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Visceral white adipose tissue and serum proteomic alternations in metabolically healthy obese patients undergoing bariatric surgery

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ABSTRACT

Metabolically healthy obesity is characterized as a comorbidity-free obesity status, however the exact pathogenetic mechanisms implicated in its transition to unhealthy obesity have not yet been unveiled. Our aim was to investigate the effect of metabolic health on the proteomic profile both in serum and visceral fat of morbidly obese subjects. 28 patients undergoing bariatric surgery were prospectively enrolled. They were divided into two groups: metabolically healthy (MHO, n = 18) and unhealthy (MUO, n = 10) obese patients. 30 biomarkers were measured in serum and visceral adipose tissue with the use of targeted proteomic analysis (Luminex assays). TNF weak inducer of apoptosis (TWEAK) (p = 0.043), TNF related apoptosis inducing ligand (TRAIL) (p = 0.037), Growth differentiation factor-15 (GDF-15) (p = 0.04), Resistin (RETN) (p = 0.047), Matrix metalloproteinase-9 (MMP-9) (p = 0.011) and C-terminal telopeptide (ICTP) (p = 0.022) were up-regulated in the MUO group in the visceral white adipose tissue. Moreover, C-C motif ligand-3 (CCL-3) (p = 0.056), Interleukin-20 (IL-20) (p = 0.04), Prokineticin-1 (PROK-1) (p = 0.028) and TWEAK (p = 0.016) were found to be suppressed in the serum of MHO group. Significant correlations between serum and adipose tissue levels of certain cytokines were also observed, while 16 biomarkers were associated with BMI. Our results indicate metabolic health substantially attenuates the expression of TWEAK, TRAIL, GDF-15, RETN, MMP-9 and ICTP expression locally, in the visceral white adipose tissue, and the expression of CCL-3, IL-20, PROK-1 and TWEAK in the peripheral blood. Intriguingly, different cytokines –except for TWEAK- are up-regulated in each site, suggesting that obesity is not a homogenous but a multi-dimensional disease.

1. Introduction

Adipose tissue is no longer considered an energy storage depot and its endocrine functions are well-documented. Dysfunction of visceral white adipose tissue is central to the pathology associated with obesity, insulin resistance, metabolic syndrome and its compartments [1]. White fat is considered detrimental for the health since it is characterized by adipocytes that contain large lipid droplets and participate in various pathways, including insulin sensitivity, lipid metabolism and low grade inflammation [2]. This dysregulated homeostasis of the adipose tissue is phenotypically manifested as obesity and its related comorbidities, such as hypertension, dyslipidemia and diabetes. However, not all obese subjects will develop this phenotype of high cardiometabolic risk. These subjects have been described as metabolically healthy obese [3].

The term "metabolically healthy obesity" was first introduced more than fifteen years ago [4], however there is still inconsistency and debate regarding its exact definition [5]. Nonetheless, it is broadly accepted that it is characterized by body mass index (BMI) > 30 kg/m^2 without the presence of any of the components of the metabolic syndrome. It has been reported that metabolically unhealthy obese subjects have hypertrophic adipocytes which lead to insulin resistance and the

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production of a wide range of pro-inflammatory adipokines. This dysmetabolic phenotype is aggravated in visceral obesity and it is associated with increased rate of comorbidities [6]. Current data suggest that the white adipose tissue and especially visceral fat is the main mediator for the pathogenetic mechanisms implicated in obesity and its comorbidities, while demonstration of these alternations may be attenuated or amended on a systematic level.

The exact molecular mechanisms implicated in the transition from healthy to unhealthy phenotype of obesity remains unclear. Proteomic technology has been proved itself a useful tool for identifying key components of the adipose tissue and peripheral blood proteome [7]. Proteomic analysis has identified markers for insulin resistance and low-grade inflammation which exert a major role in obesity and its complications [8].

Taking into consideration that visceral adipose tissue is the major site of obesity related metabolic dysregulation, we sought to investigate the effect of metabolic health in morbid obesity using a targeted proteomic approach both in serum and visceral adipose tissue.

2. Material and methods

2.1. Study population

We prospectively enrolled 28 patients undergoing bariatric surgery, namely laparoscopic sleeve gastrectomy (LSG), Roux En Y gastric bypass (RYGB) and biliopancreatic diversion (BPD). Prior to operation, all patients provided medical history and underwent physical examination. These patients were divided into two groups according to their metabolic health status, which was defined by the presence (Metabolically Unhealthy Obese, MUO) or absence (Metabolically Healthy Obese, MHO) of comorbidities such as hypertension, dyslipidemia and diabetes mellitus. During recruitment, similar baseline characteristics of the patients of each group were used as criteria for legibility so that propensity score matching would not by necessary for adjusting to confounding factors (Table 1). Thus said, the patients of the two groups are matched for gender, age, BMI, lipidemic and glycemic profile. A stricter definition of the metabolic health was not chosen in our cases intendedly, since our primary aim was to discover proteomic markers that would be altered even in the presence of one comorbidity and would not require the full spectrum of metabolic syndrome. The exclusion criteria included the presence of acute inflammation, infection or malignancy. The local ethics committee of "Evgenidion" Hospital approved our study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and both patients and controls provided written inform consent.

