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Composition of Plasma Fatty Acids and Non-Cholesterol Sterols in Anorexia Nervosa

A. ŽÁK, M. VECKA, E. TVRZICKÁ, M. HRUBÝ, F. NOVÁK, H. PAPEŽOVÁ[1], H. LUBANDA, L. VESELÁ, B. STAŇKOVÁ

Fourth Department of Medicine and [1]Department of Psychiatry, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Anorexia nervosa is a model of simple starvation accompanied by secondary hyperlipoproteinemia. The pattern of plasma fatty acids influences the levels of plasma lipids and lipoproteins. The concentration of plasma lathosterol is a surrogate marker of cholesterol synthesis *de novo*, concentrations of campesterol and β-sitosterol reflect resorption of exogenous cholesterol. The aim of the study was to evaluate fatty acids in plasma lipid classes and their relationship to plasma lipids, lipoproteins, cholesterol precursors and plant sterols. We examined 16 women with anorexia nervosa and 25 healthy ones. Patients with anorexia nervosa revealed increased concentrations of total cholesterol, triglycerides, HDL-cholesterol, campesterol and β-sitosterol. Moreover, a decreased content of n-6 polyunsaturated fatty acids was found in all lipid classes. These changes were compensated by an increased content of monounsaturated fatty acids in cholesteryl esters, saturated fatty acids in triglycerides and both monounsaturated and saturated fatty acids in phosphatidylcholine. The most consistent finding in the fatty acid pattern concerned a decreased content of linoleic acid and a raised content of palmitoleic acid in all lipid classes. The changes of plasma lipids and lipoproteins in anorexia nervosa are the result of complex mechanisms including decreased catabolism of triglyceride-rich lipoproteins, normal rate of cholesterol synthesis and increased resorption of exogenous cholesterol.

Key words

Anorexia nervosa • Plasma lipids • Lipoproteins • Lathosterol • Fatty acids • Plant sterols

Introduction

Anorexia nervosa is defined as a psychiatric disorder of unknown etiology, characterized by a diminished intake of food, pathological pursuit of slimness, and severe weight loss. Concomitantly, this disorder represents a pathophysiological model of simple starvation that is accompanied by a variety of endocrine and metabolic abnormalities.

More than half of the patients with anorexia nervosa may be hypercholesterolemic. Secondary hyperlipoproteinemia with lipoprotein phenotype IIa has been described by several authors (Mordasini *et al*. 1978, Mira *et al.* 1987, Gotto and Pownall 1999).

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Pathophysiological mechanisms underlying hypercholesterolemia in anorexia nervosa are not yet completely understood; these can include increased synthesis of cholesterol, especially of LDL, as well as decreased catabolism (Thompson 1990).

Patients with anorexia nervosa have shown a deficiency in polyunsaturated fatty acids (PUFA) of the n-6 family with compensatory changes in non-essential fatty acids (FA) and decreased fluidity of plasma lipids (Holman *et al.* 1995). The composition of fatty acids in the plasma and adipose tissue has generally been used as surrogate markers of dietary fat intake (Ma *et al.* 1995). Many studies described the effects of FA composition in dietary fat on plasma total cholesterol (TC) and triglycerides (TG), respectively, on their distribution in individual lipoproteins (LP) (Katan *et al.* 1995).

Subjects with anorexia nervosa showed PUFA deficiencies in plasma phospholipids (PL), which were different from those in persons with a simple nutritional deficiency of essential fatty acids. Patients with anorexia nervosa revealed significantly lower n-6 and n-3 elongation and desaturation products, and elevated short-chain saturated, short-chain monounsaturated, branched-chain and odd-chain fatty acids in plasma phospholipids. These changes indicated enhanced biosynthesis of alternative fatty acids that were only partially able to compensate for the loss of PUFA in the maintenance of membrane "fluidity" (Holman *et al.* 1995).

Plasma concentrations of mevalonic acid and lathosterol, and their relation to plasma TC have been shown to correlate closely with direct measurements of whole body cholesterol synthesis in men and also in some experimental animals (Kempen *et al.* 1988, Riches *et al*. 1997). Only one study has dealt with the relationship between levels of plasma lathosterol and the parameters of lipid metabolism in anorexia nervosa (Feillet *et al.* 2000).

Concentrations of plant sterols (campesterol and β-sitosterol) reflect resorption of exogenous cholesterol, thus being surrogate markers of exogenous cholesterol intake (Watts *et al.* 2002). Relationships between FA composition in individual lipid classes and levels of plasma lipids, lipoproteins and cholesterol precursors in anorexia nervosa has not yet been described.

The aim of our study was to evaluate FA in plasma cholesteryl esters (CE), TG and phosphatidylcholine (PC) in women with anorexia nervosa, and their relationship to plasma lipids, lipoproteins, apolipoproteins (apo), and non-cholesterol sterols.

