Lipids and lipoproteins in Friedreich's ataxia

J. L. WALKER, S. CHAMBERLAIN, AND N. ROBINSON

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SUMMARY Friedreich's ataxia is an autosomal recessively inherited disease affecting the nervous system with a high incidence of heart involvement. Abnormalities of lipid metabolism are known to be associated with several progressive ataxic conditions. In this study of 46 Friedreich's ataxia patients, serum lipids, fatty acids and lipoproteins were assayed and compared with some earlier findings on Friedreich's ataxia and related disorders. Abnormalities of low and high density lipoproteins suggestive of a major defect have been reported; in the present study the level and chemical composition of high density lipoprotein has been assessed in 20 Friedreich's ataxia patients but previous abnormalities could not be substantiated. Lipid compositional analysis of Friedreich's ataxia central nervous tissue and heart, which has not been previously reported, did not markedly differ from control tissue.

Friedreich's ataxia is one of the more common hereditary diseases of the nervous system which may be defined by restrictive criteria as outlined by Geoffroy et al.

Certain diseases having neurological symptoms are known to be associated with lipid metabolism and transport errors. A major group of inherited metabolic disorders that are characterised by profound deleterious effects on the central nervous system are distinguished by the accumulation of excessive quantities of lipids in various organs and tissues throughout the body.

A progressive demyelinating disorder and a kindred with signs and symptoms of a spinalcerebellar degeneration similar to Friedreich's ataxia have both been reported to exhibit low levels of low density lipoprotein (LDL). Further, the cardiac disturbances and ataxia present in Friedreich's ataxia are also found in abetalipoproteinaemia, a condition in which little or no LDL is found in serum. Recently, Wastiaux et al. and Huang et al. have reported decreased levels of low and high density lipoproteins in Friedreich's ataxia and other ataxic conditions and major differences in the composition of high density lipoproteins (HDL).

Fatty acid abnormalities have been reported in other ataxic conditions. Refsum's disease which is an hereditary form of ataxia and polyneuritis, is associated with an unusually large amount of a 20-carbon methylated fatty acid in plasma and tissue. Adrenoleucodystrophy, an X-linked hereditary neurological disorder, shows abnormal very long chain fatty acids, principally in the cholesterol esters of brain, suprarenal glands and serum.

Nervous tissue lipids are important both as structural constituents and as participants in the functional activity of the central nervous system. Identification of abnormally stored lipids in some genetically determined metabolic disorders has introduced the possibility that an abnormality in the chemistry or metabolism of one of the unique nervous system lipids may be involved in Friedreich's ataxia. Previously Robinson has shown that the affected regions of the spinal cord in Friedreich's ataxia do show lipid loss from proteolipids. The three major categories of brain lipids have been investigated in this study.

In view of the association of progressive ataxic disease and abnormalities in lipids and lipoproteins, the present study was undertaken to investigate some of these components and ascertain their possible involvement in Friedreich's ataxia.

Methods

Forty-six patients were used in the study, all of whom had been seen by a neurologist within the last 2 years and the diagnosis confirmed. All
patients met the essential criteria of Geoffroy et al: ataxia began before the end of puberty and was unrelentingly progressive, the patients exhibited dysarthria, muscle weakness, a decrease of position or vibratory sense and deep tendon areflexia in the lower limbs. Recessive inheritance was suggested in all cases. Table 1 shows the age range and disabilities of the patients involved in the study.

Neurological controls had various diagnoses, mainly of multiple sclerosis (12), muscular dystrophy (3), spasticity (3), Parkinsonism (2), and quadraplegia (2). Normal control blood was obtained from healthy volunteers. As far as possible Friedreich's ataxia patients and controls were matched for age, sex, physical activity and body weight.

**EXPERIMENTAL PROCEDURES**

Venous blood was obtained after an overnight fast. The blood was allowed to clot and after centrifugation the serum removed. Serum was then either used immediately or frozen at $-20^\circ$C until analysis within one month of collection. Controls of similar age and disability were used throughout the study, unless normal ranges were well established.

