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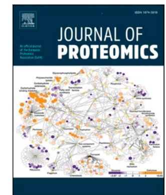

Circulating proteomic profiles in women with morbid obesity compared to normal-weight women

Laia Bertran [a], Elena Cristina Rusu [b], Maria Guirro [c], Carmen Aguilar [a,b], Teresa Auguet [a,b], Cristóbal Richart [a,*]

[a] Departament de Medicina i Cirurgia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili (URV), 43201 Reus, Spain
[b] GEMMAIR Research Group, Institut d'Investigació Sanitària Pere Virgili (IISPV), 43005 Tarragona, Spain
[c] Centre for Omic Sciences (COS), Joint Unit URV-EURECAT, Unique Scientific and Technical Infrastructures (ICTS), Eurecat, Centre Tecnològic de Catalunya, 43204 Reus, Spain

A R T I C L E I N F O

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A B S T R A C T

In this study, we aimed to evaluate circulating proteomic levels in women with morbid obesity (MO) compared to normal-weight (NW) women. Moreover, we have compared the proteomic profile between women with metabolically healthy (MH) MO and those with type 2 diabetes mellitus (T2DM). The study included 66 normal-weight (NW) women and 129 women with MO (54 MH and 75 with T2DM). Blood samples were processed for proteomics, involving protein extraction, quantification, digestion with peptide labelling and Nano (liquid chromatography (LC)-(Orbitrap) coupled to mass/mass spectrometry (MS/MS) analysis. Statistical analyses were performed. We identified 257 proteins. Women with MO showed significantly increased levels of 35 proteins and decreased levels of 45 proteins compared to NW women. Enrichment analysis of metabolic pathways revealed significant findings. Women with MO have an altered proteomic profile compared to normal-weight women, involving proteins significantly related to chylomicron assembly, complement cascade, clotting pathways and the insulin growth factor system. Regarding women with MO and T2DM compared to MHMO women, the proteomic profile shows alterations in mostly the same pathways associated with obesity. These findings confirmed in previous reports can help us better understand the pathophysiology of obesity and associated diseases.

Significance: Women with morbid obesity (MO) exhibit substantial proteomic alterations compared to normal-weight (NW) women, involving 80 proteins. These alterations are linked to significant metabolic pathways, including chylomicron assembly, complement cascade, clotting pathways and the insulin growth factor system. Additionally, women with MO and type 2 diabetes mellitus (T2DM) compared to metabolically healthy MO women share similar proteomic changes than the first comparison. These findings enhance our understanding of the pathophysiology of obesity and associated diseases, offering potential targets for therapeutic intervention.

1. Introduction

Nowadays, obesity continues to have an increasing prevalence, making this pandemic a significant economic burden on current societies worldwide [1]. The transition from overweight to obesity is an increasingly rapid process linked to poor eating habits and sedentary lifestyles [2,3]. Obesity is associated with cardiovascular risk factors such as dyslipidemia, type 2 diabetes mellitus (T2DM) and hypertension [4]. When a patient with obesity has a body mass index (BMI) greater than 40 kg/m^2, they are considered to have entered a stage called morbid obesity (MO), where the cardiometabolic risk is even higher [5]. In any case, subjects with obesity who have a low cardiovascular risk associated with a normal metabolic profile (normal glucose and lipid levels and adequate blood pressure) have been defined as having metabolically healthy obesity [6]. However, the idea of considering any type of obesity as healthy is controversial, and there is currently a challenge to understand this obesity profile and how inducing it may help in the treatment and reduce the cardiovascular risk of common patients with obesity [7,8].

Since obesity is a multifactorial condition, proteomic studies, as a

* Corresponding author.
E-mail addresses: laia.bertran@fundacio.urv.cat (L. Bertran), elena.rusu@iispv.cat (E.C. Rusu), maria.guirro@eurecat.cat (M. Guirro), carmenisabel.aguilar@urv.cat (C. Aguilar), mariateresa.auguet@urv.cat (T. Auguet), crichartjurado@gmail.com (C. Richart).

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Table 1
Anthropometric and biochemical characteristics of the study cohort.

Variables	Normal weight (BMI 19–25 kg/m^2) (n = 66)	Morbid obesity (BMI >40 kg/m^2) (n = 129)	Morbid obesity	
			Metabolically healthy (n = 54)	Type 2 diabetes mellitus (n = 75)
Age (years)	40 (35–48)	44 (41–51)	43 (39–51)	46 (41.7–51)
BMI (kg/m^2)	22.4 (21.1–23.8)	45.2 (42.1–49.4)*	44.1 (41.6–48.1)*	45.5 (42.5–49.5)*
Waist-hip (m) ratio	0.79 (0.73–0.85)	0.92 (0.86–0.97)*	0.91 (0.84–0.94)*	0.93 (0.87–0.98)*
SBP (mm Hg)	118 (108.5–125)	123 (111.2–137.5)*	118 (107.7–125)	130 (114.2–144.5)*,^
DBP (mm Hg)	70 (62.5–76)	66.5 (59.2–75.7)	65 (60–73)	69.5 (59–80.7)
Glucose (mg/dl)	81 (71–89)	104 (87–147)*	88 (80–94.5)*	137.5 (117–185.7)*,^
Insulin (mUI/L)	5.8 (4.6–8.7)	14 (7.8–23)*	11.1 (6.7–17)*	17.6 (9.1–31.5)*,^
HbA1c (%)	5.1 (4.9–5.3)	6.1 (5.3–7.9)*	5.1 (5–5.5)	7 (6.2–8.6)*,^
Triglycerides (mg/dl)	63 (52.2–86.5)	128.5 (98.7–156.2)*	104.5 (80–133.7)*	111.7 (140.5–200.2)*,^
Cholesterol (mg/dl)	180 (161–204.5)	164 (143–187)*	164 (147.8–186.7)*	166 (141–188)*
HDL-C (mg/dl)	64 (54–70)	40.6 (34–48)*	42 (34.9–55)*	38 (33.3–46)*
LDL-C (mg/dl)	105 (84–123)	95.3 (78.4–114)	100 (85–116)	91.7 (73.2–113.5)*

