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Postprandial changes in the proteome are modulated by dietary fat in patients with metabolic syndrome☆,☆☆,★

Antonio Camargo[a,1], Oriol Alberto Rangel-Zúñiga[a,1], Patricia Peña-Orihuela[a], Carmen Marín[a], Pablo Pérez-Martínez[a], Javier Delgado-Lista[a], Francisco Miguel Gutierrez-Mariscal[a], María M. Malagón[b], Helen M. Roche[c], Francisco José Tinahones[d], Francisco Perez-Jimenez[a,2], José Lopez-Miranda[a,*,2]

[a]*Lipids and Atherosclerosis Unit, IMIBIC/Reina Sofia University Hospital/University of Córdoba and CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III. 14004, Córdoba, Spain*

[b]*Department of Cell Biology, Physiology and Immunology, IMIBIC, University of Córdoba and CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III. 14014, Córdoba, Spain*

[c]*Nutrigenomics Research Group, UCD School of Public Health and Population Science, UCD Conway Institute, University College Dublin, Befield, Dublin 4, Ireland*

[d]*Hospital Virgen de la Victoria, Malaga, Spain, and CIBER Fisiopatologia de la Obesidad y Nutricion, Instituto de Salud Carlos III, Málaga, Spain*

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Abstract

Metabolic syndrome is a multicomponent disorder whose etiology is the result of a complex interaction between genetic, metabolic and environmental factors including dietary habits. Our aim was to identify proteome–diet interactions during the postprandial state after the acute intake of four meals with different qualities of fat in the proteome of peripheral blood mononuclear cells. A randomized controlled trial conducted within the LIPGENE study assigned 39 metabolic syndrome patients to one of four meals: a high-saturated-fatty-acid (HSFA) meal, a high-monounsaturated-fatty-acid (HMUFA) meal and two high-polyunsaturated-fatty-acid (from walnut) (HPUFA) meals supplemented with n-3 PUFA or placebo. We analyzed the postprandial changes in the whole proteome of both nuclear and cytoplasmic fractions of peripheral blood mononuclear cells by two-dimensional proteomics. Twenty-three proteins were differentially expressed. HSFA intake caused the postprandial increase of proteins responding to oxidative stress (HSPA1A, PDIA3 and PSME1) and DNA damage (SMC6), whereas HMUFA intake led to the up-regulation of HSPA1A and PDIA3. HPUFA meal supplementation with n-3 PUFA produced peroxisomal beta-oxidation inhibition by down-regulation of ECH1, a process related to insulin signaling improvement. In conclusion, HSFA meal intake causes deleterious postprandial changes in the proteome in terms of DNA damage and procoagulant state, which reflect a higher postprandial oxidative stress after HSFA meal intake as compared to intake of HMUFA and HPUFA meals. Moreover, the addition of long-chain n-3 PUFA to an HPUFA meal may improve insulin signaling and exerts an anti-inflammatory effect when compared to an HPUFA meal.

Keywords: Proteomic; Diet; Oxidative stress; Atherothrombosis; Metabolic syndrome

1. Introduction

The metabolic syndrome (MetS) is a multicomponent disorder associated with an increased risk of type 2 diabetes and cardiovascular diseases [1]. The etiology of MetS is the result of a complex interaction between genetic, metabolic and environmental factors, including dietary habits and, probably, the quality of dietary fat [2].

The postprandial state causes an important stress on the homeostasis due to the increase in lipid-derived proinflammatory molecules, oxidative stress and a transient increase in proinflammatory molecules released by human white blood cells and endothelial cells [3]. Likewise, the changes in postprandial metabolism occurring every time we eat a meal and alterations in this state play an important role in the development of cardiovascular disease and associated pathologies [4].

An inflammatory response is a feature of the complex proatherogenic phenotype occurring during the postprandial state [5], which is especially important in patients with MetS since they are

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* Corresponding author. Reina Sofia University Hospital, Lipids and Atherosclerosis Research Unit, 14004 Córdoba, Spain. Tel.: +34 957 012882; fax: +34 957 204763.

E-mail address: jlopezmir@uco.es (J. Lopez-Miranda).

[1] The first two authors (A.C. and O.A.R.-Z.) contributed equally to this study.

[2] The last two authors (F.P.-J and J.L.-M.) contributed equally to this study.

