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

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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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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.

2.7. Imaging acquisition and spots detection

After staining, gels were visualized with UV light by using ChemiDoc XRS System (Bio-Rad Laboratories). Images were acquired using the software Quantity One 16.0 (Bio-Rad Laboratories). The spots were detected by the PDQuest software 8.0.1 (Bio-Rad Laboratories). Detection parameters set: spot detection sensitivity=6.0; size scale=5; mini peak=2000. Speckle filter enable and sensitivity=70.0. Image smoothing enabled, filter median and smooth kernel size 3×3. Background subtraction enabled, method floating ball and background radius=67. Streak removal enabled vertical and horizontal streak radius=111. The normalization method chosen was the total quantity in valid spots.

2.8. MALDI-TOF MS analysis

Spots were excised automatically in a ProPic station (Genomic Solutions, Huntingdon, UK) and subjected to MS analysis. For matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry (MALDI-TOF-MS) analysis, gel specimens were unstained twice (30 min, 37°C) with 200 mM ammonium bicarbonate/40% acetonitrile. Gel pieces dehydrated for 5 min with pure acetonitrile and dried out over 4 h were automatically digested with trypsin according to the standard protocols in a ProGest station (Genomic Solutions). MS and MS/MS analyses of peptides of each sample were performed in a 4700 Proteomics Station (Applied Biosystems, CA, USA). Mass spectrometry was performed at the Proteomics Facility (SCAI) of the University of Cordoba, which is Node 6 of the ProteoRed Consortium financed by Genoma España and belongs to the Andalusian Platform for Genomics, Proteomics and Bioinformatics.

2.9. Pathway analysis

In order to investigate functional relationships in the set of differentially expressed proteins, we used the Ingenuity Pathway Analysis Software (Ingenuity Systems, Redwood City, CA USA) [23] which employs a predefined knowledge base containing over 10,000 curated human genes.

Network analysis yielded 15 different subnetworks. From the 10 proteins found differentially expressed after the intake of HSFA, 2 proteins (PSME1, ZFP2) were not eligible for network analysis, which yielded 6 subnetworks. For HMUFA meal, of the six proteins, two proteins (CCDC150, Serpin B1) were not eligible for network analysis which yielded four subnetworks. For HPUFA meal, of the five proteins, two proteins (HSPA6, MYL12A) were not eligible and yielded three subnetworks. For HPUFA n-3 meal, of the five proteins, three proteins (ALDH2, CAPZB, and ECH1) were not eligible and yielded two subnetworks. After that, we built two networks merging those subnetworks sharing one or more proteins.

2.10. Western blot validation experiments

Commercial antibodies used for Western blot validation experiments were provided by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Forty micrograms of protein from each individual subject (8 patients who ingested an HSFA meal, 9 patients who ingested an HMUFA, 12 patients who ingested an HPUFA meal and 10 patients who ingested an HPUFA n-3 meal) was loaded in gels for SDS-PAGE. Proteins were transferred to nitrocellulose membranes, which were blocked with 1× tris-buffered saline and tween 20 (TBS-T) containing 2% of bovine serum albumin, followed by incubation with the corresponding primary antibody diluted in TBS-T at the concentrations indicated by the manufacturer. After washing, membranes were incubated with the appropriate IgG–HRP-conjugated secondary antibody. Membranes were incubated for 2 min with SuperSignal West Pico ECL Substrate (Thermo Scientific) for protein detection. Images were acquired on a Molecular Imager ChemyDoc XRS (Bio-Rad Laboratories), and band density analysis was performed with QuantityOne Software V (Bio-Rad Laboratories). Band intensity signal was normalized by total protein in membranes, which was measured by Ponceau S stain method.