Data are expressed as the median and interquartile range. (*) Significant differences compared to normal weight group and (^) significant differences compared to the metabolically healthy morbidly obese group were considered when p-value <0.05 using the Mann-Whitney test. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high density lipoprotein – cholesterol; LDL-C, low density lipoprotein – cholesterol.

powerful branch of omics sciences, are of interest for research to understand the processes involved in the disease through the study of its implicated proteins [9]. Currently, there are certain proteomic studies in serum and plasma samples that identify proteins in patients with obesity mainly associated with lipid metabolism and inflammatory responses [9,10]. There are also proteomic studies in patients with T2DM associated with obesity [11]. However, proteomic studies related to patients with metabolically healthy obesity are scarce, heterogenic in terms of obesity degree and gender, and involve small patient groups [12]. This is why we aimed to study a considerable homogeneous cohort of women to evaluate circulating proteomic levels in women with MO and differentiate the proteomic profile between women with metabolically healthy MO and those MO with T2DM, to elucidate the physiopathological pathways.

2. Materials and methods

2.1. Participants

To perform this study, we included a control group of 66 volunteer normal-weight women (BMI = 19-25 kg/m^2) and 129 women with MO (BMI > 40 kg/m^2) scheduled to undergo laparoscopic bariatric surgery. Only women were included to avoid heterogeneity, as men and women present differences in metabolic parameters [13,14].

Of the 129 patients with MO, 54 were metabolically healthy, meaning they did not present metabolic alterations such as T2DM, dyslipidemia or hypertension, despite having MO. On the other hand, 75 had been diagnosed with T2DM, meeting the diagnostic criteria of the American Diabetes Association (ADA) [15]. Subjects without T2DM but presenting other metabolic alterations were excluded from this study since they comprised a small and heterogeneous group.

Using the GRANMO sample size calculator (v.7.04), accepting an alpha risk of 0.05 and a beta risk less than 0.2 in a bilateral contrast, we need at least 174 cases (MO) and 57 controls (normal-weight) to detect a minimum odds ratio of 0.25. It is assumed that the exposure rate in the control group will be 0.25. A loss of follow-up rate of 0 has been estimated. The POISSON approximation was used.

Exclusion criteria included acute illness, acute or chronic inflammatory or infective diseases, end-stage malignant disease, menopausal status, contraceptive treatment, alcohol intake exceeding 20 g per day and recurrent smoking.

This study was approved by the Ethics Committee of IISPV (CEIm; 23c/2015). All participants gave written informed consent. This study was conducted retrospectively by accessing the patient data collected from 2014 until 2023. During data collection and analysis, the authors did not have access to patient identifiers, working with blind and encrypted data in a RedCap database.

2.2. Biochemical, anthropometric and clinical parameters

Anthropometrical variables such as weight, height, BMI, waist-hip ratio, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained from each participant. Biochemical variables were measured from blood samples obtained by specialized nurses using a BD Vacutainer® system after overnight fasting and just before bariatric surgery in subjects with MO. Blood samples were collected and processed into plasma and serum aliquots. Serum aliquots were obtained using tubes without anticoagulant after allowing the blood to clot and stored at −80 °C until proteomics processing. Plasma aliquots were obtained from ethylenediaminetetraacetic acid (EDTA) tubes via centrifugation at 3500 rpm for 15 min at 4 °C. Biochemical variables included glucose, insulin, glycosylated hemoglobin A1c (HbA1c), triglycerides, total cholesterol, high density lipoprotein-cholesterol (HDL—C) and low density lipoprotein-cholesterol (LDL-C) measured using a conventional automated analyzer.

2.3. Proteomic analysis

2.3.1. Protein extraction and quantification for serum samples

Before proteomic analysis, the most abundant plasma proteins (albumin, immunoglobulin (Ig)G, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3 and transthyretin) were depleted to increase the number of identified/quantified proteins. Ten microliters of each sample were passed twice through the Human-14 Multiple Affinity Removal Spin (MARS) cartridge (Agilent Technologies, *Catalog Number 5188–6560*) following the manufacturer's protocol. Flow-through fractions were concentrated and buffer exchanged to about 100 μl of 6 M urea in 50 mM ammonium bicarbonate (ABC) using 5 K MWCO spin columns (Agilent 5185–5991).