Methods

Experimental subjects

The studied group consisted of 16 women with anorexia nervosa and 25 sex- and age-matched controls. The patients and controls did not use any drugs known to affect lipid and lipoprotein metabolism, vitamins, antioxidants and supplements enriched in n-3 and n-6 PUFA. None of them took any form of hormonal contraception. Neither family history of premature coronary heart disease, nor familial hyperlipoproteinemia were detected. Fully informed written consent was obtained from all participants after the institutional ethical committee had approved the study protocol.

Anorexia nervosa was diagnosed using criteria required by the Diagnostic and Statistical Manual, 4th edition (APA 1994). Eleven women had a restrictive form of anorexia nervosa, four had a combined form and one woman revealed a purgative type of anorexia nervosa. Anthropometric measurements (body weight, height, waist, hip, midarm muscle circumference, and four skinfold thickness) were carried out in all subjects (Lochman *et al*. 1989).

The body density and percentage of body fat were estimated on the basis of the sum of four skinfold thickness measurements (biceps, triceps, subscapularis, suprailiac) and using age-specific prediction equations (Durnin and Womersley 1974). The fat mass was obtained by multiplying the percentage of body fat by body weight.

Blood samples

Blood was sampled by venepuncture after an overnight fasting. Blood samples, containing EDTA (1 mg/ml), were immediately cooled (4-6 °C); serum and plasma was separated within 30 min at the same temperature (1000 x *g*, 10 min). Samples for routine laboratory analyses were processed immediately, for other analyses were stored at –70 °C until assayed.

Lipid and apolipoprotein analyses

Concentrations of plasma TC and TG were measured using enzymatic-colorimetric methods (Boehringer, Mannheim, Germany). Concentrations of cholesterol in high-density lipoprotein (HDL-C) were determined in the supernatant after precipitation of lipoproteins-B with PTA/Mg^{2+} using a kit of the same producer; those of LDL-C were calculated (Friedewald *et al.* 1972). Concentration of non-esterified fatty acids

(NEFA) was determined using an enzymatic-colorimetric method (NEFA, Randox Laboratories, U.K.). Concentrations of apo were measured by Laurell rocket electroimmunoassay, using standards and specific antibodies [apo B, apo A-I, apo A-II (Behring Werke AG, Marburg, Germany), Lp[a] (Immuno AG, Wien, Austria)]. Profiles of FA in main lipid classes of plasma CE, TG, PC) and plasma non-cholesterol sterols (cholestanol, lathosterol, campesterol, β-sitosterol) were analyzed by capillary gas chromatography as described previously (Agren *et al.* 2001, Tvrzická *et al.* 2002).

Table 1. Demographic data of anorexia nervosa and control group.

	Control group (n = 25)	Anorexia nervosa (n = 16)
Age (years)	22.5 ± 0.6	22.4 ± 1.1
Duration of anorexia (years)	NA	3.1 ± 0.6
Body weight (kg)	58.0 ± 1.7	42.7 ± 2.7***
Ideal body weight (per cent)	96.5 ± 2.0	68.8 ± 2.9**
Weight loss from usual body weight (kg)	NA	2.3 - 17.0[b]
Change in body weight (% in last 3 months)	NA	5.5 ± 0.6
Body mass index (kg/m^2)	20.9 ± 0.4	15.4 ± 0.7***
Percentage of fat mass (anthropometric)	25.2 ± 1.0	15.1 ± 1.5***
Fat mass (kg)	14.8 ± 0.9	5.3 ± 1.0***
Lean body mass (kg)	43.1 ± 1.2	37.4 ± 1.9**
Sum of four skinfold thickness [a] (mm)	48.3 ± 2.8	16.2 ± 2.3***
Waist (cm)	69.4 ± 1.1	57.7 ± 1.4**
Waist-to hip circum-ference (ratio)	0.77 ± 0.02	0.73 ± 0.01*
Midarm muscle circumference (cm)	20.21 ± 0.3	17.5 ± 0.6***

Data are mean ± S.E.M. [a] the sum of four skinfold thickness (biceps, triceps, subscapularis, suprailiac); [b] range. NA – non-applicable. Significantly different (Mann-Whitney U-test) from controls: *P<0.05; **P<0.01; ***P<0.001.

Routine laboratory tests

Serum concentrations of glucose, total protein, albumin, creatinine, liver function tests (total bilirubin concentration, activity of ALT, AST, GMT), urea, and creatinine were determined using commercially available kits using an automatic analyzer HITACHI (model 717, Tokyo, Japan).

Statistical analysis

Data expressed as mean ± S.E.M. were evaluated using non-parametric test (Mann-Whitney U-test). The differences between anorexia nervosa and controls for all FA in individual lipid classes were analyzed by the multiple Hotelling´s t-test. All statistics were calculated using commercial software Statistica (Tulsa, OK, U.S.A, StatSoft, Inc., 1999).