(a) Total serum cholesterol in 20 Friedreich's ataxia patients was extracted into isopropanol and measured on a Technicon AA1 system by the method of Zlatkis et al and the proportion of free to esterified cholesterol was estimated using the Boehringer Mannheim Test Combination. Total triglyceride was measured in 20 Friedreich's ataxia patients according to Eggstein et al.

(b) For the measurement of the plasma fatty acid profile, the total lipid was extracted according to Folch et al as modified by Danon et al. Cholesterol esters, triglycerides and phospholipids were separated and their methylated fatty acids prepared by incubation with 14% boron trifluoride in methanol at $65^\circ$C for 3 hours and subsequently estimated on a Pye Unicam Model 104 Gas Liquid Chromatograph; 10 Friedreich's ataxia and 10 control patients were assessed in each lipid category.

(c) Low density lipoprotein levels (LDL) were measured by radial immunodiffusion in sera of 25 Friedreich's ataxia and 17 control patients, using Behring Werke M-partigen plates, (purported to have negligible non specific activity) and human standard low density lipoprotein serum. Agarose gel electrophoresis was used to estimate the proportion of LDL and HDL in whole serum in 10 Friedreich's ataxia and seven control patients. Using this proportional method, pooled serum from 20 normal controls was used to produce a standard HDL serum. The high density lipoprotein (HDL) level was measured by rocket immunoelectrophoresis essentially as described by Laurell. Twenty-four Friedreich's ataxia patients, four neurological controls and 20 normal controls were tested. Human high density lipoprotein antiserum was obtained from Behring Werke having a titre of 0·2 U/ml.

(d) The dual precipitation method of Demacker et al was used to prepare HDL for measurement of cholesterol after extraction with isopropanol and phospholipids in 20 Friedreich's ataxia patients, 10 neurological and 20 normal controls. Phosphatidylcholine (Sigma Chem. Co.) was the reference standard in the latter method.

(e) Ultracentrifugation techniques were based on those of Lindgren and Nichols. Three ml of serum was raised to a density of 1·063 with 4 ml of aqueous NaBr and spun for 16 hours at 86,000 g and 14°C in an MSE Superspeed 65 with Rotor 59113. The top 1·5 ml supernatant fluid was removed and discarded. Two successive spins were then performed for 29 hours at 143,000 g and 14°C raising the solution density to 1·125 and then 1·210 with solid NaBr. The top 1 ml from each spin containing HDL2 and HDL3 respectively was removed by careful pipetting. The protein component was measured by the methods of Lowry et al and Pesce and Strande. Total and free cholesterol were estimated using the Boehringer Mannheim Test Combinations and phospholipids as in (d). Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was carried out for 4 to 5 hours on HDL2 and HDL3 after exhaustive dialysis of the lipoproteins in a dialysate of EDTA (0·05%) and NaCl (0·15 mM) adjusted to pH 7·0 with NaOH using a 7·5-% gel with a phosphate buffer system. Isoelectricfocusing was performed with the LKB ampholine system, using narrow range pH 4–6·5 plates and electrofocusing for 3 to 3·5 hours at 25 watts.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>*Disability</th>
<th>Diabetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 17</td>
<td>2</td>
<td>3</td>
<td>2A 3C</td>
<td>1</td>
</tr>
<tr>
<td>17–26</td>
<td>7</td>
<td>5</td>
<td>2A 3B 7C</td>
<td>1</td>
</tr>
<tr>
<td>27–36</td>
<td>9</td>
<td>5</td>
<td>1A 3B 10C</td>
<td></td>
</tr>
<tr>
<td>37–46</td>
<td>8</td>
<td>2</td>
<td>9C 1D</td>
<td>1</td>
</tr>
<tr>
<td>47–56</td>
<td>1</td>
<td>4</td>
<td>4C 1D</td>
<td>1</td>
</tr>
</tbody>
</table>

