Relationship between Systemic Biomarker of Lipid Peroxidation 4-Hydroxynonenal and Lipidomic Profile of Morbidly Obese Patients undergoing Bariatric Surgery

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Abstract

Obesity is characterized by fat accumulation, impaired metabolism and oxidative stress, frequently associated with lipid peroxidation and generation of bioactive 4-hydroxynonenal (4-HNE). This study aimed to evaluate the impact of bariatric surgery-induced weight loss on lipid peroxidation and associated perturbations in lipid profile. Plasma samples of twenty obese individuals before and 6 months after bariatric surgery were collected in addition to samples of ten healthy controls. HILIC-LC-MS/MS platform was used to characterize phospholipid profile, while lipid peroxidation markers 15-F2t-IsoP, 10-F4t-IsoP and reactive aldehyde 4-HNE were quantified by RP-LC-MS/MS and GC-MS, respectively. Six months post-surgery lipid peroxidation markers decreased significantly and the BMI of morbidly obese patients decreased by 13 on average. Lipidomics analysis, identified 117 phospholipid species from seven classes, and showed obesity-associated lipidome perturbations, particularly in ether-linked phosphatidylethanolamines (PEo). A total of 45 lipid species were found to be significantly altered with obesity, while 10 lipid species correlated with lipid peroxidation markers. Sample pairwise analyses indicated an interesting link between 4-HNE and the amount of two ether-linked phosphatidylethanolamines PEo(38:2) and PEo(36:2). The results indicate that weight loss-induced improvement of redox homeostasis together with changes in lipid metabolites may serve as markers of metabolic improvement. However, further studies are needed to understand the role of obesity-induced oxidative stress on ether lipid biosynthesis and lipidome perturbations, as well as the impact of bariatric surgery on metabolic improvement.

Keywords: obesity, bariatric surgery, lipid peroxidation, ether lipids, lipidomics

1. Introduction

Obesity is a complex and chronic health condition characterized by the accumulation of excess body fat [1]. It increases the risk of several health conditions, including cardiovascular disease, type 2 diabetes, certain cancers and musculoskeletal disorders [2,3], and thus remains a global health challenge with profound effects on metabolic homeostasis. Obesity is associated with dysregulated lipid metabolism and oxidative stress, namely adipose tissue dysfunction and a shift towards the development of proinflammatory adipose tissue, which contributes to the occurrence of dyslipidemia [4]. In addition, adiponectin, insulin sensitivity, elevated adipose tissue macrophage infiltration, altered redox balance favoring prooxidants, inflammation, and impaired antioxidant response are the hallmarks of obesity [5]. These processes ultimately lead to excessive amounts of reactive oxygen species (ROS) and oxidative stress. NADPH oxidase is thought to be the primary source of ROS in adipocytes, with its increased activity likely contributing to elevated ROS production in adipose tissue during obesity [6].

Oxidative stress disrupts lipid metabolism through both ROS-dependent and enzyme-dependent mechanisms. ROS-dependent lipid peroxidation leads to changes in the composition and levels of various lipid species. Polyunsaturated fatty acids (PUFAs) are particularly susceptible to ROS triggering a chain reaction of lipid peroxidation and generating oxidative degradation products [7,8]. Lipid peroxidation derived products, by interacting with metabolically active proteins, may contribute, among others, to the development of inflammatory reactions [9,10]. Lipid peroxidation products commonly occur in biological fluids and are considered reliable indicators of oxidative stress [11] and are often used as biomarkers of disease onset and progression [12,13]. The type of PUFA that is oxidized determines the lipid peroxidation products formed. Thus, ROS induced peroxidation of arachidonic acid, an omega-6 PUFA, yields isoprostanes and isofurrans, whereas peroxidation of omega-3 PUFA docosahexaenoic acid produces neuroprostanes [14]. Among the end products of lipid peroxidation are reactive aldehydes, among which the 4-hydroxynonenal (4-HNE) is extensively studied due to its bioactive characteristics [15]. Owing to its high reactivity, 4-HNE has the ability to interact with a wide range of macromolecules, such are proteins, influencing numerous cellular functions and signaling pathways [16–18]. At physiological concentrations, 4-HNE functions as a metabolic regulator; however, at pathological concentrations it exhibits deleterious effect that dependent on the time and dose of exposure. We have earlier shown that with obesity, 4-HNE modified proteins accumulate in both subcutaneous and omental adipose tissue [19,20], with highest accumulation observed in adipocytes. The 4-HNE impairs redox homeostasis of preadipocytes, affects adipogenesis and insulin signaling, promoting insulin resistance [19].

Bariatric surgery, also called metabolic surgery, has proven to be an effective intervention in the treatment of morbid obesity, resulting in significant weight loss and improvement in metabolic parameters [5]. Bariatric surgery often induces significant changes in lipid profiles. Morbidly obese patients have distinct metabolic signatures compared to lean individuals, and bariatric surgery may lead to metabolic improvement already 6 months post-surgery [21]. Substantial weight reduction following the bariatric surgery is associated with changes in the fatty acid profile, namely in total percentage of PUFAs, which are involved in the regulation of glycemic control, lipid metabolism, and inflammation [22]. The current lipid analyses used to diagnose dyslipidemia are limited to measuring total lipid concentrations. Detection and identification of specific lipid species that contribute to dyslipidemia is essential as they may modulate immune response and promote obesity-related pathologies.

Multiple pathophysiological processes, each with unique characteristics, contribute to obesity-induced dyslipidemia [23]. It is evident that oxidative stress and the resulting lipid peroxidation, play an important role in the development of obesity and metabolic syndrome and that the obesity-induced dyslipidemia is at least in part attributed to oxidative stress. Thus, understanding the underlying mechanisms of obese pathology is essential. This work aimed to determine bariatric surgery induced changes in the oxidative stress markers, by monitoring changes of 15-F2t-IsoP (commonly referred to as 8-iso-PGF2α), 10-F4t-IsoP and 4-HNE as well as to identify lipid profile and specific lipids in the circulation that accompany metabolic and redox homeostasis improvement of obese patients.

