsomes) are altered in the dystrophic hamsters. Hence the present study was undertaken in order to determine if there were changes in the lipid composition of the microsomes of the diseased animals.

Since phospholipids are basic constituents of membranes and the proper assembly of these compounds is essential for optimal membrane function, we directed our efforts towards examining if there were changes in the phospholipid content and the fatty acid composition of liver and adipose tissue microsomes in the dystrophic hamsters and age-matched randomly bred controls. Because the changes that were observed in the various analysis were more pronounced in the liver than adipose tissue, the discussion is centered around that organ.

The results shown in Table I reveal a significant increase in the concentration of the total PL and the PC and PE fractions in liver microsomes of the dystrophic hamsters. This increase in the microsomal phospholipid content follows the same pattern of increased lipid content in the livers that we previously reported (4). These results may be a reflection of changes in turnover rates of lipids, since, as we previously reported (4), the rate of incorporation of glycerol-3-phosphate into glycerides by liver homogenates of dystrophic hamsters was not different from that in normal controls. The fact that there was an increase in the phospholipid content of liver microsomes and no change in the incorporation of G3P into the glycerides suggests that there is a decrease in the rate of breakdown of these compounds.

Other changes in the liver microsomal membranes are apparent from the analysis of the fatty acid composition (Table II). The concentrations of the fatty acids that are synthesized *de novo* either from small molecules via lipogenesis (16:0) or desaturation (16:1, 18:1) are lower in the diseased animals. It appears that the livers of the dystrophic hamsters compensate for the decrease in the concentrations of monounsaturated fatty acids synthesized *de novo* by incorporating into the microsomes polyunsaturated fatty acids derived directly (18:2) or indirectly (18:3, 20:4, 22:6) from

TABLE III. RATIO OF SATURATED/MONOUNSATURATED AND SATURATED/UNSATURATED FATTY ACIDS OF LIVER AND ADIPOSE TISSUE MICROSOMAL LIPID EXTRACT OF RANDOMLY BRED (RB) AND DYSTROPHIC (BIO) HAMSTERS

	Saturated/monounsaturated fatty acids	Saturated/unsaturated fatty acids
Liver		
RB	1.694 ± 0.127[a]	0.599 ± 0.020
BIO	2.262 ± 0.084*	0.493 ± 0.012*
Adipose tissue		
RB	1.019 ± 0.023[b]	0.528 ± 0.007
BIO	1.228 ± 0.057*	0.543 ± 0.026

[a] Values are of $\bar{X}$ ± SEM of at least eight samples.
[b] Values are of $\bar{X}$ ± SEM of three samples. Each sample is composed of microsomes isolated from fat pads of three animals.
* Denotes statistically significant differences, $P < 0.05$.

the diet. This conclusion is based on the observation that the ratio of saturated/monounsaturated fatty acids is higher in liver microsomes of the diseased animals than in the normal controls, whereas the ratio of saturated/unsaturated fatty acids in the dystrophic hamsters is lower than that of the controls (Table III). This may imply, then, that the diseased animals incorporate more diet derived polyunsaturated fatty acids into their microsomal membranes so that the ratio of saturated/unsaturated fatty acids is maintained at a value nearly equivalent to that of the normal controls.

The results of this study show that microsomal membranes of livers from the dystrophic hamsters differ from those of the normal controls in their phospholipid content and their fatty acid composition. These changes may result in a change in the physical state of the membranes which in turn may affect the orientation of membrane proteins, and thus impair enzymatic function.

Summary. Analysis of liver microsomal phospholipids of dystrophic and normal hamsters showed changes in the content and fatty acid composition of these compounds in the diseased animals. The total phospholipid, phosphatidylcholine, and phosphatidylethanolamine concentrations were elevated. Determination of the fatty acid composition revealed a decrease in the concentration of monounsaturated fatty acids with a concomitant increase in the concentration of polyunsaturated fatty acids. It is concluded that these changes may affect the physical nature of membranes. Such a change in the physical state of the membrane may affect the orientation of membrane proteins, which in turn may cause alterations in enzymatic activity.

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