Results

The women with anorexia nervosa show typical features of undernutrition – significantly lower body weight, BMI, percentage of body fat, absolute fat mass, and midarm muscle circumference (all P<0.001, Table 1). There were no significant differences in the concentrations of plasma total protein, prealbumin, and albumin. Only the activities of serum cholinesterase were significantly decreased in anorexia nervosa, but remained within the physiological range. Slightly, but significantly elevated liver function tests (ALT, AST, GMT) were found in anorexia nervosa (Table 2).

Table 2. Biochemical parameters of anorexia nervosa and control group.

	Control group (n = 25)	Anorexia nervosa (n = 16)
Glucose (mmol/l)	4.67 ± 0.06	4.60 ± 0.14
Bilirubin (μmol/l)	11.4 ± 0.8	10.2 ± 1.0
ALT (μkat/l)	0.34 ± 0.03	0.76 ± 0.29*
AST (μkat/l)	0.45 ± 0.06	0.71 ± 0.20**
GMT (μkat/l)	0.23 ± 0.01	0.37 ± 0.07**
ALP (μkat/l)	1.12 ± 0.03	1.24 ± 0.15
Total plasma protein (g/l)	79.3 ± 0.7	77.4 ± 2.6
Albumin (g/l)	46.2 ± 0.4	45.2 ± 1.3
Prealbumin (g/l)	0.28 ± 0.01	0.22 ± 0.09
Cholinesterase (μkat/l)	157.3 ± 3.8	140.3 ± 8.9*
Urea (mmol/l)	4.1 ± 0.2	4.4 ± 0.4
Creatinine (μmol/l)	83.4 ± 1.6	82.4 ± 2.8
Uric acid (μmol/l)	251.9 ± 8.1	235.8 ± 22.1

Data are mean ± S.E.M., significantly different (Mann-Whitney U-test) from controls: *P<0.05; **P<0.01; ***P<0.001.

Table 3. Concentrations of plasma lipids, non-cholesterol sterols, lipoproteins and apolipoproteins.

	Control group (n = 25)	Anorexia nervosa (n = 16)
Total cholesterol (mmol/l)	4.51 ± 0.11	5.52 ± 0.41 ** [b]
Triglycerides (mmol/l)	0.85 ± 0.09	1.21 ± 0.08 ***
Phospholipids (mmol/l)	2.65 ± 0.07	3.15 ± 0.15 **
HDL-cholesterol (mmol/l)	1.22 ± 0.05	1.58 ± 0.05 ***
LDL-cholesterol (mmol/l)	2.91 ± 0.11	3.49 ± 0.50
non-HDL-cholesterol (mmol/l)	3.30 ± 0.11	4.04 ± 0.49
Apolipoprotein B (g/l)	0.69 ± 0.04	0.76 ± 0.05
Apolipoprotein A-I (g/l)	1.58 ± 0.04	1.78 ± 0.05
Lipoprotein [a] (g/l)	0.16 ± 0.05	0.22 ± 0.08
Non-esterified fatty acids (mmol/l)	0.44 ± 0.05	0.78 ± 0.09 ***
Lathosterol (μmol/l)	4.84 ± 0.53	3.70 ± 0.69
Campesterol (μmol/l)	14.50 ± 0.89	17.77 ± 2.69 *
β-sitosterol (μmol/l)	11.28 ± 0.46	17.46 ± 2.66 **
Cholestanol (μmol/l)	7.00 ± 0.41	7.38 ± 0.57
Lathosterol/total Cholesterol (ratio x 10^3)	1.05 ± 0.09	0.81 ± 0.16
Campesterol/total Cholesterol (ratio x 10^3)	3.20 ± 0.21	4.35 ± 0.59
β-sitosterol/total Cholesterol (ratio x 10^3)	2.51 ± 0.14	3.14 ± 0.41

Data are mean ± S.E.M., significantly different (Mann-Whitney U-test) from controls: *P<0.05; **P<0.01; ***P<0.001.

Women with anorexia nervosa had increased levels of plasma TC, TG, PL and HDL-C. The levels of LDL-C, apo B, apo A-I and Lp[a] did not differ significantly from the control group. Concomitantly, a rise in the levels of NEFA, campesterol and β-sitosterol was observed in the anorexia nervosa group. The concentration of lathosterol and the ratio of lathosterol/TC did not differ significantly between both groups, as well as the ratios of campesterol/TC and β-sitosterol/TC (Table 3). A significant correlation of lathosterol to TC was found only in the group of control women (r=0.537, P<0.01), but not in the group of anorexia nervosa (r=0.300, NS), as evaluated by Spearman's rank correlation coefficient.