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characterized as exhibiting a state of low-grade inflammation. MetS patients are particularly vulnerable since they exhibit an exacerbated hypertriglyceridemia response [6] and abnormalities in the postprandial metabolism of lipoproteins [7]. In addition, postprandial hypertriglyceridemia has been related to the proinflammatory state [3].

Peripheral blood mononuclear cells (PBMCs) are a subset of white blood cells consisting of lymphocytes and monocytes/macrophages. They are relatively easily accessible in humans by isolation from a blood sample, and they can be used to assess biological responses and are a potential source to discover new biomarkers of response to environmental cues, including nutrition [8–10]. Despite the increasing number of studies that utilize PBMCs for diagnosis or disease-associated purposes [8], most of them analyze gene expression, and, while relevant, they have the intrinsic limitation of not focusing on the final products that perform the biological function. However, in the area of nutritional research, these studies have shown, for example, that diet modulates the gene expression of inflammatory genes [11,12], metabolism-related genes [13] and oxidative stress [14].

Proteomics is a central platform in nutrigenomics that describes how our genome expresses itself as a response to diet [15]. However, while proteomics represents a novel, promising tool to uncover the mechanisms of action of nutrients as well as to identify potential biomarkers of health or disease, the actual use of this technique in dietary intervention trials is still rather limited [16]. Fuchs et al. [17] have shown the PBMC proteome response to a dietary intervention with isoflavone-enriched soy extract in postmenopausal women in the fasting state. However, no studies to date have addressed the postprandial modulation of PBMC proteome by diet. Additionally, an observational study has shown that the plasma proteomic profile differs between young people of diverse ethnocultural groups with different dietary habits [18].

In this study, we present data on changes in the proteome of PBMCs isolated from MetS patients in response to the acute intake of four diets with different quantities and qualities of fat. The nutritional regulation of the postprandial proteome was analyzed to identify fast-response proteins to the quality of dietary fat and to pave the way for future research into the molecular mechanism underlying gene–nutrient interaction. To the best of our knowledge, our study is the first one focusing on postprandial proteome modulation by diet.

2. Methods and materials

2.1. Participants and recruitment

This study was conducted within the framework of the LIPGENE study (Diet, genomics and metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis), a Framework 6 Integrated Projected funded by the European Union. All participants gave written informed consent and underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. This study was carried out at the Lipid and Atherosclerosis Unit of the Reina Sofia University Hospital from February 2005 to April 2006. The experimental protocol was approved by the local ethic committee according to the Helsinki Declaration.

From this LIPGENE cohort, we analyzed the PBMC proteome from 24 patients, 6 patients per meal (3 women and 3 men), by two-dimensional (2-D) proteomic analysis. After that, we used the PBMC proteins from the whole subgroup, which comprised 39 patients (Supplemental Table 1) [8 patients ingested a high-saturated-fatty-acid (HSFA) meal, 9 patients ingested a high-monounsaturated-fatty-acid (HMUFA) meal, 12 patients ingested a high-polyunsaturated-fatty-acid (HPUFA) meal, and 10 patients ingested an HPUFA n-3 meal] to validate proteomic data by Western blot.

2.2. Design, randomization and intervention

MetS patients were randomly stratified to one of four test meal intakes. MetS was defined by published criteria [19], which conformed to the LIPGENE inclusion and exclusion criteria [20]. Randomization was completed centrally according to age, gender and fasting plasma glucose concentration using the Minimization Program for Allocating Patients to Clinical Trials (Department of Clinical Epidemiology, London Hospital Medical College, UK) randomization program. The meals differed in fat quality while remaining isoenergetic (Supplemental Table 2).

Briefly, HSFA meal provided 38% E from SFA, 21% from MUFA and 6% from PUFA; HMUFA meal provided 12% E from SFA, 43% from MUFA and 10% from PUFA; and HPUFA meals provided 21% E from SFA, 28% from MUFA and 16% from PUFA. HPUFA with placebo, meal included 1.2 g supplement of control high-oleic sunflower seed oil capsules; HPUFA with long chain (LC) n-3 PUFA, meal included 1.24 g/d of LC n-3 PUFA (ratio 1.4 EPA:1 DHA).

Patients arrived at the clinical center at 08:00 h following a 12-h fast, refrained from smoking during the fasting period and abstained from alcohol intake during the preceding 7 days. In the laboratory and after cannulation, a fasting blood sample was taken before the test meal, which then was ingested within 20 min under supervision.

