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Fatty Acid Composition of Serum Lipids in Diabetic Children and their Matched Healthy Controls

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ABSTRACT

The fatty acid compositions of the serum cholesterol esters, phospholipids and triglycerides were determined in 28 insulin-dependent diabetic children and 13 healthy controls. The diabetic children were on a regulated diet and the disease was under good control. The relative contents of linoleic and arachidonic acids were higher in the serum lipids of the diabetics than in the controls as could be expected from the dietary advice given. However, the degree of diabetic control was not significantly correlated to the fatty acid content of any lipid fraction. The serum concentration of apolipoprotein (apo) A-I was directly correlated to the content of polyunsaturated fat and the ratio between polyunsaturated and saturated fatty acids in the triglycerides. The apo A-II concentration was significantly correlated to the ratio between homo-gamma-linolenic and arachidonic acid in all serum lipid esters. No similar relationships were seen among the healthy controls. The different relationships between serum apolipoprotein concentrations and the fatty acid composition in serum in diabetics and controls is compatible with the hypothesis that not only the quantity but also the quality of the serum lipoproteins are different in these two categories of children.

INTRODUCTION

Juvenile onset, insulin-dependent diabetics show an increased incidence of early atherosclerotic cardiovascular disease. An increased amount of polyunsaturated fatty acids (PUFA) in the diet contributes to a reduction of atherosclerotic cardiovascular disease, at least in nondiabetics (3, 14, 16). A recent study suggests that an increased intake of PUFA decreases the incidence of vascular complications in adult onset diabetics (10). The mechanism for this beneficial effect is not clear. The polyunsaturated fatty acid arachidonic acid (20:4) is the precursor of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), and eicosapentaenoic acid (20:5) is the precursor of the corresponding TXA_3 and PGI_3. In escimoes a very low incidence of atherosclerosis was associated with a change in serum fatty acid pattern in favour of 20:5 and a subsequent change in PG/TX formation in favour of TXA_3/PGI_3 (5).

Increased platelet aggregation associated with a disturbed prostaglandin/thromboxane formation has been shown in diabetics with vascular disorder (8). We have recently shown that diabetic children have a diminished vascular response to post-ischaemic hyperaemia (6). This response is to a major part dependent on PGI_2 formation (6). A first step in studying these interactions is a description of the serum fatty acid composition in a group of diabetic children without atherosclerosis or diabetic vascular disease compared with the pattern in healthy matched controls, which is the aim of this work.

SUBJECTS

The study comprised 28 children aged 4 to 17 years (17 girls and 11 boys) with insulin dependent diabetes mellitus (IDDM). The height, body weight and growth velocity were in all cases within ± 2 SD for Swedish children (12). None of the children had albuminuria, hypertension or ophthalmological or other clinical signs of vascular disease. They were being treated with two doses of intermediate monocomponent porcine insulin daily. Some of them were also receiving a rapid monocomponent insulin in the morning and/or afternoon. Their total daily insulin dosage was 0.5 - 1.2 IU/kg body weight. They were all treated according to the same general principles, including prescriptions of regulated diet, aiming at a fat consumption of 30-33% of the energy intake and a P/S (polyunsaturated/saturated) ratio of about 0.5. Judging from clinical and laboratory findings the diabetic disease was under good control (haemoglobin A_1 10.8 ± 1.8%, fasting serum glucose 9.6 ± 0.5 mmol/l, serum triglyceride concentations (TG) 0.72 ± 0.23 mmol/l, and cholesterol concentrations (chol) 4.59 ± 0.78 mmol/l. Thirteen of the diabetic children had peers of the same age and sex, living in the same area and most often attending the same class at school, participating as matched healthy controls. This study is part of an investigation approved by the Ethical Committee of the Medical Faculty of the University of Uppsala.

METHODS

All blood specimens were taken fasting at 08.00-08.30 in the morning before breakfast and insulin injection. Serum glucose and HbA_1 were determined by routine methods (Yellow Spring Instruments 23 AM glucose analyzer and HbA_1 microcolumns, Bio-Rad Laboratories). TG and chol in serum were determined by semiautomated methods in an LKB system (2071, 2074, 2086), using commercial test kits (Test Combination from Boehringer-Mannheim and Nyco-test cholesterol from Nyegaard). The concentrations of serum apolipoproteins (apo) B, A-I and A-II were determined by electroimmunoassay using monospecific antibodies as earlier described in detail (21).

