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Abnormal polyunsaturated fatty acid patterns of serum lipids in Reye's syndrome

(phospholipids/free fatty acids/phospholipase A_2/lipolysis)

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ABSTRACT **Fatty acid patterns of the serum lipids were measured in 17 children with Reye's syndrome (RS). Serial measurements of total serum free fatty acids (FFA) showed that levels were increased during RS and, after recovery, were significantly lower in the patients who survived. Fatty acid patterns of serum FFA, triglycerides, and phospholipids in patients with RS were significantly different from those in controls. In RS the polyunsaturated fatty acid content of phospholipids was less than control values; in the FFA, it was higher. This was consistent with the possible involvement of increased phospholipase activity. The increase in polyunsaturated fatty acids in FFA, the precursors of prostaglandins, suggests that a grossly disturbed prostaglandin pattern may occur in RS. These changes in lipid metabolism may be related to the abnormal hepatic and neurological functions observed in RS.**

Abnormal serum lipid patterns have been reported in children with encephalopathy and fatty degeneration of the viscera [Reye's syndrome (RS)], but the increases of serum total free fatty acids (FFA) have received the most study (1–3). Pollack *et al.* (4) found a correlation between the increased initial values of FFA (>0.85 mEq/liter) and poor patient outcome. The increased FFA may result from hepatic dysfunction in RS and may also be the toxin responsible for the hepatic dysfunction (5–9).

RS usually follows a viral illness and is associated with nausea, vomiting, headache, lethargy, and, finally, coma and death. Increased intracranial pressure, seizures, and an abnormal electroencephalogram are frequently noted. Transient increases of liver enzymes and ammonia, denoting significant liver dysfunction, are regularly present. In this laboratory, studies of patterns of polyunsaturated fatty acids (PUFA), measured by gas chromatography, have revealed abnormalities in their availability, utilization, or metabolism in patients maintained on total parenteral nutrition without fat (10), in chronic malnutrition (11), in cystic fibrosis (12), in linolenic acid deficiency involving neuropathy (13), in Sjögren–Larsson syndrome involving neuropathy (14), in a congenital syndrome involving multisystem neuronal degeneration (15), and in rats fed isomeric monounsaturated acids present in partially hydrogenated vegetable oils (16).

These experiences, together with the fact that RS is a metabolic disturbance, suggested the possible importance of evaluation of essential fatty acid status in RS. Therefore, a comprehensive analysis of fatty acid composition of FFA, phospholipids (PL), triglycerides (TG), and cholesteryl esters (CE) in serum was undertaken during RS and after recovery.

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METHODS

Serum samples were collected from 17 patients with RS. All patients had acute encephalopathy and laboratory evidence of hepatic dysfunction. Liver biopsies were performed on six of the patients; they confirmed the diagnosis and revealed diffuse cellular swelling and lipid accumulation. All patients received glucose intravenously and supportive care. Patients 5–7, 11, and 13, whose subdural pressure was monitored, were given mannitol, dexamethasone, and artificial hyperventilation to decrease intracranial pressure. Eight patients received exchange transfusions. Levels of individual and total FFA were also determined in seven patients who recovered from RS.

The methods of lipid separation and fatty acid analyses were those used in our studies of effect of age and sex upon fatty acid patterns in serum lipids (17) and in recent studies on polyunsaturated fatty acids (PUFA) in disease (11–16). Samples of serum drawn from patients undergoing treatment for RS were collected, frozen, and sent to the Hormel Institute for analysis.

The samples were thawed and a known quantity of heptadecanoic acid was added to a known volume of serum as an internal standard for quantification of FFA. Lipids were extracted from the samples with methanol/chloroform, 1:1:1, (vol/vol). The extract was dried under a gentle stream of nitrogen at room temperature to minimize loss of the volatile shorter chain FFA (<12:0), and the lipids were redissolved in 0.1 ml of chloroform and spotted onto a 20 × 20 cm sheet of silicic acid-impregnated ITLC paper (Gelman Sciences, Ann Arbor, MI). Chromatograms were developed in petroleum ether (30–60°C)/diethyl ether/acetic acid, 90:10:1 (vol/vol), sprayed with 0.1% dichlorofluorescein, and illuminated with UV light. FFA, PL, TG, and CE appeared as distinct bands which were cut apart and put into glass tubes with Teflon-lined caps. These were transesterified with 2 ml of 14% BF_3 in methanol (wt/vol) at 75°C for 1 hr. After esterification, the methyl esters were extracted with petroleum ether (30–60°C); the samples were taken to dryness under a stream of nitrogen at room temperature and