2. Materials and methods

2.1. Subjects recruitment and biological samples

Twenty clinically well characterized, obese patients with BMI > 35 kg/m^2 awaiting weight reduction surgery were recruited from University Hospital "Sveti Duh" and General Hospital Varazdin. Additional, ten healthy lean controls were recruited from University Hospital "Sveti Duh". The study was approved by the University Hospital "Sveti Duh" Ethics Committee and General Hospital Varazdin Ethics Committee, and written informed consent was obtained from each person involved. All procedures were in accordance with recommendations in the Helsinki Declaration of 1975.

Blood samples of obese individuals were collected before surgery and six months after bariatric surgery, in addition to blood samples of healthy lean controls. Plasma samples were isolated and stored at -80°C until further analyses.

2.2. Standards and reagents

All solvents were of LC–MS grade and all chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Lipid extraction and quantification of phospholipid content

The modified Folch method [24] was used for total lipid extraction from plasma samples. In short, 200 μl of plasma sample was mixed with 1.5 ml of ice-cold methanol and 3 ml of chloroform. Next, the samples were vortexed and allowed to sit on ice for 60 minutes. Following the incubation time, 1.25 ml H_2O (MiliQ) was added, the samples were incubated on ice for 10 minutes and then centrifuged at 2500× g for 10 min at room temperature. Lipid extracts were recovered from the bottom organic phase and kept at −20° C until analysis. Blank samples were also prepared and were analyzed with the sequence, for detection of possible contaminations. The quantification of phospholipids in each extract followed the Bartlett and Lewis method [25]. The procedure involved the use of perchloric acid for acidic hydrolysis, followed by incubation with ammonium molybdate in the presence of ascorbic acid. The absorbance recorded at 800 nm was used to compute the total quantity of phospholipids present in each sample.

2.4. Characterization of the phospholipid profile by HILIC-MS/MS

Hydrophilic interaction liquid chromatography (HILIC), using a UPLC system (Agilent 1290; Agilent Technologies, Santa Clara, CA, USA) coupled with a QTOF mass spectrometer (Agilent 6540; Agilent Technologies, Santa Clara, CA, USA), was carried out to obtain the phospholipid profile. Internal standards of PC(14:0/14:0), LPC(19:0), PE(14:0/14:0), PI(16:0/16:0) and PS(14:0/14:0) (Avanti Polar Lipids, Inc., Alabaster, AL, USA) were used for quantification and evaluation of the ion fluctuations. The mobile phase consisted of the mixture of solvents A (ACN/MeOH/water 50:25:25 (v/v/v) with 1 mM ammonium acetate) and B (ACN/MeOH 60:40 (v/v) with 1 mM ammonium acetate). Starting at 0% A, gradient elution was administered, increasing linearly to 100% A in 20 minutes, held for 35 minutes, and then returned to 0% A in 5 minutes. Mobile phase A was used to dilute the total lipid extracts normalized to 25 µg of phospholipids. Samples were randomly injected into an Ascentis Si HPLC Pore column (15 cm x 1.0 mm, 3 μm; Sigma–Aldrich), with an injection volume set to 5ul and the flow rate set to 30ul/min. All the samples were analyzed in negative ESI mode, with the electrospray voltage set to - 3000 V, the capillary temperature set to 250 °C and the sheath gas flow set to 13L/min. By normalizing the area of each peak to the peak area of an internal standard, the relative abundances of each ion were determined.

2.5. Data treatment and statistical analysis

The filtering, peak detection, alignment and integration as well as the assignment of each PL species was carried out by the MZmine 2.30 software for the data obtained [26]. Univariate and multivariate statistical analyses were performed using Metaboanalyst version 6.0 online tool (https://www.metaboanalyst.ca/) [27]. The data obtained by MS/MS analysis were autoscaled before Principal component analysis (PCA). In order to estimate the importance of each variable autoscaled data were also used to a partial least squares-discriminate analysis (PLS-DA) and variable importance in projection (VIP) scores. Univariate statistical analysis was carried out using the ANOVA test with Tukey's post hoc test with $p < 0.05$ considered statistically significant. The heatmaps were created using "Euclidean" as the clustering distance and "Ward" as the clustering algorithm.

2.6. Quantification of lipid peroxidation products

Lipid peroxidation in plasma samples was determined by measuring small molecular weight reactive aldehyde 4-hydroxynonenal (4-HNE) as well as isoprostanes (15-F2t-IsoP) and neuroprostanes (10-F4t-NeuroP). In short, following the alkaline hydrolysis step, 15-F2t-IsoP and 10-F4t-NeuroP were extracted using the SPE cleansing method on C18 Sep-pak Vac columns (Waters). Using modified LC-MS methods of Coolen and Dupuy, respectively, total 15-F2t-IsoP and 10-F4t-NeuroP were quantified [28,29]. 15-F2t-IsoP and 10-F4t-NeuroP were analyzed in negative ion mode with multiple reaction monitoring (MRM), using Agilent 1290 LC coupled with Agilent 6460 mass spectrometer. Agilent Zorbax SB C18 analytical column (2.1 × 100 mm, 1.8 µm particle size) with a 5 µL injection volume was used for separation. The mobile phase comprised 0.1% acetic acid in MilliQ water (A) and acetonitrile (B). The separation was carried out with linear gradient of water/acetic acid (99.5:0.5, v/v) and acetonitrile. 15-F2t-IsoP-d4 was used as an internal standard. For 15-F2t-IsoP, 15-F2t-IsoP-d4, and 10-F4t-NeuroP, respectively, transitions of the precursor to the production m/z 353.2-193.1; 357.2-197.1 and 377.0-153.0 were employed.

Tsikas method [30] with minor modification based on gas chromatography coupled with mass spectrometry (Agilent Technologies, Palo Alto, CA, USA) was used to determine the reactive aldehyde as the O-pentafluorobenzyl-oxime-trimethyl silane (O-PFB-oxime-TMS) derivatives, with 4-hydroxynonenal-d_3 as an internal standard. Aldehyde derivatives were separated on an HP-5 ms capillary column with a 0.25-mm internal diameter, 0.25-µm film thickness, and a 30-m

length, and were evaluated using selected ion monitoring mode (SIM). The following ions were monitored: m/z 242.0 for 4-HNE-PFB-TMS and m/z 245.0 for the ISTD derivative.