*A Walking without aid  B Walking only with substantial aid  C Confined to a wheelchair  D Bedridden*
Lipids and lipoproteins in Friedreich's ataxia

(f) The basic analytical scheme of Suzuki\(^ {26}\) employing thin layer chromatographic techniques, was used to examine the lipids of the grey and white cerebrum, cerebellum, spinal cord and heart. Tissue from two Friedreich's ataxia patients and one control (cancer of the ovary) obtained within 6 hours of death was frozen immediately under liquid nitrogen and subsequently stored at \(-20^\circ\)C until use. The three major categories of nervous tissue lipids investigated by this method were cholesterol, sphingolipids and glycerophosphates.

Results

(a) The total serum cholesterol, triglycerides and ratio of esterified to free cholesterol were within acceptable limits of the hospital normal ranges and compared well with other published values.\(^ {27, 28}\) Two patients were on the upper limit of the normal cholesterol range, while one patient was above the normal triglyceride range (table 2).

(b) The fatty acid profiles in total serum, phospholipids, triglycerides and cholesterol esters (table 3) showed no significant abnormalities. The percentage of the major fatty acids were normal compared to controls and no abnormal fatty acids were observed. Slightly low values of linoleic and arachidonic acids were initially observed in the Friedreich's ataxia patients but comparison with controls of equal age and disability revealed no differences.

(c) Serum lipoprotein levels in the Friedreich's ataxia patients and controls are shown in table 4. Although there are only a few people in each age and sex range, no decreased levels of LDL were found, but slightly increased LDL levels compared to the normal American adult population of Lindgren et al\(^ {26}\) were seen in six Friedreich's ataxia patients out of the 29 studied and in three of the 17 controls. HDL values were normal in 13 of the 20 Friedreich's ataxia patients and 15 of the 20 controls, but increased levels were found in five Friedreich's ataxia patients and

| Table 2 Serum cholesterol and triglycerides (mean ± SD) |
|----------------------------------|------------------|------------------|
| Lipid                           | Friedreich's     | Normal range     |
| Total cholesterol m mol/l       | 5.3 ± 1.5 (20)*  | 3.6–7.0          |
| Total triglycerides m mol/l     | 1.2 ± 0.5 (20)*  | 0.3–1.8          |
| Esterified cholesterol %        | 71.2 ± 6.6 (10)* | 65–75            |
| Free cholesterol %              | 28.8 ± 6.6 (10)* | 25–35            |

\(\ast\) Number of patients

<table>
<thead>
<tr>
<th>Table 3 Fatty acid profile of serum lipids</th>
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</thead>
<tbody>
<tr>
<td>Fatty acid</td>
</tr>
<tr>
<td>Serum lipids %</td>
</tr>
<tr>
<td>F. ataxia(\ast)</td>
</tr>
<tr>
<td>14:0 1.8 ± 0.5 1.7 ± 0.6 0.8 ± 0.4 1.4 ± 0.3</td>
</tr>
<tr>
<td>16:0 28.0 ± 3.7 27.4 ± 6.8 30.1 ± 3.4 30.1 ± 2.8</td>
</tr>
<tr>
<td>16:1 3.9 ± 1.5 3.7 ± 1.7 1.2 ± 0.8 2.2 ± 1.0</td>
</tr>
<tr>
<td>18:0 8.9 ± 2.3 8.1 ± 2.6 17.2 ± 2.3 17.1 ± 3.1</td>
</tr>
<tr>
<td>18:1 26.5 ± 3.9 27.5 ± 3.5 17.8 ± 4.2 16.8 ± 3.9</td>
</tr>
<tr>
<td>18:2 23.3 ± 3.2 23.8 ± 4.9 25.7 ± 3.6 25.1 ± 3.1</td>
</tr>
<tr>
<td>20:4 7.6 ± 3.2 7.8 ± 2.1 6.9 ± 2.3 7.1 ± 2.0</td>
</tr>
</tbody>
</table>

\* Means of 10 Friedreich's ataxia patients ± SD
\(\ddagger\) Means of 10 controls ± SD