The fatty acid compositions of serum triglycerides (TG) cholesterol esters (CE) and phospholipids (PL) were determined in blood serum. The blood samples were allowed to coagulate for two hours and serum was collected by low speed centrifugation at $+4^{o}C$. The

serum lipids were extracted with chloroform-methanol and separated by thin layer chromatography according to a method previously described (1). The fatty acids of the isolated lipid fractions, triglycerides, cholesterol esters and phospholipids, were transesterified to methyl esters (17) and separated isothermally at 185°C on a 10% SP-2340 column in a Pye Unicam 104 gas-liquid chromatograph equipped with an automatic sample injector (Hewlett Packard Co.) Retention times of known fatty acid standards (Supelco inc.,) were used for identifying the peaks of the chromatogram (9). The relative compositions of the different identified fatty acids (expressed in %) and their retention times were calculated by a HP 3380A digital integrator.

The statistical calculations were performed by the SAS statistical program package on IBM 155-158 at Uppsala University. In comparisons between diabetics and non-diabetics only patients with matched controls were included and tested against their controls in two-tailed paired t-tests. When correlations within the diabetic group were performed, all the 28 diabetic subjects were included.

RESULTS

TABLE 1: Relative concentration of fatty acids and P/S ratios in serum lipid esters of 13 diabetic children compared with their matched healthy controls (mean ± SD), (* = $p<0.05$, ** = $p<0.01$).

	CE		TG		PL	
	Controls	Diabetics	Controls	Diabetics	Controls	Diabetics
14:0	0.8±0.2	0.7±0.2	2.4±0.8	1.7±0.7	0.4±0.1	0.3±0.1
16:0	11.9±1.9	11.4±1.0	26.5±5.3	25.3±1.9	32.8±4.4	32.7±4.0
16:1	3.5±1.02	2.9±0.4*	5.1±1.4	4.6±0.9	0.4±0.4	0.5±0.3
18:0	1.0±0.2	1.0±0.3	4.6±1.5	4.6±0.9	16.3±2.2	15.5±2.3 *
18:1	20.6±3.0	18.0±1.6 **	43.0±2.7	41.2±2.4	13.7±1.8	12.3±1.4 **
18:2	56.4±5.9	59.9±3.0 *	15.5±4.9	19.3±2.9 *	24.1±4.1	25.6±3.5 *
18.3	1.1±1.1	0.7±0.8	1.7±0.7	1.8±0.5	0.8±0.5	0.5±0.3**
20:3	0.8±0.7	0.7±0.7	0.4±0.7	0.2±0.3	4.3±1.5	4.6±0.6
20:4	3.6±1.4	4.2±1.3*	0.8±0.5	1.2±0.6**	6.4±1.7	7.3±1.8**
20:5	0.5±0.5	0.7±1.1	0.0±0.1	0.0±0.0	0.9±1.4	0.7±0.7
P/S	4.5±1.0	5.0±0.6*	0.5±0.2	0.7±0.1	0.6±1.1	0.7±0.1**

The fatty acid composition of CE, TG and PL of the diabetic children and their controls are given in Table 1. The most striking differences between diabetics and controls were found within the long chained unsaturated fatty acids. The linoleic acid (18:2) and 20:4 content in all lipid fractions were significantly higher in the diabetics than in the controls.

18:2 in CE was thus about 60% in diabetics compared with 56% in the controls. On the other hand, oleic acid (18:1) and linolenic acid (18:3) were significantly lower in diabetics in CE and PL. The 18:2/18:1 ratio was significantly higher in diabetics in all serum lipid esters. The P/S ratio was higher in PL and CE.

The degree of diabetic control, measured as HbA_1, was not significantly correlated to the fatty acid content of any lipid fraction.

The concentration of apo A-I in serum of the diabetic children was significantly positively correlated to the content of homo-gamma-linolenic acid (20:3), 20:4 and the ratio 18:2/18:1 and negatively correlated to the content of 18:1 in the triglyceride fraction while no such correlation was seen in CE or PL. The concentration of serum apo A-II was directly correlated to the content of in 20:3 TG and to the ratio 20:3/20:4 in CE and TG. The concentrations of serum triglycerides were inversely correlated to the content of 20:4 in PL and directly correlated to the 20:3/20:4 ratio in the same fraction. The serum cholesterol was negatively correlated with the content of 20:5 in PL. Table 2.