3. Results

This study included 20 morbidly obese patients undergoing bariatric surgery and 10 lean controls with aim to evaluate the impact of bariatric surgery on the reversal of redox dyshomeostasis by the assessment of lipid peroxidation derived 15-F2t-IsoP, 10-F4t-NeuroP and 4-HNE and consequently their impact on plasma phospholipidome. After clinical evaluation of patients by a multidisciplinary team (psychologist, psychiatrist, endocrinologist, gastroenterologist, cardiologist, physiatrist, surgeon), patients with BMI > 35 kg/m2 were selected for the purposes of this research. The general characteristics of obese patients and lean controls are presented in Table 1.

Table 1. Patients Characteristics (average ± S.E.)

	Age (years)	BMI (Pre-OP)	BMI (Post-OP)	Δ BMI
Obese patients	45 ± 2	49.7 ± 2.4	35.3 ± 1.5	13.3 ± 1.4
Control	51 ± 7	23.6 ± 0.6	n.a.	n.a.

Six months post bariatric surgery the BMI of morbidly obese patients decreased by 13 in average, with a maximum of 34.6 decrease in BMI of one patient.

The amount of 4-HNE, 15-F2t-IsoP and 10-F4t-NeuroP in respect to the amount of control lean subjects is shown on Figure 1. A significant 3-fold increase in 4-HNE was observed in the plasma of morbidly obese patients undergoing bariatric surgery ($p < 0.05$). Although the amount of 4-HNE was significantly reduced 6 months post-surgery it still did not reach the levels measured in control lean subjects. Similarly, the levels of 15-F2t-IsoP and 10-F4t-NeuroP were almost 2-fold greater prior to bariatric surgery, however, within the next six months the levels decreased and did not significantly differ from the levels measured in control lean subjects (Figure 1).

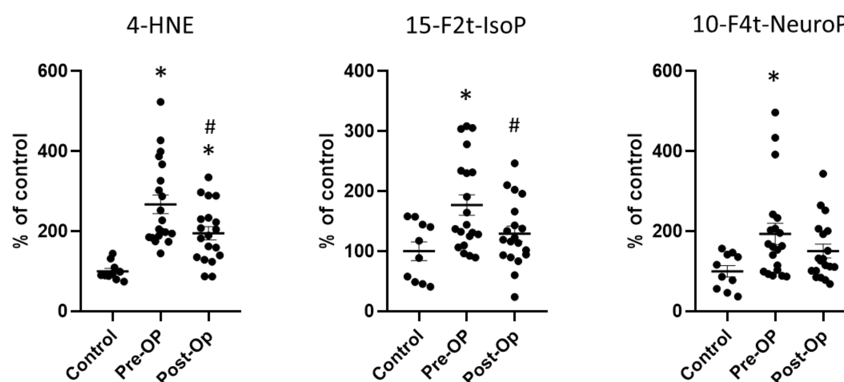

Figure 1. The 4-HNE, 15-F2t-IsoP and 10-F4t-NeuroP in plasma samples of control lean subjects, morbidly obese patients before bariatric surgery (Pre-Op) and samples obtained 6 months post bariatric surgery (Post-Op). Significance * $p < 0.05$ compared to control, # $p < 0.05$ compared to Pre-OP.

In this work, we characterized the alterations in the plasma phospholipid profile in obesity and phospholipidome improvement six months following bariatric surgery were determined by using a high-resolution HILIC-LC-MS/MS. We have identified phospholipid species from seven distinct classes, that included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositols (PI), phosphatidylserine (PS), lyso-PC (LPC), lyso-PE (LPE), and sphingomyelin (SM). The list of 117 phospholipid species that were identified and quantified, which corresponds to the most abundant species in all the detected classes, are shown in Supplementary Table S1.

Lipidomics analysis of plasma samples showed obesity associated lipidome perturbations. Almost double amount of total PIs was recorded in morbidly obese patients prior to surgery (Figure 2). Close to 40% increase was observed for total PCs, PEs, and PSs, 100% increase for PIs, while SMs showed 20% increase in morbidly obese patients compared to lean controls ($p < 0.05$). Following bariatric surgery, the levels PCs, PIs, PSs and SMs decreased to control levels. Contrary, total PE values remained elevated even 6 months post-surgery. Interestingly, a slight increase was observed in total LPCs and LPEs prior and six months post-surgery although it did not reach significance ($p > 0.05$).

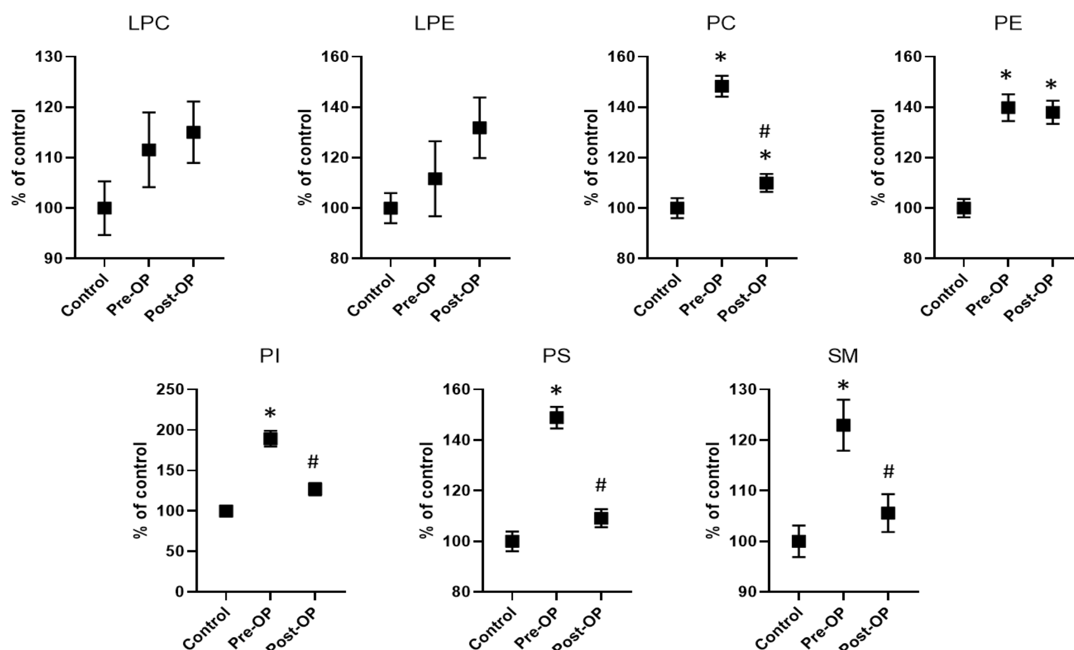

Figure 2. Bariatric surgery induced changes in different lipid classes in plasma samples of morbidly obese patients 6 months post bariatric surgery (Post-Op) compared to paired plasma samples collected before bariatric surgery (Pre-Op) and samples of control lean subjects. Significance * $p < 0.05$ compared to control, # $p < 0.05$ compared to Pre-OP.