2.11. Statistical analysis

2-D gel analysis was performed by PDQuest software (Bio-Rad Laboratories), version 8.0. Manual corrections were also performed to validate the matches automatically generated by the software. Spot volume values were normalized in each gel by dividing the raw quantity of each spot by the total volume of all the spots included in the same gel. Other normalizations provided by the PDQuest software were also performed with similar results. Variations of all the identified spots were finally confirmed and quantified by density measurements using ImageJ 1.40g software. Statistical analysis used SSPS statistical software, version 15.0 for WINDOWS (SSPS, Chicago, IL, USA). Normal distribution of variables to characterize differences in the expression of proteins under study was assessed using the Kolmogorov–Smirnov test followed by a Student's *t* test for independent samples. Differences were considered significant at $P<.05$. All data are expressed as mean±S.E.M.

3. Results

3.1. Baseline characteristics

No significant differences were observed in the baseline characteristics of the 39 subjects with MetS participating in the dietary intervention (Supplemental Table 1).

3.2. Proteomic analysis

We performed 2-D gel electrophoresis of nuclear and cytoplasmic fractions from PBMCs separately. On average, >400 different protein spots from nuclear and cytoplasmic fractions were resolved by 2-D PAGE.

Comparative proteomic analysis of the PBMC proteome from patients before (fasting state) and after the intake of the test meal (postprandial state) (Table 1) revealed that the acute intake of HSFA meal caused the postprandial up-regulation of five proteins (ZFP2, SMC6, HLA-C, THBS1 and PSME1) and the postprandial down-regulation of five proteins (REV3-like, PDIA3, HSPA1A, FGB and PLEC). HMUFA meal intake caused the postprandial up-regulation of three proteins (HSPA1A, HLA-A and PDIA3) and the postprandial down-regulation of three proteins (CCDC150, CEP290 and SERPIN B1). HPUFA meal intake caused the postprandial up-regulation of four proteins (FLNA, GRIN1, HLA-C and HSPA6) and the postprandial down-regulation of one protein (MRLC3). HPUFA n-3 meal intake caused the postprandial up-regulation of three proteins (VIM, PPIA, ALDH2) and the postprandial down-regulation of two proteins (CAPZB, ECH1).

The identified proteins could be grouped into the following categories: (a) molecular chaperones and stress response (HSPA1A, HSPA6, PDIA3); (b) cell-to-cell interaction (FGB, THBS1); (c) DNA maintenance (SMC6, REV3-like, CEP290); (d) structural filaments or components of the cytoskeleton (PLEC, FLNA, VIM, MRLC3, CAPZB); (e) enzymes and receptor (HLA-A, HLA-C, SERPIN B1, GRIN1, PPIA, ALDH2, ECH1); (f) others (ZFP2, CCDC150, PSME1) (Fig. 1).

3.3. 2-D proteomic result validation by Western blot

To confirm proteomic analysis results using an independent technique, we analyzed eight differentially expressed proteins (fold change ranging from 2.53 to 0.43) by Western blot, which corresponded to the following categories: three chaperones, two enzymes, two cell structural components and one cell-to-cell interaction. Quantification of the immunoreactive bands revealed the same diet-induced changes as those observed by 2-D proteomics (Supplemental Figure 1).

3.4. Pathway analysis

Ingenuity Pathway Analysis showed that the top function associated with proteins differentially expressed after HSFA meal intake was inflammatory response (HLA-C, THBS1 and PSME1, up-regulated; PLEC, FGB and HSPA1A, down-regulated). Likewise, different genetic disorders were the top functions associated to proteins differentially expressed after acute intake of HMUFA, HPUFA and HPUFA n-3 meals.

In terms of molecular function, cell-to-cell signaling and interaction (HSFA five proteins, HMUFA two proteins and HPUFA zero protein), together with DNA replication, recombination and repair (HSFA two proteins, HMUFA two proteins, and HPUFA zero protein), showed higher representation after the intake of HSFA and HMUFA than after the intake of HPUFA meal, but not after the intake of HPUFA n-3 (cell-to-cell signaling and interaction, two proteins; DNA replication, recombination and repair, two proteins). On the other

Table 1
Quantity and quality of dietary fat effect in the postprandial changes on proteome

