Lipid Composition and de novo Lipid Biosynthesis of Human Palmar Fat in Dupuytren's Disease

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ABSTRACT

Seventy-two surgically obtained Dupuytren's disease palmar-fat (DDPF) specimens and 18 location-matched specimens from patients not suffering from this disease (controls) were studied for their total lipid composition and de novo lipogenic activity. Incubation of "DDPF" with 1-[^{14}C]acetate in oxygen produced [^{14}C]palmitate and [^{14}C]stearate in approximately equal yields as those obtained from "controls." No [^{14}C]octanate was formed in any of the palmar-fat preparations. The lipids and fatty acid analysis revealed differences: (a) DDPF specimens were richer in free fatty acids, methyl esters of fatty acids and free-cholesterol than specimens of controls. (b) DDPF specimens contained less phospholipids. (c) DDPF specimens showed a significantly higher content of octanoate and other short-chain fatty acids than specimens of controls. The above findings are not incompatible with the results expected if some mild hypoxia occurred in DDPF; this has been suggested in the statistical correlations observed for this disease and alcoholism with liver involvement.

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INTRODUCTION

Dupuytren's disease (DD) is an affliction characterized by a degeneration of elastic fibers (1) and a progressive irreversible contraction of one or more fingers (2). Several articles and reviews have recently been published (1,3,4) but no total lipid assays or lipogenic activity have been reported for the palmar-fat tissues.

Published reports (1,3,5) have shown statistically significant evidence linking chronic liver disease (from alcoholism) and the incidence of DD). Several Alcoholic Research Centers have demonstrated the presence of volatile (short to medium chain) fatty acids in breath and in the sera of patients with liver cirrhosis (6-11). This fact has been explained by the suggestion that, in alcoholism, a liver hypoxia is responsible for the incomplete oxidation of some of the long-chain fatty acids, and thus shorter chain fatty acids (6) are found in larger than normal amounts (8); since these fatty acids are water-soluble and volatile, they will be found in all tissues and in breath.

The associations of alcoholism and local palmar hypoxia with DD prompted us to investigate whether higher than normal levels of volatile fatty acids may be present in the palmar fat of DD patients, and if this result may be because of a special biosynthesis.

MATERIALS AND METHODS

Specimens of palmar fat were obtained from surgically hospitalized patients. Seventy-two DD and 18 location-matched surgical specimens from palms of patients not suffering DD or other metabolic disease were studied (controls). The tissues were kept chilled (1 C) and immediately taken to the laboratory, padded dry with filter paper and weighed.

The individual tissue was chopped into small pieces with fine scissors and the tissue divided into various weighed portions. One of the weighed aliquots was mixed with a 30× vol of chloroform/methanol (2:1, v/v). Lipid extraction was performed at 5 C for 48 hr, with agitation (wrist-action shaker). The lipid extract was then used for chemical analysis.

Neutral Lipid Analysis

Neutral lipids were separated from phospholipids by silicic acid column chromatography using sequential chloroform and methanol elution. The individual neutral lipids were separated by thin layer chromatography (TLC) using Silica Gel G on glass plates. The solvent system was petroleum ether/ethyl ether/acetic acid (90:10:1, v/v/v). Neutral lipids were identified by staining with iodine vapor.

Phospholipid Analysis

Phospholipids were separated by two-dimensional TLC using Silica Gel H on glass plates. The solvents used for the first and second dimension separation were chloroform/methanol/acetic acid/water (200:120:25:15, v/v/v/v) and chloroform/methanol/acetic acid/water (100:20:40:20, v/v/

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v/v), respectively. The R_f values were determined by the use of known standards (Supelco, Bellefonte, PA). Lipid fractions were estimated by the charring procedure. All of these analytical procedures, extraction methods, and quantitations have been previously reported in great detail (12-13).

Fatty Acid Analysis

Potassium salts of the fatty acids were prepared by saponification of the palmar fat under nitrogen with 20% KOH in 90% ethanol for 3 hr at 100 C (fraction S). An aliquot of fraction S was acidified and then extracted with diethyl ether. The recovered fatty acids were esterified with 14% boron trifluoride in methanol. The methylated fatty acids obtained were then purified by TLC using the neutral lipids procedure. Gas liquid chromatography (GLC) was performed using a Packard Model 846 with a 6-ft glass column packed with 10% DEGS-PS (Supelco, Bellefonte, PA) and a nondestructive mass detector (argon ionization). Argon was used as a carrier gas. Temperature programming was carried out from 150 to 200 C at 1 C/min and then maintained at 200 C for an additional 45 min. The retention time was used for identification; quantitation was by comparison with known amounts of authentic fatty acid methyl esters (Applied Sciences, State College, PA).