The identified differences in the phospholipid profiles among groups studied both univariate and multivariate statistical analysis were used. The principal component analysis (PCA) was performed after the data were autoscaled in order to show the experimental groups' clustering trends. Hence, fingerprinting of plasma lipidome revealed distinct lipidome signatures between obese and lean cohort. To explore the differences in overall lipidomic profiles of obese and lean subjects the PCA of all samples was used. The PCA showed a clear distribution between the three study groups (Control, Pre-OP and Post-OP). Although an improvement in the phospholipid profile may be noticed 6 months post bariatric surgery, still strong perturbations in phospholipids persist (Figure 3A).

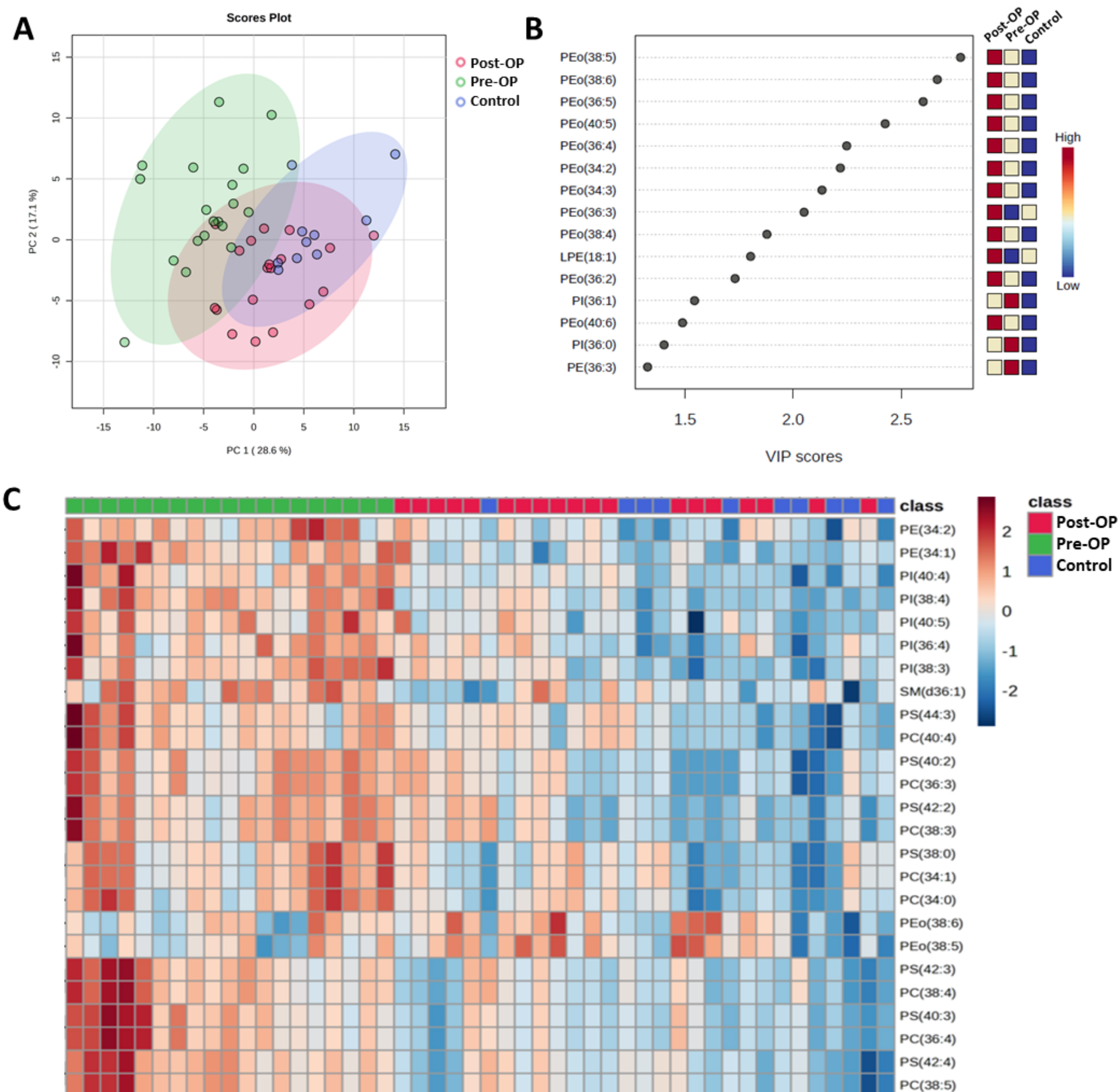

Figure 3. The lipidome profile of control lean subjects, morbidly obese patients before bariatric surgery (Pre-Op) and 6 months post bariatric surgery (Post-Op). A. Principal Component Analysis (PCA) of plasma samples, B. Identified Variable importance in projection (VIP) for group clustering with the relative concentrations of the corresponding lipid in each group under study, and C. Hierarchical Clustering Heatmaps of top 25 phospholipid species.

