

# Serum Lipoprotein Fatty Acid Profile in Hereditary Ataxias

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**ABSTRACT:** We investigated the serum fatty acid profiles of cholesterol esters, phospholipids and triglycerides in 24 patients with Friedreich's disease and 16 patients with other forms of spinocerebellar degeneration. In 8 patients with Friedreich's disease we also analyzed the fatty acid profile of the lipoprotein fractions. We found no major differences in fatty acid profiles between ataxic patients and sex and age-matched controls; in particular there was no decrease of linoleic acid in Friedreich's disease. The level of linoleic acid in serum cholesterol esters decreased with increasing disability of patients.

**RÉSUMÉ:** Nous avons étudié le profil d'acides gras des esters du cholestérol, des phospholipides et des triglycérides dans le sérum de 24 patients atteints de la maladie de Friedreich et de 16 patients atteints d'autres formes de dégénérescence spinocérébelleuse. Chez 8 patients atteints de la maladie de Friedreich, nous avons étudié aussi le profil d'acides gras des fractions lipoprotéiques. Nous n'avons pas trouvé de différences majeures entre les patients ataxiques et des contrôles appariés pour le sexe et l'âge. En particulier, nous n'avons pas trouvé de diminution de l'acide linoléique chez les patients atteints de la maladie de Friedreich. Le niveau sérique de l'acide linoléique des esters du cholestérol montrait une diminution qui était corrélée avec la sévérité de l'atteinte des patients.

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The term "hereditary ataxias" encompasses many heterogeneous disorders which comprise "early onset", usually autosomal recessive, and "late onset", usually autosomal dominant, forms.<sup>1</sup> Among the former, Friedreich's disease (FD) is the most common, accounting for 44% of all cases.<sup>2</sup> The genetic locus for FD had recently been assigned to the centromeric region of chromosome 9.<sup>3</sup> Autosomal dominant cerebellar ataxia (ADCA) is the most common among the adult onset forms. The ADCA gene has been mapped on the short arm of chromosome 6 in some families, whereas linkage was excluded in other families.<sup>4</sup> Despite advances of molecular genetics, the pathogenesis of most inherited ataxias remains unknown.

The Quebec Cooperative Study of Friedreich's Ataxia suggested three main metabolic abnormalities in FD: transport and handling of some aminoacids, oxidative metabolism and membrane lipid composition.<sup>5</sup> Yao et al.<sup>6</sup> described a decrease of serum linoleic acid in FD. Davignon et al.<sup>7</sup> reported the same finding in both total plasma and HDL, and related it to the pathogenesis of the disease. However, Walker et al.<sup>8</sup> could not confirm this finding, which has also been found in other neurological disorders.<sup>6,9,10</sup>

Since the existence and the specificity of the decrease of linoleic acid in FD is debated, we analyzed the fatty acid profiles of serum lipids in 24 FD patients and in 16 patients with other forms of spinocerebellar degeneration. Fatty acid profiles of lipoprotein fractions were also analyzed in a subgroup of FD patients.

## PATIENTS AND METHODS

Clinical features of the patients are shown in Table 1. Twenty-four patients (16 males, 8 females) had FD. Mean age  $\pm$  SD was  $23 \pm 7$  years. Diagnostic criteria for FD were: autosomal recessive inheritance, onset by age 20 years, progressive ataxia of stance and gait and lower limb areflexia. At least one of the following signs was also required in the index cases: dysarthria, extensor plantar response or echocardiographic signs of hypertrophic cardiomyopathy.<sup>2</sup> Molecular genetic studies were performed in 19 FD patients. Polymorphic markers of the region 9q13-q21 cosegregated with the disease locus in all cases. FD patients were compared with 24 healthy controls (16 males, 8 females, mean age  $\pm$  SD =  $23 \pm 6$ ).

Sixteen ataxic patients (8 males, 8 females) received diagnoses different from FD. Mean age  $\pm$  SD was  $49 \pm 13$  years. Eleven were classified according to Harding<sup>1</sup> as ADCA, 1 as early onset cerebellar ataxia with retained tendon reflexes (EOCA), 2 as idiopathic late onset cerebellar ataxia (ILOCA). Two patients were not classifiable according to Harding: both had a late onset autosomal recessive cerebellar ataxia with preserved tendon reflexes. The patients were compared with 15 healthy controls (9 males, 6 females; mean age  $\pm$  SD =  $44 \pm 12$ ).

Data obtained from additional 39 healthy controls were used only for linoleic acid/age correlation.