Table 1
Demographic characteristics, lipidemic and glycemic profile of the patients included in our study. Continuous variables are expressed as mean ± standard deviation.

	MHO (n = 18)	MUO (n = 10)	p-value
Gender (f:m)	11:7	2:3	ns
Age (y)	36.7 ± 12.6	37.3 ± 13	ns
BMI (kg/m^2)	47.5 ± 8.6	48.7 ± 9.1	ns
Hypertension, n(%)	0	7 (70)	$p < 0.05$
Hyperlipidemia, n(%)	0	3 (30)	$p < 0.05$
Diabetes, n(%)	0	5 (50)	$p < 0.05$
Sleep apnea, n(%)	3 (16.7)	3 (30)	ns
GERD, n(%)	8 (44.4)	3 (30)	ns
Smoking, n(%)	9 (50)	5 (50)	ns
Alcohol consumption, n(%)	0	0	ns
Prior Surgery, n(%)	3 (16.7)	3 (30)	ns
Glucose (mg/dL)	118 ± 35	119 ± 36	ns
Total Cholesterol	134 ± 13	133 ± 14	ns
HDL-C (mg/dL)	39 ± 7	38 ± 7	ns
LDL-C (mg/dL)	65 ± 11	65 ± 11	ns
Triglycerides	149 ± 45	151 ± 46	ns
Hemoglobin (g/dL)	13.1 ± 2.3	13.3 ± 1.6	ns
WBC ($cells/mm^3 \times 1000$)	16.5 ± 3.6	18.1 ± 3.7	ns
Neutrophils %	83.4 ± 9.8	83.2 ± 10.1	ns
Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 01	ns

MHO: Metabolically healthy obese; MUO: Metabolically unhealthy obese; BMI: Body Mass Index; GERD: Gastroesophageal reflux disease; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; WBC: Wight blood cells; ns: not significant.

2.2. Blood and adipose tissue sampling

Blood samples were collected under fasting conditions at the time of the operation. Plasma was separated immediately and aliquots were frozen at −80 °C. Blood was also drawn one day pre-operatively and these samples were used for biochemical measurements. Total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, glucose and creatinine. Low density lipoprotein (LDL) cholesterol was calculated with Friedewald's equation. White blood cells, % neutrophils, hemoglobin and hematocrit were also measured.

Visceral adipose tissue (approximately 500 mg) was excised from the exterior surface of the fundus of the stomach. A standardized procedure of adipose tissue excision was followed in all patients. All tissues were immediately snap-frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Sample preparation

Protein extraction from human adipose tissue was performed as previously described [9]. To extract the proteins, ~100 mg of tissue was homogenized in 4 μL/mg tissue standard homogenization buffer with protease inhibitors (SHB-P: 20 mM Tris-HCl, 1 mM EDTA, 255 mM sucrose, pH 7.4, cOmplete Mini, EDTA-free, protease inhibitor cocktail from Roche) on ice using a rotor-stator for three 10 s cycles of homogenization interspersed with 10 s cycles of rest. The homogenates were centrifuged at 1000*g* for 10 min and the subnatant below the lipid cake was aspirated. Total protein concentrations of the tissue extracts were measured using Pierce BCA Protein Quantitation kit and the samples were stored at −80 °C until assayed.

Serum samples were heat-inactivated to destroy complement prior to performing dual-antibody Luminex assays. Samples were thawed at room temperature and gently mixed after thawing. Then, they were placed in a 56 °C water bath for 30 min, gently mixed after the incubation and the inactivated serum samples were used for the measurements.

2.4. Proteomics

30 custom dual-antibody Luminex assays of potential biomarkers were developed using ProtATonce (Athens, Greece) multiplex assay service. Briefly, 2 to 5 antibodies were selected and cleaned up from amine containing buffers and carrier proteins that interfere with the coupling procedure. All antibodies were tested pair-wise as capture and as detection antibody. Capture antibodies were coupled to the beads whereas detection antibodies were biotinylated. Quality control confirmed biotinylation and coupling efficiency. For each biomarker the optimal capture/detection antibody pair was selected based on signal-to-noise ratio measurement. Assays were separated in 2 multiplex panels according to their multiplexability and concentration of secondary antibody was evaluated based on its signal and its noise (off-target signal) in the bead panel. The cross reactivity performance of the 22plex and 8plex panel was evaluated with test samples that contain single recombinant proteins. Assay validation including Limit of detection (LOD) and reproducibility were performed based on the European medicines Agency EMEA/CHMP/EWP/192217/2009 guideline on bioanalytical method validation.