The plasma FA composition in main lipid classes is shown in Table 4. There were significant differences between the anorexia nervosa and control group in overall FA profiles, as evaluated by the multiple Hotelling´s t-test, only in CE (P<0.05) and PC (P<0.05). Differences in the FA profile of TG did not reach statistical significance using Hotelling´s t-test. The most consistent observations in the anorexia nervosa group, evaluated by one-dimensional statistical analysis (Mann-Whitney U test), were a decreased content of linoleic acid (LA, 18:2n-6) and raised content of palmitoleic acid (POA, 16:1n-7) in all lipid classes. Moreover, increased content of oleic (18:1n-9) and vaccenic (18:1n-7) acids, and a decreased content of docosahexaenoic acid (DHA, 22:6n-3) were found in CE. The enhanced content of palmitic acid (16:0) and diminished content of hexadecenoic acid (16:1n-9) were observed in TG and PC. A lower content of stearic acid (18:0) was observed only in PC. Table 5 shows derived parameters of FA metabolism – the sums of saturated (SFA), monounsaturated (MFA) and PUFA contents and some FA product/substrate ratios that implicate activities of Δ9 desaturase (Δ9D), Δ6D, Δ5D, as well as the elongase activity. Patients with anorexia nervosa had increased content of SFA in TG and PC, increased content of MFA in CE and PC, and lower content of n-6 PUFA in all lipid classes. No changes were observed in n-3 PUFA. A rise in activity of Δ9D was observed in CE and PC. Decreased activity of Δ5D was observed in CE of anorexia nervosa group.

Discussion

Anorexia nervosa is an eating "disorder" of unknown origin that is characterized by many medical complications, including endocrine and metabolic ones which are of primary importance (Sharp and Freeman 1993). The endocrine complications associated with anorexia nervosa concerning hypothalamic hypogonadism, hypercortisolemia (despite normal levels of the adrenocorticotropic hormone), and abnormalities in thyroid function that comprise lowered triiodothyronine and thyroxine levels, and elevated reverse triiodothyronine concentrations (Mehler *et al.* 1997). It is generally accepted that each of the mentioned endocrinological disorders can be associated with secondary hyper- or dyslipoproteinemia (Thompson 1990, Gotto and Pownall 1999).

The observed findings in the levels and composition of plasma lipids and lipoproteins implicate

some pathophysiological mechanisms of dyslipidemia in anorexia nervosa. Increased levels of TC in anorexia nervosa are likely caused, besides the rise in HDL-C, by increased concentrations of TG-rich LP (VLDL and IDL). The rise in HDL-C concentration is probably the reflection of high TC levels and non-enzymatic distribution of cholesterol between all lipoprotein classes (HDL-C in control and anorexia nervosa comprised 27 and 29 % of TC, respectively). Differences in calculated LDL-C concentrations did not differ significantly from the controls. Inconsistent findings concerning lipid and LP metabolism in anorexia nervosa have been described. Some authors (Mordasini *et al.* 1978, Mira *et al*. 1987) reported secondary hypercholesterolemia due to an elevation of LDL-C in approximately half of the women with anorexia nervosa. On the other hand, Crisp *et al*. (1968) found nearly normal levels of plasma TC, only two persons out of twelve were hypercholesterolemic. Other authors (Arden *et al.* 1990) described elevated levels of HDL-C in anorexia nervosa. This disease can be divided into several groups not only according to the mechanisms of weight loss (Sharp and Freeman 1993), but also according to the relation with LP metabolism and lipid patterns (Case *et al.* 1999). In our study, we did not find any association between the type of anorexia nervosa (restrictive, purgative, combined) and dyslipidemia. Nevertheless, the number of our patients with purgative and combined types of anorexia nervosa was too low.

We did not find significant differences between the levels of lathosterol and the ratio of TC/lathosterol between the two groups. As these parameters reflect the rate of cholesterol synthesis *"de novo"* (Kempen *et al.* 1988, Riches *et al.* 1997), we suppose that the elevation of TC is caused by decreased catabolism of TC, likely due to decreased catabolism of VLDL *via* its conversion to IDL and LDL. Our results are consistent with those of Nestel (1973), who described decreased secretion of bile acids in anorexia nervosa, and with the results of Feillet *et al.* (2000), who presented elevated concentrations of plasma TC associated with a normal rate of cholesterol synthesis in anorexia nervosa.