Progression of the disease was evaluated according to the Inherited Ataxia Progression Scale (IAPS:<sup>11</sup> stage I, affected

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**Table 1. Clinical Features of Patients**

No.	Diagnosis	Sex/Age	Disease Duration	IAPS Stage	NUDS
1	FD	M/13	1	2	50
2	FD	M/14	8	2	37
3	FD	M/17	1	2	39
4	FD	F/12	4	2	44
5	FD	M/18	4	2	44
6	FD	M/20	16	2	34
7	FD	F/15	14	2	42
8	FD	M/21	7	3	32
9	FD	M/22	16	4	23
10	FD	M/22	10	3	33
11	FD	F/17	5	2	45
12	FD	F/17	6	2	45
13	FD	M/23	11	3	28
14	FD	M/26	20	4	11
15	FD	M/27	15	4	28
16	FD	M/20	7	2	41
17	FD	F/23	5	3	36
18	FD	M/29	16	3	32
19	FD	M/29	5	2	38
20	FD	M/34	22	4	24
21	FD	F/23	16	4	22
22	FD	M/37	28	4	27
23	FD	F/30	21	3	39
24	FD	M/38	22	4	28
25	ADCA	M/32	7	2	45
26	NC	F/33	9	2	37
27	ADCA	M/31	12	2	43
28	NC	F/61	14	3	32
29	ADCA	M/45	9	2	46
30	ADCA	F/40	13	2	35
31	ADCA	M/46	4	2	41
32	ADCA	M/34	9	2	38
33	ADCA	F/57	7	2	44
34	ADCA	F/40	4	2	45
35	ILOCA	M/61	4	3	31
36	ILOCA	M/64	8	4	14
37	ADCA	M/67	5	2	41
38	ADCA	F/55	17	4	32
39	EOCA	F/43	41	3	32
40	ADCA	F/70	41	3	24

IAPS: Inherited Ataxia Progression Scale;<sup>11</sup> NUDS: Northwestern University Disability Scale;<sup>12</sup> FD: Friedreich's disease; ADCA: autosomal dominant cerebellar ataxia; EOCA: early onset cerebellar ataxia with retained tendon reflexes; ILOCA: idiopathic late onset cerebellar ataxia; NC: not classifiable according to Harding.<sup>1</sup>

asymptomatic case; stage 2, symptoms present but mild; stage 3, fully developed disease, patient unable to work; stage 4, patient confined to wheelchair). Disability was scored according to the Northwestern University Disability Scale (NUDS<sup>12</sup>; 50 = normal; 0 = maximal disability).

Venous blood was obtained after an overnight fast. Total lipids were extracted from 1 ml of serum with a 2:1 chloroform/methanol mixture according to Folch et al.<sup>13</sup> Lipid extracts were fractionated by thin layer chromatography<sup>14</sup> and the bands corresponding to cholesterol esters, phospholipids and triglycerides were scraped and methylated by incubation with methanol and sulphuric acid.<sup>15</sup> Gas chromatographic analysis was performed on a glass column packed with 10% SP-2330 on 100/120 mesh chromasorb WAW (Supelco), using a Perkin Elmer Sigma 3B apparatus equipped with a flame ionization detector. A Perkin

Elmer Sigma 15 integrator was used to measure the relative proportion of the following fatty acids: palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), eicosatrienoic acid (20:3), arachidonic acid (20:4). The fraction containing very low and low density lipoproteins (VLDL + LDL) and that containing high density lipoproteins (HDL) were prepared from 4 ml of serum by sequential ultracentrifugation at density 1.063 and 1.212 according to Hatch and Lees.<sup>16</sup> Lipid extraction and separation of cholesterol esters, phospholipids and triglycerides from lipoprotein fractions were performed as described above.

Comparisons between patients and controls were performed by unpaired Student t-test. Correlations between linoleic acid level with NUDS score and age were performed by Pearson's correlation coefficient.

## RESULTS

Serum cholesterol and triglycerides were not different in controls vs. FD (mean  $\pm$  SD = 180  $\pm$  37 mg%ml vs. 170  $\pm$  46; 104  $\pm$  40 vs. 107  $\pm$  42) and vs. patients with other spinocerebellar degenerations (207  $\pm$  36 vs. 206  $\pm$  60; 136  $\pm$  51 vs. 110  $\pm$  49).

The percent distribution of fatty acid composition in serum lipids in FD is shown in Table 2. We found an increase of arachidonic acid in phospholipids (11% more than controls) and of palmitoleic acid in triglycerides (35%).

In patients with other spinocerebellar degenerations (Table 3) linoleic acid was decreased in cholesterol esters (-13%) and phospholipids (-11%) whereas stearic (17%), oleic (27%) and arachidonic (15%) acids were increased in cholesterol esters.