To identify the lipids that contributed the most to the difference between, VIP scores were used (Figure 3B). The ether-linked phosphatidylethanolamines (PEos) were highlighted as the most important features for group clustering with the VIP scores greater than 2.0 for PEo(38:5), PEo(38:6), PEo(36:5), PEo(40:5), PEo(36:4), PEo(34:2), PEo(34:3) and PEo(36:3), while ether-linked PEs with VIP values between 1.7 and 2.0 were PEo(38:4) and PEo(36:2). Subsequent analysis of top 25 lipid species with hierarchical clustering heatmaps is shown on Figure 3C. Using the high-resolution LC-MS approach a total of 45 lipids were found to be significantly altered with obesity, the amount of 47 lipids significantly changed in obese cohort six months post-surgery, while only 13 lipids were found to significantly differ in the amount between post-surgery samples and those of lean controls (Table 2). The list of lipids with significantly different levels between the obese and lean cohort was determined by the combination of multivariate and univariate statistics.

Table 2. Lipids significantly altered in obesity, between control lean subjects, morbidly obese patients before bariatric surgery (Pre-Op) and samples obtained 6 months post bariatric surgery (Post-Op).

Compound	m/z	RT	Pre-Op (% Δ)	Post-Op (% Δ)	FC (Post-Op vs Pre-Op)	pBH Ctrl vs Pre-Op	pBH Pre-Op vs Post-Op	pBH Ctrl vs Post-Op
LPE(18:1)	478.2936	6.86	165.6 ± 46.0	220.1 ± 25.5	2.67	n.s.	n.s.	0.029
PC(32:1)	790.5565	10.48	158.3 ± 15.0	104.3 ± 8.7	0.85	n.s.	0.015	n.s.
PC(34:0)	820.6036	10.33	134.2 ± 7.5	108.6 ± 3.9	0.88	0.025	0.016	n.s.
PC(34:1)	818.5850	10.33	125.7 ± 6.4	103.6 ± 3.6	0.88	0.040	0.016	n.s.
PC(34:2)	816.5771	10.35	136.2 ± 8.2	116.6 ± 5.8	0.93	0.028	n.s.	n.s.
PC(36:3)	842.5866	10.19	145.3 ± 8.3	109.9 ± 6.0	0.83	0.014	0.007	n.s.
PC(36:4)	840.5711	10.04	188.5 ± 18.7	108 ± 6.7	0.69	0.018	0.003	n.s.
PC(36:5)	838.5570	10.06	162.7 ± 15.6	89.9 ± 10.2	1.42	0.034	0.003	n.s.
PC(38:3)	870.6217	10.01	153.0 ± 10.7	101.5 ± 7.1	0.75	0.018	0.003	n.s.
PC(38:4)	868.6038	9.90	160.3 ± 13.5	99.8 ± 5.6	0.72	0.026	0.003	n.s.
PC(38:5)	866.5879	9.93	135.5 ± 8.1	97.1 ± 4.0	0.84	0.028	0.003	n.s.
PC(38:6)	864.5738	9.94	120.4 ± 8.8	79.8 ± 5.2	0.75	n.s.	0.003	n.s.
PC(40:4)	896.6383	9.90	189.5 ± 22.6	115.6 ± 9.0	2.10	0.034	0.016	n.s.
PC(40:5)	894.6214	9.82	139.4 ± 12.5	89.9 ± 6.5	0.77	n.s.	0.007	n.s.
PC(40:6)	892.6060	9.80	117.6 ± 7.2	81.3 ± 5.6	0.76	n.s.	0.003	n.s.
PCp(34:2)	800.5814	10.32	88.4 ± 10.9	131.2 ± 9.5	2.69	n.s.	0.016	n.s.
PCp(36:3)	826.5960	10.18	136.2 ± 8.4	126 ± 6.7	1.09	0.040	n.s.	n.s.
PCp(36:4)	824.5800	10.00	143.6 ± 9.9	130 ± 9.3	1.35	0.028	n.s.	n.s.
PCp(38:3)	854.6213	10.04	158.2 ± 11.5	133.7 ± 8.4	1.01	0.023	n.s.	n.s.
PE(34:1)	716.5169	4.98	142.0 ± 5.2	111 ± 3.9	0.81	0.001	0.002	n.s.
PE(34:2)	714.5091	5.01	125.6 ± 3.0	113.8 ± 1.7	0.92	0.001	0.007	0.006
PE(36:4)	738.5111	4.80	127.4 ± 4.6	111.7 ± 2.8	0.92	0.008	0.019	n.s.
PE(38:4)	766.5341	4.65	149.3 ± 12.4	118.1 ± 8.5	0.87	0.039	n.s.	n.s.

