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The Fatty Acid Pattern of Adipose Tissue and Liver Triglycerides According to Fat Droplet Size in Liver Parenchymal Cells of Diabetic Subjects

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Summary. In 40 diabetic inpatients the fatty acid pattern of triglycerides in liver fat and in subcutaneous adipose tissue was determined by gas liquid chromatography. With rising size of fat droplets in liver parenchymal cells there was a significant increase of palmitic acid (C:16), oleic acid (C 18:1) and linoleic acid (C 18:2) as well as a decrease of myristoleic acid (C 14:1), arachidonic (C 20:4) and eicosapentaenoic acid (C 20:5) in liver triglycerides, resulting in a fatty acid composition in big droplets similar to that of adipose tissue. Fatty acid pattern of subcutaneous depot fat was strikingly constant.

Key words: Adipose tissue, diabetes mellitus, fatty acid composition, gas-liquid chromatography, liver, steatosis hepatis, triglycerides.

The composition of fatty acids in adipose tissue is the subject of numerous papers [1, 2, 4, 14, 16, 18, 20, 22, 23, 29]. Several gas-chromatographic investigations dealing with the fatty acid pattern of triglycerides in liver tissue have also been performed [3, 7, 25, 26, 31, 33, 38].

Simultaneous gas-chromatographic analyses of liver and adipose tissue were published by Takahashi and Tanaka [38], Laurell and Lundquist [26] and Schön *et al.* [33]. Most of the tissues were obtained from autopsy material.

At present there is no general concept of the relative proportions of individual fatty acids of the triglycerides in liver and adipose tissue. The predominant concept is of a direct dependence of the triglycerides in the liver on the influx of adipose depot fat via serum free fatty acids. This seems to be uncertain for several fatty acids [12, 13, 26] and an influence of ingested fat on the liver fatty acid pattern [6, 39], as well as that of adipose tissue, has been reported. For that reason Laurell and Lundquist [26] studied the mechanism in patients during starvation.

In previous gas-chromatographic studies from biopsy specimens we observed an increase of palmitic and oleic acid and a decrease of eicosapentaenoic acid (C 20:5) in liver triglycerides of diabetics, together with a rise of the fat droplet size in the liver parenchymal cells [35]. The purpose of the present paper was to determine whether these results bear any relation to the fatty acid pattern of subcutaneous adipose tissue in diabetic subjects.

Material and Methods

In 40 diabetic inpatients on standard diet simultaneous biopsies were taken from the liver, by the Menghini technique [27], and abdominal subcutaneous adipose tissue, by a small infraumbilical incision.

The liver biopsy specimen was divided. The first part was prepared for histological examination (HE, Sudan III, van Gieson, Turnbull's blue, Fouchet) in formaldehyde (10%). The second sample was stored in saline at −30° C for gas-chromatographic analysis of the triglycerides. Cases with pathological histological findings such as hepatitis and cirrhosis were excluded. The liver fat depositions were classified into 4 categories according to the size of fat droplets independent of the amount of fat by means of an ocular net with a length of 110 or 250 μm (35):

1. normal (no visible pathological changes) — 6 cases,
2. small fat droplets in parenchymal cells, 1.7 ± 0.4 μm ($\bar{x} \pm s$), maximal size 6.6 μm — 7 cases,
3. medium-sized droplets, 7.9 ± 1.5 μm, maximal size 24.0 μm — 5 cases,
4. big droplets (fat vacuoles), 20.8 ± 5.5 μm, maximal size 50.0 μm — 22 cases.

In stage four there were 5 cases with normal body weight and fatty liver. The average age in stage one was 47.2, in stage two 38.9, in stage three 54.0 and in stage four 52.0 years. There were no striking differences between the groups in sex, body weight, fasting blood sugar and serum triglycerides (Table 1). Diabetes mellitus was well compensated during the study. Diseases affecting lipid metabolism, like hyperlipoproteinemia (triglycerides above 200 mg/100 ml, cholesterol above 280/100 ml), nephrosis, hepatitis, alcoholism were excluded. The patients were kept in hospital at least two weeks before the biopsies. Two subjects in stage 4 were treated with sulfonylureas; all other patients received insulin. The subcutaneous adipose tissue was transferred to saline solution and stored at −30° C for

gas-chromatographic analysis as well as the second liver biopsy specimen.