Abbreviations: RS, Reye's syndrome; PUFA, polyunsaturated fatty acids; PL, phospholipids; TG, triglycerides; CE, cholesteryl esters; FFA, free fatty acids; GLC, gas/liquid chromatography. The abbreviated ω nomenclature for polyunsaturated acids consists of the number of carbon atoms of the fatty acid, colon, the number of double bonds, ω, and the number of carbon atoms beyond the last double bond including the terminal (ω) carbon atom. This nomenclature is used to indicate metabolic relationships because metabolic changes do not alter the terminal (ω) structure.

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Table 1. Major FFA in serum from patients with RS and after recovery

Patient	FFA, μg/ml								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:4	Total
1	5.2	24.0	104.8	15.7	74.8	114.4	20.2	—	359.1
2	8.2	11.5	90.8	26.7	33.1	169.0	80.3	3.5	423.1
3	4.5	21.4	134.3	44.0	45.8	235.1	32.6	9.2	526.9
4	2.5	7.2	80.9	23.8	39.5	189.7	66.3	5.2	415.1
5	3.2	7.2	70.8	18.6	44.2	191.5	63.5	4.4	403.4
6	0.5	3.0	41.3	10.2	18.3	80.3	31.8	1.8	187.2
7	2.6	5.1	111.3	14.7	71.7	231.3	105.9	4.3	546.9
8	3.5	4.9	46.2	6.7	21.9	63.4	25.8	1.1	173.5
9	3.4	9.1	87.2	—	28.9	122.3	39.4	7.6	297.9
10	5.1	9.5	33.7	8.8	11.7	53.2	27.3	2.7	152.0
11	1.0	5.0	84.7	15.2	81.2	227.0	66.3	6.5	486.9
12	2.5	7.4	62.8	12.2	29.3	132.6	42.7	3.5	293.0
13	1.5	8.3	85.6	18.5	34.6	165.2	47.0	4.9	365.6
14	—	8.0	72.8	16.1	52.8	148.8	24.1	16.4	339.0
15	0.6	3.6	31.6	4.6	15.8	49.4	14.9	3.0	123.5
16	0.4	2.6	25.5	0.4	17.5	38.4	12.6	0.3	97.7
17	1.0	6.3	69.8	12.2	37.9	153.1	53.1	2.7	336.1
Mean	2.7	8.5	72.6	14.6	38.8	139.1	44.3	4.5	325.1
±SD	±2.2	±5.9	±30.1	±10.5	±21.1	±65.3	±25.4	±3.9	±139.0
Recovery*	1.6	4.3	33.3	7.7	13.5	46.4	19.4	2.4	128.5
	±1.4	±3.9	±16.4	±6.4	±6.5	±19.5	±12.1	±2.0	±47.9
P†	NS	NS	<0.005	NS	<0.005	<0.001	<0.05	NS	<0.002

* Mean (±SD) after recovery in patients 1–7.
† NS, not significant.

redissolved in a minimal volume of petroleum ether for gas/liquid chromatography (GLC).

GLC was carried out on a Packard 428 gas chromatograph equipped with a flame ionization detector and a 12-foot (365 cm) × 1/8-inch (3 mm) aluminum column packed with 10% Silar 10C on 100–120 Gas Chrom Q. The temperature was programmed from 110°C to 230°C at 4°C/min with a final hold of 8 min. This procedure separated the methyl esters of fatty acids from 8:0 to 22:6ω3. Areas under peaks were measured by an electronic digital integrator (Infotronics model CRS-104). Components were identified by comparison with known methyl ester standards. A similar method was used for analysis of samples from patients 1–4 and 8–10 (3) and the responses for the components listed in Table 1 are comparable in the two methods. Children included in a study of effects of age and sex upon PUFA of serum lipids served as controls for comparisons of fatty acid patterns (17). Significant differences were determined using Student's t test.