<table>
<thead>
<tr>
<th>Table 4 Low density and high density lipoprotein levels measured immunologically (mean mg/100 ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1. LDL Under 27</td>
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<tr>
<td>27–36</td>
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<tr>
<td>37–46</td>
</tr>
<tr>
<td>All ages</td>
</tr>
<tr>
<td>2. HDL Under 27</td>
</tr>
<tr>
<td>27–36</td>
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<tr>
<td>37–46</td>
</tr>
<tr>
<td>47–56</td>
</tr>
<tr>
<td>All ages</td>
</tr>
</tbody>
</table>

\(\ast\) Number of patients.
Table 5  High density lipoprotein composition  

(\text{mean mg/100 ml \pm SD})

<table>
<thead>
<tr>
<th></th>
<th>Friedreich's ataxia</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (t)</td>
<td>166.2 ±35.8 (20)*</td>
<td>14.9 ±15.4 (20)</td>
</tr>
<tr>
<td>Protein (t)</td>
<td>178.5 ±32.4 (10)</td>
<td>162.8 ±31.2 (10)</td>
</tr>
<tr>
<td>Phospholipids (&amp;)</td>
<td>93.8 ±30.7 (20)</td>
<td>89.8 ±37.8 (20)</td>
</tr>
<tr>
<td>Phospholipids (&amp;)</td>
<td>98.5 ±16.7 (10)</td>
<td>108.2 ±16.4 (10)</td>
</tr>
<tr>
<td>Total cholesterol (&amp;)</td>
<td>51.8 ±16.9 (20)</td>
<td>27.6 ±26.1 (30)</td>
</tr>
<tr>
<td>Total cholesterol (P)</td>
<td>46.6 ±8.2 (10)</td>
<td>41.8 ±11.4 (10)</td>
</tr>
</tbody>
</table>

\(0^*\) Number of patients  
\(t\) Ultracentrifugation  
\(\&\) Dual Precipitation  
\(\&\) Ultracentrifugation  
\(P\) Ultracentrifugation  

four controls, while slightly low levels were found in two Friedreich’s ataxia patients and one control. The proportion of HDL to LDL in 10 Friedreich’s ataxia patients was 30-0% HDL to 62-0% LDL with a standard deviation of ±5.6 while in the seven controls the proportion was 34-5% HDL to 65-5% LDL with a standard deviation of ±4.0. Normal values quoted for this method are 33-0% HDL to 67-0% LDL with an approximate standard deviation of 5%. Both Friedreich’s ataxia and control values fell within this range.

Analysis of the serum HDL composition assessed using both the dual precipitation and the ultracentrifugation method is shown in Table 5. Values obtained by ultracentrifugation were estimated by summation of the individual components of HDL2 and HDL3. The percentage composition of the major components of total HDL, HDL2, and HDL3 (excluding triglycerides) are shown in Table 6. The protein levels measured by the two methods varied but no significant differences were seen between the two groups.

Good correlation was found between the HDL cholesterol and the phospholipid measurement by dual precipitation and ultracentrifugation, with slightly increased results for phospholipids and decreased results for cholesterol by the latter method. The Friedreich’s ataxia and control phospholipid and cholesterol values did not significantly differ, nor did the percentage composition of total HDL, HDL2 or HDL3.

SDS acrylamide gel electrophoresis of HDL2 and HDL3 showed two major bands which appear to correspond to Apoprotein Al and Apoprotein A1 as described by Foreman et al\(^{23}\) and Eder and Roheim.\(^{31}\) In HDL3 three additional minor bands were consistently found in 10 Friedreich’s ataxia and 10 control samples. Isoelectric focusing revealed many bands, but no differences in banding could be seen between the Friedreich’s ataxia and control gels.

\(5\) The total lipid analysis of the cerebrum, cerebellum, spinal cord and heart muscle showed no marked differences in position or intensity of banding of cholesterol, cerebrosides, sulphatide, sphingomyelin or the cholines, ethanolamine and serine phospholipids. The polyphosphoinositides were not extracted.