2.3.2. Protein digestion and peptide 11-plex tandem mass tags (TMT) labelling

Twenty-five micrograms of total protein (quantified by Bradford's method) were reduced with 4 mM 1,4-dithiothreitol (DTT) for 1 h at 37 °C and alkylated with 8 mM iodoacetamide (IAA) for 30 min at 25 °C in the dark. Samples were then digested overnight (pH 8.0, 37 °C) with sequencing-grade Trypsin/Lys-C Protease Mix (ThermoFisher Scientific, CA, USA) at an enzyme ratio of 1:50. Digestion was quenched by acidification with 1 % (v/v) formic acid, and peptides were desalted on Oasis HLB SPE columns (Waters, Massachusetts, USA) before TMT 11-plex labelling (ThermoFisher Scientific, CA, USA) following the manufacturer's instructions. Briefly, samples were resuspended with triethyl ammonium bicarbonate (TEAB) 100 mM, then each TMT channel, previously prepared with acetonitrile (CAN), was added to each sample

Table 2
Proteins significantly upregulated or downregulated in women with morbid obesity compared to normal-weight women.

Upregulated					
Protein Coding Name	Log2FC	Adj P-val	Protein Coding Name	Log2FC	Adj P-val
Complement factor H (CFH)	2.71	<0.0001	Lipopolysaccharide-binding protein (LBP)	1.13	<0.0001
All-trans-retinol dehydrogenase [NAD(+)] (ADH1B)	2.38	<0.0001	Superoxide dismutase [Cu—Zn] (SOD1)	1.12	<0.0001
C-reactive protein (CRP)	2.26	<0.0001	Complement factor H-related protein 2 (CFHR2)	1.11	0.0065
Complement factor I (CFI)	2.14	<0.0001	Attractin (ATRN)	1.09	0.0267
SAA2-SAA4 readthrough (SAA2-SAA4)	2.09	<0.0001	Alpha-1-acid glycoprotein 2 (ORM2)	1.08	0.0125
Fructose-bisphosphate aldolase (ALDOB)	2.07	0.0001	Serum amyloid P-component (APCS)	1.06	0.0004
Proteoglycan 4 (PRG4)	1.95	<0.0001	Ceruloplasmin (CP)	1.06	0.0002
C3/C5 convertase	1.95	<0.0001	C4b-binding protein alpha chain (C4BPA)	1.04	0.0003
Proteoglycan 4 (Fragment) (PRG4-F)	1.82	0.0003	C4b-binding protein beta chain (C4BPB)	0.98	0.0001
von Willebrand factor (VWF)	1.72	<0.0001	Apolipoprotein C-III (APOC3)	0.86	0.0043
Pantetheinase (VNN1)	1.62	0.0001	Haptoglobin (HP)	0.79	0.0015
RUN and FYVE domain-containing protein 1 (RUFY1)	1.50	0.0008	Complement C2 (C2)	0.7	0.0184
Apolipoprotein C-II (APOC2)	1.46	<0.0001	Insulin-like growth factor-binding protein (IGFBP4)	0.66	0.0074
Complement C4—B (C4B)	1.45	0.0003	Vitamin K-dependent protein S (PROS1)	0.57	0.0145
Pigment epithelium-derived factor (SERPINF1)	1.41	0.0004	Beta-2-microglobulin (B2M)	0.56	0.0014
CD5 antigen-like (CD5L)	1.36	<0.0001	Retinoic acid receptor responder protein 2 (RARRES2)	0.51	0.0286
Scavenger receptor cysteine-rich type 1 protein M130 (CD163)	1.35	0.0001	Cadherin-related family member 5 (CDHR5)	0.04	0.0003
Complement factor D (CFD)	1.34	0.0032			

DOWNREGULATED					
Protein Coding Name	Log2FC	Adj P-val	Protein Coding Name	Log2FC	Adj P-val
Cartilage oligomeric matrix protein (COMP)	−0.36	0.0082	Fibulin-1 (FBLN1)	−1.16	<0.0001
Tenascin-X (TNXB)	−0.41	<0.0001	Intercellular adhesion molecule 2 (ICAM2)	−1.17	0.0003
Plexin domain-containing protein 2 (PLXDC2)	−0.44	0.0324	Tetranectin (CLEC3B)	−1.18	<0.0001
Collectin-10 (COLEC10)	−0.48	0.0197	Insulin-like growth factor II (IGF2)	−1.19	<0.0001
Complement component C7 (C7)	−0.51	0.0346	Insulin-like growth factor-binding protein complex acid labile subunit (IGFALS)	−1.23	<0.0001
Clusterin (CLU)	−0.55	0.0314	Neural cell adhesion molecule 1 (NCAM1)	−1.28	<0.0001
Transthyretin (TTR)	−0.6	0.0325	Glutathione peroxidase 3 (GPX3)	−1.3	<0.0001
Filamin-A (FLNA)	−0.61	0.0032	Phosphatidylinositol 5-phosphate 4-kinase type-2 gamma (PIP4K2C)	−1.33	<0.0001
Coagulation factor V (F5)	−0.62	0.0184	Corticosteroid-binding globulin (SERPINA6)	−1.34	<0.0001
Lumican (LUM)	−0.62	0.0043	Cadherin-13 (CDH13)	−1.36	<0.0001
Serotransferrin (TF)	−0.7	0.0035	Insulin-like growth factor-binding protein 3 (IGFBP3)	−1.39	<0.0001
Interleukin-1 receptor accessory protein (IL1RAP)	−0.73	0.0046	Inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2)	−1.46	<0.0001
Cadherin-1 (CDH1	−0.77	0.0335	Gc-globulin (GC)	−1.49	<0.0001
Alpha-1-antitrypsin (SERPINA1)	−0.78	0.0043	Fibulin-1 (FBLN1)	−1.50	<0.0001
Cadherin-5 (CDH5)	−0.85	0.0003	Apolipoprotein A-IV (APOA4)	−1.54	<0.0001
Zinc-alpha-2-glycoprotein (AZGP1)	−0.88	0.0003	Kallistatin (SERPINA4)	−1.63	<0.0001
Mannose-binding protein C (MBL2)	−0.9	0.0008	Adiponectin (ADIPOQ)	−1.64	<0.0001
Carboxypeptidase N catalytic chain (CPN1)	−0.9	0.0022	Gelsolin (GSN)	−1.68	<0.0001
Target of Nesh-SH3 (ABI3BP)	−0.93	0.0007	Sex hormone-binding globulin (SHBG)	−1.87	<0.0001
Peptidase inhibitor 16 (PI16)	−1.04	<0.0001	Insulin-like growth factor-binding protein 5 (IGFBP5)	−1.98	<0.0001
L-selectin (SELL)	−1.07	<0.0001	Insulin-like growth factor I (IGF1)	−2.26	<0.0001
Apolipoprotein A-I (APOA1)	−1.11	0.0006	Antithrombin-III (SERPINC1)	−2.31	<0.0001
Neural cell adhesion molecule L1-like protein (CHL1)	−1.15	<0.0001			