2.5. Biomarkers

The following 30 biomarkers were measured in patients' serum and adipose tissue: 22 multiplex panel: C-C motif chemokine ligand 3 (CCL3), C-C motif chemokine ligand 5 (CCL5), C-X-C motif chemokine 10 (CXCL10), CXCL11, follistatin (FST), intracellular adhesion molecule-1 (ICAM-1), interferon gamma (IFN-γ), interleukin-1α (IL-1α), IL-4, IL-8, IL-12A, IL-17F, IL-20, IL-22, neuregulin 1 (NRG1), prokineticin 1(PROK1), resistin (RETN), S100A6, tumor necrosis factor alpha (TNF-a), trefoil factor 3 (TFF-3), TNF superfamily member 10/TNF related apoptosis inducing ligand (TRAIL) and TNF superfamily member 12/TNF weak inducer of apoptosis (TWEAK); 8 multiplex panel: growth/differentiation factor 15 (GDF15), Clock interacting pacemaker (CICP), C-terminal telopeptide (ICTP), tissue inhibitor of metalloproteinases 1 (TIMP-1), matrix metallopeptidase 9 (MMP-9), procollagen type III N-terminal peptide (P3NP), suppression of tumorogenicity 2 (ST2) and interleukin-6 (IL-6).

2.6. Statistical analysis

Patients' mean fluorescence intensity (MFI) values were used as input of all the downstream analyses.

2.6.1. Significance analysis

In order to assess whether if any of the measured cytokines could progress as candidate biomarkers, analysis of variance models (ANOVA) were fitted for each of them. Differential expression among the subgroups was considered using threshold models ($p = 0.05$). Unadjusted as well as risk factor adjusted (hypertension, diabetes mellitus, hypercholesterolemia) models were fitted. Obtained p-values were further corrected for multiple comparisons using the false discovery rate (FDR) method.

2.6.2. Correlation analysis

Correlations between adipose tissue and serum were computed over the Z-scores of the MFIs using coefficient of determination (R squared) after linear model fitting.

3. Results

3.1. Anthropometric, clinical and biochemical data

18 patients were characterized as MHO, while 10 as MUO. Demographic characteristics and non-proteomic blood measurements are tabulated in Table 1. Baseline characteristics were similar between the two groups except for the comorbidities used for the definition of metabolic unhealthy obesity (hypertension, diabetes and dyslipidemia). The lack of difference in these baseline characteristics was intentional in order to investigate the effect of metabolic health per se on the proteome even when compared with well-regulated metabolically unhealthy patients. In this way, potentially confounding effects of severe metabolic dysregulation of the patients were avoided. All patients were morbidly obese with an average BMI of $47.5 \pm 8.6\,kg/m^2$ and $48.7 \pm 9.1\,kg/m^2$ for MHO and MUO, respectively. Prevalence of hypertension, diabetes and dyslipidemia in the MUO group was 70%, 50% and 30%, respectively.

3.2. Proteomic alternations in adipose tissue and serum

Three different dilutions of the extracted serum were used for our analysis. The optimal dilution was selected for each biomarker (smallest p-value from ANOVA analysis) (Fig. 1). Comparison of MFIs in the visceral adipose tissue, significant differences were noted in the case of TWEAK ($p = 0.043$), TRAIL ($p = 0.037$), GDF-15 ($p = 0.04$), RETN ($p = 0.047$), MMP-9 ($p = 0.011$) and ICTP ($p = 0.022$) (Fig. 2). Statistical analysis revealed that the serum MFI of the following proteins differed between the two groups: CCL-3 ($p = 0.056$), IL-20 ($p = 0.04$), PROK-1 ($p = 0.028$) and TWEAK ($p = 0.016$) (Fig. 3).

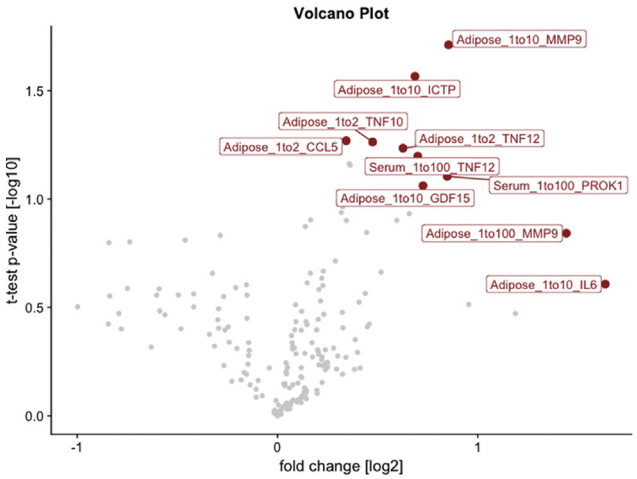

Fig. 1. Volcano plot illustrating the significant ($p < 0.05$) differences between metabolically healthy (MHO) and unhealthy (MUO) obese subjects with respect to cytokine, tissue harvested and dilution used. *CCL-5: C-C motif ligand 5; MMP-9: Matrix metalloproteinase-9; ICTP: C-terminal telopeptide; TNF10: TNF superfamily member 10/TNF related apoptosis inducing ligand (TRAIL); TNF12: TNF superfamily member 12/TNF weak inducer of apoptosis (TWEAK); PROK-1: Prokineticin-1; GDF-15: Growth differentiation factor-15; IL6: Interleukin-6.

As it was anticipated, when adjusting for hypertension, diabetes and dyslipidemia none of the aforementioned differences remained significant.