**Table 2. Serum Fatty Acid Percent Composition in Friedreich's Disease**

Fatty Acid	Patients (n = 24)	Controls (n = 24)
Cholesterol Esters		
16:0	11.8 $\pm$ 1.1	11.5 $\pm$ 1.1
16:1	2.3 $\pm$ 0.8	2.7 $\pm$ 1.1
18:0	0.7 $\pm$ 0.2	0.8 $\pm$ 0.6
18:1	19.7 $\pm$ 3.4	19.7 $\pm$ 3.0
18:2	55.1 $\pm$ 5.6	55.8 $\pm$ 4.6
20:3	0.8 $\pm$ 0.4	0.8 $\pm$ 0.3
20:4	8.7 $\pm$ 1.4	7.7 $\pm$ 2.0
Phospholipids		
16:0	32.5 $\pm$ 2.0	32.5 $\pm$ 1.7
16:1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1
18:0	15.3 $\pm$ 1.4	14.8 $\pm$ 1.2
18:1	11.9 $\pm$ 1.9	12.2 $\pm$ 1.7
18:2	22.9 $\pm$ 3.3	23.8 $\pm$ 2.9
20:3	3.6 $\pm$ 0.8	3.8 $\pm$ 0.7
20:4	13.3 $\pm$ 1.9	12.0 $\pm$ 2.0*
Triglycerides		
16:0	26.0 $\pm$ 6.0	26.7 $\pm$ 5.0
16:1	3.9 $\pm$ 1.7	2.9 $\pm$ 1.9*
18:0	2.8 $\pm$ 1.2	3.7 $\pm$ 2.3
18:1	40.1 $\pm$ 5.7	42.2 $\pm$ 5.5
18:2	23.5 $\pm$ 7.6	20.9 $\pm$ 7.7
20:3	0.3 $\pm$ 0.1	0.4 $\pm$ 0.3
20:4	1.3 $\pm$ 0.4	1.4 $\pm$ 0.5

Values are mean  $\pm$  SD

\*p < 0.05

**Table 3. Serum Fatty Acid Percent Composition in Other Spinocerebellar Degenerations**

Fatty Acid	Patients (n = 16)	Controls (n = 15)
Cholesterol Esters		
16:0	11.7 ± 1.2	11.3 ± 1.0
16:1	3.9 ± 1.1	3.2 ± 1.0
18:0	0.7 ± 0.1	0.6 ± 0.1*
18:1	22.4 ± 5.9	17.6 ± 3.7*
18:2	50.3 ± 8.2	57.7 ± 3.9**
20:3	1.1 ± 0.5	0.9 ± 0.3
20:4	9.1 ± 1.5	7.9 ± 1.5*
Phospholipids		
16:0	32.4 ± 1.8	32.0 ± 1.7
16:1	0.4 ± 0.2	0.3 ± 0.1
18:0	14.7 ± 1.6	15.2 ± 1.0
18:1	13.0 ± 2.5	11.3 ± 2.6
18:2	21.0 ± 3.7	23.7 ± 2.5*
20:3	4.6 ± 0.7	4.5 ± 0.8
20:4	13.7 ± 2.3	12.2 ± 2.3
Triglycerides		
16:0	24.5 ± 3.5	25.5 ± 2.1
16:1	4.9 ± 2.0	4.0 ± 2.6
18:0	2.9 ± 0.8	3.4 ± 0.7
18:1	43.7 ± 8.7	39.0 ± 6.8
18:2	19.9 ± 9.1	24.0 ± 6.5
20:3	0.4 ± 0.2	0.4 ± 0.1
20:4	1.8 ± 0.7	1.7 ± 0.7

Values are means ± SD  
\* p < 0.05; \*\* p < 0.01

**Table 4. HDL Fatty Acid Percent Composition in Friedreich's Disease**

Fatty Acid	Patients (n = 8)	Controls (n = 10)
Cholesterol Esters		
16:0	11.5 ± 1.1	11.4 ± 0.8
16:1	2.3 ± 0.8	3.1 ± 0.9
18:0	0.8 ± 0.2	0.8 ± 0.3
18:1	19.4 ± 4.3	19.0 ± 2.2
18:2	55.1 ± 5.6	56.4 ± 3.7
20:3	0.6 ± 0.1	0.9 ± 0.5
20:4	9.4 ± 1.8	8.0 ± 1.7
Phospholipids		
16:0	31.2 ± 1.8	30.4 ± 1.9
16:1	0.4 ± 0.1	0.5 ± 0.2
18:0	15.3 ± 1.8	15.0 ± 1.3
18:1	12.4 ± 2.1	12.2 ± 1.3
18:2	21.6 ± 2.9	23.7 ± 2.2
20:3	3.3 ± 0.7	4.0 ± 0.4*
20:4	14.9 ± 1.2	13.5 ± 1.7
Triglycerides		
16:0	27.1 ± 7.7	31.1 ± 4.2
16:1	3.8 ± 1.8	3.0 ± 1.6
18:0	3.1 ± 1.4	2.9 ± 0.6
18:1	40.8 ± 6.5	40.9 ± 3.6
18:2	22.0 ± 11.1	17.2 ± 4.0
20:3	UD	UD
20:4	1.3 ± 0.6	1.6 ± 0.7