Upregulated and downregulated proteins were identified by positive or negative log2 fold changes (log2FC). respectively. Statistical differences were considered significant when the adjusted p-value (Adj P-val) was less than 0.05. One-way ANOVA with Tukey HSD post hoc and Benjamini-Hochberg FDR correction was performed between groups. Detailed information on proteins and further statistical data are provided in Supplementary Table 3.

according to Supplementary Table 1.

To normalize all samples across different TMT-multiplexed batches, a pool containing all samples was labelled with the TMT-126 tag and included in each TMT batch. Then, each plex of samples was mixed also according to Supplementary Table 1 and the different TMT 11-plex batches were desalted on Oasis HLB SPE columns before nanoLC-MS analysis.

2.3.3. NanoLC-(Orbitrap)MS/MS analysis

Labelled and multiplexed peptides were loaded on a trap nanocolumn (75 μm I.D.; 1.5 cm length; 3 μm particle diameter, ThermoFisher Scientific, CA, USA) and separated onto a C-18 reversed phase (RP) μPAC™ Neo high-performance liquid chromatography (HPLC) column (180 μm bed; 50 cm length; 2.5 × 16 μm pillar diameter, ThermoFisher Scientific, CA, USA) on a Vanquish Neo VN-S10 System (ThermoFisher Scientific). Chromatographic separation was performed with a 180-min gradient using water LC-MS grade (0.1 % formic acid) and acetonitrile (0.1 % formic acid) as mobile phases at a flow rate of 300 nl/min.

Mass spectrometry (MS) analyses were performed on an Orbitrap Eclipse from ThermoFisher Scientific by an enhanced FT-resolution MS spectrum (R = 60,000 FHMW) followed by data-dependent FT-MS/MS acquisition (R = 50,000 FHMW, 30 % NCE HCD) from the ten most intense parent ions with a charge state acquisition from two to six and dynamic exclusion of 0.7 min.

2.3.4. Protein identification and quantification

Protein identification and quantification were performed using Proteome Discoverer software v.2.5 (ThermoFisher Scientific, CA, USA) with the Mascot search engine (v2.8, Matrix Science) combining raw data files obtained from each plex. For protein identification, the workflow used the Mascot node combining *Homo sapiens* and

Table 3
Proteins significantly upregulated or downregulated in women with morbid obesity and T2DM compared to women with metabolically healthy morbid obesity.

Upregulated		
Protein Coding Name	Log2FC	Adj P-val
Collectin-10 (COLEC10)	1.72	0.0015
Fructose-bisphosphate aldolase (ALDOB)	1.57	0.0022
Vitronectin (VTN)	1.51	0.0015
Poliovirus receptor (PVR)	1.46	0.0024
Apolipoprotein A-IV (APOA4)	1.23	0.0284
Attractin (ATRN)	1.20	0.0168
Clusterin (CLU)	1.09	0.0022
Complement component C7 (C7)	1.08	0.0292
Collectin-11 (COLEC11)	1.01	0.0424
Scavenger receptor cysteine-rich type 1 protein M130 (CD163)	0.99	0.0189
Leukocyte immunoglobulin-like receptor subfamily A member 3 (LILRA3)	0.95	0.0311
Retinoic acid receptor responder protein 2 (RARRES2)	0.83	0.0325
Kininogen-1 (KNG1)	0.83	0.0053
Vitamin K-dependent protein S (PROS1)	0.58	0.0495