Test meals provided an equal amount of fat (0.7 g/kg body weight), E content (40.2 kJ/kg body weight), cholesterol (5 mg/kg of body weight), fiber and vitamin A [62.9 mmol vitamin A (retinol)/m^2 body surface area]. The test meal provided 65% of E as fat, 10% as protein and 25% as carbohydrates. During the postprandial assessment, participants rested and did not consume any other food for 9 h but were allowed to drink water.

The natural foods used in the meals were as follows: HSFA, 38% E from SFA, based on butter, whole milk, white bread and eggs intake; HMUFA, 43% E from MUFA, based on olive oil, skimmed milk, white bread, eggs, yolk eggs and tomatoes intake; HPUFA (21% SFA, 28% MUFA), based on butter, olive oil, skimmed milk, white bread, eggs, yolk eggs and walnuts.

2.3. Blood sample collection

Venous blood samples were obtained in the fasting state, after a 12-h fast, before and at 4 h after the ingestion of the breakfast. Samples from the fasting and postprandial states were collected in tubes containing 1 g EDTA/L and were stored in containers with iced water in the dark. Special care was taken to avoid exposure to air, light and ambient temperature. Plasma was separated from whole blood by low-speed centrifugation at 1500*g* for 15 min at 4°C within 1 h of extraction.

2.4. Isolation of peripheral blood mononuclear cells

PBMCs were isolated within 2 h after blood draw from 30-ml EDTA anticoagulated blood samples. Buffy coats were diluted 1:2 in phosphate-buffered saline (PBS), and cells were separated in 5 ml Ficoll gradient (lymphocyte isolation solution, Rafer) by centrifugation at 2000*g* for 30 min. PBMCs were collected and washed twice with cold PBS. PBMCs were stored in lysis buffer A (10 mM HEPES; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 0.5 mM PMSF; 1 mM DTT; 10 μg/ml CLAP; 1% NP-40) at −80°C prior to protein extraction.

2.5. Protein extraction from peripheral blood mononuclear cells

Protein extracts from nuclear and cytoplasmic fractions from PBMCs were obtained following the procedure previously described by Hernandez-Presa et al. [21]. Briefly, samples were thawed for 15 min in ice, and then they were vortexed for 20 min and centrifuged at 15,000*g* for 5 min at 4°C, and the cytoplasmic fraction was collected on the supernatant. Pellet was dissolved in 100-μl lysis buffer C (20 mM HEPES; 400 mM NaCl; 1 mM EDTA; 1 mM EGTA; 1 mM PMSF; 1 mM DTT; 10 μg/ml CLAP) and incubated for 20 min in ice. Samples were vortexed every 5 min for 30 s during the incubation period. After centrifugation at 10,000*g* for 5 min at 4°C, the nuclear fraction was collected on the supernatant. Protein samples were quantified by Bradford method using Dye Reagent Protein (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions.

2.6. 2-D gel electrophoresis

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was performed as described by Görg et al. [22]. Protein fractions were cleaned using the Ready Prep 2-D Cleanup Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. A total of 200 μg of protein were diluted in 200 μl of rehydration buffer (8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte and 0.002% bromophenol blue). Immobilized pH gradient strips (11 cm, pH 3–10) were rehydrated overnight in a Protean IEF Cell (Bio-Rad Laboratories) following a stepwise voltage: slow ramp, 250 V (15 min), 8000 V (5 h, linear gradient), 8000 V (26,000 Vh), total Vh 40,000. Strips were equilibrated in equilibration buffer I [6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% sodium dodecyl sulfate (SDS), 20% glycerol, 2% (w/v) DTT] for 10 min and then for another 20 min in equilibration buffer II [6 M urea, 0.375 M Tris–HCl, pH 8,8, 2% glycerol, 2.5% (w/v) iodoacetamide]. Thereafter, proteins were separated in 12% Bis-Tris Criterion XT Precast Gels (Bio-Rad Laboratories) using a Criterion Dodeca Cell system (Bio-Rad Laboratories) in MOPS buffer 1× at 180 V.

Gel staining was performed overnight in darkness with SYPRO Ruby after protein fixing by using 40% methanol and 10% acetic acid for 2 h. Gels were washed in 40% methanol and 10% acetic acid twice for 1 h and once in distilled water for 30 min before imaging acquisition.