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Chronic pancreatitis and the composition of plasma phosphatidylcholine fatty acids

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ABSTRACT

Chronic pancreatitis (CP) is an irreversible inflammatory disorder characterized by the destruction of both exocrine and endocrine tissue. There is growing evidence that dysregulation of fatty acid (FA) metabolism is connected with many diseases; however, there are few data concerning FA composition in CP. Therefore, we analyzed FA profiles in plasma phosphatidylcholines in 96 patients with CP and in 108 control subjects (CON).

The patients with CP had, in comparison with CON, increased sum of monounsaturated FA (ΣMUFA) and decreased content of polyunsaturated FA (PUFA) in both n-6 and n-3 families. Moreover, CP patients had increased indexes for delta-9, delta-6 desaturases, and fall in activity of delta-5 desaturase. Increased ratio of 16:1n-7/18:2n-6 (marker of essential n-6 FA deficiency), was more prevalent among CP patients.

These changes implicated decreased fat intake, including n-3 as well as n-6 PUFA, and intrinsic changes in FA metabolism due to the alteration of delta desaturase activities.

1. Introduction

Chronic pancreatitis (CP) is a condition characterized by progressive and irreversible damage to both the exocrine and endocrine parts of the gland. In addition to many complications, CP patients are at increased risk of developing pancreatic cancer. The pathological findings of CP are characterized by progressive pancreatic inflammation, fibrosis, acinar atrophy and distorted and blocked ducts. The basic etiopathogenetic mechanism responsible for the development of CP is inflammation-led fibrosis. Pancreatic fibrosis in CP is caused by recurrent episodes of acute pancreatitis, which causes interstitial acinar and fatty tissue necrosis and consequently leads to acinar fibrosis and distorted and/or blocked pancreatic ducts. Fibrotic destruction of the pancreatic gland is irreversible, and morphological and structural changes lead to the functional impairment of both exocrine and endocrine functions, eventually leading to malnutrition and/or diabetes [1,2].

Annual incidence of CP worldwide ranges between 1.8 and 14.0/100,000 subjects. CP prevalence varies at an average of about 50/100,000 persons. Approximately 70% of CP cases are caused by alcohol abuse, and the remaining cases are associated with genetic disorders, pancreatic duct obstruction, recurrent acute pancreatitis, autoimmune pancreatitis and unknown mechanisms [1].

There is growing evidence that dysregulation of fatty acid (FA) metabolism is connected with chronic diseases such as metabolic syndrome (MS) [3], obesity [4], diabetes mellitus type 2 (DM2T) [5], cardiovascular diseases [6], neuro-psychiatric disorders [7], various cancers [8], inflammation [6], allergies and autoimmune diseases [9].

In addition to the abovementioned diseases, the role of fat (and FA) has been extensively studied in many gastrointestinal diseases, such as non-alcoholic fatty liver (NAFLD)/non-alcoholic steatohepatitis (NASH) [10] and acute pancreatitis (AP), as well as in connection with AP severity and AP outcomes (reviewed in [11]).

Abbreviations: AP, acute pancreatitis; BMI, body mass index; CF, cystic fibrosis; CON, healthy controls; CP, chronic pancreatitis; CRP, C-reactive protein; CT, computer tomography; DD, delta desaturases; DGLA, dihomo-γ-linolenic acid (20:3n-6); DHA, docosahexaenoic acid (22:6n-3); DM, diabetes mellitus; DM2T, diabetes mellitus type 2; EFAD, essential fatty acid deficiency; EPA, eicosapentaenoic acid (20:5n-3); ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; FA, fatty acid; FELA, fecal elastase activity; HOMA-IR, homeostasis model assessment method; LDL-C, low density lipoprotein cholesterol; MRCP, magnetic resonance cholnagiopancreatography; MS, metabolic syndrome; MUFA, monounsaturated fatty acids; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEFA, non-esterified fatty acids; PUFA, polyunsaturated fatty acids; SCD-1, steraoyl-CoA desaturase, synonym for delta 9 desaturase (D9D); SFA, saturated fatty acids; TG, triacylglycerols.

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Although there is growing evidence that dysregulation of FA metabolism is connected with a number of diseases, there are few data concerning FA composition in pancreatitis. The composition of FA in plasma lipids and the RBC membrane has been evaluated both in acute [12] and chronic pancreatitis [13–16], as well as in CP and with and without diabetes mellitus (DM) [14,15].

FA composition in plasma phospholipids and cholesteryl esters reflects both dietary intake of FA over a six-week to three-month period as well as endogenous FA metabolism (synthesis of FA *de novo*, β-oxidation, enzymatic desaturation and elongation, conversion of polyunsaturated FA to eicosanoids and lipoperoxidation) [17].

The aim of the study was to evaluate changes in the composition of plasma phosphatidylcholines FA in patients with (i) CP in comparison with healthy controls (CON) and in relation to (ii) concomitant diabetes mellitus and (iii) malnutrition. Furthermore, multivariate linear discriminant analysis was used to evaluate the discriminative power of different FAs as independent variables (iv) capable of differentiating CP from healthy controls.