Discussion

Familial ataxia is known to occur in a variety of inborn errors of metabolism. Intermittent ataxia is seen in several specific aminoacidurias such as Hartnup disease\(^{32}\) and in certain defects of carbohydrate metabolism, for example, pyruvate dehydrogenase deficiency.\(^{32}\) Progressive unrelenting ataxia is more common in lipid metabolic disorders, such as phytanic acid storage disease\(^{9}\) and abetalipoproteinemia.\(^{6}\) Friedreich’s ataxia is a progressive unrelenting ataxia and it is therefore possible that it is a lipid disorder.

Table 6  Percentage composition of total HDL, HDL2 and HDL3

<table>
<thead>
<tr>
<th>Total HDL%</th>
<th>HDL2</th>
<th>HDL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. ataxia*</td>
<td>Control(&amp;)</td>
<td>F. ataxia(&amp;)</td>
</tr>
<tr>
<td>Protein(&amp;)</td>
<td>52.2</td>
<td>53.2</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>29.6</td>
<td>27.4</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>16.4</td>
<td>17.6</td>
</tr>
<tr>
<td>Free cholesterol(&amp;)</td>
<td>1.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(0^*\) Means of 20 Friedreich’s ataxia patients  
\(\&\) Means of 10 Friedreich’s ataxia patients  
\(\&\) Means of 20 controls  
\(\&\) Means of 10 controls  
\(t\) Ultracentrifugation  
\(\&\) Ultracentrifugation  
\(\&\) Dual Precipitation  
\(\&\) Ultracentrifugation  
\(\&\) Ultracentrifugation  

Boehringer Test Combination

\(t\) Means of 10 Friedreich’s ataxia patients  
\(\&\) Means of 20 controls  
\(\&\) Means of 20 controls  
\(t\) Ultracentrifugation  
\(\&\) Ultracentrifugation  
\(\&\) Dual Precipitation  
\(\&\) Ultracentrifugation  
\(\&\) Dual Precipitation  
\(\&\) Dual Precipitation  

Boehringer Test Combination
In 20 Friedreich’s ataxia patients, serum cholesterol and triglyceride levels were predominately normal confirming the results of Podolsky et al.,4 and Butterworth et al.,9 but contrary to the elevated serum cholesterol levels reported by Krongrad et al.8 and elevated triglyceride levels reported by Badiu and Cherciulescu.7 Two of the Friedreich’s ataxia patients did show increased cholesterol values but with no corresponding increase in triglyceride values; this may be due to one of the patients being diabetic and the other obese. The findings indicate that none of the patients have hypobetalipoproteinemia as, in hypobetalipoproteinemia, low LDL levels are associated with low serum cholesterol values.48

It has been shown that unusual fatty acid profiles can cause neurological disease as in Refsum’s disease where large amounts of phytic acid cause amongst other symptoms, ataxia and polyneuritis.9 Igarashi et al.10 have shown abnormal very long chain fatty acids in adrenoleucodystrophy, an X-linked hereditary neurological disorder particularly affecting cerebral white matter, adrenal cortex and testes in young boys. The neurological symptoms are often the only manifestations of this disease.

The fatty acid profiles in Friedreich’s ataxia did not show any abnormalities; the percentages were not significantly different from those of similarly disabled controls and there were no unusual peaks, confirming the findings of Huang et al5 and Yao and Dyck.39 Serum fatty acids vary in neurological diseases40 so it was essential to compare groups of equal disability. It is possible that unnatural fatty acids such as trans-trans or trans-cis linoleic acid were present which cannot be discriminated by gas liquid chromatography, but it does not seem likely that this would cause any adverse effects when adequate amounts of the essential fatty acids are present in the diet.41