DOWNREGULATED		
Protein Coding Name	Log2FC	Adj P-val
Complement component C6 (C6)	−0.82	0.0189
Complement factor H-related protein 3 (CFHR3)	−0.86	0.0238
Sex hormone-binding globulin (SHBG)	−0.89	0.0189
Ceruloplasmin (CP)	−0.94	0.0053
Inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1)	−1.16	0.0366
Insulin-like growth factor-binding protein complex acid labile subunit (IGFALS)	−1.34	0.0168
Adiponectin (ADIPOQ)	−1.62	0.0024

Upregulated and downregulated proteins were identified by positive or negative log2 fold changes (log2FC). respectively. Statistical differences were considered significant when the adjusted p-value (Adj P-val) was less than 0.05. One-way ANOVA with Tukey HSD post hoc and Benjamini-Hochberg FDR correction was performed between groups. Detailed information on proteins and further statistical data are provided in Supplementary Table 4.

contaminants databases, assuming trypsin digestion. The fragment ion mass tolerance assumed an error of 20 mmu for FT-MS/MS fragmentation mass and 10 ppm for FT-MS precursor ion mass. Oxidation of methionine and acetylation of the N-terminal were set as dynamic modifications, carbamidomethylation as a static modification, and TMT-11plex as a quantitation method. The false discovery rate (FDR) and protein probabilities were calculated by Percolator, with peptide identification set to a maximum of 1 %. Peptide quantitation data were retrieved from the 'Reporter ions quantifier' node in Proteome Discoverer, using the area of unique and razor peptides and total peptide amount as normalization. Peptide and protein results are expressed in abundance area and are dimensionless.

2.4. Statistical analysis

Descriptive data of the patients (anthropometric and biochemical variables) were analyzed using the SPSS/PC+ for Windows statistical package (version 27.0; SPSS, Chicago, Illinois, USA). The Kolmogorov-Smirnov test assessed the distribution of variables. Variables were presented as the median and interquartile range because they exhibited a non-normal distribution. Comparative analyses were conducted using the nonparametric Mann-Whitney *U* test. *P*-values <0.05 were considered statistically significant.

For the proteomics data obtained, only proteins identified in >60 % of samples in at least one of the experimental groups were considered. Missing value estimation after data filtering was performed using the KNN (k-nearest neighbors) algorithm from MetaboAnalyst software v5.0 for univariate and multivariate statistical analysis. For statistical analysis of the proteomics data, a log base 2 data transformation was applied, and one-way ANOVA with Tukey HSD post hoc and Benjamini-Hochberg FDR correction was performed between groups, with a *p*-value cut-off of <0.05. Hierarchical clustering heatmaps for the top 25 proteins in each comparison were performed with Euclidean distance measure and Ward clustering method using MetaboAnalyst software v5.0.

Cytoscape software v3.10.2, with the ClueGO plugin, was used to perform gene enrichment analysis and pathway clustering. STRING was used as the database to obtain information on the included proteins, focusing exclusively on *Homo sapiens*, and for conducting the gene enrichment analysis. ClueGO served as the plugin to perform functional analysis of the proteins and their interactions. We selected the Reactome database to identify pathways developed by the included proteins, setting a medium network specificity and displaying only pathways with a *p*-value less than 0.05, with Benjamini-Hochberg p-value correction applied. The remaining parameters were kept at their default settings, including GO term grouping and preferred layout.

3. Results

3.1. Participants

In this work, we classified our cohort depending on their BMI into normal-weight subjects as the control group, and subjects with MO, as shown in Table 1. In this case, subjects were comparable in terms of sex, age, DBP and LDL-C levels. Subjects with MO presented higher BMI, waist-hip ratio, SBP and levels of glucose, insulin, hbA1c and triglycerides than normal-weight women. Moreover, women with MO presented decreased levels of HDL-C but also cholesterol levels, probably given that the 26 % of subjects with MO received treatment for dyslipidemia.

Then, the subjects with MO were subclassified as either metabolically healthy (n = 54) or having T2DM (n = 75), as also shown in Table 1. In this case, subjects were comparable in terms of sex, age, BMI, waist-hip ratio, DBP and lipid profile. Women with MO and T2DM had increased SBP, glucose, insulin, hbA1c and triglyceride levels compared to subjects with metabolically healthy MO.

3.2. Proteomics analysis in serum samples

After proteomics analysis in serum samples of the whole cohort (n = 195), a total of 537 proteins were identified. Information regarding relative quantification and identification such as Mascot score, protein FDR confidence and other details such as samples ID, are provided in Supplementary Table 2. Samples were filtered to include only those proteins present in >60 % of samples in at least one experimental group, and the KNN method was used for estimation, leaving a total of 257 proteins.

First, we studied which proteins differed in concentration between normal-weight subjects and subjects with MO. We found that women with MO had significantly increased levels of 35 proteins and significantly decreased levels of other 45 proteins compared to normal-weight women (Table 2). The correlation between protein coding names and UniProt IDs is detailed in Supplementary Table 3.

Next, we compared the differential proteins between MO women with T2DM and women with metabolically healthy MO. We found 14 proteins with increased concentration and 7 proteins with decreased concentration in the T2DM cohort compared to the metabolically healthy group (Table 3). The correlation between protein coding names and UniProt IDs is detailed in Supplementary Table 4.

