Chemical-physical properties of lipoproteins in anorexia nervosa

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Abstract

Background Anorexia nervosa (AN), a psychosomatic disorder, has serious negative effects on multiple organs and systems of the human body. Anorexia nervosa usually runs a chronic course and is associated with significant morbidity and mortality.

In order to elucidate the role played by lipids in AN, in the present study we compared the plasma lipid profile and the chemical-physical properties of lipoproteins obtained from subjects affected by AN.

Materials and methods The study was performed on lipoproteins of AN subjects and of age-matched healthy subjects used as controls. We tested the susceptibility to oxidative stress in vitro, the fatty acid content, the fluidity using 2-dimethylamino-(6-lauroyl)-naphthalene (Laurdan) and 1,6-difenil-1,3,5-esatriene (DPH) probes.

Results Present results indicate that AN patients present a deep alteration of the composition and of chemical-physical properties in circulating lipoproteins, even in the absence of significant modifications to clinical metabolic parameters. A significantly decreased body mass index (BMI) was found in AN patients in comparison with controls. Anorexia nervosa patients showed a significant modification of phospholipids to protein ratio and a significantly increased percentage of unsaturated fatty acids compared with control subjects as well as a decreased fluidity, a significantly increased percentage of liquid-crystalline phase in VLDL, and a significantly reduced susceptibility to oxidative stress, more pronounced in LDL.

Conclusions These results confirm the hypothesis that anorexia is accompanied by changes of lipid metabolism in the central nervous system (CNS).

Keywords Anorexia nervosa, fatty acids, lipoproteins, membrane fluidity, oxidative stress.

Introduction

Anorexia nervosa (AN) is a chronic disorder characterized by the patient’s refusal to maintain body weight accompanied by many medical complications [1].

Beumont first divided AN patients into two groups: ‘dieters’ and ‘vomiters and purgers’ [2]. Later Garfinkel et al. [3] reviewed the classification and diagnosis of eating disorders. Regarding its pathogenesis, AN is a multifactorial disorder. The role of biological and psychological, familial and cultural predisposing factors is presumed to vary across this heterogeneous patient population. Precipitants of the disorder are less clearly understood, except that food restriction is invariably an early element [4].

Controversies still exist also about the lipid pattern in AN. Holman et al. demonstrated that patients with AN have deficiencies of selected essential fatty acids, compensatory changes in non essential fatty acids and decreased fluidity of plasma lipids [5]. Mehler et al. recently observed that total and LDL cholesterol were in the normal range and HDL levels were high in patients affected by the AN type characterized by alimentary restriction [6]. Case et al. demonstrated that low plasma lipid levels were present in AN patients [7].

In order to further elucidate the role played by lipids in AN, we compared the plasma lipid profile and chemical-physical properties of lipoproteins obtained from subjects with AN.
Patients and methods

Thirty AN subjects (25 females and five men) and 30 healthy subjects (25 females and five men) age-matched (being 23.2 ± 4.7 years for AN subjects and 23.8 ± 3.7 years for subjects) as controls were included in the present study. Anorexic nervosa subjects were of the restricting subtype, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [8].

Body mass index (BMI) was 16.5 ± 3.4 for AN subjects and 21.8 ± 2.3 for controls.

Each subject gave informed consent before the investigation. The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 1996. Blood was drawn in the fasting state in anticoagulant citrate dextrose (ACD buffer: 36 mL of citric acid, 5 mM KCl, 90 mM NaCl, 5 mM glucose, 10 mM EDTA, pH 6.8).

Lipoprotein isolation

LDL, VLDL and HDL were isolated from plasma by density gradient ultracentrifugation using a vertical rotor TV-865 B according to the method of Chung and Segrest [9]. The HDL and VLDL obtained by ultracentrifugation were dialyzed against 0.9% NaCl at 4°C for 24 h.

Susceptibility to oxidative stress in vitro

Lipoproteins were incubated at 37°C for 18 h with a freshly prepared CuSO4 solution added to a final concentration of 10 μM per 1 mg mL−1 of VLDL or HDL protein. The degree of lipoprotein oxidation was determined by the measurement of hydroperoxide levels and thiobarbituric acid-reactive substances (TBARS) before and after the peroxidative stress [10]. Thiobarbituric acid-reactive substances were determined using the Yagi method [11]. Results are expressed in nmol malondialdehyde 100 μg−1 protein. Proteins were measured by the Bradford method [12]. Oxidizability was measured as differential TBARS content before and after incubation with CuSO4.