This observation reinforces the hypothesis that metabolic health protects against up-regulation of these detrimental factors.

3.3. Correlation of protein expression between serum and visceral fat

Two sites of sampling were opted in order to obtain information regarding the proteomic alternations both on local and systematic level. Correlation analysis for each dilution was performed and 1:2 dilution was deemed optimal for revealing possible correlation between the protein levels in serum and adipose tissue (Supplemental Fig. 1). MFIs of IL-22, TNF-α, IFNG, CXCL-11, IL-17 and IL-4 were statistically correlated between the serum and fat (Fig. 4, Table 2). Additionally, a correlation of protein levels with BMI was attempted. In order to provide a clinical insight, this analysis was divided into two sub-analyses with respect to the value of the BMI (lower or higher than the average BMI) (Table 3). In this circumstance, 1:100 dilution was selected. Interestingly enough, different biomarkers were correlated with BMI for values over and below mean BMI. Namely, PROK-1, IL-12, IL-20, GDF-15 and CCL-3 exhibited a significant correlation for the lower BMI values, while IFNG, IL-8, IL-22, IL-4, TIMP-1, TNF-α, CXCL-10, TRAIL, CXCL-11, IL-17 and FST achieved statistical significance for BMI values above average.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.cyto.2018.11.017.

4. Discussion

White adipose tissue has gained considerable interest due to its fundamental role in the pathogenesis of obesity. However, the exact regulating pathways, the genetic and epigenetic factors implicated are not yet fully elucidated. This is the first targeted-proteomics study investigating the impact of metabolic health on protein expression in the serum and visceral adipose tissue of morbidly obese patients. Our results suggest that TWEAK, TRAIL, GDF-15, RETN, MMP-9 and ICTP are downregulated in visceral fat and IL-20, PROK-1, TWEAK and CCL-3

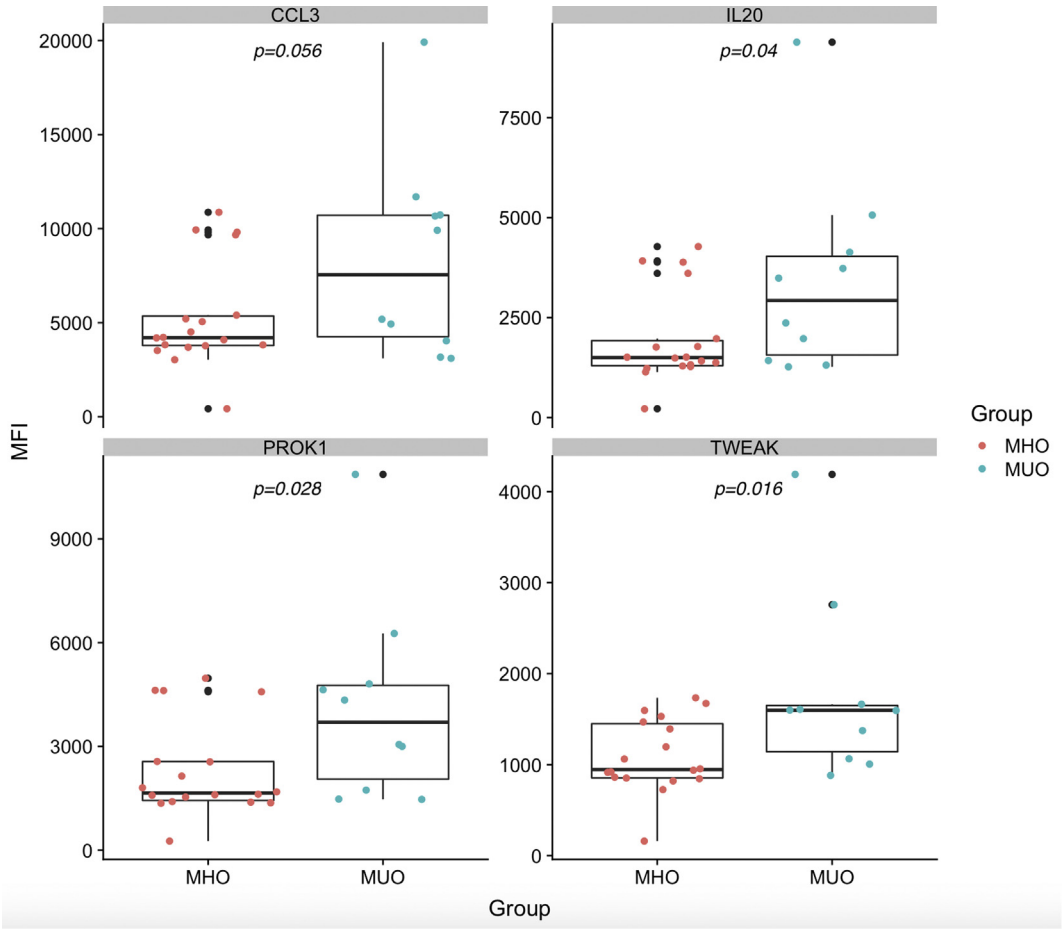

Fig. 2. Statistically significant differences of visceral adipose tissue MFI levels of TWEAK (A), TRAIL (B), GDF-15 (C), RETN (D), MMP-9 (E) and ICTP (F) between metabolically healthy (MHO) and unhealthy (MUO) obese subjects. Distributions expressed in the form of boxplots. P-values annotated on the figure. Comparisons of cytokines' MFI levels in the adipose tissue that did not achieve statistical significance ($p < 0.05$) are not illustrated. *TWEAK: TNF superfamily member 12/TNF weak inducer of apoptosis; TRAIL: TNF superfamily member 10/TNF related apoptosis inducing ligand; GDF-15: Growth differentiation factor-15; RETN: Resistin; MMP-9: Matrix metalloproteinase-9.*

