Adipose Fatty Acid Composition in a Case of Generalized Deficiency of Cytochrome b_5 Reductase in Congenital Methemoglobinemia with Mental Retardation

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HIRONO, H. *Adipose Fatty Acid Composition in a Case of Generalized Deficiency of Cytochrome b_5 Reductase in Congenital Methemoglobinemia with Mental Retardation.* Tohoku J. exp. Med., 1983, **140** (4), 391-394 — The fatty acid composition of the triglyceride in adipose tissue in a case of generalized deficiency of cytochrome b_5 reductase in congenital methemoglobinemia with mental retardation was analyzed and compared with age-matched controls. The proportions of linoleic acid, linolenic acid and arachidonic acid were decreased to less than half of normal level. There was a decrease in total unsaturated fatty acids and an increase in palmitic acid. These results revealed an undeveloped pattern of fatty acid composition in adipose tissue in the patient. ——— adipose fatty acid composition; generalized deficiency of cytochrome b_5 reductase; fatty acid desaturation

A generalized deficiency of cytochrome b_5 reductase is expected to produce a systemic impairment of the metabolic process linked to cytochrome b_5 , such as fatty acid desaturation (Leroux, Junien and Kaplan 1975). It would be very important to analyze the fatty acid composition of body lipids as well as brain lipids in such patients. Previous studies (Hirono 1980) on the brain have shown a reduction in percentage of nervonic acid and the diminution of monoenes and hydroxy fatty acids in cerebrosides. The contents of cholesterol and phospholipids in white matter were reduced to 80% of the normal, whereas cerebroside was reduced to 48% of the normal.

In the present work, the fatty acid composition of the triglyceride in adipose tissue in a patient was determined and compared with age-matched controls.

MATERIALS AND METHODS

Materials

A 33-month-old girl had died from congenital methemoglobinemia with mental retardation and bilateral athetoid syndrome caused by generalized deficiency of NADH

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cytochrome b_5 reductase. The adipose tissue specimens were removed 4 hr after death and immediately frozen in dry ice and stored at -80°C .

The controls were a 30-month-old boy weighing 14 kg and a 44-month-old boy with a weight of 10.6 kg. The adipose tissue specimens were obtained during surgical operation.

Lipid extraction and fatty acid analysis

About 100 mg of minced adipose tissue was kept in 5 ml of chloroform-methanol (2:1, v/v) in a stoppered tube with occasional squeezing with a glass bar and left overnight. The sample was added with 1 ml of 0.1% sodium chloride in 0.004 M sulfuric acid, and the mixture was shaken vigorously and then centrifuged. The upper phase was discarded and the lower phase was diluted up to 10 ml with chloroform-methanol (1:1, v/v). A 100 μl portion of the extract was applied to a TLC plate in 5 cm band and developed with petroleum ether-ethyl ether-acetic acid (85:15:1, v/v/v) for 1 hr. The triglyceride band was scraped off into a screw capped tube and kept overnight in a desiccator. Two milliliter of methanol and 50 μl of sulfuric acid were added, the tube was capped tightly and placed in a boiling water for 1 hr. Extraction of fatty acid methyl esters was made with 1.5 ml, 1 ml and 1 ml of petroleum ether successively and washed with 2 ml of 2% sodium bicarbonate solution and 1 ml of water twice. After centrifugation, the extract was evaporated carefully under a stream of nitrogen. Fatty acid composition analysis was performed according to the method described earlier (Karlsson 1975).

RESULTS AND DISCUSSION

There were considerable decreases in the proportion of linoleic acid, linolenic acid and arachidonic acid to less than half of the control value, and a slight decrease in oleic acid in adipose triglyceride in the patient of generalized deficiency of cytochrome b_5 reductase (Table 1). Diminished proportions of unsaturated fatty acids were observed whereas saturated fatty acids, palmitic and myristic acids, increased when compared with controls.