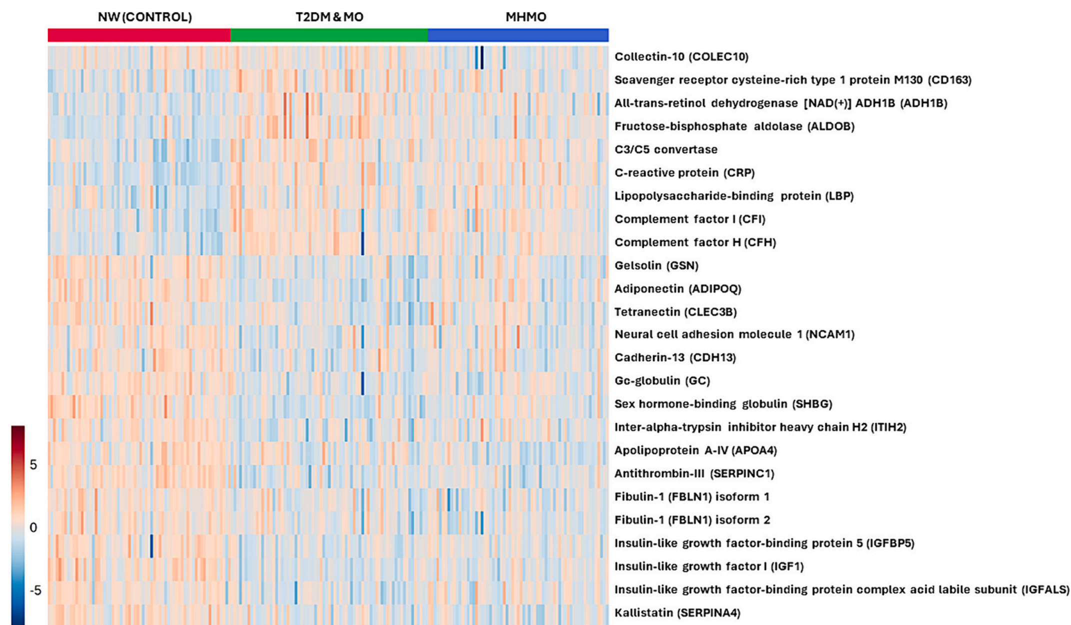

Fig. 1. Hierarchic-clustered Heatmap for the top 25 proteins differently expressed between groups: control group of normal-weight subjects (NW). morbidly obese with type 2 diabetes mellitus (T2DM & MO) and metabolically healthy morbidly obese (MHMO).
The more intense the red. The more overexpressed the protein is. while the more intense the blue. The more underexpressed the protein is. This illustration was generated using MetaboAnalyst software. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Proteomic profile comparison between groups

A hierarchical clustering analysis of the top 25 proteins revealed distinct profiles of upregulation and downregulation across each group (Fig. 1). In this analysis, we identified several proteins that are commonly overexpressed in MO subjects (with or without T2DM), including scavenger receptor cysteine-rich type 1 protein M130 (CD163), all-trans-retinol dehydrogenase [NAD(+)] ADH1B (ADH1B), fructose-bisphosphate aldolase (ALDOB), C3/C5 convertase, C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), complement factor I (CFI), and complement factor H (CFH). These proteins tend to be more highly expressed in MO subjects with T2DM than in metabolically healthy MO subjects when compared to the normal-weight group, though this trend is subtle. Additionally, we observed that some proteins were significantly downregulated in the MO group compared to the normal-weight group, as shown in Fig. 1. Notably, collectin-10 (COLEC10) appears to be slightly downregulated in metabolically healthy MO subjects but upregulated in normal-weight and MO subjects with T2DM. However, no significant differences were observed between the two groups of obese subjects.

3.4. Biological processes enrichment analysis

To understand the pathways associated with the significantly altered proteins in each comparison, we conducted an enrichment analysis of the metabolic pathways, organized according to the percentage of altered proteins involved. The altered proteins significantly different between the MO and normal-weight groups correspond to various pathways, mostly including chylomicron assembly, the complement cascade, platelet degranulation and the clotting cascade (Fig. 2A). Finally, the altered proteins significantly different between the T2DM and metabolically healthy groups correspond to the complement cascade and platelet deregulation, as well as insulin-like growth factor regulation pathways (Fig. 2B).

4. Discussion

In this study, we conducted a proteomic analysis on serum samples from a substantial number of women with MO, comparing them with a control group of normal-weight women. Moreover, we have analyzed differences between MO women with T2DM and those who are metabolically healthy. We identified 45 proteins decreased in MO women compared to normal-weight women, of which the most notable were Gc-globulin (GC), apolipoprotein A-IV (APOA4), kallistatin (SERPINA4), adiponectin (ADIPOQ), gelsolin (GSN), sex hormone-binding globulin (SHBG), insulin-like growth factor I (IGF1) and antithrombin III (SERPINC1). Additionally, we found 35 circulating proteins significantly increased in MO women, highlighting the CFH and the CRP. Further analysis of all these proteins revealed that 34.62 % of these proteins are involved in the chylomicron assembly pathway, 15.38 % in the complement cascade, 11.54 % in the clotting cascade, and another 11.54 % in platelet degranulation. A smaller portion of proteins was implicated in pathways such as regulation, transport and uptake by insulin-like growth factor binding proteins, extracellular matrix proteoglycans, scavenging of heme from plasma and cell junction organization.