PE(40:6)	790.5369	4.58	154.2 ± 20.2	90.9 ± 13.1	0.73	n.s.	0.034	n.s.
PEo(34:2)	700.5261	4.96	123.2 ± 17.7	211.1 ± 17.6	2.23	n.s.	0.007	0.006
PEo(34:3)	698.5135	4.97	130.8 ± 26.7	222.9 ± 22.8	2.40	n.s.	0.034	0.015
PEo(36:3)	726.5410	4.85	108.2 ± 16.7	185.7 ± 15.5	2.15	n.s.	0.007	0.015
PEo(36:4)	724.5281	4.84	163.7 ± 24.0	235.9 ± 19.9	2.05	n.s.	n.s.	0.003
PEo(36:5)	722.5101	4.73	152.5 ± 14.4	210.4 ± 15.2	1.63	n.s.	0.024	0.003
PEo(38:4)	752.5616	4.70	133.5 ± 14.9	180.5 ± 16.4	1.53	n.s.	n.s.	0.029
PEo(38:5)	750.5423	4.61	146.0 ± 12.0	213.1 ± 16.3	1.63	n.s.	0.008	0.003
PEo(38:6)	748.5263	4.64	148.3 ± 11.8	197.6 ± 14.6	1.46	0.035	0.033	0.003
PEo(40:5)	778.5754	4.54	195.0 ± 39.0	328.2 ± 39.1	3.63	n.s.	n.s.	0.006
PEo(40:6)	776.5602	4.58	131.7 ± 12.8	150.7 ± 10.0	1.58	n.s.	n.s.	0.039
PI(32:0)	809.5160	2.31	270.2 ± 39.3	194.2 ± 35.7	2.55	0.028	n.s.	n.s.
PI(34:0)	837.5506	2.30	195.4 ± 25.5	148.8 ± 19.9	0.96	0.045	n.s.	n.s.
PI(34:1)	835.5317	2.30	177.6 ± 18.7	146 ± 15.5	1.03	0.034	n.s.	n.s.
PI(34:2)	833.5145	2.30	169.7 ± 16.1	154.7 ± 18.0	1.06	0.028	n.s.	n.s.
PI(36:1)	863.5629	2.30	185.0 ± 20.6	180.5 ± 23.2	1.17	0.028	n.s.	n.s.
PI(36:2)	861.5437	2.29	160.9 ± 13.3	145.3 ± 18.1	1.04	0.026	n.s.	n.s.
PI(36:4)	857.5179	2.28	206.5 ± 21.5	138.3 ± 10.8	0.85	0.018	0.023	n.s.
PI(38:2)	889.5785	2.29	166.3 ± 15.0	110.1 ± 10.4	0.76	0.026	0.015	n.s.
PI(38:3)	887.5590	2.28	170.3 ± 12.4	107 ± 7.3	0.71	0.009	0.003	n.s.
PI(38:4)	885.5507	2.28	198.8 ± 13.6	119.9 ± 5.7	0.71	0.001	0.001	n.s.
PI(38:5)	883.5355	2.28	155.3 ± 10.9	134.8 ± 8.0	0.95	0.018	n.s.	n.s.
PI(40:4)	913.5752	2.28	222.9 ± 20.0	138.3 ± 5.8	0.73	0.005	0.003	0.010
PI(40:5)	911.5594	2.28	168.0 ± 12.9	117.7 ± 9.0	0.92	0.014	0.012	n.s.
PI(40:6)	909.5462	2.27	135.5 ± 9.3	98.2 ± 12.1	0.81	n.s.	0.046	n.s.
PS(36:0)	790.5565	9.88	147.9 ± 13.0	96.3 ± 7.1	0.83	n.s.	0.007	n.s.
PS(38:0)	818.5850	10.33	125.7 ± 6.4	103.6 ± 3.6	0.88	0.040	0.016	n.s.
PS(38:1)	816.5771	10.35	136.2 ± 8.2	116.6 ± 5.8	0.93	0.028	n.s.	n.s.
PS(40:2)	842.5866	10.19	145.3 ± 8.3	109.9 ± 6.0	0.83	0.014	0.007	n.s.
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PS(42:3)	868.6038	9.90	160.3 ± 13.5	99.8 ± 5.6	0.72	0.026	0.003	n.s.
PS(42:4)	866.5879	9.93	135.5 ± 8.1	97.1 ± 4.0	0.84	0.028	0.003	n.s.
PS(42:5)	864.5738	9.94	120.4 ± 8.8	79.8 ± 5.2	0.75	n.s.	0.003	n.s.
PS(44:3)	896.6383	9.90	189.5 ± 22.6	115.6 ± 9.0	2.10	0.034	0.016	n.s.
PS(44:4)	894.6214	9.82	139.4 ± 12.5	89.9 ± 6.5	0.77	n.s.	0.007	n.s.
PS(44:5)	892.6060	9.80	117.6 ± 7.2	81.3 ± 5.6	0.76	n.s.	0.003	n.s.
SM(d34:0)	763.5923	11.72	142.5 ± 8.0	124.9 ± 9.3	0.93	0.018	n.s.	n.s.
SM(d34:2)	759.5646	11.78	153.5 ± 8.1	118.6 ± 6.0	0.81	0.004	0.007	n.s.
SM(d36:1)	789.6091	11.57	143.9 ± 7.5	107.8 ± 6.5	0.81	0.011	0.006	n.s.
SM(d36:2)	787.5984	11.57	153.7 ± 12.9	108.8 ± 9.0	0.79	0.028	0.022	n.s.
SM(d38:1)	817.6406	10.54	152.3 ± 10.5	123.4 ± 6.4	0.92	0.020	n.s.	n.s.
SM(d41:2)	857.6708	11.16	123 ± 10.7	79.6 ± 7.5	0.72	n.s.	0.009	n.s.

%Δ, percentage of change; FC, fold change; n.s., non significant; pBH, Benjamini-Hochberg adjusted p-value; RT, retention time

The amount of LPE(18:1) was found to be significantly elevated post-surgery, although total amount of LPEs did not significantly differ from control. In addition to LPE(18:1), lipids PEo(36:4), PEo(40:5) and PEo(40:6) were found to be significantly upregulated only in the Post-OP samples compared to control and Pre-OP samples. Obesity associated alterations in specific LPCs were not identified. Total of 14 PCs, 4 PCps, 5 PEs, 10 PEos, 14 PIs, 13 PSs and 6 SMs were found to be altered in obesity.

The potential impact of elevated levels of 4-HNE, 15-F2t-IsoP and 10-F4t-NeuroP in plasma samples on plasma lipidome was further examined. Hence, the 4-HNE level was found to have moderate negative correlation with 10 lipids in Pre-OP samples, and 1 lipid in Post-OP samples. An amount of 15-F2t-IsoP showed moderate negative correlation with 9 lipids in Pre-OP samples and 4 lipids in Post-OP samples. Contrary, 10-F4t-NeuroP concentration had moderate positive correlation with 5 lipids in the Pre-OP samples and moderate negative correlation with 3 lipids in Post-OP samples. The correlation analysis of significantly altered lipids with levels of oxidative stress markers is presented in Table 3.

Table 3. Correlation between altered phospholipids and levels of 4-HNE, 15-F2t-IsoP and 10-F4t-NeuroP in plasma of obese patients before bariatric surgery (Pre-Op) and 6 months post bariatric surgery (Post-Op). Lipids marked in bold are those found to be altered with obesity. Spearman correlation coefficients are shown and significance marked: * $p < 0.05$; ** $p < 0.01$.