Table 4. Fatty acid composition of plasma lipids

Fatty acid[b]	Cholesteryl esters		Triacylglycerols		Phosphatidylcholine	
	Control group	Anorexia nervosa+ [a]	Control group	Anorexia nervosa	Control group	Anorexia nervosa+[a]
14:0	1.03±0.07	0.87±0.10	1.62±0.16	2.00±0.20	0.27±0.02	0.29±0.03
16:0	10.71±0.26	11.65±0.42	21.73±0.67	24.75±0.66**[b]	25.90±0.34	28.11±0.52**
16:1n-9	0.79±0.03	0.68±0.05	0.98±0.03	0.81±0.04**	0.20±0.01	0.17±0.01
16:1n-7	2.37±0.15	3.37±0.34**	3.53±0.20	4.62±0.41**	0.62±0.03	0.84±0.07**
18:0	2.39±0.08	2.53±0.19	3.29±0.16	3.31±0.14	13.67±0.27	12.59±0.37
18:1n-9	16.93±0.39	20.76±0.98**	39.47±0.55	39.96±1.11	10.72±0.27	12.48±0.48*
18:1n-7	1.40±0.04	1.57±0.06*	3.19±0.13	3.35±0.19	2.18±0.05	2.18±0.07
18:2n-6	53.59±0.82	47.96±1.35***	20.03±0.96	15.16±0.90**	25.61±0.56	23.16±0.86*
18:3n-6	0.76±0.04	0.95±0.16	0.44±0.03	0.39±0.06	0.05±0.01	0.10±0.02
18:3n-3	0.76±0.03	0.78±0.04	1.69±0.09	1.45±0.18	0.34±0.02	0.35±0.02
20:1n-9	0.06±0.01	0.06±0.01	0.33±0.01	0.37±0.03	0.18±0.01	0.18±0.01
20:2n-6	0.15±0.01	0.13±0.01	0.36±0.02	0.35±0.02	0.43±0.01	0.48±0.03
20:3n-6	0.61±0.20	0.67±0.04	0.31±0.02	0.33±0.02	2.86±0.10	3.15±0.23
20:4n-6	6.93±0.27	6.09±0.31	1.46±0.08	1.33±0.15	10.82±0.30	10.16±0.55
20:5n-3	0.68±0.05	0.64±0.01	0.23±0.02	0.22±0.05	0.77±0.06	0.77±0.03
22:4n-6	0.01±0.01	0.02±0.01	0.18±0.01	0.17±0.02	0.39±0.01	0.40±0.02
22:5n-6	0.06±0.01	0.03±0.01	0.12±0.04	0.13±0.02	0.26±0.01	0.34±0.03
22:5n-3	0.15±0.02	0.13±0.02	0.41±0.02	0.37±0.04	1.06±0.05	1.06±0.08
22:6n-3	0.66±0.30	0.52±0.05*	0.56±0.05	0.43±0.05	3.58±0.19	3.12±0.23

Data are mean ± S.E.M. (in mol %), Mann-Whitney U test: * P<0.05,** P<0.01, *** P<0.001, [a] Hotelling´s multiple t-test: + P<0.05
[b] Short hand notation of fatty acids: number of carbon atoms: number of double bonds n-number of carbon atom from the methyl end to the nearest double bond.

Table 5. Derived parameters of fatty acid metabolism.

Derived indices	Cholesteryl esters		Triacylglycerols		Phosphatidylcholine	
	Control group	Anorexia nervosa[a]	Control group	Anorexia nervosa[a]	Control group	Anorexia nervosa[a]
ΣSFA	14.23±0.34	15.14±0.55	26.71±0.84	30.12±0.70[b]**	39.90±0.23	41.05±0.32**
ΣMFA	21.44±0.51	26.45±1.19***	47.50±0.74	49.11±1.19	13.90±0.31	15.86±0.52**
Σn-6PUFA	62.09±0.79	55.85±1.44***	22.90±1.00	18.29±1.03*	40.46±0.43	37.79±0.58**
Σn-3PUFA	2.24±0.08	2.07±0.13	2.89±0.14	2.47±0.23	5.74±0.23	5.30±0.25
16:1n-7/16:0[b]	0.21±0.01	0.29±0.03*	0.16±0.01	0.19±0.02	0.02±0.01	0.03±0.01*
18:1n-9/18:0[b]	7.23±0.27	8.74±0.65*	12.76±0.72	12.31±0.49	0.79±0.03	1.01±0.05***
18:3n-6/18:2n-6[c]	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01	ND	ND
20:3n-6/18:2n-6[d]	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.11±0.01	0.14±0.01
20:4n-6/20:3n-6[e]	11.61±0.53	9.54±0.77*	4.95±0.26	4.41±0.53	3.92±0.19	3.63±0.43
20:3n-6/18:3n-6[f]	0.84±0.04	0.92±0.12	0.77±0.07	1.18±0.14*	37.90±2.16	37.97±4.56
20:4n-6/18:2n-6[g]	0.13±0.01	0.13±0.01	0.08±0.01	0.09±0.01	0.43±0.02	0.46±0.05

Data are mean ± S.E.M., (in mol %), Mann-Whitney U-test:* P<0.05,** P<0.01,*** P<0.001; [a] Hotelling´s multiple t-test: * P<0.05 (calculated only for sums of SFA, MFA, PUFA) [b] Δ9 Desaturase (Δ9D), [c] Δ6D, [d] Δ6D+Elongase(E), [e] Δ5D, [f] E, [g] Δ6D+Δ5D+E, ND – not determined..