Within the group of healthy controls there were no significant correlations between the apo A-I concentration and any fatty acid concentration . The serum concentration of apo A-II and apo B were significantly negatively correlated to the content of 14:0 in TG.

TABLE 2: Correlations between plasma lipid and apolipoprotein concentrations and the relative concentration of some fatty acids in serum lipid esters of 28 diabetic children.Only the significant correlations are presented..

TG	vs	PL	20:4	r = -0.52	p <0.01
		PL	20:3/20:4	r = +0.56	p <0.01
Chol	vs	PL	20:5	r = -0.53	p <0.01
Apo A-I	vs	TG	18:1	r = -0.55	p <0.01
			20:3	r = +0.54	p <0.01
			20:4	r = +0.50	p <0.01
			18:2/18:1	r = +0.51	p <0.01
apo A-II	vs	CE	20:3/20:4	r = +0.60	p <0.001
		TG	20:3	r = +0.62	p <0.001
		TG	20:3/20:4	r = +0.57	p <0.01

DISCUSSION

A regulated diet is the basis for all treatment programmes for diabetes mellitus. During recent years the high fat, low carbohydrate diet has been abandoned in favour of a diet containing a high amount of complex carbohydrates with a relatively low fat content. With regard to the dietary fatty acids the relative content of polyunsaturated fats should be moderately increased (15).

The healthy controls showed a proportion of linoleic acid which was similar to that

earlier reported in an urban population of healthy Swedish children (13). The higher content of PUFA in the serum lipid esters in the diabetic children reflects the dietary advice given. The fatty acid content of the triglycerides merely mirrors the fat composition of the diet during the days preceding the test, while the change of the fatty acid pattern in the cholesterol esters and phospholipids is more gradual over a period of several weeks (2).

In the present study there was no significant correlation between the degree of diabetic control and the fatty acid pattern in the serum lipid esters. Assuming that the P/S-ratio in the serum lipids is correlated to the dietary adherence, this indicates that there was no significant relationship between the degree of dietary adherence and the degree of diabetic control. However, this does certainly not mean that the dietary adherence is of no importance for the diabetic control. Rather, the lack of correlation may be due to the fact that the diabetic control is influenced not only by the fat quality of the diet but also by other properties of the diet as well as by the administration of exogenous insulin.

A new finding was the positive relationship between the serum concentration of apo A-I and the relative content of polyunsaturated fat and the P/S-ratio in the serum triglycerides. Apo A-I is the major protein component of the high density lipoproteins (11). Insulin dependent diabetic children have higher A-I concentrations than matched healthy controls (7). In spite of high HDL levels the incidence of atherosclerotic vascular disease in diabetics is high. It has been postulated that the HDL composition may be qualitatively different in diabetics compared with that of healthy controls (4). The positive correlation between the apo A-I concentration and the content of polyunsaturated fat in TG may indicate such a difference. In non-diabetics a high intake of polyunsaturated fats has been shown to decrease the serum apo A-I levels, at least during short term dietary treatment (19, 20). The lack of correlation between the serum apo A-I values and the P/S-ratio in the other serum lipid esters in this study indicates that the A-I concentration was not related to the intake of polyunsaturated fats during longer time periods. The positive correlation between A-I and the content of PUFA in the serum triglycerides indicates a positive relationship between the polyunsaturated dietary fat intake during the last days before the sample was taken and the serum apo A-I levels. Possibly a high intake of PUFA mirrors a high total fat intake. Introduction of neutral fat in the intestine induces synthesis of apo A-I in the intestinal cells (18).

We have earlier described a positive relationship between the serum apo A-II concentration and the serum triglyceride concentration in diabetic children (7). The present study shows that both A-II and TG are directly correlated to the ratio 20:3/20:4 in the serum lipid esters. The TG concentration was inversely correlated with 20:4 in TG. We have no explanation for these findings or for the negative correlation between the serum cholesterol concentration and the 20:5 content in PL.

One main finding in the present study was the confirmation of the difference between diabetic children and healthy controls with regard to the relationships between serum apolipoprotein concentrations and serum lipid composition. We have earlier shown

diverging relationships in insulin treated diabetic children and healthy controls between serum apolipoprotein concentrations and serum lipid concentrations (7). This study shows that the two groups differ also with regard to the relationships between serum apolipoprotein concentrations and the fatty acid composition of the serum lipid esters. This is compatible with the view that not only the quantity but also the quality of the serum lipoproteins are different in these two categories of children (4).

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