The fatty acid compositions of controls 1 and 2 were very similar reflecting the closeness in age of the subjects. The values of these controls in the present work agreed well with Baker's results (1969) although the former subjects were Swedish

TABLE 1. *Fatty acid composition of triglyceride in adipose tissue*

	Patient	Control 1	Control 2
(Age in months) 33	30	44	
12:0		1.7	0.9
14:0	7.8	5.6	4.7
16:0	31.8	22.2	22.2
16:1	6.8	7.3	5.5
18:0	7.2	6.4	8.1
18:1	39.9	43.1	45.7
18:2 (n-6)	5.3	10.4	9.1
18:3 (n-3)	1.2	2.0	2.6
20:4 (n-6)	0.1	1.4	1.2
Monoenes	46.7	50.4	51.2
Unsaturated/Saturated	53.2/46.8	64.1/35.9	64.1/35.9

Values are molar percentages of fatty acid methyl esters.

while the latter subjects were American. Widdowson et al. (1975), however, reported that the difference between the composition of the fat of the infants in Netherlands and England is attributed to the nature of the fat in the milk they received. It seemed likely that very good accordance between those in Sweden and American might have resulted from similar diet which was a regular meal but not milk after weaning. Comparison between the patient who was French and the controls, Swedish, therefore could be made.

In the full-term newborn infant, adipose tissue contains approximately twice as much palmitic acid (40–50%) and only roughly one-tenth as much linoleic acid (1–3%), compared with adult tissue (Hirsch 1965; Fosbrooke and Wharton 1975). The data in controls of the present work would seem to show an increase in linoleic acid to 10% and a decrease in palmitic acid to 22% at 2 or 3 years of the normal development. The results on the patient of the present case represent a value between newborn infants and age-matched controls, namely an undeveloped pattern.

Very few studies on adipose fatty acid composition in various disease conditions have been done. Hirsch (1965) has found no significant alterations in adipose composition in obesity, pregnancy, diabetes and myocardial infarction and pointed out that the dominant factor establishing adipose fatty acid composition is the fatty acid pattern of dietary fat. An elevated level of palmitoleic acid in a small number of young children who had extreme steatorrhea, hyperlipemia or Von Gierke's disease was reported. However, there was no change in palmitoleic acid in the patient of the present case. Kaunitz et al. (1961) reported that linoleate regulates the type of fat deposited; it leads to a decrease in neutral fat in relation to body weight and facilitates the laying down of a depot fat more representative of that in the diet.

Hirsch (1965) suggested that the presence of a trienoic acid in newborn adipose tissue was a reflection of low availability of linoleic acid in the developing of this tissue. It might have been a low availability of linoleic acid that led to the undeveloped pattern of adipose fatty acids in the present case. The increase in 5, 8, 11 eicosatrienoic acid is one of the earliest biochemical manifestations of essential fatty acid deficiency in linoleic acid-deficient animals (Holman 1968). However, there was no increase of this acid level in the patient in spite of reductions in linoleic acid, linolenic acid and arachidonic acid. This observation and the lack of increase in oleic acid suggests that a situation differing from essential fatty acid deficiency is associated with the fatty acid pattern occurring in the generalized deficiency of cytochrome b_5 reductase in congenital methemoglobinemia in the present case.

Oshino et al. (1971) have detected cytochrome b_5 reductase not only in liver but also in adipose tissue microsome. Under normal conditions, linoleic acid serves as substrate for the elongation and desaturation enzymes (Lee et al. 1977; Okayasu et al. 1977; Stegink et al. 1977). Since cytochrome b_5 reductase is involved in desaturation of fatty acid (Ozols 1976), generalized deficiency of this enzyme might have caused the reduction of unsaturated fatty acid synthesis. This can explain

the marked reduction of arachidonic acid. However, influence of this enzyme deficiency on desaturation was unexpectedly rather small with a reduction of unsaturated fatty acid only 10%. It seems likely that the generalized deficiency of this enzyme might have caused reduced absorption of essential fatty acids from digestive organ or reduced uptake of extracellular essential fatty acids into adipose cell. Considering the presence of essential fatty acid deficiency, increases in monoenoic fatty acid and 5,8,11-eicosatrienoic acid should take place in this patient. But the diminished capacity in desaturation mechanism seems to have restricted the increase of these fatty acids of n-9 series.

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