	FC	*P* value	MW	pI	Accession no.	Score CI %	Pep count	Ion CI %
Postprandial changes of proteins induced after acute HSFA meal consumption								
Nuclear fraction								
1. ZFP2: zinc finger protein 2 homolog	3.27	.036	54359.7	8.91	gi|47271457		8	
2. SMC6: structural maintenance of chromosomes 6	2.35	.006	35828	7.59	gi:122070455	99.919	11	
3. REV3-like: catalytic subunit of DNA polymerase zeta (yeast), isoform CRA	0.44	.031	37.5	9.6	gi:119568677	99.093		
Cytoplasm fraction								
4. HLA-C: HLA class I histocompatibility antigen. Cw-1 alpha chain	3.90	.030	24053.8	6.75	gi|599786	30.637	6	
5. THBS1: thrombospondin	2.53	.043	42215.2	6.6	gi|553801	99.99	5	99.997
6. PSME1: proteasome activator subunit 1 isoform 1	1.90	.031	28754.9	6.28	gi|30581141	99.982	28	81.644
7. PDIA3: protein disulfide-isomerase	0.58	.043	52884.4	4.76	gi:2507460	100	8	100
8. HSPA1A: heat shock 70-kDa protein 1A	0.43	.047	70280.1	5.48	gi|5123454	100	16	100
9. FGB: fibrinogen beta chain. isoform CRA_f	0.33	.026	44723.6	8.84	gi|119625340	100	9	99.834
10. PLEC: plectin-1	0.32	.049	518510.8	5.60	gi:209572726	99.322	33	
Postprandial changes of proteins induced after acute HMUFA meal consumption								
Nuclear fraction								
11. CCDC150 protein	0.25	.032	44114.5	9.47	gi|38541637	83.74	9	
12. CEP290: centrosomal protein of 290 kDa	UE	.018	268104.8	5.75	gi:116241294	99.941	26	
Cytoplasm fraction								
8. HSPA1A: heat shock 70-kDa protein 1A	2.20	.045	70110.0	5.42	gi|386785	100	16	100
13. HLA-A: MHC class I antigen	1.81	.042	21139.1	5.77	gi|89152359	44.903	6	
7. PDIA3: protein disulfide isomerase	1.40	.043	54453.6	6.78	gi|119597640	100	15	99.95
14. SERPIN B1: serpin peptidase inhibitor, clade B (ovalbumin), member 1	0.36	.036	42889.8	6.22	gi:266344	99.999	9	99.512
Postprandial changes of proteins induced after acute HPUFA meal consumption								
Cytoplasm fraction								
15. FLNA: filamin alpha (actin binding protein 280), isoform CRA_e	7.94	.0434	266548.5	5.79	gi|119593154	100	15	100
16. GRIN1: glutamate receptor subunit zeta-1	2.73	.0469	105373	8.5	gi:548377	99.274	14	
4. HLA-C: HLA class I histocompatibility antigen. Cw-17 alpha chain	1.38	.029	19045.2	6.95	gi:34395506	39.587	6	
17. HSPA6: heat shock 70-kDa protein 6, variant	1.35	.046	71416.3	5.81	gi|62898285	99.874	5	99.992
18. MRLC3: myosin regulatory light chain 12A.	0.49	.0397	19456.2	4.74	gi|127169	99.992	5	99.916
Postprandial changes of proteins induced after acute HPUFA n-3 meal consumption								
Nuclear fraction								
19. VIM: vimentin	1.94	.031	53604.1	5.09	gi|47115317	53.109	6	73.579
20. PPIA: cyclophilin A	1.84	.047	18039.9	8.37	gi|75766275	100	5	100
Cytoplasm fraction								
21. ALDH2: aldehyde dehydrogenase 2 family (mitochondrial)	1.78	.042	56932.9	6.63	gi|48146099	92.902	2	99.944
22. CAPZB: capping protein (actin filament) muscle Z-line, beta	0.69	.045	29561.9	6.45	gi|55665440	100	7	100
23. ECH1: enoyl Coenzyme A hydratase 1, peroxisomal	UE	.014	36077.5	8.47	gi|16924265	100	6	100

FC, fold change (proteins level at postprandial/proteins level at fasting). *P* value, *t* test *P* value; MW, theoretical molecular weight; pI, theoretical isoelectric point. Pep count, peptide count; CI, confidence index; UE, underexpressed, not detected at postprandial.

hand, cellular assembly and organization (HSFA one protein, HMUFA one protein, HPUFA two proteins and HPUFA n-3 three proteins) as well as cell morphology (HSFA zero protein, HMUFA zero protein, HPUFA two proteins and HPUFA n-3 two proteins) showed higher representation after the intake of HPUFA meals than after the intake of HSFA and HMUFA meals.