Fatty acid analysis

To determine the fatty acid content in LDL, VLDL and HDL, lipids were extracted by the method of Folch [13] and transmethylated as described by Lillington et al. [14] in the presence of nonadecanoic acid as an internal standard. Methyl esters were analyzed by gas chromatography.

Fluorescent studies

2-dimethylamino-(6-lauroyl)-naphthalene (Laudan) was purchased from Molecular Probes, Inc. (Eugene, OR).

Isolated lipoproteins were labelled by 1 mM Laudan by adding a solution of probe in ethanol to the sample under rigorous stirring at 25°C. Final protein concentration was 0.4 mg mL−1.

Laudan generalized polarization (GP340) (λex = 340 nm) was calculated according to Parasassi et al. by the following equation:

\[ GP = \frac{(IB - IR)}{(IB + IR)} \]

where IB and IR are the emission intensities at the blue (440 nm) and red (490 nm) edges of the emission spectrum and correspond to the fluorescent emission maximal in the gel and liquid-crystalline phases, respectively [15].

Characteristic Laurdan GP values in phospholipid vesicles were determined to be, respectively, \( G_{pp} = 0.6 \) and \( G_{pl} = -0.2 \) for the gel and liquid-crystalline phases and independently of the phospholipid composition and pH value; therefore, it was proposed to use GP value to quantify the two phases, following the equation:

\[ GP = x \times G_{pp} + (1 - x) \times G_{pl} \]

where \( x \) is the fractional intensity of the gel phase [16].

Lipoprotein fluidity was studied by determining the fluorescence anisotropies (reciprocal of fluidity) of the probes 1,6-difenil-1,3,5-esatriene (DPH) (purchased from Molecular Probes), which is incorporated in the deeper hydrophobic part. Incubation with DPH was performed as described by Mazzanti et al. [17].

Fluorescence intensities (100 readings each) of the vertical and horizontal components of the emitted light were measured on a Perkin-Elmer MPF66 spectrofluorometer equipped with two glass prism polarizes (excitation wavelength 365 nm, emission wavelength 430 nm).

Statistical analysis

Results are expressed as means ± standard deviation. Statistical evaluations were performed using ANOVA, unpaired t-test.

Results

The average BMI was significantly decreased in the AN patients in comparison with the controls (Table 1 and 16.5 ± 3.4 vs. 21.8 ± 2.3 kg m−2, \( P < 0.001 \)), while plasma lipid parameters showed no significant differences either in total cholesterol or in HDL cholesterol.

A significant modification of the phospholipids to protein ratio was observed in lipoproteins of the AN patients (Fig. 1. \( P < 0.05 \)). A significantly increased percentage of unsaturated fatty acids was observed in every lipoprotein fraction (Table 2, \( P < 0.001 \)).

A decreased fluidity tested using the fluorescent probe DPH was observed in the inner part of the lipoproteins (Fig. 2. \( P < 0.001 \)).

The steady-state fluorescent studies performed by the Laurdan probe showed a significant increased percentage of liquid-crystalline phase in VLDL isolated from the AN
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Table 1 Characteristics of studied subjects

<table>
<thead>
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<th>Control subjects, n = 30</th>
<th>Anorexia nervosa subjects, n = 30</th>
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<tbody>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>21·8 ± 2·3</td>
<td>16·5 ± 3·4*</td>
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<tr>
<td>Blood glucose (mg dL(^{-1}))</td>
<td>84·0 ± 5·6</td>
<td>74·9 ± 8·6</td>
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<tr>
<td>Total cholesterol (mg dL(^{-1}))</td>
<td>184·3 ± 25·7</td>
<td>167·6 ± 30·4</td>
</tr>
<tr>
<td>HDL cholesterol (mg dL(^{-1}))</td>
<td>36·0 ± 15·1</td>
<td>52·6 ± 14·3</td>
</tr>
<tr>
<td>Triacyl-glycerols (mg dL(^{-1}))</td>
<td>78·0 ± 26·9</td>
<td>84·3 ± 28·8</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.
Significant differences (using ANOVA); *P < 0·001.