(marginally) in serum in MHO subjects compared with the MUO ones. Although, different proteins were influenced by metabolic health in peripheral blood and adipose tissue –except for TWEAK-, a robust correlation between MFIs in serum and visceral fat was observed in the case of IL-22, TNF-α, IFNG, CXCL-11, IL-17 and IL-4. Moreover, 16 proteins were associated with BMI for either lower or higher BMI values.

Obesity is a multifactorial disease associated with a wide range of comorbidities such as hypertension, dyslipidemia and diabetes, imposing, thus, a substantial cardiometabolic risk. Apart from the quantification of obesity (BMI, waist circumference, total % body fat), scientific interest also focuses also on the "quality" of the obesity. The term "metabolically healthy obesity" was introduced to characterize obese subjects without any other obesity-related comorbidities. It is presumed that MHO has lower risk for target organ damage and complications associated with their excess adiposity.

4.1. Visceral adipose tissue biomarkers

TWEAK, TRAIL, GDF-15, RETN, MMP-9 and ICTP differed significantly between MHO and MUO in visceral fat. TRAIL, like TWEAK, is a promoter of apoptosis and it is involved in adipocyte differentiation via caspase-mediated downregulation of adipogenic transcription factors [10,11]. Apart from its inflammatory component, TRAIL has been reported to engage in the mechanisms of insulin resistance in obese subjects [12]. GDF-15 has been extensively studied for its role in cardiovascular disease as both a prognostic and diagnostic biomarker [13]. Even though a study did not report a significant impact of obesity on GDF-15 levels in heart failure patients [14], it is advocated that thermogenesis, lipolysis, oxidative and glucose metabolism are influenced by GDF-15 in obese patients [15–17]. Despite the diverse roles of GDF-15 in inflammatory and apoptotic pathways, the macrophage inhibitory cytokine 1 (MIC-1)/GDF-15 pathway has been proposed to be implicated in obesity and its comorbidities [18,19]. RETN is secreted by the adipose tissue and its role in insulin resistance and diabetes progression in obese subjects is well-documented [2,20,21]. MMP-9 is a metalloproteinase involved in the degradation of collagen promoting neoangiogenesis and attraction of neutrophils among others [22]. It has been linked to increased inflammation and interacts with pathways leading to atherosclerosis and coronary artery disease, while recent data suggest its participation in obesity [22,23]. In specific, MMP-9 levels are increased in obesity and may play a role in insulin resistance [24,25]. ICTP is a marker for bone turnover, reflecting the allegedly protective role of obesity against osteoporosis [26]. Although, ICTP is reported to be increased after bariatric surgery, in our study it was higher in the MUO than MHO patients, suggesting, thus, that different mechanisms may be involved in each case [27–29].

4.2. Serum proteomic biomarkers

In our study serum MFIs of IL-20, PROK-1 and TWEAK were lower in MHO. IL-20 is a cytokine structurally related to IL-10 activated by