In order to investigate whether the differentially expressed proteins after the intake of different quality of fat interact with each other, we studied the relationships between the differentially expressed proteins at the postprandial state after the acute intake of four test meals by using the Ingenuity Pathway Analysis Software. Thus, the network analysis yielded 15 different subnetworks, which could be grouped into two networks by merging those subnetworks sharing one or more proteins. The top functions associated to network 1 were cellular growth, proliferation, movement and development. For network 2, they were inflammatory response, cell death, cellular growth and proliferation. Finally, we removed those proteins not connecting the identified proteins directly (Fig. 2).

4. Discussion

Herein, we report for the first time the postprandial proteomes of PBMC in response to the quality of fat in diet. Moreover, this is the first study dealing with postprandial response to different dietary models in MetS patients, whose etiology is related to dietary habits [2].

Our proteomic approach yielded 23 differentially expressed proteins at the postprandial state after the acute intake of four test meals with different qualities of fat. HSFA and HMUFA meals caused postprandial proteome changes in processes such as cell signaling and interaction and DNA repair. In contrast, an HPUFA postprandial challenge led to postprandial changes in proteins involved in processes such as cellular assembly and organization, or cell morphology. Additionally, the supplementation of an HPUFA meal with LC n-3 PUFA caused proteome changes related to cell signaling and interaction, DNA repair, cellular assembly and organization, and cell morphology.

The current study showed that despite the heterogeneity of the fatty meals administered, in terms of fat quality, most of the postprandial short-term response proteins could be grouped in an interconnected network, associated to cellular functions such as cellular growth and proliferation (network 1), which may be important for the development of atherosclerosis since it has been demonstrated that the progression of this disease is associated with the clonal expansion of T cells [24].

However, none of the dietary models tested showed a proteome profile favoring or repressing the growth and proliferation of PBMC. The fact that each meal intake caused the up-regulation or down-regulation of only one or two proteins in the network suggests that dietary fat may affect cellular growth and proliferation processes at the postprandial state but not in the fatty acid proportions or doses administered in our study.