Compound	Pre-OP			Post-OP		
	HNE	15-F2t-IsoP	10-F4t-NeuroP	HNE	15-F2t-IsoP	10-F4t-NeuroP
LPC(16:0)	-0.58 **	-0.43	0.08	0.2	0.31	-0.35
LPC(18:0)	-0.45 *	-0.29	0.02	0.27	0.35	-0.32
LPC(20:4)	-0.43	-0.51 *	0.04	0.08	0.08	-0.08
LPC(16:1)	-0.52 *	-0.23	0.19	-0.05	0.4	-0.01
LPE(20:4)	-0.62 **	-0.35	0.11	0.11	0.16	-0.1
LPE(18:0)	-0.54 *	-0.39	-0.03	-0.02	0.23	-0.27
LPE(16:0)	-0.5 *	-0.18	0.23	-0.09	0.38	0.02
LPE(22:6)	-0.34	-0.1	0.45 *	-0.32	0.16	0.32
LPE(18:1)	-0.51 *	-0.23	0.02	-0.16	-0.18	-0.1
PC(38:6)	-0.02	-0.24	0.62 **	-0.08	0.15	0.28
PC(38:5)	**-0.29**	**-0.45 ***	**0.23**	**0.19**	**-0.07**	**-0.23**
PC(40:6)	-0.11	-0.13	0.54 *	-0.02	0.08	0.33
PCp(38:4)	0.06	-0.36	0.18	0.12	0.19	-0.56 *
PCp(36:3)	**-0.07**	**-0.54 ***	**-0.17**	**0.06**	**0.08**	**-0.48 ***
PC(32:0)	-0.49 *	-0.1	-0.09	-0.22	0.1	-0.31

PCp(38:3)	**-0.07**	**-0.29**	**-0.1**	**-0.15**	**-0.5 ***	**-0.49 ***
PC(36:5)	**-0.12**	**-0.42**	**-0.05**	**-0.08**	**-0.54 ***	**0.04**
PC(34:3)	-0.38	-0.58 **	-0.35	-0.1	0.06	-0.04
PE(36:2)	-0.25	-0.45 *	-0.22	0.17	-0.26	0.05
PE(34:1)	**-0.21**	**-0.01**	**0.03**	**-0.5 ***	**-0.44**	**0.28**
PE(36:1)	-0.31	-0.16	-0.11	-0.07	-0.53 *	0.1
PI(36:1)	**-0.23**	**-0.5 ***	**-0.04**	**0**	**-0.1**	**-0.17**
PI(40:5)	**-0.47 ***	**-0.16**	**0.06**	**0.11**	**0.2**	**-0.13**
PI(40:4)	**-0.46 ***	**-0.26**	**-0.11**	**-0.18**	**-0.12**	**-0.14**
PI(40:2)	-0.2	-0.45 *	-0.13	-0.05	0.09	0.24
PS(42:5)	-0.02	-0.24	0.62 **	-0.08	0.15	0.28
PS(42:4)	**-0.29**	**-0.45 ***	**0.23**	**0.19**	**-0.07**	**-0.23**
PS(44:5)	-0.11	-0.13	0.54 *	-0.02	0.08	0.33
PS(40:4)	**-0.12**	**-0.42**	**-0.05**	**-0.08**	**-0.54 ***	**0.04**
PS(38:2)	-0.38	-0.58 **	-0.35	-0.1	0.06	-0.04

Of the lipids that were found to correlate with lipid peroxidation markers, only 10 were identified to be altered with obesity. Among those 2 showed moderate negative correlation with 4-HNE, and 4 with 15-F2t-IsoP, while none correlated with 10-F4t-NeuroP in the Pre-OP samples. Furthermore, we tested whether the changes in the amount of specific lipids in paired Pre-OP and Post-OP samples correlates with lipid peroxidation and found an interesting link between 4-HNE and amount of two ether-linked phosphoethanolamines PEo(38:2) and PEo(36:2) (Figure 4).

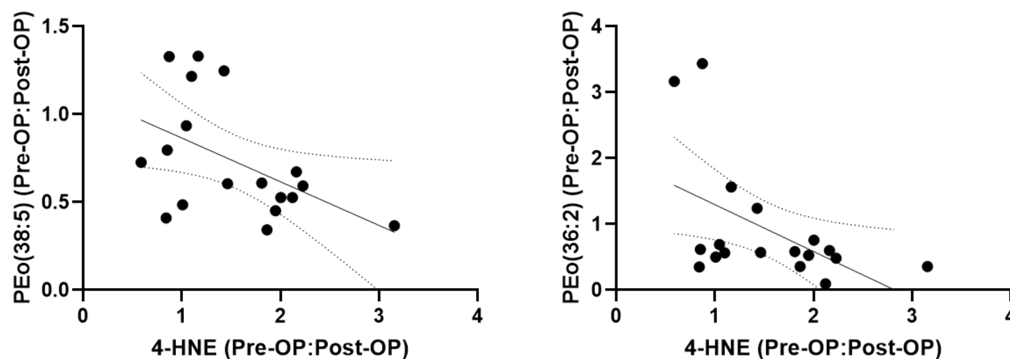

Figure 4. Relation between changes in 4-HNE and ether-linked phosphoethanolamines PEo(38:5) and PEo(36:2).

For both, PEo(38:2) and PEo(36:2), the increase of their amount in plasma samples followed the decrease of 4-HNE. However, the same was not observed for 15-F2t-IsoP and 10-F4t-NeuroP s (data not shown).

4. Discussion

Obesity is accompanied by the excessive ROS production, reduced antioxidant defenses and inflammation. Obesity itself can trigger systemic oxidative stress through multiple biochemical pathways, including superoxide production by NADPH oxidases, oxidative phosphorylation, glyceraldehyde auto-oxidation, activation of protein kinase C, and the polyol and hexosamine pathways [31,32]. Additional contributors to oxidative stress in obesity include elevated leptin levels [33], tissue dysfunction [32], reduced antioxidant defenses [34], chronic inflammation [35], and the generation of reactive oxygen species after meals [36]. Vitamin E is a major factor in the non-enzymatic antioxidant defenses. An inverse relationship between obesity and serum vitamin E level has been previously demonstrated [37]. Moreover, it has been revealed that patients with metabolic syndrome had significantly lower serum levels of vitamin E [38]. Chronic low-grade inflammation in obesity is a major contributor to oxidative stress [39]. Obesity-induced oxidative stress is implicated in the development of various obesity-related complications, such as insulin resistance and diabetes. Regardless of the metabolic syndrome, oxidative damage to lipids is present in obese individuals [40]. Bariatric surgery offers numerous benefits for obese patients, including weight loss, BMI reduction, improved metabolic status, and reduction of inflammation. Bariatric surgery results in elevated levels of anti-inflammatory IL-10 and antioxidants, as well as lower levels of lipid peroxidation markers and oxidized proteins in the bloodstream [40–43]. In addition to significant weight loss, this study also confirmed that bariatric surgery leads to a decrease in lipid peroxidation after six months. To our knowledge, this is the first study to monitor the changes in 15-F2t-IsoP, 10-F4t-NeuroP and 4-HNE in parallel in the same samples. The strong decrease in systemic oxidative stress markers indicates an important beneficial effect of bariatric surgery on body's redox homeostasis and an overall metabolic improvement.