Volatile Fatty Acids

Another aliquot of fraction S was assayed by steam distillation in a closed glass system and assayed for volatile free fatty acids in a special GLC system (8).

Lipogenetic Activity

From fresh surgical samples of DDPF, weighed aliquots were obtained. The weighed tissue aliquot was homogenized in 2 1/2 vol of buffer containing K_2HPO_4, 0.067 M; KH_2PO_4, 0.042 at pH 7.0 at 0 C. The mixture was centrifuged for 7 min at 500 × g to remove unbroken cells, nuclei, and cell debris. For each test, 5 mg of 500 × g supernatant protein was added to a flask containing 1 mg of 1-[^{14}C]acetate (1 mCi/mmol) in pH 7.2 buffer. Final volume of each reaction was 5 ml. The flasks were incubated at 37 C for 3 hr with gentle shaking; the gaseous phase was 100% oxygen. After incubation, 2 mg of a carrier mixture of fatty acids (16:0, 16:1, 18:0, 18:1 and 8:0) was added to each flask. Solid pellets of metaphosphoric acid were added to bring the mixture to pH 2. The material was extracted in a closed glass container continuously with ether for 24 hr. Aliquots of the ether extract were evaporated to dryness and placed in a desiccator over KOH. One ml of 10% acetic acid was added to each residue which was again dried in a desiccator over KOH. Each residue was dissolved in absolute ether in a 1 ml volumetric flask. Aliquots were methylated (12), then taken for GLC radioassay (6). Other aliquots were used to obtain the volatile fatty acids by steam distillation (8) and then quantitated by GLC radioassay (6).

Sodium 1-[^{14}C]acetate (sp act 15 mCi/mmol) was obtained from New England Nuclear Corporation, Boston, MA. A Packard Tri-Carb Spectrometer was used for determination of radioactivity. Radioactive assay of the GLC products was by oxidation and subsequent assay in a proportional radioactivity counter attached to the GLC (Packard 894). Counting was to ± SD. These techniques have been reported previously (14-18).

Protein

The biuret technique was utilized for protein assays of tissue aliquots. Bovine serum albumin (ICN-Nutritional Biochemicals, Cleveland, OH) was used as the standard (19,20).

RESULTS

In Table 1 are shown the lipid assays obtained from DD and control palmar-fat specimens. The studies indicate that the differences between DD and controls are not statistically significant for most classes of lipids. Although some minor differences could be shown, DD samples yielded higher values compared to controls for only some of the neutral lipid components: free fatty acids, methyl esters and cholesterol, yet these were not outstanding. Lower values for all of the phospholipids were also seen in the DD specimens; but the levels found were so small that statisties are hard-pressed to indicate significance. The observed patterns for both types of specimens are not that much different than those observed for many fat-rich areas or lipid pads of the body (14).

In Table 2, we have presented the results of fatty acid analysis by GLC after saponification and esterification of aliquots of the samples. Results of the GLC assays indicated a similar pattern of fatty acid distribution for both the control and DD specimens in most of the medium- and long-chain fatty acids. But, in the assay of volatile fatty acids obtained by closed-vessel steam distillation and then GLC, the short-chain fatty acids (octanoate and smaller sizes) showed a statistically significantly higher value for the DD specimens than for the controls.

In Table 3, the results are presented for the 3-hr incubations of palmar-fat tissue homogenates under a gaseous phase of 100% oxygen. The incorporation of 1-[^{14}C]acetate into the fatty acids was small and in the range reported for similar tissues (14). No differences were observed in the lipo-