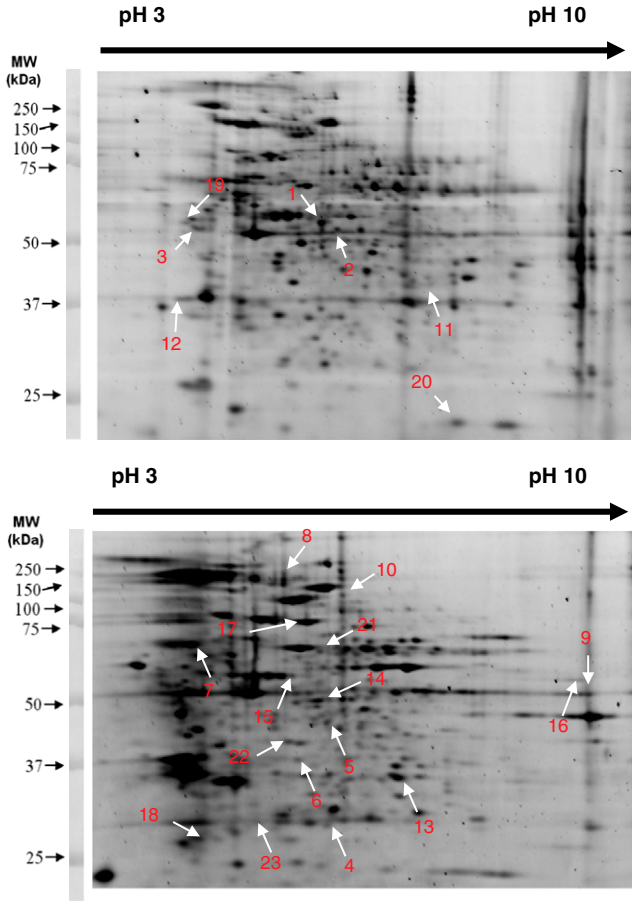

Fig. 1. 2-D PAGE of PBMC whole proteome, nuclear fraction [1] and cytoplasmic fraction [2]. Proteins were separated on a 2-DE gel using 18-cm pH 3–10 strips in the first dimension and 12% SDS-PAGE gels in the second dimension, as described in Material and Methods. Differentially expressed proteins are indicated with arrows. The numbers corresponding to the spot numbers are listed in Table 1.

Notably, several of the differentially expressed proteins or protein isoforms found in our study [heat shock protein (HSPA1A, HSPA6), myosin regulatory chain, proteasome activator subunit, filamin alpha, fibrinogen beta chain and thrombospondin] have been shown to be modulated by soy isoflavones in the fasting state after a long-term intervention [17]. This study showed the chronic effect of a dietary intervention with isoflavone-enriched soy extract in postmenopausal women. Together, these and our findings support the idea that these proteins are sensitive to modulation by nutritional intervention.

The postprandial state involves an increase in the production of reactive oxygen species, which can cause damage to biological macromolecules such as proteins and DNA [25,26]. HSPA1A is a member of the heat shock protein family [27], whose gene expression is repressed by p53 when activated by DNA damage [28]. Previous studies from our laboratory have shown that an HMUFA meal following a long-term consumption of a MUFA-rich diet lowered several postprandial oxidative stress biomarkers as compared to that evoked by an HSFA meal after long-term consumption of an SFA-rich diet [29]. Taking into account that p53 has antioxidant functions protecting the genome from oxidation produced by oxidative stress [30,31], our results suggest that p53 activation by postprandial oxidative stress after an HSFA meal intake — probably by DNA damage, as shown previously in an elderly population [32] — could be responsible for HSPA1A down-regulation and, for the opposite effect, up-regulation after an HMUFA meal, which may be accounted for by a lack of p53 postprandial activation.

Interestingly, THBS-1, an adhesive glycoprotein whose gene expression is increased by p53 [33], was up-regulated after the HSFA meal intake and remained unchanged after the intake of the other three meals. This glycoprotein has been shown to promote platelet aggregation [34], a physiologic process of defense against uncontrolled hemorrhage, although it also occludes blood vessels with thrombi, thus leading to cardiovascular events, including unstable angina and myocardial infarction [35]. In line with this, we also observed a postprandial decrease in fibrinogen beta chain (FGB), whose synthesis is the limiting step in the production of mature fibrinogen [36]. Fibrinogen is cleaved by thrombin to form insoluble fibrin which is the most abundant component of blood clots [37]. Thus, FGB postprandial decrease after an HSFA meal intake suggests an increased fibrin production by cleavage and release, which would reduce the substrate amount, FGB.

Both THSB1 increase and FGB decrease suggest a procoagulant state after an HSFA meal when compared to the other meals investigated herein. In this regard, an imbalance between procoagulant and profibrinolytic activity has been linked to coronary heart disease [38]. This is especially important in MetS patients as they exhibit a prothrombotic state in the fasting state [1], which seems to be increased in the postprandial state after an SFA-rich meal intake.

The notion that several of the postprandial proteome changes observed are due to increased postprandial oxidative stress after an SFA meal intake is also supported by the observation that SMC6, a protein involved in the structural maintenance of chromosomes [39], and PSME1, a proteasome activator [40] that has been recently related to oxidative stress protection [41], were up-regulated in patients following this diet. Moreover, REV3, a protein involved in translesion DNA synthesis [42], was down-regulated after an HSFA meal intake. Remarkably, protein levels of SMC6, PSME1 and REV3 remained unchanged after the intake of the other three meals.