Values are means ± SD  
\* p < 0.05  
UD = undetectable

**Table 5. LDL-VLDL Fatty Acid Percent Composition in Friedreich's Disease**

Fatty Acid	Patients (n = 8)	Controls (n = 11)
Cholesterol Esters		
16:0	11.4 ± 0.9	11.4 ± 0.5
16:1	2.6 ± 0.8	3.1 ± 0.8
18:0	0.7 ± 0.1	0.8 ± 0.1
18:1	120.1 ± 3.9	21.0 ± 3.0
18:2	53.9 ± 5.5	54.3 ± 3.8
20:3	0.7 ± 0.2	0.7 ± 0.1
20:4	10.0 ± 1.3	7.8 ± 1.7**
Phospholipids		
16:0	32.9 ± 2.2	33.2 ± 1.2
16:1	0.4 ± 0.1	0.4 ± 0.2
18:0	16.0 ± 1.4	15.3 ± 1.0
18:1	11.7 ± 2.2	12.2 ± 1.6
18:2	21.8 ± 3.0	22.6 ± 1.8
20:3	4.6 ± 1.2	4.9 ± 1.0
20:4	11.9 ± 1.7	10.9 ± 1.5*
Triglycerides		
16:0	24.4 ± 5.1	27.6 ± 3.0
16:1	4.2 ± 1.4	3.7 ± 1.9
18:0	3.2 ± 1.3	3.9 ± 1.2
18:1	39.1 ± 6.5	41.7 ± 4.9
18:2	25.6 ± 10.4	17.7 ± 4.4*
20:3	0.4 ± 0.2	0.4 ± 0.2
20:4	1.6 ± 0.5	1.8 ± 0.7

Values are means ± SD  
\* p < 0.05; \*\* p < 0.01

Fatty acid composition of serum HDL and VLDL+LDL in a subgroup of FD patients and controls is shown in Tables 4 and 5. In the HDL fraction a decrease of eicosatrienoic acid (-17%) was found in phospholipids, in the VLDL+LDL fraction arachidonic acid was decreased in both cholesterol esters (-28%) and phospholipids (-9%) and linoleic acid was increased in triglycerides (45%).

There was a correlation between linoleic acid levels in cholesterol esters and NUDS scores in ataxic patients ( $r_p = 0.40$ ;  $df = 38$ ;  $p < 0.05$ ) with lower linoleic acid values in more disabled patients. This correlation was absent in phospholipids ( $r_p = 0.24$ ;  $df = 38$ ). Linoleic acid levels did not correlate with age in 78 normal controls either in cholesterol esters ( $r_p = 0.13$ ;  $df = 76$ ) or in phospholipids ( $r_p = 0.05$ ;  $df = 76$ ).

## DISCUSSION

Yao et al.<sup>6</sup> found a decrease of linoleic acid in cholesterol esters and triglycerides from 6 FD patients. This finding was also present in other patients with different hereditary neuropathies. Davignon et al.<sup>7</sup> reported a decrease of linoleic acid in both total plasma (29.11 vs. 45.50%) and HDL cholesterol esters (26.78 vs. 45.18%) and in HDL phospholipids (19.28 vs. 28.38%) in 11 FD patients.

Our study failed to confirm a decrease of linoleic acid in serum of 24 FD patients. On the other hand, we found a moderate linoleic acid decrease in cholesterol esters and phospholipids from 16 patients with other spinocerebellar degenerations.

These data on the whole suggest that a linoleic acid decrease is not a specific or consistent finding in FD; in fact, besides

hereditary ataxias, it can also be found in inherited neuropathies,<sup>6</sup> demyelinating disorders<sup>9</sup> and muscular diseases.<sup>10</sup>

We also demonstrated that linoleic acid levels in cholesterol esters inversely correlate with disability in patients. Since no correlation with age was present in our group of 78 normal controls, a decrease of linoleic acid seems to be an effect not of normal ageing, but rather of progressing disability. Disability of patients should definitely be considered in planning studies on linoleic acid levels.

Arachidonic, oleic and stearic acids were increased in cholesterol esters of patients with other spinocerebellar degenerations. These increases are probably compensatory to the linoleic acid decrease. It is more difficult to explain the arachidonic acid increase in phospholipids of FD patients and the other differences from the study on lipoprotein fractions. Although these changes are statistically significant, their magnitude is not biologically relevant, they were not reported in previous studies and were not searched for in our study. Therefore, they might be considered chance results due to the great number of comparisons performed.

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