Lipid Composition of Adipose Tissue from “Membranous Lipodystrophy”

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and MOTOMIYA, M. *Lipid Composition of Adipose Tissue from “Membranous
Lipodystrophy”*. Tohoku J. exp. Med., 1981, 133 (4), 451-456—The lipid composi-
tion of the adipose tissue from the bone marrow and that of the subcutaneous
depot fat of four patients of membranous lipodystrophy were investigated. It
was found that the lipid composition of the samples from the cases of membranous
lipodystrophy was almost similar to that in controls. Also discussions were
made on mechanism related to the accumulation of abnormal depot fat. ———
lipids; lipomatosis; bone marrow; membranous lipodystrophy

Membranous lipodystrophy was first described as a pathological entity by Nasu et al. (1973). Its characteristics consist of cystic dysplasia in the long bone, membranous structures not only in the bone marrow but also in the adipose tissue, and of sudanophilic leucodystrophy of the cerebrum. About 30 cases of membranous lipodystrophy have been reported up to the present in Japan (Sato et al. 1976; Tashiro et al. 1976; Akai et al. 1977). On the other hand, Hakola (1972) reported 9 patients with the signs and symptoms almost similar to those of membranous lipodystrophy and diagnosed them as lipomembranous polycystic osteodysplasia. These two diseases are likely to belong to the same entity as evaluated by clinicopathological findings. Up to the present, disturbance of lipid metabolism has been suggested as a cause of membranous lipodystrophy. However, there have been only a few reports on the detailed analysis of lipids from the membranous lipodystrophy (Nasu et al. 1973; Tashiro et al. 1976). The present paper deals with the analysis of lipid composition of the adipose tissue of four cases of membranous lipodystrophy together with relevant chemical and laboratory data.

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CLINICAL

The details of the clinicopathological finding of the four cases of the membranous lipodystrophy in the present communication were published elsewhere (Sato et al. 1976). Hence only a brief summary of the clinical and laboratory data will be presented here. Table 1 shows the history and results of the neuro-psychiatric evaluation of the above four cases. The principal histological changes in the bone were atrophy and cystic radiolucency. As shown in Table 2 cholesterol was somewhat lower and the β -lipoprotein level was somewhat higher than usual in Cases 3 and 4. The serum lipase level as determined by the method of Tietz and Fiereck (1966) was somewhat higher than usual in Case 4. Other laboratory data were almost within normal limits.

TABLE 1. *Clinical data of the patients with membranous lipodystrophy*

Case No.	Sex	Age at the first examination	Year of the first examination	History of repeated fracture	Mental disturbance	Outcome
1	F	41	1970	+	None	Suicide at the age of 43
2	M	29	1970	+	Epilepsy	Death at the age of 30
3	M	19	1973	+	Euphoria	Alive
4	M	19	1974	+	Euphoria	Alive

TABLE 2. *Results of quantitation of lipids, lipase and lipoprotein in serum*

	Case 1	Case 2	Case 3	Case 4
Total lipid (mg/100 ml)	-	-	497	359
Total cholesterol (mg/100 ml)	130	160	193	157
Cholesterol ester (mg/100 ml)	-	-	68	77
Neutral fat (mg/100 ml)	105	62	99	66
Phospholipid (mg/100 ml)	159	140	209	177
Free fatty acid (mEq/liter)	-	-	0.41	0.44
Lipase*	-	-	0.6	1.6
α -Lipoprotein	-	-	17.9	21.8
Pre β + β -lipoprotein	-	-	77.0	74.1

* Determined by the method of Tietz and Fiereck (1966).

EXPERIMENTAL

Materials. The specimens from the bone marrow and from the subcutaneous depot fat of the cases of membranous lipodystrophy were obtained by operation. The operation comprised curettage of the bone marrow and the yellow-brown colored adipose tissue therein, and subsequent bone grafting.

Fractionation of lipids. All the samples were frozen immediately after operation and kept at -20°C until used for biochemical analysis. The fraction of lipid was freed from non-lipid contaminations as described by Folch et al. (1957), concentrated in vacuo and was dried completely in a desiccator.

Thin layer chromatography. Samples were applied on 20×20 cm plates of Silicagel G (Merck). Irrigation on Silicagel G for the detection of glycolipid was effected in a solvent system of chloroform: methanol: water (65:25:4) (solvent 1). Detection was made with anthron reagent (Roe 1955). For the chromatographic separation of sphingolipids, irrigation was effected in a solvent system of chloroform: methanol: water (60:35:8) (solvent 2). Detection was made by the reagent of Dittmer and Lester (1964). Irrigation in a solvent

system of petroleum ether: ether: acetic acid (80:20:1) (solvent 3) was carried out for the separation of lipids by classes. Thereafter the plates were sprayed within 50% H_2SO_4 and charred for revelation of spots. In two-dimensional thin layer chromatography for the detection of sphingolipids, the first run was effected in solvent 3 and the second in solvent 1. For the quantitative determination of different classes of lipids, the plates after irrigation in solvent 3 were sprayed with water and the band corresponding to each class of lipids was eluted with a 2:1 mixture of chloroform and methanol and ether. The eluate was dried and the ratio of individual fraction was determined by gravity.