On the other hand, PDIA3 (also called ERp57) belongs to a family of oxidoreductases, including PDI, ERp72, P5 and PDIR, which are involved in the formation of native disulfide bonds, and under conditions of oxidative stress, the oxidoreductases are quickly reduced and remain active. Interestingly, PDIA3 has been shown to be reduced directly by glutathione [43], a molecule that increases at the postprandial state after an HMUFA meal following long-term consumption of an HMUFA-rich diet, and decreases after an HSFA meal intake following the consumption of a long-term SFA-rich diet in our population [29]. In all, these findings indicate that HSFA diet would decrease PDIA3 levels in the short term and repress the activity of the enzyme when the component responsible for its reduction (glutathione) is scarce (i.e., after long-term consumption of an HSFA diet). In contrast, it was up-regulated after HMUFA intake, as glutathione levels may be higher than after an HSFA meal intake, as has been shown by Perez-Martinez et al. [29].

Taken together, these findings suggest that the proteome, at least partially, seems to be responding to the oxidative status at the postprandial state after the intake of the different meals, directly and through DNA damage. In addition to the relationship with oxidative stress, the top function associated with the proteins differentially expressed after an HSFA meal intake was inflammatory response, which supports the link between oxidative stress and inflammation [44,45]. Notably, although both HSPA1A and PDIA3 belong to protein families associated with endoplasmic reticulum stress (HSP70 and PDI), these isoforms seem to respond to oxidative stress rather than to ER stress, which is also related to oxidative stress [46], and this supports the notion that unfolded protein response fulfils a wide spectrum of physiological roles [47]. Thus, in conditions of high postprandial oxidative stress (i.e., after intake of an HSFA meal), it seem to have a lack of unfolding protein response in MetS patients, which could also contribute to impair the postprandial state of these patients.

