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Short communication

Effect of a high-fat challenge on the proteome of human postprandial plasma☆

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SUMMARY

Background & aims: Postprandial lipemia has been associated with inflammation, oxidative stress and vascular dysfunction. This metabolic disturbance represents a complex process only partly understood. The purpose of this study was to identify variations in plasma proteome after a high-fat challenge in healthy middle-aged men.
Methods: Two-dimensional electrophoresis was used to compare plasma from seven subjects, drawn before and 4 h after a high-fat challenge.
Results: Among the 231 spots detected and analyzed, 22 were present at different levels in postprandial hyperlipemic plasma compared to preprandial plasma. For 10 of them, corresponding proteins were identified by mass spectrometry. Some of them are related to the hemostatic system (tetranectin and fibrinogen) or the complement system (complement component 3 and 4 and ficollin-3) and have been previously associated to atherothrombosis.
Conclusion: These results provide new perspectives and broaden our understanding of the biological processes of postprandial metabolic stress, as well as its links with the development of atherosclerosis.

1. Introduction

Since the early report of Zilversmit in 1979,[1] the postprandial increase in blood lipids and triacylglycerol-rich lipoproteins has been proposed to play a causal role in the etiology of cardiovascular disease. Indeed, human studies have shown that a transient increase in blood triacylglycerol and fatty acid concentrations impairs endothelium-dependent vasodilation in healthy subjects[2] which is an early event in the development of atherogenesis.[3] Postprandial lipemia has also been associated with elevated concentrations of circulating proinflammatory and prooxidative molecules such as tumor necrosis factor-alpha, interleukin-6, interleukin-8 and nitrotyrosine.[4] We hypothesize that postprandial lipemia could induce modification of blood proteins involved in inflammation, coagulation, signaling or proteases activation. These variations could have an involvement in the complex metabolic stress associated to postprandial hyperlipemia and could constitute future potential biomarkers for the development of diseases associated to fat consumption. To identify such modifications, we used two-dimensional electrophoresis (2-DE) on proteins of plasma withdrawn before and after a high-fat challenge in middle-aged healthy men.

2. Subjects and methods

2.1. Study design

The study group consisted of seven healthy normolipemic, non-smoking male subjects. The physical characteristics of the subjects were (mean ± SD): age (yr) 49.3 ± 7.6; body weight (kg) 73.9 ± 9.4; BMI (kg/m^2) 25.0 ± 2.8; cholesterolemia (mmol/L) 5.15 ± 0.52; triacylglyceridemia (mmol/L) 0.84 ± 0.22. The purpose and potential risks of the study were explained to all subjects, and their written consents were obtained before participation. The study was carried out in accordance with the guidelines of the Declaration of

Abbreviations: C3, complement component 3; C4, complement component 4; FB, fibrinogen; Hp, haptoglobin; MS, mass spectrometry; PMF, peptide mass fingerprints; TN, tetranectin; 2-DE, two-dimensional electrophoresis.

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Helsinki and after approval by the Ethics Committee of the Auvergne area. After an overnight fast of at least 12 h, blood was drawn in the morning (baseline, 0 h). Blood samples were collected into 6 ml EDTA Vacutainer tubes. The volunteers then received an oral fat challenge consisting of commercial fresh cream (50 g fat per 1 m^2 of body surface) (total lipid content 42 g/100 wet wt., with the percentage composition SFA/MUFA/PUFA of about 68/29/3). Consumption of the cream was completed within 15 min. Plasma samples were then drawn 4 h after the cream was consumed. This time was selected as it corresponds to the postprandial peaks of plasma triacylglycerol and free fatty acid concentrations.[5,6] The volunteers abstained from consuming any food and drinks except water during the experiment duration.

2.2. Analytical procedures

Cholesterol and triacylglycerol concentrations were determined enzymatically using commercial kits from BioMerieux (Charbonnieres-les-Bains, France) and from Wako (NEFA-C kit Unipath, Dardilly, France) for free fatty acids.

2.3. Sample preparation for 2-DE

15 μl of each plasma sample was used for electrophoresis. Albumin and immunoglobulin G, the two most abundant proteins in plasma, were removed from these 15 μl sample, using an Albumin and IgG Removal Kit (GE Healthcare, Saclay, Orsay, France). 2-DE and image analysis were performed as previously described[7] with minor modifications. For analytical gels, 100 μg protein were loaded. For preparative gels, 5 albumin and immunoglobulin G depleted plasma samples were mixed to obtain 1000 μg protein, and then loaded. Analytical gels were used for image analysis and preparative gel for spot extraction and MS identification. The proteins were loaded onto 17-cm Bio-Rad ReadyStrips (Marnes La Coquette, France), pH 3–10 nonlinear. After passive rehydration of the strips and isoelectric focusing (IEF), the IPG strips were equilibrated. Then, the strips were loaded onto vertical 12% polyacrylamide gels and sealed with 0.5% (w/v) agarose. Proteins were visualized by silver staining, and gel images acquired and analyzed using the PDQuest software (Bio-Rad). For MS identification, spots were excised from one preparative gel using pipette tips.