TABLE I

Percentage of Lipid Composition of Human Palmar-Fat Surgical Specimens

	72 Specimens Dupuytren disease		18 Specimens (location-matched) controls	
		±SEM		±SEM
Total lipids (% net weight)	98.3	±2.1[a]	99.0	±1.7
Neutral lipids	96.5	±1.4	95.3	±1.2
Polar lipids	2.5	±0.6	3.5	±0.7
Undetermined lipids	1.0		1.2	
Neutral lipids:				
Mono- and diglycerides	1.7	±0.4	0.9	±0.2
Cholesterol	1.9	±0.7	0.3	±0.1
Free fatty acids	4.4	±0.9	2.1	±0.7
Triglycerides	78.7	±2.4	86.6	±3.1
Methyl esters	3.8	±0.9	0.9	±0.1
Cholesteryl esters	3.9	±0.3	3.7	±0.8
Undetermined	2.1		0.8	
Phospholipids:				
Phosphatidylethanolamine	0.4	±0.1	0.5	±0.1
Phosphatidylinositol	0.1	±0.1	0.2	±0.1
Phosphatidylserine	0.1	±0.1	0.2	±0.1
Phosphatidylcholine	0.2	±0.2	0.4	±0.2
Lysophosphatidylcholine	0.1	±0.1	0.3	±0.1
Sphingomyelin	0.2	±0.1	0.4	±0.1
Phosphatidic acid	0.7	±0.3	0.8	±0.2
Cardiolipin	0.3	±0.2	0.4	±0.1
Undetermined	0.4		0.3	

[a]Standard error of the mean.

genetic activity of DD samples from that found in controls based on protein content.

When incubations were carried out using hypoxic conditions (a gaseous phase of 100% nitrogen), no incorporations of 1-[^{14}C]acetate were observed in any of the recovered fatty acids. These observations were repeated several times (four) for both DD and control homogenates.

DISCUSSION

Daris (3) and others (1,5) have made a case for the statistical relationship between the incidences of Dupuytren's disease and alcoholism. A hypothesis in Dupuytren's disease is that a mild hypoxic state may develop inside the tissues of the lipid pad of the palm of the hand. This hypoxic state may be caused by poor circulation to the area and the abnormal connective tissue (4,21) distribution and local accumulation in the area may further contribute to the local formation of an hypoxic state, especially in a traumatized palm.

In alcoholism, it has been shown (22) that the octanoate serum concentrations of fasting patients are much higher than in those who did not have hepatic involvement or in nondrinkers (7,8). The origin of the octanoate was attributed to incomplete oxidation of long-chain fatty acids in the liver (6). The biosynthetic ability under oxygenated conditions of tissue homogenates of palmar fat was shown in this study to be about the same for both DD and controls; yet, the analysis of fatty acids revealed a larger content of short-chain fatty acids in the DD specimens. Since serum octanoate levels were shown (8,23) to be directly proportional to the extent of liver impairment (in alcoholic cirrhosis), the great increase (3-4 times over the control values) for octanoate and other short-chain fatty acids in the total lipids of palmar fat of DD specimens is not inconsistent with the concept of a locally present mild hypoxic state exacerbated or induced by alcoholism or trauma in the palms of such patients.

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TABLE 2

Percentage Fatty Acid Composition of Human Palmar Fat

	Dupuytren	Control
Less than 8[a] and 8[c]	2.3±0.4[b]	0.5±0.2
10[c]	1.4±0.2	0.8±0.2
12[c]	2.7±0.4	2.9±0.3
14	2.6±0.6	2.7±0.2
14:1	0.4±0.3	-
16	29.2±2.4	27.2±1.1
16:1	1.7±0.3	6.3±0.7
18:0	8.7±0.5	7.2±0.9
18:1	34.1±4.7	36.7±3.9
18:2	5.4±2.1	7.2±2.0
20	1.2±0.3	1.4±0.9
18:3	3.0±0.7	2.0±1.1
20:2	1.9±0.4	1.7±0.6
20:3	1.3±0.5	7.4±0.5
Over 20	1.8±0.2	1.0±0.3
Undetermined	2.3	1.0

[a]Determined by closed-vessel steam distillation and then GLC (8).
[b]Standard error of the mean.
[c]Determined by GLC of esterified fatty acids (fatty acids of 12-20 carbons in chain).

TABLE 3

Recovered [^{14}C]Fatty Acids Obtained from 1-[^{14}C]Acetate by Homogenates of Palmar Fat (dpm/mg C × 10^{-3})/mg Protein (Average of 10 Assays; Protein Calculated from Wet Weight of Sample; Gaseous Phase 100% Oxygen)

Fatty acid chain length	DDPF	Controls
Less than 8 and 8:0	0	0
10:0	0	0
12:0	0	0
14:0	0	0
16:0	387±23[a]	365±31
10:1	trace	trace
18:0	185±37	231±43
18:1	trace	trace
Over 18	0	0

[a]Standard error of the mean.

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