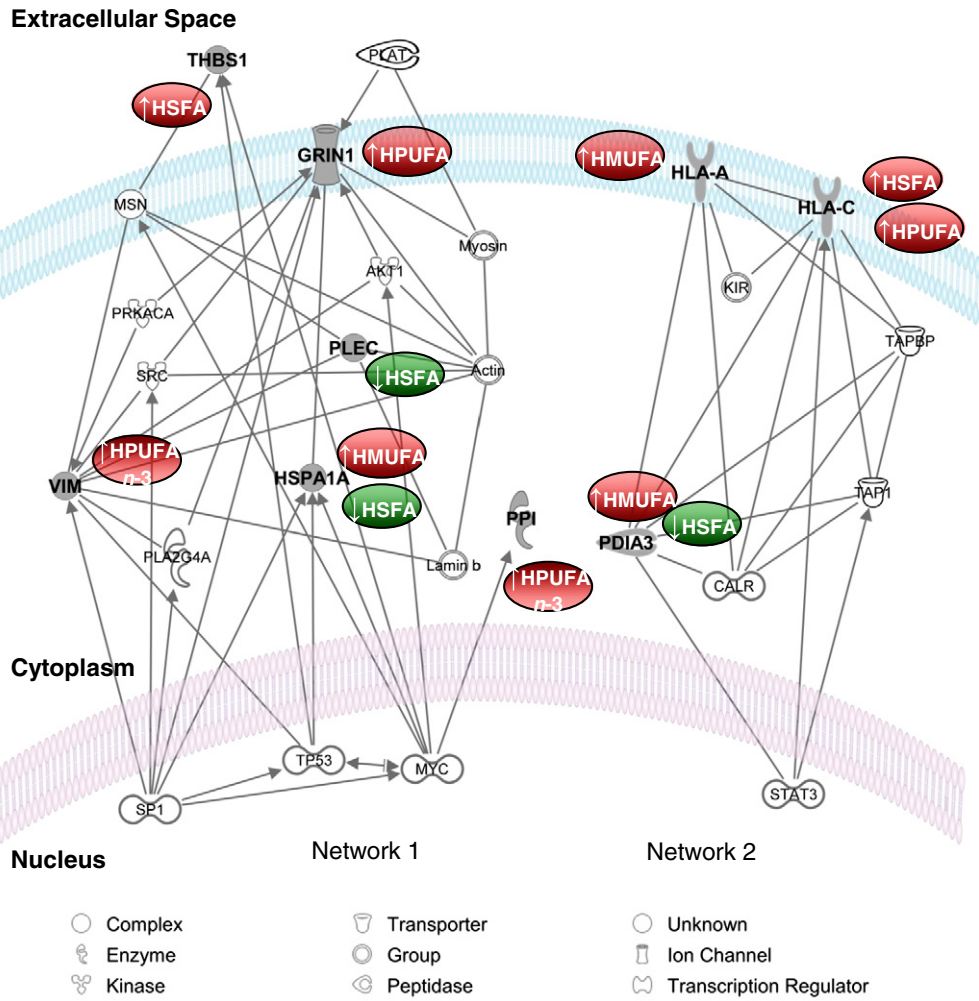

Fig. 2. Interaction between induced proteins at the postprandial state. Networks of differentially expressed proteins at the postprandial state after the acute intake of four diets with different quantities and qualities of fat that were found to be interconnected and interacting with each other. Gray symbols denote that the proteins were found overexpressed or underexpressed by dietary fat at the postprandial state.

When meals contained close percentages of SFA and MUFA (HPUFA meals), no proteome changes related to oxidative stress were observed, probably due to an intermediate situation between HSFA and HMUFA meals. However, the intake of HPUFA meals (with or without n-3 supplementation) seems to cause postprandial changes in cell morphology, as suggested by the dysregulation observed in FLNA (filamin alpha) and MRLC3 (myosin regulatory light chain) after HPUFA intake, and VIM (vimentin) and CAPZB (capping protein, actin filament) after HPUFA intake supplemented with LC n-3 fatty acids.

In addition, the supplementation of an HPUFA meal with LC n-3 PUFA led to a proteome change related to fatty acid metabolism. Fatty acid beta-oxidation occurs in both mitochondria and peroxisomes. Mitochondria catalyze the beta-oxidation of the bulk of fatty acids derived from diet, and peroxisomes are involved, preferentially, in the beta-oxidation chain shortening of very long chain fatty acids [48]. It has been suggested that increased intakes or supplements of n-3 fatty acids may improve defects in insulin signaling [49]. Since peroxisomal beta-oxidation has been shown to be inhibited by insulin [50], the fact that our study has shown that ECH1, an enzyme involved in the peroxisomal beta-oxidation pathway [51], was down-regulated after an HPUFA n-3 meal intake suggests that ECH1 down-regulation and the subsequent reduction of peroxisomal beta-oxidation may underlie the beneficial effects in insulin signaling observed after intake of n-3 fatty acids, and suggests that peroxisomal beta-oxidation inhibition by insulin could be caused by ECH1 down-regulation.

In addition, n-3 PUFA supplementation of an HPUFA meal led to the postprandial up-regulation of PPIA (cyclophilin A), a protein that binds to cyclosporine, an immunosuppressant which is usually used to suppress rejection after internal organ transplants, by halting the production of the proinflammatory molecules TNF-α and interleukin-2 [52] and was also involved in the anti-inflammatory effects associated to n-3 fatty acid consumption [53]. Thus, n-3 PUFA consumption could increase the effect of cyclosporine which would support the therapeutic administration of n-3 PUFA in combination with cyclosporine after organ transplantation.

In conclusion, HSFA meal intake causes deleterious postprandial changes in the proteome, in terms of DNA damage and the procoagulant state, which reflects higher postprandial oxidative stress after an HSFA meal intake when compared to the intake of HMUFA and HPUFA meals. Moreover, the addition of LC n-3 PUFA to an HPUFA meal may improve insulin signaling and exerts an anti-inflammatory effect when compared to an HPUFA meal.

Further research is needed to extend our knowledge about the nutritional regulation of the postprandial events related to cardiovascular diseases. In addition, it would be interesting to compare the effect of dietary fat in pathological and nonpathological conditions.

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