RESULTS

Free Fatty Acids. Table 1 shows the values for the major individual FFA and the total FFA in the 17 patients with RS. Only the major components that constitute the bulk of the FFA are listed. Serial samples were obtained from patients 1–11. Patients 1–7 survived, and levels of individual and total serum FFA values at their complete recovery were averaged to give the mean recovery values. Patients 8–11 and 13 did not survive. Patients 1–5 and 8–10 were treated with exchange transfusions, and patients 6, 7, and 11 were monitored for intracranial pressure and treated with sedation and hyperventilation. Fatty acid analyses were made only once during the course of the disease in patients 12–17. Table 1 also shows that the mean values of individual FFA as well as the total FFA were much higher before or during treatment for RS than they were after recovery and that all the major acids present in FFA showed this phenomenon.

In survivors, recovery levels of serum FFA were significantly lower ($P < 0.001$) than initial levels (Table 2). FFA levels were uniformly lower at the end of the first exchange transfusion than prior to the transfusion ($P < 0.02$). Nonsurvivors had significantly lower initial values, and their mean values for FFA increased rather than decreased after exchange transfusion. In these patients, levels just before death varied from extremely

Table 2. Serum total FFA in response to treatment in RS

Patient	FFA, μg/ml				
	Initial	1st exchange transfusion		After recovery*	Highest
		Start	End		
Survivors:					
1	359.1	359.1	274.0	104.5	359.1
2	286.4	423.1	279.4	176.1	423.1
3	457.0	526.9	373.5	197.2	526.9
4	377.6	415.1	162.2	52.9	415.1
5	403.4	403.4	337.5	115.0	403.4
6	187.2	†	†	116.5	187.2
7	546.9	†	†	138.1	546.9
Mean	373.9	425.5	285.3	128.5	408.8
Nonsurvivors:					
8	173.5	173.5	632.8	57.0	856.0
9	206.9	297.9	440.5	800.7	800.7
10	152.0	152.0	87.0	87.0	147.0
11	510.0	†	†	69.5	510.8
Mean	260.8	207.8	386.8	253.6	578.6

* In nonsurvivors, the value at death.
† Patients not treated with exchange transfusion.

high to very low, indicating that the high FFA concentration was not the immediate cause of death.

PUFA Patterns. For the 10 most recently studied cases of RS, serum lipids were analyzed at the Hormel Institute by a method of GLC designed for separation and measurement of PUFA, and these cases were used for comparisons involving PUFA in FFA (Fig. 1). In the samples from these patients, the fatty acids with chain length less than 12:0 were not detected, but 12:0 was relatively more plentiful in RS than in the control population (0.41 ± 0.08 vs. 0.04 ± 0.03%; $P < 0.001$). The 18:2ω6 was significantly lower in the RS patients than in the controls, but the PUFA derived from it were present in significantly higher proportions than normal. Other increases were: 20:2ω6, 3-fold; 20:3ω6, 5-fold; 20:4ω6 (arachidonic acid), 2-fold; 22:4ω6, 10-fold; and 22:5ω6, 10-fold. These acids together comprised 3.3% of the FFA in patients with RS, and this was a 3-fold increase over the control group. Many of these acids are often undetectable in serum FFA from normal controls (17). The normally undetectable 20:3ω9 increased to 1.35% of the FFA, but 20:5ω3 (eicosapentaenoic acid) decreased to 9% of its usual values. That is, although generally there was an increase in PUFA content of FFA, not all PUFA took part in the increase, skewing the pattern of the PUFA present in the FFA pool. The fatty acid pattern of serum TG closely resembled that of FFA.