In-gel digestion and matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) identification of proteins; All these steps were performed as previously described.[8] For MALDI-TOF analysis, the peptide solutions were loaded directly onto the MALDI target. Peptide masses were determined in positive-ion reflectron mode using a Voyager DE-Pro model MALDI-TOF mass spectrometer (PerSeptive BioSystems, Framingham, MA). External calibration was performed with a standard peptide solution (Proteomix, LaserBio Labs, Sophia-Antipolis, France). Internal calibration was performed using peptides resulting from auto-digestion of porcine trypsin, with protonated masses of 842.509, 1045.564, and 2211.104 Da. Peptide mass fingerprints (PMF) were compared with human protein databases (NCBI nonredundant and UniProtKB/SwissProt), using mascot software (www.matrixscience.com).[9] The database search in Mascot was conducted using a mass accuracy of 30 ppm, 1 missing trypsin cleavage site, partial carbamidomethylation of cysteine and partial methionine oxidation. A protein was successfully identified when a significant Mascot score was achieved ($p < 0.05$).

Table 1
Changes in postprandial plasma lipid concentrations after high fat ingestion.

Plasma parameters	0 h	4 h	*p*-value
Triacylglycerols, mmol/L	0.75 ± 0.18	1.30 ± 0.36	<0.01
Total cholesterol, mmol/L	6.03 ± 0.43	6.32 ± 0.38	NS
Free fatty acids, mmol/L	0.41 ± 0.15	0.68 ± 0.12	<0.01

The mean and standard deviation of plasma cholesterol, triacylglycerols and free fatty acids drawn before and 4 h after the high-fat challenge are presented. $n = 7$. NS, $p > 0.05$.

2.4. Data analyses

Statistical analysis was carried out using the SAS software (version 8.1, SAS Institute Inc., Cary, NC, USA). For biochemical

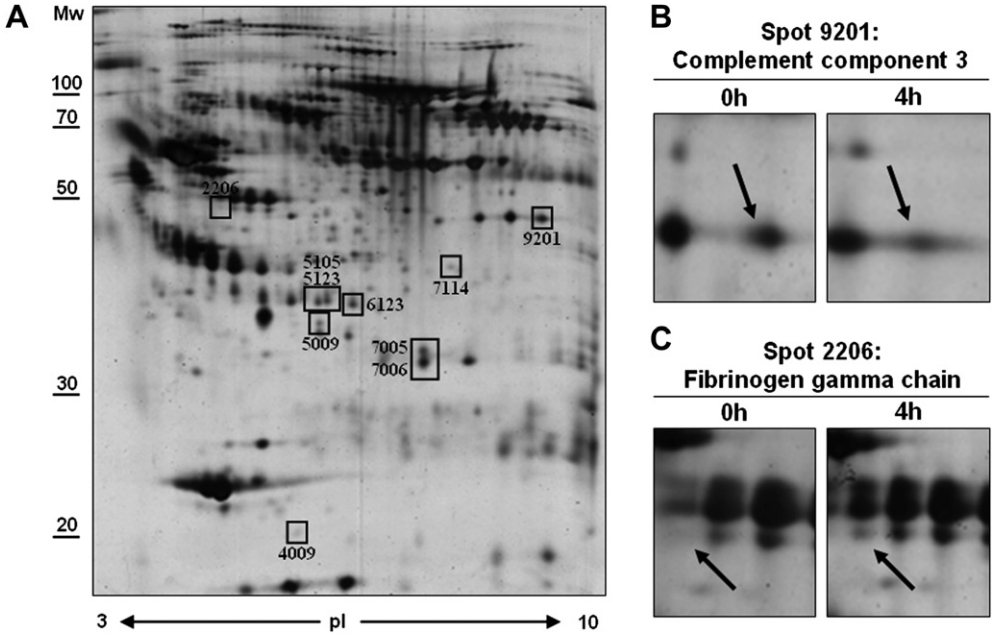

Fig. 1. 2-DE of pre- and postprandial plasma. (A) Densitometry scan of a typical silver-stained 2-DE gel with plasma proteins (depleted of albumin and immunoglobulin G). The boxes frame the 10 spots identified by MS (Numbers = index number assigned to the spots by PDQuest). (B, C) Examples of spot intensity from the 2-DE analysis showing differential relative abundance in pre- (0 h) and postprandial (4 h) plasma. Arrows point to protein spots with differential relative abundance.