In this study, we found a substantial correlation between BMI and the plasma lipidome and that weight loss causes a shift in the plasma lipidome toward the lipidome of the lean cohort, indicating specific physiological changes and suggesting that lipid metabolites, in particular ether lipids, may serve as markers of weight loss-induced metabolic reprogramming.

The obese cohort studied with BMI > 35 had greatly increased total PCs, PEs, PIs, PSs and SMs, while no significant difference was found for total LPEs and LPCs. Similarly, total PEs and SMs were reported to be elevated in obese cohort with BMI > 30, while no difference was observed for total LPCs [44]. In contrast to our results, the same study reported a decrease in total LPEs and no significant difference in PCs [44], which could be due to differences in BMI in the obese cohort. In this study, weight loss six months after bariatric surgery was accompanied by the decrease in PCs, PIs, PSs and SMs, while no reduction was observed for total LPCs, LPEs and PEs. However,

quantification of specific lipid species revealed that the levels of PEo(38:5), PEo(38:6), PEo(36:5), PEo(40:5), PEo(36:4), PEo(34:2), PEo(34:3), PEo(36:3), PEo(38:4) and PEo(36:2) change with obesity. This underscores the importance of detecting and quantifying specific lipid species that contribute to dyslipidemia to elucidate the mechanisms underlying obesity-related pathologies. Phospholipids are crucial for the control of cell metabolism and also serve as a primary structural element of cell membranes [45]. Detailed analysis of specific lipids has identified several ether phospholipids that are altered in obesity. The role of ether lipids is not fully understood, although they make up almost 20% of the human phospholipidome. A recent study has shown that ether phospholipids are essential for mitochondrial ROS homeostasis [46]. This study identified ten ether-linked phosphoethanolamines (PEo) and four phosphatidylcholine plasmalogens (PCp) that are altered in both obesity and weight loss. PCs essential components of cellular membranes, are crucial for plasma lipoproteins and for the formation of acetylcholine. The levels of ten PCs and three PCps were elevated in obesity, while they returned to normal levels after weight loss, suggesting a remodeling of the membrane lipidome that may be related to metabolic improvement. Although the biological functions of PCps are not completely understood, due to the presence of an ester bond in sn-2 and a vinyl ether moiety in the sn-1 position of the glycerol backbone, a number of different functions have been postulated, such as modulation of membrane fluidity and intracellular trafficking, storage of precursor fatty acids for the synthesis of inflammatory mediators, and possible action as endogenous antioxidants and scavengers against lipid peroxidation [47,48]. In addition, ether lipids in breastmilk have been shown to be efficient in preventing childhood obesity [49]. Moreover, dysfunctional exocytosis in obesity is likely the result of altered lipid trafficking [50]. Thus, the reduction in PCp that followed the decrease in BMI and reduced lipid peroxidation, could be at least in part the result of cellular adaptation to the altered redox balance and normalized cellular trafficking. In addition, PCs by the action of PS synthase 1 in the endoplasmic reticulum in the regions close to mitochondria may be converted to PS and transferred to mitochondria, while further decarboxylation of PS in the mitochondria generates PEs [51]. In our cohort, both PE and PS were increased in obesity, whereas the amount returned to normal after weight loss. In contrast, as many as ten PEos were increased with significant weight loss, however the exact function of specific PEos remains to be elucidated. Interestingly, although we did not observe a direct correlation between the specific PEos and the measured markers of oxidative stress, pairwise analysis of patient samples obtained before and after surgery showed that as redox homeostasis improved and 4-HNE, lipid peroxidation derived aldehyde, decreased, the amount of PEo(38:2) and PEo(36:2) increased. However, the same was not observed for 15-F2t-IsoP and 10-F4t-NeuroP, suggesting a selective role of the specific lipid peroxidation markers

studied. In addition, although moderate correlation of more than 20 lipid species with lipid peroxidation markers was observed, not all lipids that significantly correlated were found to be associated with obesity. Potential limitations of our study refer to the sample size, which is relatively small and focused to a specific population, limiting the general application of the results. Additionally, selection bias may occur due to participants lifestyle choices and behavioral factors that also contribute significantly to obesity and its management. For example, patients who are younger and have higher BMI levels are also more likely to have bariatric surgery. Furthermore, the results could have been skewed by unidentified variations between the groups, as information regarding the participants' social state, dietary, and physical activity status was not accessible for the surgery group. Changes in comorbidities after surgery may also be influenced by factors other than weight loss, such as changes in medication or lifestyle. Individual differences in metabolism, genetics and other biological factors can contribute to variability in oxidative stress levels, making it challenging to draw definitive conclusions. Thus, in order to better understand the involvement of oxidative stress in lipidome remodeling, studies involving larger cohort are necessary. Finally, our data point to the importance of specific ether lipids in metabolic improvement, highlighting the need for further functional studies on oxidative stress modulated ether lipids in order to understand underlying mechanism and beneficial effects of bariatric-surgery induced weight loss.

Author Contributions

ES and MJ designed the study. MH, WL, IJK, AS, IS, AP, AMS, TG, MS and TK performed the experiments and analyzed the data. ES and MJ supervised the progress of the work. MH, NŽ, ES and MJ wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

We would like to acknowledge the Ministry of Science and Higher Education in Poland, Ruder Boskovic Institute in Croatia and COST Action 19105-Pan-European Network in Lipidomics and EpiLipidomics (EpiLipidNET).

Funding

This work was financially supported by the Ministry of Science and Higher Education in Poland under the program scientific activity of the Medical University of Białystok and by the Ruder

Boskovic Institute in Croatia. The funding body did not participate in the design of the study, analysis, interpretation of data or in writing the manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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