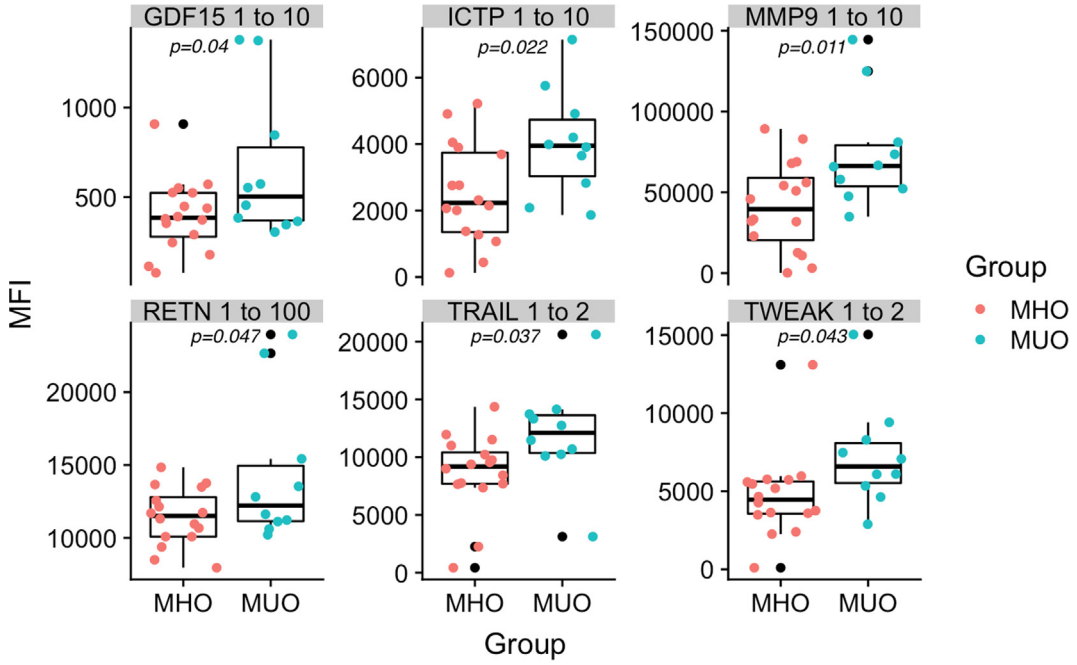

Fig. 3. Statistically significant differences of serum MFI levels of CCL-3 (A), IL-20 (B), PROK-1 (C) and TWEAK (D) between metabolically healthy (MHO) and unhealthy (MUO) obese subjects. Distributions expressed in the form of boxplots. P-values annotated on the figure. Comparisons of cytokines' MFI levels in the serum that did not achieve statistical significance ($p < 0.05$) are not illustrated. *CCL-3: C-C motif ligand 3; IL-20: Interleukin-20; PROK-1: Prokineticin-1; TWEAK: TNF superfamily member 12/TNF weak inducer of apoptosis.*

monocytes and keratinocytes and exerts pro-inflammatory activities. Its role has been investigated in atherosclerosis and stroke and it seems to contribute to low grade inflammation during obesity, while its expression is suppressed with weight-loss [30]. Although, it appears that IL-20 is involved in the pathogenesis of obesity, current evidence about its association with metabolic health is scarce [31,32]. Prokineticins are released by monocytes, macrophages and reproductive organs [33]. Their levels have been reported to be increased in obese subjects, while PROK-1, in specific, has been proposed to be implicated in the pathogenesis of insulin resistance [34]. It plays a significant role in adipocyte