Gas chromatography of fatty acids. Samples were hydrolyzed with 0.5 N KOH in 55% ethanol. This saponification mixture received an addition of 2 N HCl. The acidified aqueous phase was extracted with petroleum ether. The organic phase was repeatedly washed with water, dried with Na_2SO_4 and was used for the preparation of the methyl ester of fatty acid in the reaction with diazomethane. A gas chromatograph (JCG-750, JEOL Co.) equipped with a hydrogen-flame ionization detector was used for the detection and tentative identification for fatty acids based on the equivalent chain length method throughout this series of experiments. (The temperature of a 2-m column of 1% diethyleneglycol succinate (DEGS) on Chromosorb W was kept at 200°C and the pressure of the carrier gas 0.7 kg/cm².) For the identification of hydroxy fatty acids, a mixture of methyl esters of fatty acids was chromatographed on a plate of Silicagel G in a solvent system of *n*-hexane: ethyl ether (60:40) (solvent 4). 2-OH-methyl myristate were used as references of hydroxy fatty acids. Detection was made by spraying a 0.01% solution of Rhodamine B (10 ml of a 0.1% ethanolic solution of Rhodamine B mixed with 90 ml of a 0.25 M solution of NaH_2PO_4).

RESULTS

After irrigation in solvent 1, no spot of glycolipids could be detected with anthron reagent even when 1 mg of the lipid fraction was applied. After irrigation in solvent 2, a faint spot which had the same R_f as that of phosphatidyl choline from egg yolk was detected. However, the quantity of the phospholipid by visual evaluation of the charred chromatogram was very small as compared with that of the other-than-phospholipid.

TABLE 3. *Lipid classes detected in specimens from the cases of membranous lipodystrophy*

	Case 1 BM, f, 43y*	Case 1 SC, f, 43y	Case 2 BM, m, 31y	Case 3 BM, m, 21y	Case 4 SC, m, 20y	Control 1 SC, m, 8y	Control 1 BM, m, 8y	Control 2 SC, f, 42y	Control 3 SC, m, 24y	Control 4 BM, m, 25y
CHE (%)	6.7	3.6	3.7	8.1	3.1	0.7	3.8	2.5	5.7	4.9
TG (%)	54.7	42.8	63.0	57.6	57.6	93.2	70.6	63.0	60.2	54.2
FFA (%)	5.6	6.8	8.6	7.8	5.6	1.6	4.9	4.0	6.2	5.6
DG (%)	12.5	14.0	8.0	12.1	20.2	1.6	9.3	9.8	9.2	14.2
CH (%)	8.2	15.2	5.2	6.8	3.0	1.3	6.2	8.4	8.4	14.2
Other (%)	12.9	17.6	11.5	7.6	16.0	1.5	5.2	12.3	9.8	6.9
Total lipid (%)	45.1	28.6	37.5	-	-	-	-	27.6	56.2	10.2

CHE, cholesterol ester; TG, triglyceride (simple lipid); FFA, free fatty acid; DG, diglyceride; CH, cholesterol; Other, other components (unidentified). BM, bone marrow; SC, subcutaneous depot fat.

Separation by thin layer chromatography in a solvent system of petroleum ether: ether: acetic acid (80:20:1).

Determination of the ratio of individual lipid classes by gravimetry.

*f, 43y=female, 43-year-old.

Quantitation of lipids eluted from the chromatogram after irrigation in solvent 3 indicated that of the lipid classes detected (cholesterol ester, triglyceride, free fatty acids, diglyceride and cholesterol). Triglyceride was the major component (Table 3). Also it was found that the composition of the lipid classes from the samples of the cases of membranous lipodystrophy was almost similar to that of the controls.

In two-dimensional chromatography no sphingolipid could be detected. Irrigation in solvent 4 failed to reveal any OH-fatty acid.

There was a case-to-case difference in the fatty acid composition of triglycerides (simple lipid) of the cases of membranous lipodystrophy. However,

TABLE 4. *Fatty acid composition of simple lipids (triglycerides)*

	Case 1 SC, f, 43y*	Case 2 BM, m, 31y	Case 3 BM, m, 21y	Case 4 SC, m, 20y	Control 1 SC, m, 8y	Control 1 BM, m, 8y	Control 2 SC, f, 42y	Control 3 SC, m, 24y	Control 4 BM, m, 25y
13:0†		0.3			0.2		0.3		
14:0	1.2	4.8	9.4	11.7	1.7	2.4	2.9	2.4	1.6
14:1	0.5	1.7	1.3	0.3	0.6	0.9	0.8	0.9	
16:0	29.1	20.7	46.1	58.3	24.3	21.6	26.8	21.0	26.3
16:1	19.3	15.5	12.2	4.2	10.5	7.2	12.9	7.2	9.8
16:2					0.3	0.4	0.8	0.4	
18:0	0.5	1.4	7.3	4.7	1.1	1.3	0.8	1.3	3.1
18:1	42.3	52.0	25.1	16.1	56.7	58.2	39.4	58.2	58.9
18:2	7.1	2.6			4.2	6.6	14.4	6.6	3.5
20:0		1.0			0.4	2.0	0.9	2.0	1.8

* f, 43y=female, 43-year-old. SC, subcutaneous depot fat; BM, bone marrow.