Disorders with decreased serum low density lipoprotein (LDL) levels are known to be associated with ataxia. In abetalipoproteinemia the symptoms include ataxia neuropathy and cardiac disturbances associated with complete or almost complete absence of LDL.8 Mars et al42 have reported 13 members of a kindred with familial hypobetalipoproteinemia, some of whom showed symptoms of a progressive demyelinating disorder affecting the central nervous system. Badiu and Cherciulescu7 and more recently Wastiaux et al43 have reported low LDL levels in Friedreich’s ataxia and other ataxic conditions. Our investigations of 29 Friedreich’s ataxia patients revealed no values below the normal lower limits of Lindgren et al29 in fact, six patients had slightly elevated results. It is difficult to estimate the significance of the previous findings; Badiu and Cherciulescu have not published actual values whilst Wastiaux et al showed normal control values considerably above the quoted mean values for this radial immunodiffusion method. It is not stated whether the patients were fasted; if they were not, this may account for the discrepancies between the results. The increased levels of LDL shown by six Friedreich’s ataxia patients and three controls, are at the upper limit of the normal range, but this may only be an indication of increased risk of developing coronary disease.

Only Huang et al5 have reported HDL abnormalities in Friedreich’s ataxia. In their study of 11 patients they showed considerably reduced protein, slightly low cholesterol, elevated triglyceride levels and an overall low level of HDL in Friedreich’s ataxia serum. Severe deficiency or absence of HDL in plasma is found in Tangier disease but this rare condition bears little resemblance to Friedreich’s ataxia. Careful attention was given to the HDL determinations in view of the striking findings of Huang et al5 and therefore each component of HDL was effectively measured twice. However, we were unable to substantiate any abnormal finding in HDL. Total levels of HDL in serum measured in 20 Friedreich’s ataxia patients showed almost exactly the same range of values and average results as 20 controls. Quantitative estimations of the major components of HDL, cholesterol, phospholipid and protein in 20 Friedreich’s ataxia patients did not differ significantly from 20 control values. The protein component of HDL2 and HDL3 examined by SDS gel electrophoresis and isoelectric focusing showed no qualitative differences between the Friedreich’s ataxia and control groups.

The group of patients examined by Huang et al5 show some differences from our own, three of his 11 Friedreich’s ataxia cases are known to have abnormalities of glucose metabolism which may cause the lipoprotein pattern to change44; abnormality of glucose tolerance has been reported to cause an elevation in very low density lipoproteins and chylomicrons with a corresponding decrease of LDL and HDL. Of his remaining eight Friedreich’s ataxia patients four out of five had elevated bilirubin levels; the other three patients were not measured. Hamel et al45 estimate that approximately 30% of Friedreich’s ataxia patients have elevated serum bilirubin levels, but these values may represent the top end of the normal range, and therefore, may not
be significant.\textsuperscript{4} High bilirubin levels found in obstructive jaundice, however, are associated with very low concentrations of HDL\textsuperscript{11} but whether there is any relationship between such abnormalities and the HDL levels in this group of 11 patients, is difficult to elucidate. Direct and indirect bilirubin levels have been measured in 25 of our Friedreich's ataxia patients and no elevated levels were found (unpublished observation). As an alternative hypothesis the results of Huang et al may be due to a preponderance of an abnormal gene causing a low level of HDL in a genetically fairly homogenous population. Such a population is found in French Canada where it has been suggested that the Friedreich's ataxia gene was present within a core of no more than 10 families,\textsuperscript{46} but the U.K. has a considerably more heterogeneous population.

The gross analysis of tissue lipids showed no qualitative abnormality. The cerebral white and grey matter, cerebellum, spinal cord and heart lipid extracts in the two Friedreich's ataxia patients compared to the control showed absolutely identical bands and intensity of banding on thin layer chromatography plates. The procedure is sufficiently quantitative for most purposes.\textsuperscript{25}

Further studies of specific lipid types may elucidate abnormalities in other lipids which are present in considerably lower concentrations.

In conclusion, results from this study and comparisons with the findings of the other workers infer that there is no clear indication of a significant defect of major lipid and lipoprotein components in Friedreich's ataxia.

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