Increased levels of plant sterols (campesterol, β-sitosterol) in anorexia nervosa, which are surrogate markers of dietary cholesterol absorption (Watts *et al.* 2002), implicate that dyslipidemia in our patients may result partly from increased absorption of exogenous cholesterol. This assumption is supported by the fact that we did not find significant differences in levels of apo B, which reflects the number of VLDL, IDL and LDL particles. Increased resorption of exogenous cholesterol that is carried in chylomicrons and their remnant particles would probably be reflected by an increased level of apo B 48 (Gotto and Pownall 1999). The method used for apo B determination in our study is based only on apo B 100 measurement. Secretion rate of hypertriglyceridemic VLDL ($VLDL_1$) depends on the concentration of FFA and TG, as well as on the basal and post-load insulin concentrations. On the other hand, normotriglyceridemic VLDL ($VLDL_2$) secretion rate is more likely regulated by the hepatic availability of cholesterol and is closely correlated with mevalonic acid and lathosterol concentrations (Pietzsch and Julius 1999). Increased levels of plasma TG observed in anorexia nervosa group are probably also caused by decreased catabolism of VLDL and IDL. These results are consistent with findings of several studies (Feillet et *al.* 2000, Jaguenaud *et al*. 1996). Rise in plasma TG is likely due to decreased activity of lipoprotein lipase, as well as of hepatic triglyceride lipase (Mordasini *et al.* 1978, Jaguenaud *et al.* 1996). Putative decreased activity of apo B/E receptors, due to diminished levels of estrogens in anorexia nervosa (Campos *et al.* 1997), can also contribute to raised levels of TG as well as of VLDL and IDL. Arden *et al.* (1990) described dyslipidemia in anorexia nervosa with normal TC, increased TG and low HDL-C. In our study, the rise in the levels of Lp[a] in anorexia nervosa did not reach statistical significance.

The low levels of insulin that are achieved during prolonged starvation increase the levels of cAMP in the adipose tissue and therefore the activity of hormone-sensitive lipase responsible of triglyceride lipolysis and raised FFA levels. Other hormones such as glucagon, ACTH, TSH, and GH also increase adipose tissue lipolysis but they are of minor importance (Cahill 1970).

The most consistent finding of this study is the drop of LA content and rise of POA content in all lipid classes of anorexia nervosa group. Increased marker of Δ9D (measured as product/substrate ratio) was found in CE and PC. Linoleic acid represents the most buoyant PUFA in plasma lipids (Thompson 1990); POA is suggested as a surrogate marker of lipogenesis (Aarsland and Wolfe 1998). The activities of Δ9D and FA synthase were described to correlate tightly and significantly with the same metabolic and hormonal stimuli (Brenner 1989). In this context, increased TG levels are caused not only by decreased catabolism of TG-rich LP, but also by

raised lipogenesis. Both protein-energetic malnutrition (PEM) and anorexia nervosa represent nutritional and metabolic disorders connected with deficiency of essential FA (Holman *et al.* 1995, Leichsenring *et al.* 1995, Franco *et al.* 1999, Decsi and Koletzko 2000). Changes in FA pattern in anorexia nervosa and different types of PEM may be the consequences of different pathogenic factors or their combinations: (i) reduced intake of essential FA – LA, α-linolenic acid (ALA), (ii) impaired conversion of LA and ALA to their metabolites, (iii) increased requirements of long-chain PUFA for PL and eicosanoid synthesis, (iv) increased degradation of FA by β-oxidation and lipid peroxidation (Decsi *et al.* 1998).

The typical diet consumed by anorexia nervosa patients is low-caloric, especially low in fat, which results in a lower intake of both n-3 and n-6 PUFA families (Sharp and Freeman 1993, Holman *et al.* 1995, Mehler *et al.* 1997). Decreased concentrations of naturally occurring antioxidants and increased lipoperoxidation in different types of PEM have been repeatedly described (Sive *et al.* 1993, Lenhartz *et al.* 1998, Fechner *et al.* 2001). In our patients with anorexia nervosa, we found only a decreased content of LA with an unchanged content of ALA and other PUFA. Moreover, we did not find substantial changes in the elongation and desaturation of PUFA. It is known that LA, in comparison to ALA (and other n-3 PUFA), is more sensitive to lipoperoxidation (Yazu *et al.* 1996). We suggest that the decrease in LA content is preferentially due to increased oxidative degradation. Thus, reduced antioxidative capacity and decreased levels of estrogens may also contribute to this phenomenon in anorexia nervosa (Sack *et al.* 1994).

Our results are basically in agreement with those of Holman *et al.* (1995) for total SFA, MFA and PUFA in individual lipid classes, and products of Δ9D in CE and PL. The character of changes in individual FA is also similar, however, different changes were observed for LA, γ-linolenic acid and FA 20:2n-6. Both studies differed in the number and clinical characteristics of patients [n = 8 vs. 16, percentage of ideal body weight (range) 68-97 vs. 54-89, plasma lipids were not specified in Holman´s study] as well as in statistical evaluation (Student´s T-test vs. Mann-Whitney´s U test). In another study (Langan and Farrell 1999), 15 patients with anorexia nervosa were studied; their ideal body weight was between 62 and 98 kg. The authors found a decreased content of LA and DHA in PL. Patients with a loss of ideal body weight higher than 25 % had, besides a decreased content of LA, also an increased content of oleic acid and unchanged content of arachidonic acid (AA). Thus increased AA/LA ratio reflects a decreased content of LA rather than increased elongation and desaturation activity. The results of our study and those of both above mentioned studies (Holman *et al.* 1995, Langan and Farrell 1999) reveal only small differences in the content of individual FA. These differences may result from different number and clinical characteristics of patients, as well as from a different methodology and statistical evaluation. All the three studies deal with relative concentration of FA; elongation and desaturation activities are derived values, which would not reflect real concentrations and activities.