Total lipids were extracted from liver or fat tissue by means of chloroform-methanol (2:1, v/v). The triglyceride fraction was isolated by thin layer chromatography on silica gel G (Merck, Darmstadt). The plates were developed in hexane-diethylether-acetic acid (73:25:2, v/v). The lipid fractions were visualized under UV-light after the plates had been sprayed with a solution of 0.1% dichlorfluorescein in ethanol. The triglyceride fraction was scrabbled directly into a test tube. The esterification was achieved by 0,5 N sodium methylate at room temperature. After evaporation under nitrogen the sample in hexane solution was injected directly into a gas chromatograph (GCHF 18.3, VEB Chromatron, Berlin, flame ionization detector). Analyses of fatty acid methylesters were performed on columns (2 m × 4 mm) of 10% diethyleneglycol succinate, stabilized (Analabs, North Haven, USA) on Gas Chrom P (100—120 mesh), isothermal at 200° C. Components were identified by their retention time relative to authentic standards (Applied Science Laboratories, State College, USA). The quantity of each fatty acid was calculated from the product of the peak height and width in the half height. The method has been published in detail previously [15].

Statistical analysis was performed using Student's t-test.

Table 1. *Relative body weight, fasting blood sugar, triglycerides and cholesterol in the 4 patient groups (mean ± S.E.M.)*

	normals	small droplets	medium-sized droplets	big droplets
relative body weight[a]	105± 9	107±16	106± 9	109±14
fasting blood sugar[b]	161±21	151±22	158±33	150±35
serum triglycerides[a]	138±13	144±58	151±27	145±25
serum cholesterol[b]	223±21	220±30	214±29	217±30
number of patients (n)	6	7	5	22

[a] Metropolitan Life Insurance Company tables — (28)
[b] by Flow Stream Analyzer — (24)
[a] by Stolz *et al.* 1968 — (36)
[b] by DAB 7 — (10)

Table 2. *Comparison of triglyceride fatty acid pattern in subcutaneous and liver fat according to fat droplet size in liver parenchymal cells* (x = $p < 0.01$, o = $p < 0.05$)

	subcutaneous fat								liver fat							
fatty acid	normal liver		small droplets		medium droplets		big droplets		normal liver		small droplets		medium droplets		big droplets	
	x̄	s	x̄	s	x̄	s	x̄	s	x̄	s	x̄	s	x̄	s	x̄	s
C 8	<0.1	—	0.1	0.4	0.6	1.3	0.1	0.3	0.9	1.6	0.9	0.8	0.0	—	0.7	1.3
C 10	0.1	0.1	<0.1	—	0.0	—	0.1	0.8	1.2	2.5	0.2	0.4	0.1	0.3	0.8	1.4
C 12	0.6	0.5	0.5	0.4	0.2	0.1	0.3	0.5	2.9	4.8	0.8	0.4	0.5	0.5	1.7	2.8
C 12:1	0.1	0.5	<0.1	—	0.0	—	0.2	0.8	2.4	4.9	0.1	0.4	0.2	0.4	1.6	3.0
C 12:2	<0.1	—	<0.1	—	0.0	—	<0.1	—	0.3	0.3	0.2	0.5	0.0	—	0.1	0.2
C 14	4.1	2.3	5.2	4.1	2.9	0.8	2.5	0.8	3.7	2.3	2.6	0.8	3.2	1.1	2.6	1.5
C 14:1	1.0	0.6	0.8	0.6	0.9	0.4	0.7	0.5	3.2	1.8	2.2	1.2	2.8	1.4	1.3	0.9x
C 14:2	<0.1	—	0.2	0.2	<0.1	—	0.1	0.5	<0.1	—	0.4	0.5	0.0	—	<0.1	0.3
C 16	23.8	2.8	25.5	5.9	25.7	1.1	22.9	3.0	16.5	3.2	18.4	3.3	23.1	7.9	26.2	4.9x
C 16:1	6.7	1.8	7.6	2.3	6.4	2.4	8.3	2.4	6.0	6.3	5.0	3.1	7.0	1.1	5.5	1.9
C 16:2	0.1	0.2	0.3	0.2	0.2	0.2	0.3	0.7	0.4	1.1	0.5	0.8	0.0	—	0.2	0.4
C 18	6.0	4.5	4.1	1.2	5.1	1.0	3.7	1.4	6.6	3.7	6.2	2.4	7.9	3.1	5.4	1.7
C 18:1	44.7	4.0	43.2	7.0	45.2	2.3	50.0	4.6	24.7	8.2	28.1	6.2	31.2	5.0	41.6	6.6x
C 18:2	6.7	2.1	6.3	2.6	7.8	1.6	6.6	3.0	3.0	1.5	4.2	2.5	3.5	1.1	5.0	2.1o
C 18:3	2.6	0.7	2.1	0.8	2.3	0.7	1.9	0.8	1.7	1.1	1.9	1.3	1.3	0.7	1.1	0.7
C 20	<0.1	—	0.0	—	0.0	—	0.0	—	1.4	2.3	0.0	—	0.1	0.3	0.1	0.1
C 20:1	<0.1	—	0.0	—	<0.1	—	<0.1	—	0.2	0.3	0.1	0.3	0.3	0.6	<0.1	0.1
C 20:2	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.3	0.6	0.8	1.0	1.3	1.5	<0.1	—
C 20:4	1.9	1.2	1.6	1.3	1.0	0.3	0.9	0.5	2.8	3.2	1.8	2.0	0.9	1.1	0.4	0.4x
C 20:5	0.7	0.8	0.4	0.3	0.3	0.2	0.4	0.3	18.1	10.0	23.7	9.4	13.4	9.6	4.4	3.8x
C 22	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.4	1.4	2.1	1.0	1.6	1.9	2.1	0.5	0.7
C 24	<0.1	—	0.1	0.2	0.1	0.1	0.0	—	0.7	1.8	0.6	1.0	0.0	—	0.1	0.4