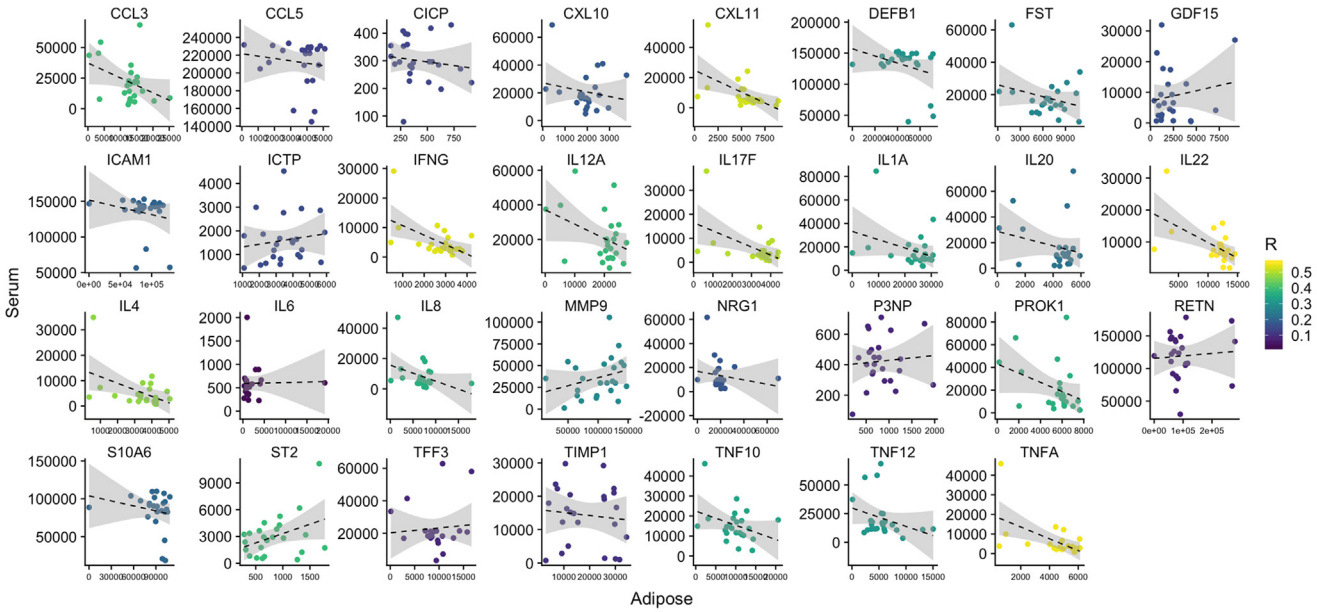

Fig. 4. Correlation of MFIs between serum and adipose tissue. 1 to 2 dilution was utilized. The color of the dots indicates the "degree" of correlation (absolute value of R). The more yellow the more robust the correlation, while the more blue the less robust. Significant correlations were noted in the case of IL-22 ($p = 0.004$), TNF-α ($p = 0.005$), IFNG ($p = 0.007$), CXCL-11 ($p = 0.008$), IL-17 ($p = 0.012$) and IL-4 ($p = 0.019$). *CCL: C-C motif chemokine ligand; CXCL: C-X-C motif chemokine; CXCL11, FST: follistatin; ICAM-1: intracellular adhesion molecule-1; IFN-γ: interferon gamma; IL: interleukin; NRG1: neuregulin 1; PROK1: prokineticin 1; RETN: resistin; TNF-α: tumor necrosis factor alpha; TFF-3: trefoil factor 3; TRAIL: TNF superfamily member 10/TNF related apoptosis inducing ligand; TWEAK: TNF superfamily member 12/TNF weak inducer of apoptosis; GDF15: growth/differentiation factor 15; CICP: Clock interacting pacemaker; ICTP: C-terminal telopeptide; TIMP-1: tissue inhibitor of metalloproteinases 1; MMP-9: matrix metallopeptidase 9; P3NP: procollagen type III N-terminal peptide; ST2: suppression of tumorogenicity 2.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Correlation between protein MFIs in serum and visceral adipose tissue when 1:2 dilution used. This dilution was preferred over 1:10 and 1:100 dilution due to the overall strongest correlation achieved between the cytokines' levels in serum and fat. R-square (coefficient of determination) indicates the proportion of the variance in the dependent variable (adipose tissue levels) that is predictable from the independent variable (serum levels). The fitted linear models have the form of: Adipose(MFI) = a * Serum(MFI) + b. A is represented by the "Estimate". Standard error of the fitting curve also presented. The * detonates the p-values that are lower than 0.05.

Cytokine	R^2	Estimate	S.E.	p-value
CCL3	0.152	−0.12	0.06	0.060
CCL5	0.017	−0.01	0.01	0.541
CICP	0.018	−0.34	0.53	0.528
CXL10	0.032	−0.01	0.01	0.404
CXL11	0.277	−0.10	0.03	0.008*
DEFB1	0.092	−0.16	0.11	0.149
FST	0.075	−0.06	0.05	0.194
GDF15	0.026	0.04	0.05	0.448
ICAM1	0.038	−0.19	0.20	0.359
ICTP	0.022	0.20	0.28	0.488
IFNG	0.289	−0.09	0.03	0.007*
IL12A	0.149	−0.18	0.09	0.063
IL17F	0.255	−0.08	0.03	0.012*
IL1A	0.113	−0.16	0.10	0.108
IL20	0.053	−0.02	0.02	0.280
IL22	0.316	−0.33	0.10	0.004*
IL4	0.224	−0.09	0.03	0.019*
IL6	0.001	0.27	2.33	0.910
IL8	0.133	−0.13	0.07	0.080
MMP9	0.080	0.43	0.31	0.181
NRG1	0.029	0.16	0.20	0.426
P3NP	0.007	0.22	0.55	0.699
PROK1	0.135	−0.03	0.02	0.078
RETN	0.006	0.15	0.43	0.722
S10A6	0.041	−0.19	0.19	0.341
ST2	0.151	0.07	0.04	0.061
TFF3	0.007	0.02	0.06	0.707
TIMP1	0.011	−0.12	0.24	0.632
TNF10	0.115	−0.16	0.10	0.105
TNF12	0.096	−0.06	0.04	0.141
TNFA	0.303	−0.10	0.03	0.005*

CCL: C-C motif chemokine ligand; CXCL: C-X-C motif chemokine; CXCL11, FST: follistatin; ICAM-1: intracellular adhesion molecule-1; IFN-γ: interferon gamma; IL: interleukin; NRG1: neuregulin 1; PROK1: prokineticin 1; RETN: resistin; TNF-α: tumor necrosis factor alpha; TFF-3: trefoil factor 3; TRAIL: TNF superfamily member 10/TNF related apoptosis inducing ligand; TWEAK: TNF superfamily member 12/TNF weak inducer of apoptosis; GDF15: growth/differentiation factor 15; CICP: Clock interacting pacemaker; ICTP: C-terminal telopeptide; TIMP-1: tissue inhibitor of metalloproteinases 1; MMP-9: matrix metallopeptidase 9; P3NP: procollagen type III N-terminal peptide; ST2: suppression of tumorogenicity 2.

differentiation and it is up-regulated by insulin [35]. Despite its novelty, PROK-1 is a promising biomarker for insulin resistance and may be the link between obesity and cardiovascular disease [36]. TWEAK has gained attention as a potentially important regulator of the inflammatory/anti-inflammatory equilibrium which takes place in the insulin-resistant milieu [37]. It participates in differentiation, metabolism and secretory function of adipocytes, while it has also been associated with diabetes and non-alcoholic fatty liver disease [38,39]. Its implication with triglycerides accumulation and insulin resistance justify its relationship with metabolic health in obese subjects [37,38,40]. Albeit marginally, MFI of CCL-3 differed between the two groups. CCL-3 is a pro-inflammatory marker initiating the recruitment and activation of macrophages, monocytes and especially neutrophils. It is proposed that it plays a role in the adipocyte-macrophage crosstalk in obesity, hence association with metabolic health has not been yet established [41].