PUFA patterns of serum PL in patients with RS and in normal children are compared in Fig. 2. The PUFA pattern of this lipid class was grossly abnormal in children with RS, and it was different from that of FFA and of TG. Serum PL have been analyzed most commonly for evaluation of essential fatty acid status, because PLs are a major structural lipid in membranes, because the polyunsaturated essential fatty acids are more abundant in this serum lipid class, and because the responses to nutritional or metabolic deficiencies of essential fatty acids cause greater changes in PL than in FFA, CE, or TG (10). In serum PL from RS the most significant changes from control values were the decreases in 18:3ω6 (a Δ6 desaturase product), 20:3ω6, and 22:4ω6, all of which were increased in FFA. Thus, at least some of the PUFA present in the FFA may have come from the serum PL through increased phospholipase action. The fatty acid patterns of serum CE in patients with RS were relatively normal.

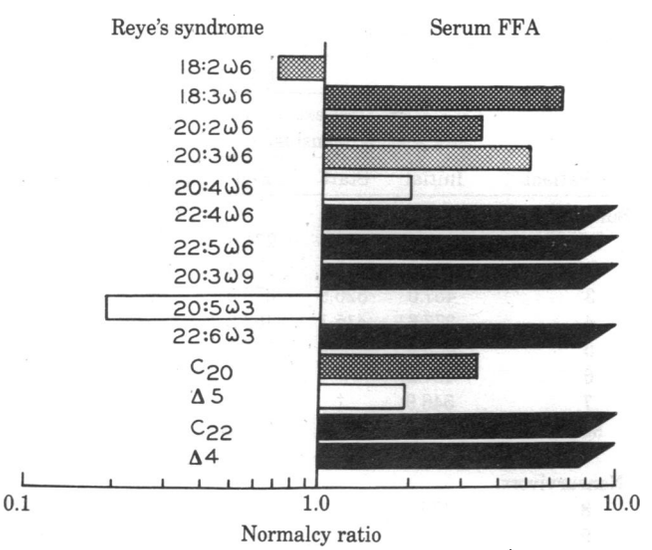

FIG. 1. PUFA profile for serum FFA in RS. Normalcy ratio is the observed value divided by the control value; the ratio is plotted on a logarithmic scale. Black bars, $P < 0.001$; dark crosshatch, $P < 0.01$; light crosshatch, $P < 0.05$; open bars, not significant. Pointed bars extend beyond limits of the chart.

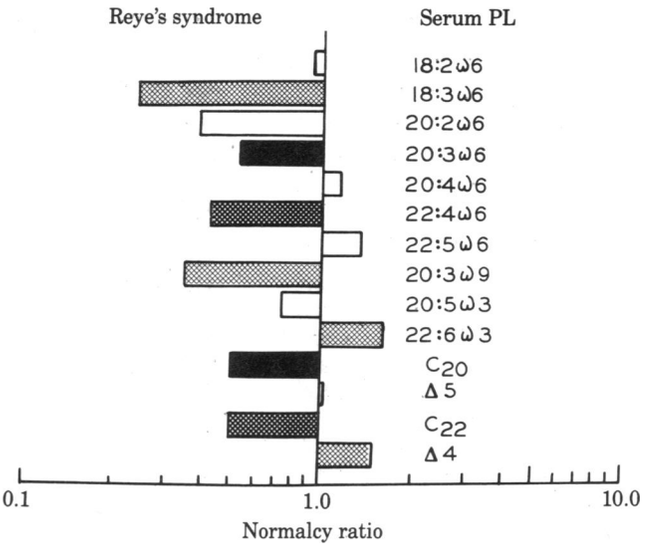

FIG. 2. PUFA profile for serum PL in RS.

DISCUSSION

Increases in serum FFA associated with encephalopathy and fatty degeneration of the viscera in children have been reported by Bourgeois *et al.* (5). Brown *et al.* reported a case of RS in which FFA were increased 2- to 3-fold initially, but after treatment with intravenous glucose and insulin the patient recovered and FFA levels decreased to normal (1). In another reported (3) case of RS, after exchange transfusion there was a remission of symptoms, and at recovery the serum FFA value had decreased to 1/4 the value. In the present study, the seven patients who survived had decreases of total serum FFA from a mean of 408.8 μg/ml initially to 128.5 μg/ml at complete recovery ($P < 0.001$).