Subsequently, we found that MO women with T2DM presented increased levels of 14 proteins, of which the most notable were COLEC10, ALDOB, vitronectin (VTN), poliovirus receptor (PVR), clusterin (CLU) and kininogen 1 (KNG1). Additionally, 7 proteins showed decreased levels, highlighting the ceruloplasmin (CP) and the adiponectin (ADIPOQ), compared to women with metabolically healthy MO. Most of these proteins are involved in pathways related to the complement cascade (33.33 %), platelet degranulation (33.33 %), regulation, transport and uptake by insulin-like growth factor binding proteins (22.22 %) and the initial triggering of the complement pathway (11.11 %).

First, we compared our results with another proteomics study in a cohort of men and women with MO that uses another group of normal-weight subjects as a control. They only found significantly increased levels of complement C3 protein in subjects with MO compared to normal-weight individuals [16], but we did not find significant differences. However, they did not find significant differences in proteins that

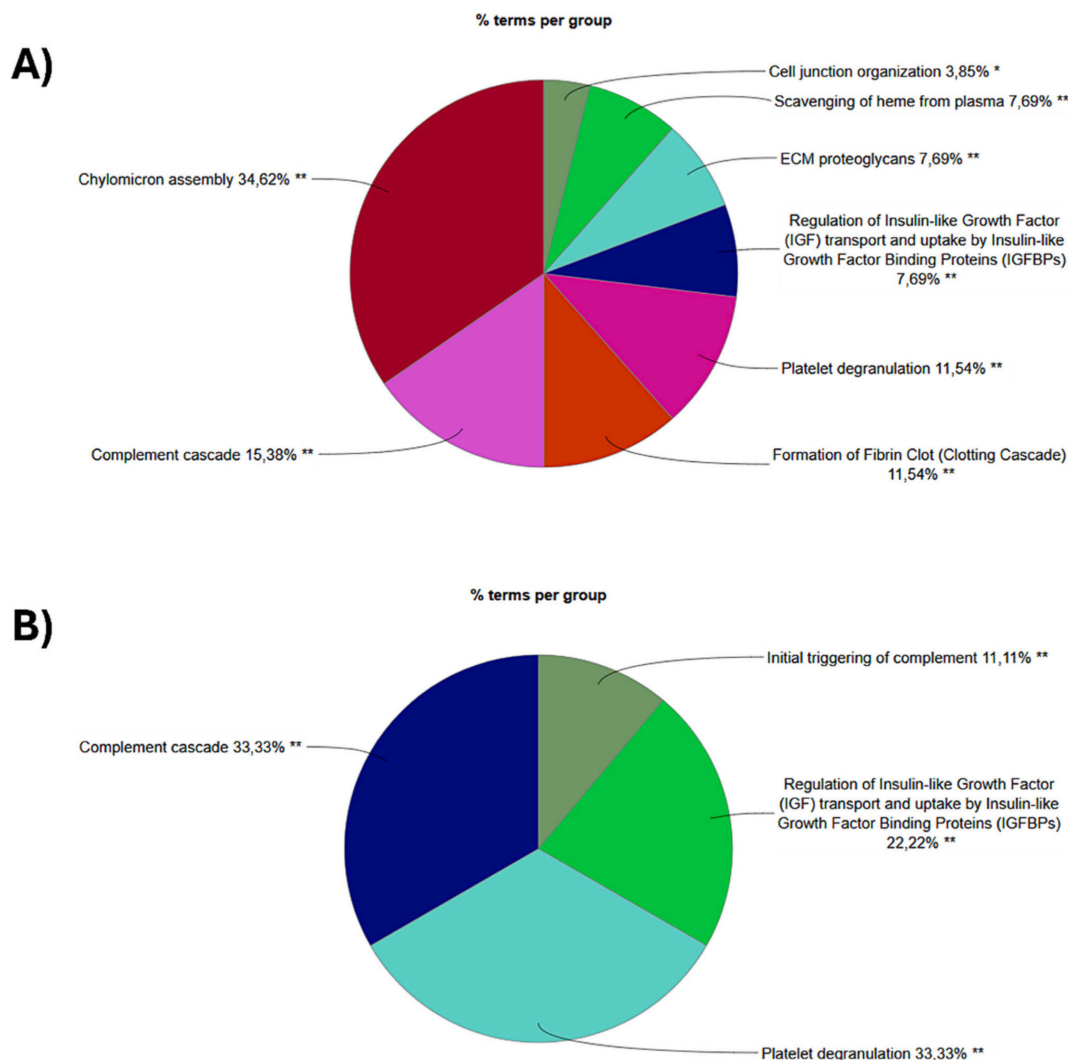

Fig. 2. Overrepresentation analyses of the involved pathways (Reactome) most representative between A) normal-weight women and women with morbid obesity; B) women with morbid obesity and type 2 diabetes mellitus and women with metabolically healthy morbid obesity.
This illustration was performed using Cytoscape software.

we found increased such as apolipoprotein C-II (APOC2) and apolipoprotein C-III (APOC3) or decreased like alpha-1-antitrypsin (SERPINA1), apolipoprotein A-I (APOA1), APOA4 or transthyretin (TTR) in our women with MO compared to the normal-weight group.