In conclusion, the changes of plasma lipids and lipoproteins in anorexia nervosa are likely associated with increased lipogenesis and absorption of exogenous cholesterol, decreased catabolism of TG-rich LP and normal rate of cholesterol synthesis. Changes in FA pattern are the result of complex mechanisms including low dietary intake of PUFA, probably increased lipoperoxidation and Δ9 desaturation due to nutritional and hormonal disturbances. Understanding of the problems of lipid and FA metabolism in anorexia nervosa will require a more detailed study, which should include more relevant parameters, e.g. absolute concentrations of FA, direct estimation of elongation and desaturation activities, direct estimation of lipoperoxidation markers, and polymorphism of genes controlling LP metabolism.

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References

AARSLAND A, WOLFE RR: Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. *J Lipid Res* **39**: 1280-1286, 1998.

AGREN, JJ, TVRZICKÁ E, NENONEN MT, HELVE T, HÄNNINEN O: Divergent changes in serum sterols during a strict uncooked vegan diet in patients with rheumatoid arthritis. *Br J Nutr* **85**: 137-139, 2001.

APA: *Diagnostic and Statistical Manual of Mental Disorders.* American Psychiatric Association, Washington, DC, 1994.

ARDEN MR, WEISELBERG EC, NUSSBAUM MP, SHENKER IR, JACOBSON MS: Effect of weight restoration on the dyslipoproteinemia of anorexia nervosa. *J Adolesc Health Care* **11**: 199-202, 1990.

BRENNER RO: Factors influencing fatty acid chain elongation and desaturation. In: *The Role of Fats in Human Nutrition.* AJ VERGROESEN, M CRAWFORD (eds), Academic Press, London, 1989, pp 45-79.

CAHILL GF: Starvation in man. *N Engl J Med* **282**: 668- 675, 1970.

CAMPOS H, WALSH BW, JUDGE H, SACKS F: Effects of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. *J Clin Endocrinol Metab* **82**: 3955-3963, 1997.

CASE T, LEMIEUX S, KENNEDY SH, LEWIS GF: Elevated plasma lipids in patients with binge eating disorders are found only in those who are anorexic. *Int J Eat Disord* **25**: 187-193, 1999.

CRISP AH, BLENDIS LM, PAWAN GL: Aspects of fat metabolism in anorexia nervosa. *Metabolism* **17**: 1109-1118, 1968.

DECSI T, KOLETZKO B: Effects of protein-energy malnutrition and human immunodeficiency virus-1 infection of essential fatty acid metabolism in children. *Nutrition* **16**: 447-453, 2000.

DECSI T, MOLNAR D, KOLETZKO B: The effect of under- and overnutrition on essential fatty acid metabolism in childhood. *Eur J Clin Nutr* **52**: 541-548, 1998.

DURNIN JV, WOMERSLEY J: Body fat assessed from the total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 71 years. *Br J Nutr* **32**: 77-97, 1974.

FECHNER A, BOHME CC, GROMER S, FUNK M, SCHIRMER RH, BECKER K: Antioxidant status and nitric oxide in the malnutrition syndrome kwashiorkor. *Pediatr Res* **49**: 237-243, 2001.

FEILLET F, FEILLET-COUDRAY C, BARD J, PARRA H, FAVRE E, KABUTH B, FRUCHART J, VIDAILHET M: Plasma cholesterol and endogenous cholesterol synthesis during refeeding in anorexia nervosa. *Clin Chim Acta* **294**: 45-56, 2000.

FRANCO VHM, HOTTA JKS, JORGE SM, DOS SANTOS JE: Plasma fatty acids in children with grade III protein-energy malnutrition in its different clinical forms: marasmus, marasmic kwashiorkor, and kwashiorkor. *J Trop Pediatr* **45**: 71-75, 1999.

FRIEDEWALD WT, LEVY RI, FREDRICKSON DS: Estimation of the concentration of low density cholesterol without use of the preparative ultracentrifuge. *Clin Chem* **18**: 499-503, 1972.

GOTTO AM JR, POWNALL HJ: *Manual of Lipid Disorders*. Williams & Wilkins, Baltimore, MD, 1999.

HOLMAN RT, ADAMS CE, NELSON RA, GRATER SJE, JASKIEWICZ JA, JOHNSON SB, ERDMAN JW: Patients with anorexia nervosa demonstrate deficiencies of selected essential fatty acids, compensatory changes in nonessential fatty acids and decreased fluidity of plasma lipids. *J Nutr* **125**: 901-907, 1995.