Table 2 Chemical-physical parameters in lipoprotein fractions obtained from both controls and AN patients

<table>
<thead>
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<th>Control subjects, n = 30</th>
<th>Anorexia nervosa subjects, n = 30</th>
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<tr>
<td>Saturated/unsaturated fatty acids ratio</td>
<td>LDL</td>
<td>1·93 ± 0·15</td>
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<td></td>
<td>LDL, oxidative stress (TBARS nmol 100 µg(^{-1}) prot.)</td>
<td>0·18 ± 0·09</td>
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<td></td>
<td>VLDL, % LC (Laurdan)</td>
<td>22·5 ± 3·92</td>
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<td></td>
<td>VLDL, % Gel (Laurdan)</td>
<td>77·5 ± 4·03</td>
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</table>

Data are expressed as mean ± SD.
Significant differences (using ANOVA); *P < 0·001.

Lipoproteins interact with the circulating cells, such as platelets and erythrocytes, which depend, lacking any synthetic apparatus, on lipoproteins for their lipid turnover.

Altered lipoprotein-cell exchange can be responsible in determining alterations in the development and maintenance of pathological states.

Lipoproteins play a role in the regulation of the cholesterol content of platelet membranes. Anorexia nervosa alteration of the lipid composition of the membranes, induced by lipoproteins, may modify the bilayer fluidity and influence cellular processes, including the transport mechanisms and the functionality of enzymes and receptors [19].

The question regarding cholesterol content in anorexia nervosa remains controversial, but in accordance with Mehler et al. [20] we reported no significant differences either in total cholesterol or in HDL cholesterol even if HDL levels were favourably high.

Discussion

The pathogenesis of AN remains uncertain, but evidence exists that the lipoprotein profile and cholesterol are modified [18].

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Regarding the fatty acid content, in every lipoprotein fraction we observed a significantly increased percentage of unsaturated fatty acids, increased phospholipid to protein ratio, and a significantly increased percentage of liquid-crystalline phase in VLDL, i.e. increased fluidity of the membrane. This phenomenon can result either from increased phospholipids or increased unsaturated fatty acid content [21,22].

Unsaturated fatty acids are the target of peroxidation, which occurs in natural cellular membranes. Previously we observed in Type 2 diabetes mellitus that VLDL and HDL showed a decrease in the saturated fatty acid content with a concomitant increase in unsaturated fatty acids and higher basal peroxide levels compared with healthy subjects [23].

In the present work in AN patients we found a significantly reduced susceptibility to oxidative stress which was more pronounced in LDL. This phenomenon may be owing to the increased levels of plasma retinols and alpha-tocopherol observed in anorexic patients [24], and this increase in antioxidant defences might explain the decreased susceptibility to oxidative stress reported for the LDL fraction.

Alterations of the chemical-physical properties of the plasma membrane have also been associated with a decreased activity of enzymes, i.e. adenylate cyclase (AC), the Na+/K+-ATPase, the Na+/H+ pump and alkaline phosphatase [25].

It has been shown that different cellular functions can be influenced by one or more lipoprotein classes. For instance, VLDL and/or LDL stimulate the activity of membrane enzymes, such as Mg2+-ATPase in erythrocytes or adenylate cyclase in liver or adipocytes.

The present data indicate in AN patients a deep alteration of the composition and chemical-physical properties in circulating lipoproteins. This phenomenon seems to occur even in the absence of significant modifications in clinical metabolic parameters and can be a marker of the disease. These results are consistent with the hypothesis that anorexia is accompanied by changes in the lipid metabolism in CNS. In effect, a significant decrease in various fatty acids prior to gas-liquid chromatography. Anal Biochem 1976;72:248–54.

The altered brain metabolism may be related to the circulating lipoprotein modifications. Structural alterations of phospholipids and integral constituents of myelin and synaptosomes may be related to psychotic disorders and body image distortion common to AN [27]. Lipid deregulation induces a disorder in bioenergetic homeostasis. Movement of lipids from adipose cells into the bloodstream affects carbohydrate and lipid metabolism, which in turn modifies the blood brain barrier (BBB). The derangement at the appetite centre may provoke a constant sensation of satiety and replacement of the correct body image that encourages poor judgement concerning food intake and self-support [28], interfering with an appropriate body image further and contributing to a reduced caloric intake.

In conclusion, we can hypothesize that the present work can represent, for the first time in the literature, a suitable model for studying anorexia nervosa in the derangement of lipid moiety.

References


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