Another study of proteomics in a similar cohort of subjects with obesity and T2DM reported in serum samples different proteins with significant changes between subjects with obesity and T2DM and non-diabetics that do not match with ours. They found that adiponectin levels, the only protein that we also found, were decreased in their subjects with obesity without T2DM and in their subjects with obesity and T2DM compared to those without obesity and without T2DM. However, they did not find significant differences regarding adiponectin compared between subjects with obesity and T2DM and subjects without obesity and without T2DM [11]. In this study, we reported decreased levels of adiponectin in our women with MO compared to the normal-weight group and also in women with MO and T2DM compared to women with metabolically healthy MO. Thus, we agree with them in terms of adiponectin in the effect of obesity, but not in the effect of T2DM inside the obesity. These discrepancies can be explained by the difference in sample size, since they evaluated heterogeneous sexes and a small cohort [11], whereas we studied a homogeneous and considerable cohort of women, and in the case of subjects with MO, they studied subjects with a different degree of obesity [11]. In this sense, we previously reported decreased levels of adiponectin in women with MO compared to the normal-weight group [17], and it was also found to be decreased in subjects with obesity and T2DM, since it has antidiabetic effects [18].

Another proteomic study reported proteins differently concentrated between subjects with obesity with or without T2DM [19]. The only protein they found comparable to our comparison is ceruloplasmin (CP), however, they did not find significant differences between groups. Meanwhile, we reported decreased levels of this protein in our subjects with MO and T2DM compared to subjects with metabolically healthy MO. These discrepancies can be explained because we compared subjects with metabolically healthy MO with subjects with MO and T2DM, whereas they did between subjects with obesity, but not subjects with MO, with subjects with MO and T2DM [19]. In addition, although both studies used mass spectrometry, they performed 2D difference gel electrophoresis (DIGE) matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF), while we assessed HPLC-MS.

Later, we compared our results with a proteomics study where they evaluated the proteome in accordance with metabolically healthy obesity or metabolically unhealthy obesity [12], but our results did not agree. We found increased levels of complement C7 in our T2DM group compared to the metabolically healthy group, while they reported increased levels of this protein in their subjects with metabolically

healthy obesity compared to individuals with metabolically unhealthy obesity. The discrepancies can be explained because they do not study a MO cohort, and also because they studied an African-American cohort [12], while we studied Caucasian MO women.

In another previous report, authors suggested a panel of 15 proteins that can distinguish between degrees of obesity, mostly between moderate and severe or morbid degrees of obesity [20], including proteins that we found in this study increased in our subjects with MO, such as vitamin K-dependent protein S (PROS1) and CD5 antigen-like (CD5L), or decreased, just like zinc-alpha-2-glycoprotein (AZGP1), complement component C6 (C6) and kallistatin (SERPINA4). These findings suggested a potential role for these proteins in the MO. To highlight this, most of these proteins are integrated in complement cascades [20].

Furthermore, there is another proteomic study in Russian subjects that proposes some proteins as a predictor model of BMI [21], that agree with some proteins that we reported in our subjects with MO, such as increased levels of complement factor H (CFH), C4b-binding protein alpha chain (C4BPA), pigment epithelium-derived factor (SERPINF1) and vitamin K-dependent protein S (PROS1), and decreased levels of complement component C7 (C7), alpha-1-antitrypsin (SERPINA1) and APOA4. They stated that their proposed proteins are mostly involved in coagulation and complement cascades [21], such as in our study.

To sum up, most of the proteins that we found significantly altered in obesity are implicated in chylomicron assembly, which makes sense, as alterations in several proteins of phospholipid synthesis are associated with diseases including fatty liver and obesity [22]. Moreover, chylomicron assembly is one of the key pathways relevant to obesity [23]. Then, most of the proteins found in both comparisons are involved in the complement cascade, which also makes sense since complement activation is a common process in metabolic pathology linked to inflammation and immune response [24]. Additionally, another common cascade in both comparatives is the activation of platelets and clotting processes, which has been previously related to obesity and T2DM as a higher risk of thrombosis [25,26]. Furthermore, the insulin growth factor system is another pathway that some proteins are involved in, particularly in the T2DM comparative but also in the MO comparative. The insulin growth factor system has been previously related to obesity and insulin resistance alterations due to the key role of insulin in these pathologies [27].

5. Conclusions

In conclusion, we have identified a proteomic profile in women with MO compared to normal-weight women, consisting of proteins involved in chylomicron assembly, the complement cascade, clotting pathways and the insulin growth factor system. Additionally, we have found a proteomic profile in women with MO and T2DM, compared to metabolically healthy MO women, composed of proteins implicated in mostly the same pathways as MO. These findings confirmed in previous reports can help us better understand the pathophysiology of obesity and associated diseases.

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CRediT authorship contribution statement

Laia Bertran: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Elena Cristina Rusu:** Writing – original draft, Investigation, Formal analysis, Data curation. **Maria Guirro:** Writing – original draft, Software, Methodology, Data curation. **Carmen Aguilar:** Writing – review & editing, Resources, Methodology. **Teresa Auguet:** Writing – review & editing, Validation, Resources. **Cristóbal Richart:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

Laia Bertran declares no conflicts of interest.
Elena Cristina Rusu declares no conflicts of interest.
Maria Guirro declares no conflicts of interest.
Carmen Aguilar declares no conflicts of interest.
Teresa Auguet declares no conflicts of interest.
Cristóbal Richart declares no conflicts of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2024.105317.

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