Results

In liver biopsy specimens an increase of palmitic acid (C 16:0) and oleic acid (C 18:1) as well as a decrease of myristoleic (C 14:1) and eicosapentaenoic acid (C 20:5) in association with a rising size of fat droplets in parenchymal cells have been confirmed. The significant increase of palmitic acid was from

16.5% to 26.2% and that of oleic acid from 24.7 to 41.6%. Linoleic acid rose significantly from 3.0 to 5.0%. Myristoleic acid diminished significantly from 3.2 to 1.3% and arachidonic acid (C 20:4) from 2.8 to 0.4%, while eicosapentaenoic acid decreased from 18.1% to 4.4% (Table 2).

With increasing droplet size, the fatty acid pattern of liver triglycerides becomes more similar to that of adipose tissue.

The fatty acid composition of triglycerides in adipose tissue was not altered in the 4 stages, showing no significant differences with age, sex and body weight. The predominant fatty acids in subcutaneous fat were palmitic and oleic acid.

Discussion

The fatty acid composition of liver triglycerides found in this study confirms previous results [35]. It is similar to the findings of Laurell and Lundquist [26] in obese nondiabetics. A comparison of the fatty acid pattern in adipose tissue of our subjects with the contradictory results in the literature is problematic and will not be discussed here. It seems to be uncertain whether sex, age and body weight have a significant influence on fat composition [2, 4, 17], even though statistically significant differences have been reported in some papers [2, 16, 20, 23]. Diet appears to be the dominant factor determining the fatty acid pattern, superimposing the relatively small and dissimilar effects of age, sex and body weight [20, 21, 34]. Because of the lack of significant age differences in our 4 groups, the increase of oleic acid could not be due to advancing years or increasing body weight, as was described by Insull and Bartsch [20]. The standard procedure makes it improbable that the results could be affected by diet regimen.

As stated earlier [35] and by other authors [3, 33] oleic acid significantly increased with increasing fat deposition in liver. In our specimens a close correlation with fat droplet size could be found independently of the extent of fat deposition. Miscellaneous findings with various sized droplets were excluded. Consideration of this problem will be published elsewhere. In accordance with Takahashi and Tanaka [38] a significant increase of linoleic acid occurred with increasing fat deposition in the liver, confirming the interpretation of these authors that the inflow of fatty acids from adipose tissue is an important source of triglycerides accumulating in fatty liver. The decrease of C 20:5 fatty acid is unexplained, for the metabolic importance of this fatty acid is yet unclear. It is noteworthy, however, that arachidonic and eicosapentaenoic acid are precursors of prostaglandins [5, 11], which have a lipolytic effect. The striking result of our investigations is the growing resemblance of liver fat to adipose tissue with rising droplet size; thus the fatty acid patterns would be equal if the liver fat droplets reached the size of fat cells. The speculation is obvious that oleic acid and palmitic acid in triglycerides are the dominant depot fatty acids in all organs (inclusive blood vessels) which can accumulate fat [8, 30].

Liver diseases such as hepatitis, cirrhosis and hepatic coma lead to a significant increase of free fatty acids in serum, especially oleic acid [9, 19, 32, 37], which are thought not to be absorbed from liver parenchyma. The amount of liver fat has not been taken into consideration in those studies. Liver damage may, however, influence the results and, as was done in our investigations, patients with liver damage should be excluded from the analyses.

Antonini *et al.* [2] have described a significant rise of oleic acid in adipose tissue of diabetic men and a decrease in diabetic women. Gellhorn and Benjamin [17] found an increased synthesis of oleic acid in diabetic rats. In diabetic women a significant increase of palmitic acid in adipose tissue has been demonstrated [2]. On the other hand several authors [1, 16, 18] found no differences of adipose tissue composition between diabetics and normals. This does not affect our own observations, which are confined to diabetic subjects. Whether the correlations are also discernible in nondiabetic subjects has to be investigated.

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