† The number of carbon atoms; the number of double bonds. For example, 16:0 is hexadecanoic acid (palmitic acid) and 18:2 is octadecadienoic acid.

TABLE 5. *Composition of fatty acids from cholesterol ester*

	Case 1 BM, f, 43y*	Case 1 SC, f, 43y	Control 1 SC, m, 8y	Control 1 BM, m, 8y
14:0	6.0	12.0		1.1
14:1	1.2	0.5		3.5
15:0				
15:1	41.0	60.0		63.5
16:0	11.6	6.9		15.9
16:1				1.8
17:0				1.8
18:0	7.1	4.9		1.0
18:1	31.5	15.7		1.0
19:0	1.6			8.5
20:0				1.8

After saponification with ethanolic KOH. Preparation of methyl ester with diazomethane. Gas-chromatographic analysis as in Table 4. Samples from Control 1 BM and Control 1 SC were united and used for analysis because only a small amount of material was available.

* f, 43y=female, 43-year-old; SC, subcutaneous depot fat; BM, bone marrow.

Gas-chromatographic analysis of the fatty acids from other samples could not be carried out because of the limited amount of material.

TABLE 6. *Composition of free fatty acids*

	Case 1 SC, f. 43y	Case 1 BM, f. 43y	Control 1 SC, m, 8y	Control 1 BM, m, 8y
14:0	6.2	11.4	4.7	4.7
14:1	1.7	4.2	0.3	1.9
15:0	1.7			
15:1				
16:6	46.8	58.8	58.6	49.3
16:1	3.9	2.6	4.7	11.2
17:0	2.9	4.2	0.1	0.7
17:1	0.8		2.3	0.4
18:0	15.8	7.0	3.5	9.4
18:1	12.2	10.4	25.8	11.2
19:0	4.0	1.4		11.2
19:1	4.0			

Preparation of methyl ester with diazomethane. Gas-chromatographic analysis as in Table 4. SC, subcutaneous depot fat; BM, bone marrow.

Gas-chromatographic analysis of fatty acids from other samples could not be carried out because of the limited amount of material.

the data, as a whole, were similar to those of the control specimens. Both in samples from the cases of membranous lipodystrophy and in those from the controls, palmitic acid (hexadecanoic acid) and octadecamonoenoic acid were the major constituents (Table 4). No pattern characteristic of membranous lipodystrophy could be confirmed. No difference was found either in fatty acid composition of cholesterol ester (Table 5) or that of free fatty acids (Table 6) between the samples of membranous lipodystrophy (Cases 3 and 4) and control specimens.

DISCUSSION

The yellow bone marrow in animals which is the important depot fat reflects the functional and metabolic status of the host (Lund et al. 1962). Usually the fatty acid composition of the yellow bone marrow is similar to that of the adipose tissue of the same individual (Zakaria and Shafrir 1976). The lipid in the normal bone marrow primarily consists of triglyceride (simple lipids) that amounts to as high as 96 to 98% of the total lipids according to Zach (Zakaria) and Shafrir (1974). Since the depot fat of an organism shows an almost similar pattern with respect to composition of lipid irrespective of age (Krut and Bronte-Stewart 1964), the control specimens were taken from the subjects whose ages were somewhat different from those of the four cases of membranous lipodystrophy in the present study. It was found that the relative ratio of triglyceride in the present experiments was mostly lower than the ratio reported in the literature by other investigators. None the less it was the principal constituent of the lipids. It has been accepted that palmitic acid and octadecamonoenoic acid (oleic acid) are the major constituents of the fatty acids of simple lipid in bone marrow and depot fat (Hefferman 1963; Zach and Shafrir 1974). In the report of Lund et al. (1962) the percentage of palmitic acid was 26% and that of octadecamonoenoic acid (oleic

acid) was 46%. Oleic acid was the major fatty acid according to Hefferman (1963). Our results were almost in agreement with the results of the above investigators. Thus no chemical data suggestive of the findings that are characteristics of membranous lipodystrophy could be detected.

On the other hand, a histological study has revealed the presence of membranous structures. This finding has been ascribed to the inability of the fatty cells to maintain their structural integrity (Sato et al. 1976). Accumulation of unusual lipids in the bone marrow may be due to the lack of lipase or to an excess of hydroxy fatty acids. In our study, however, no evidence for the presence of hydroxy fatty acids could be obtained. Further chemical studies are required in order to arrive at conclusion concerning the pathogenesis of membranous lipodystrophy.

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