Acute liver dysfunction may be the cause of increased FFA in RS. Total hepatectomy in dogs leads to convulsions and death in 3–8 hr (18), and Zieve *et al.* (19) have found a 2-fold increase in short-chain FFA in hepatectomized dogs maintained with intravenous glucose for at least 24 hr. In patients with acute hepatitis, total serum FFA and short-chain FFA are increased compared to control patients (20, 21).

Hepatic dysfunction may cause an increase in FFA; conversely, an increase in FFA may injure hepatocytes (1, 22). Administration of FFA to rabbits quickly causes tachypnea and loss of consciousness, and postmortem findings include fatty infiltration of the liver (23). FFA inhibit mitochondrial metabolism by uncoupling oxidative phosphorylation at low concentrations and by inhibiting oxidation of β-hydroxybutyrate and succinate at higher concentrations (24). Swelling of liver mitochondria *in vitro* has been induced by FFA of various chain lengths, and the most potent agents studied were lauric (12:0), myristic (14:0), and oleic (18:1) acids (9). Liver mitochondrial injury induced by FFA may play an important part in RS; three separate studies have shown mitochondrial swelling to be present in liver biopsies from patients with RS, as judged by electron microscopy (8, 25, 26).

Our results confirm reports that individual FFA and total FFA are increased in RS (Table 1). Levels of FFA that were increased initially were significantly lower at the time of recovery in the patients who survived. Unlike other investigators (4), however, we could not correlate the severity of the disease with the initial concentrations of FFA. We did find the significant increases in the 12:0 described by Mamunes *et al.* (7) but

did not observe increases in shorter chain acids. All the major fatty acid components of the FFA participated in the increase during RS, although for several of them the increase was not significant.

The importance of long-chain fatty acids to the increase of FFA is apparent from the analysis of the 10 most recent samples for their individual PUFA contents (Fig. 1). The ratio of the content of each fatty acid in FFA during the episode of RS to the content after recovery was 1.69 for 12:0, 1.98 for 14:0, 2.18 for 16:0, 1.90 for 16:1, 2.87 for 18:0, 3.7 for 18:1, 2.28 for 18:2ω6, and 1.81 for 20:4ω6. The arachidonic acid content during RS was extremely variable, ranging from not detectable to 16.4 μg/ml and making the difference between RS and recovery nonsignificant. Our RS patients had 2.53-fold increases in total FFA concentration ($P < 0.002$) and, in addition, had increases in the proportions of long-chain ω6 acids within the FFA which varied from 2- to 10-fold. Thus, some of the PUFA increased in concentration as much as 25-fold (i.e., 22:4ω6 and 22:5ω6) whereas 20:5ω3 decreased to half of the control concentration. These drastic changes in the metabolic pool from which PUFA are converted to prostaglandins and other biologically active oxidation products could have profound effects upon prostaglandin balance and therefore upon metabolic control. The abnormal patterns seen in FFA were reflected in the TG pattern, because TG are derived from the circulating FFA.

The altered patterns of PUFA in PL were not those of an essential fatty acid deficiency but rather indicated an altered metabolism of PUFA, perhaps caused by cell injury. Lipolytic injury to the liver or to the central nervous system, both of which are rich in PUFA, could explain the decrease of ω6 acids in serum PL and their increase in serum FFA. The patterns of PUFA in PL and FFA would probably be altered as observed if phospholipase A_2 were released during the onset of RS and if tissue or serum PL were involved in the rapid release of PUFA into the FFA pool. Recently, Malewicz *et al.* (27) observed that, during invasion of baby hamster kidney cells by dengue virus, a marked transient increase in phospholipase A_2 occurred. The transient nature of the marked increase in FFA in RS is evident in survivors. The long-chain ω6 acids which occur predominantly at the 2 position of PL, and which in free form are the immediate precursors of prostaglandins and other biologically active oxidation products, were found to be decreased significantly in serum PL and to be increased significantly in the FFA (Figs. 1 and 2) in RS. Prostaglandins released by membrane disruption have been linked to the development of experimental brain edema (28). Therefore, we postulate that the viral infection which precedes RS releases phospholipase A_2, which in turn releases PUFA from tissue PL into the FFA pool, stimulating prostaglandin synthesis and the consequent changes in metabolic control, leading to liver and brain dysfunction.

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