Table 3
Correlation between protein serum MFIs and BMI for BMI below and higher than the average (Low BMI and High BMI, respectively). R-square (coefficient of determination) indicates the proportion of the variance in the dependent variable (serum protein levels) that is predictable from the independent variable (BMI value). Only the correlations that are significant ($p < 0.05$) are illustrated.

Low BMI			High BMI		
Biomarker	R^2	p-value	Biomarker	R^2	p-value
PROK-1	0.49	0.002	IFNG	0.81	0.002
IL-12	0.43	0.005	IL-8	0.65	0.015
IL-20	0.33	0.019	IL-22	0.62	0.02
GDF-15	0.25	0.046	IL-4	0.62	0.02
CCL-3	0.23	0.05	TIMP-1	0.6	0.025
			TNF-α	0.58	0.027
			CXCL-10	0.58	0.027
			TRAIL	0.55	0.035
			CXCL-11	0.5	0.04
			IL-17	0.52	0.044
			FST	0.5	0.048

*PROK-1: Prokineticin-1; IL-12: Interleukin-12; IL-20: Interleukin-20; GDF-15: Growth differentiation factor-15; CCL-3: C-C motif ligand-3; IFNG: Interferon-gamma; IL-8: Interleukin-8; IL-22: Interleukin-22; IL-4: Interleukin-4; TIMP-1: Tissue inhibitor of matrix metalloproteinases-1; TNF-α: Tumor necrosis factor-α; CXCL-10: C-X-C motif ligand-10; TRAIL: TNF superfamily member 10/TNF related apoptosis inducing ligand; CXCL-11: C-X-C motif ligand-11; IL-17: Interleukin-17; FST: Follistatin.

4.3. Correlation between blood and fat measurements

Apart from identifying biomarkers for metabolically healthy obesity, our aim was also to examine if visceral fat and peripheral blood adapt unanimously to obesity and its comorbidities. Since, only TWEAK differed in both tissues between the two groups, a correlation analysis between the MFIs in serum and fat of each protein was performed. IL-22, TNF-α, IFNG, CXCL-11, IL-17 and IL-4 were found to exhibit significant correlation. All these proteins are known to participate in the inflammatory cascade [42–48]. Inflammation is undoubtedly a component of obesity and as supported by our results it is not restricted locally in the adipose tissue but it affects the whole circulation. Intriguingly, an inverse correlation between adipose tissue and serum levels of these proteins was noted, indicating their implication in the pathogenesis of metabolically unhealthy status only on a focal (adipose tissue) or a systematic (serum) level and not on both. Additionally, the lack of correlation in the markers that differed in either serum or fat indicates that certain proteins exert different roles with regard to their site of action. Their discrete role in metabolic health during obesity is a major finding suggesting the complexity of this entity.

4.4. Association of biomarkers with BMI

Regardless of the metabolic health status, 16 proteins were correlated with BMI. The correlation analysis was split for BMI values above and below the mean BMI value. Intriguingly, different proteins were found to be significantly correlated with BMI in each case. This observation provides information regarding the different response of the organism during the initial stages of obesity and the established morbid obesity. Consequently, it can be assumed that obesity is a dynamic state in which distinct proteins are upregulated with respect to its severity. The above stated proteomic biomarkers exert divergent roles, however the pro-inflammatory action seems to be their most common characteristic. The interplay between obesity and inflammation is well-established, yet inflammatory pathways may be involved in each stage of obesity [49].

4.5. Limitations

Albeit their novelty, the outcomes of our study should be interpreted under a specific point view. The lack of follow-up and the relatively small size of our population may add up to drawbacks of our study. Yet, the homogeneity of the study population, the detailed patient history, the sampling from both serum and adipose tissue as well as the sufficient level of statistical significance of our outcomes outweigh any possible limitations. As such, our study's limitations do not hinder the extraction of reliable and reproducible conclusions.

4.6. 4.6 Future perspectives

This study can be used as a stepping stone for further clinical and basic research studies emphasizing in the pathophysiology of both obesity in general and metabolically healthy obesity in specific. Our results provide a novel insight in the aforementioned metabolic pathways for patients with morbid obesity, being eligible for bariatric surgery. Better understanding of the role of the molecules implicated in obesity metabolism is necessary for a more effective treatment approach of obesity. Moreover, large population cohort studies should be conducted in combination with prospective studies in patients following weight loss treatment plans or undergoing bariatric surgery including baseline and follow-up data.

5. Conclusions

This is the first study to report data from targeted proteomic analysis of serum and visceral adipose tissue in morbidly obese patients with or without metabolic health. Our results suggest increased inflammation levels and up-regulation of pro-inflammatory markers in MUO subjects not only focally on the visceral fat but also systematically in the peripheral blood. Moreover, despite the fact that the expression of certain proteins is correlated between these two tissues, other proteins seem to exert different roles with regard to their site of action. Our data set a milestone for further multiplex proteomics approach in the discovery of new biomarkers for metabolically healthy obesity and verification of the proposed ones.

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None.

Conflict of interest

None.

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