2. Patients and methods

2.1. Study design and participants

This study was carried out at the 4th Department of Medicine of the General University Hospital from January 2010 to September 2013. The study group consisted of 108 (55M/53F) controls (CON) and 96 consecutively diagnosed patients (70M/26F) with chronic pancreatitis (CP). All samples were marked with unique anonymized identification numbers, and the data was merged only after the assays had been completed. The study protocol was approved by the Joint Ethics Committee of the General University Hospital and the 1st Medical Faculty of Charles University in Prague (decision no. 3311/2011) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical diagnosis of CP was based on clinical features (abdominal pain, nausea and/or vomiting, anorexia and/or malnutrition and steatorrhea) confirmed by two or more imaging methods (abdominal – USG, contrast-enhanced computed tomography – CT), endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP) and endoscopic ultrasonography (EUS). Only patients with definite CP were included. The grade of CP (mild – moderate – severe) was assessed according to M-ANNHEIM pancreatic imaging criteria. All patients were assessed using a combination of EUS and other imaging methods (CT, or USG, or MRCP), since EUS does not differentiate between moderate and severe grades, and other methods (CT, USG or MRCP) cannot differentiate between mild and moderate changes [18].

The exclusion criteria for both groups were: ongoing antioxidant therapy (e.g. vitamin C, vitamin E, allopurinol, N-acetylcysteine), lipid-lowering treatment (fibrates, statins), supplementation with n-3 polyunsaturated fatty acids, kidney disease (creatinine $>$ 150 μmol/l), clinically manifest proteinuria (urinary protein $>$ 500 mg/l), liver cirrhosis, decompensated diabetes mellitus, concomitant malignancies, chronic immunosuppressive and anti-inflammatory therapy as well as chemotherapy. Further criteria for exclusion were: endocrine disease, acute pancreatitis or acute relapse of CP, unstable angina pectoris, acute myocardial infarction $<$ 1 year prior to enrollment, either coronary artery bypass grafting or percutaneous coronary intervention, and stroke. Persons who were operated on in the upper gastrointestinal tract (in the previous 12 months) and subjects who exhibited systemic inflammation in the previous 6 months were also excluded. Patients with CP enrolled in the study were re-examined after 2 years in order to exclude the development of pancreatic cancer and thus to avoid enrolling patients with the initial stages of pancreatic cancer in the study.

The diagnosis of T2DM was based on recommended guidelines [19]. In the CP group, there were 34 DM2T patients (30M/4F). Among all of the 34 DM2T patients, 12 (11M/1F) were treated with insulin therapy and 22 (19M/3F) were placed under peroral antidiabetic therapy or on a diet.

Alcoholic CP was diagnosed in 72 patients, obstructive CP in 9 subjects; 15 patients experienced idiopathic CP. Among the 96 patients with CP, 58 patients had severe grade, 22 had moderate and 16 had mild grade changes in pancreatic morphology according to the imaging methods used. Severe exocrine dysfunction [FELA (fecal elastase activity) $<$ 200 ng/g] was found in 61 patients with CP.

Complications (ascites, bleeding, obstruction and/or *ductus choledochus* stricture, pancreatic fistula, duodenal stenosis, splenic and/or portal vein thrombosis, segmental portal hypertension) were found in 43 patients with CP. According to the M-ANNHEIM severity index of CP, 26 patients were categorized as being at a minor level, 27 subjects at an increased level, 30 cases at an advanced level and 13 at a marked level of CP [18]. A total of 70 patients in the CP group were on pancreatic enzyme replacement therapy.

Among the CP group, 9 subjects were underweight (BMI $<$ 18.5 kg/m^2), 5 patients had plasma albumin lower than 35 g/l and 19 subjects had CRP higher than 10 mg/l. All CP patients were screened for nutritional risk. Among all of the CP patients, 15 patients were nutritionally at-risk (NRS score $\geq$ 3) [20]. The patients were asked to maintain their regular diet without using any supplements that might affect the intake of FA and they were recommended: (1) to avoid alcohol consumption and smoking; (2) not to skip meals; (3) to take small (low volume) and frequent meals; (4) to minimize high-sugar (high-glycemic, resp.) index food or fluids. Recommended energy intake was 30–35 Kcal/kg/day, with a protein 1–1.5 g/kg/day, and approximately 30% of calories may (should) be given as fat.

2.2. Blood sampling and anthropometry

Blood samples were taken after 12 h of fasting. Routine biochemical and hematological analyses were performed immediately and samples for special analyses were stored at –80 °C until use. Basic clinical and anthropometrical data, including assessment of body fat, were examined using standard methods, as described previously [3].

2.3. Laboratory measurements

Plasma concentrations of total cholesterol and triglycerides were measured using enzymatic-colorimetric methods (Boehringer, Mannheim, Germany). HDL-C was determined in supernatant after precipitation of B lipoproteins by PTA/Mg^{2+}, using a kit from the same manufacturer. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula. Concentration of apolipoprotein (apo) *B* was measured by Laurell rocket electroimmunoassay using standard and specific antibodies (Behringwerke, Marburg, Germany). Immunoreactive insulin was determined using the RIA method and double monoclonal antibodies (Insulin IRMA, Immunotech Prague, Czech Republic). The concentrations of non-esterified fatty acids (NEFA) were determined using an enzymatic-colorimetric method (NEFA, Randox Laboratories, UK).

Fatty acid patterns in the main plasma lipid classes were examined using analytical procedures described previously and