JAGUENAUD P, MALON D, COSNES J, REVEILLARD V, APFELBAUM M, RIGAUD D: Lipoprotéines plasmatiques et anorexia nervosa. Effect de la réalimentation. *Can Nutr Diet* **31**: 45-50, 1996.

KATAN MB, ZOCK PI, MENSINK RP: Dietary oils, serum lipoproteins, and coronary heart disease. *Am J Clin Nutr* **61 (**Suppl): 1368S-1373S, 1995.

KEMPEN HJM, GLATZ JFC, LEUVEN JAG, VAN DER VOORT HA, KATAN MB: A serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J Lipid Res* **29**: 1149-1155, 1988.

LANGAN SM, FARRELL PM: Vitamin E, vitamin A and essential fatty acid status of patients hospitalised for anorexia nervosa. *Am J Clin Nutr* **69**: 672-678, 1999.

LEICHSENRING M, SUTTERLIN N, LESS S, BAUMANN K, ANNINOS A, BECKER K: Polyunsaturated fatty acids in erythrocyte and plasma lipids of children with severe protein-energy malnutrition. *Acta Paediat* **84**: 516-520, 1995.

LENHARTZ H, NDASI R, ANNINOS A, BOTTICHER D, MAYATEPEK E, TETANYE E, LEICHSENRING M: The clinical manifestation of the kwashiorkor syndrome is related to increased lipid peroxidation. *J Pediatr* **132**: 879-881, 1998.

LOCHMAN T, ROCHE A, MARTOREL R (eds): *Standardization of Anthropometric Measurements.* Human Kinetics Publishers, Champaign, IL, 1989, pp 39-80.

MA J, FOLSOM AR, ECKFELDT JH, LEWIS I, CHAMBLES LE: Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Clin Nutr* **62**: 572-578, 1995.

MEHLER PS, GRAY MC, SCHULTE M: Medical complications of anorexia nervosa. *J Women´s Health* **6**: 533-541, 1997.

MIRA M, STEWART PM, WIZZARD J, ABRAHAM S: Biochemical abnormalities in anorexia nervosa and bulimia. *Ann Clin Biochem* **24**: 29-35, 1987.

MORDASINI R, KLOSE G, GRETEN H: Secondary type hyperlipoproteinemia in patients with anorexia nervosa. *Metabolism* **27**: 71-79, 1978.

NESTEL PS: Cholesterol metabolism in anorexia nervosa and hypercholesterolemia. *J Clin Endocrinol Metab* **38**: 325-328, 1974.

PIETZSCH J, JULIUS U: Regulation of VLDL oversecretion: insight from stable isotope studies. In: *Advances in Lipoprotein and Atherosclerosis Research, Diagnostics and Treatment.* W JAROSS, S BERGMANN, M HANEFELD, M MENSCHIKOWSKI (eds), Proceedings of the 10th International Dresden Lipid Symposium, Dresden, 1999, pp 185-190.

RICHES FM, WATTS GF, NAOUMOVA RP, KELLY JM, CROFT KD, THOMPSON GR: Direct association between the hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 and plasma mevalonic acid and lathosterol concentration in man. *Atherosclerosis* **135**: 83-91, 1997.

SACK MN, RADER DJ, CANNON III RO: Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopasal women. *Lancet* **343**: 269-270, 1994.

SHARP CW, FREEMAN CPL: The medical complications of anorexia nervosa. *Br J Psychiat* **162**: 452-462, 1993.

SIVE AA, SUBOTZKY EF, MALAN H, DEMPSTER WS, HEESE HD: Red blood cell antioxidant enzyme concentrations in kwashiorkor and marasmus. *Ann Trop Paediatr* **13**: 33-38, 1993.

THOMPSON GR: *A Handbook of Hyperlipidaemia*. Current Science, London, 1990.

TVRZICKÁ E, VECKA M, STAŇKOVÁ B, ŽÁK A: Analysis of fatty acids in plasma lipoproteins by gas chromatography-flame ionisation detection. Quantitative aspects. *Anal Chim Acta* **465**: 337-350, 2002.

WATTS GF, CHAN DC, BARRETT PHR, O´NEIL FH, THOMPSON GR: Effects of HMG CoA reductase inhibitor on cholesterol absorption in obesity: implications for the regulation of very-low-density lipoprotein B-100 metabolism. *Atherosclerosis* **3 (**Suppl): S235, 2002.

YAZU K, YAMAMOTO Y, UKEGAWA K, NIKI E: Mechanism of lower oxidability of eicosapentaenoate than linoleate in aqueous micelles. *Lipids* **31**: 337-340, 1996.

Reprint requests

A. Žák, Fourth Department of Medicine, First Faculty of Medicine, Charles University, U nemocnice 2, 128 08 Prague 2, Czech Republic. E-mail: azak@vfn.cz