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Lipidomics-Based Cartography of Membrane Phospholipids Remodeling Uncovers Enzymatic Underpinnings of Cellular Dysfunction in Metabolic Syndrome Patients

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Abstract

Background: Metabolic syndrome (MetS) is linked with insulin resistance and low-grade chronic inflammation. **Emerging** evidence indicates that these features are associated with the disturbed membrane phospholipid composition but the enzymatic drivers of membrane remodeling in real-life MetS are still too poorly mapped.

Objective: The overall design is to map membrane phospholipid remodeling through lipidomics and identify the enzymatic causes of cellular dysfunction of patients who have MetS.

Methods: This is a hospital-based case-control study, 100 adults were witnessed (MetS, n=50; controls, n=50). Fasting clinical and biochemical parameters were measured, which included lipid profile, hs-CRP, fasting insulin and HOMA-IR. Plasma and PBMC lipid extracts were analysed by UHPLC -MS/MS lipidomics. Remodeling indices (LPC/PC, LPE/PE, saturation index, PUFA-enrichment) derived. PBMC enzymatic activities important to Lands' cycle (PLA2, LPCAT, ACSL4 and MBOAT7) were measured. Group comparisons, correction for multiple testing (fdr) correction for lipidomics, correlation and regression analysis were performed in multivariable.

Results: MetS cases had greater insulin resistance and inflammation (HOMA-IR and hs-CRP; p<0.001). Lipidomics revealed an increase in the level of lysophospholipids (total LPC and LPE; p < 0.001; the level was statistically significant after a False Discovery 0.001) with a decrease in the level of structural phospholipids (PC and PE; p < 0.025; the level was statistically significant at False Discovery <= 0.025). Remodeling indices showed high LPC/PC and LPE/PE ratios and high saturation indices with depletion of the phospholipid species with the higher UFA content (p<0.001). Through enzymatic profiling, higher PLA2 and lower LPCAT, ACSL4 and MBOAT7 activities (p<=0.002) were found to be increased. PLA2 activity showed a positive correlation with LPC/PC (rho equal to 0.72 and HOMA-IR (rho equal to 0.59) and ACSL4 activity was positively correlated with arachidonoyl enrichment (rho equal to 0.66) (all p less than 0.001). LPC/PC ratio and PLA2 activity were independent predictors of HOMA-IR and ACSL4 activity was an inverse predictor of hs-CRP in the regression analysis. Conclusions: MetS is represented by a lipidomics signature of membrane phospholipid remodeling resulting from a lipid remodeling deacylation/reacylation imbalance - PLA2 activation _impaired reacylation capacity (LPCAT/ACSL4/MBOAT7). These enzymatically anchored remodeling indices have a strong link to insulin resistance and inflammation, providing support for their therapists or mechanistic biomarker value.

Keywords: Lipidomics Metabolic syndrome; PLA2; Lysophospholipid acetyltransferase (LPCAT); ACSL4 and MBOAT7

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1. Introduction

Metabolic syndrome (MetS) comes as a multi-factorial metabolic disorder with a condition of cluster of inter-related risk factors that accompany one another including central obesity, dyslipidemia, insulin resistance and high blood pressure, representing a high risk in development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Grundy *et al*., 2023). The global prevalence of MetS has increased remarkably affecting up to one-third of adults in the world (Saklayen, 2018). Beyond abnormalities of the body systems, recent evidence suggests that MetS reflects a deep-seeded change in the structure and function of the cellular membranes, most notably in the remodeling of membrane phospholipids that conserve biophysical and biochemical homeostasis (Han *et al*., 2022). The building block of the structure of biological membranes are phospholipids. The composition in fatty acids determines the membrane curvature, fluidity and the organization of proteins, and thus regulates important cellular processes such as signal transduction, vesicular transport, and mitochondrial function (Wymann & Schneiter, 2008). Disturbances to this composition have been implicated in metabolic inflexibility, oxidative stress and inflammatory activation in obesity and insulin resistance (Huang *et al*., 2021). Such disturbances are driven much by the dysregulation of the processes of phospholipid remodeling that are controlled by the Lands' cycle, in which phospholipids are sequentially deacylated (by phospholipase A2, or PLA2) and reacylated (by acyl-CoA:lysophospholipid acyltransferases, or LPLATs), including LPCAT and MBOAT PHP families (Shindou *et al*., 2022). Under physiological conditions, the cycle of Lands keeps the proper balance between the saturated or polyunsaturated acyl chains present in phospholipids optimizing the integrity of the membranes and including lipid signaling. However, in MetS, increased oxidative stress and excess of saturated fatty acids has led to modification of the substrate pool and enzyme selectivity resulting in accumulation of lysophospholipids, and loss of polyunsaturated phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species (Inoue et al., 2024). These changes compromise the relationship of insulin receptors and insulin signaling and support insulin resistance (Kawanishi et al., 2023). Furthermore, consequently abnormal functions of several enzymes including LPCAT3 and ACSL4 have been implicated with aberrant inflammatory responses and mitochondrial dysfunction (Hsu et al., 2020). The discovery of the field of lipidomics allows for system-wide quantification of lipid species by high-resolution liquid chromatography - mass spectrometry (LC-MS) (Quehenberger *et al*., 2023). Lipidomic studies have shown that certain lipid classes, such as phospholipids, sphingolipids and ceramides, show certain features of alteration in patients with MetS, which correspond to underlying metabolic derangements (Friedman *et al*., 2022; Meikle *et al*., 2021). Noteworthy is, distinct plasma phospholipid signatures are linked to markers of inflammation, hepatic steatosis and endothelial dysfunction, implying that remodeling of lipids plays a central role in the pathophysiology of MetS (Suhre *et al*., 2021). There is experimental evidence to support these clinical findings. Animal models of high-fat feeding indicate extensive remodeling of membrane phospholipids in the liver and adipose tissues resulting in elevated lyso-PC and reduced species of PC enriched in polyunsaturated fatty acids with impaired mitochondrial respiration and oxidative phosphorylation (Mendez and Mora, 2024). The genetic manipulation of remodeling enzymes like ACSL4 or MBOAT7 has a significant effect on tissue phospholipid composition as well as metabolic outcomes highlighting the causal connection between the remodeling enzyme and metabolic dysfunction (Li *et al*., 2023). Despite these advances, the structure of phospholipid remodeling at the lipidome scale - where information on structural lipid data, enzymatic activity and clinical phenotypes are integrated - still leaves ulcer. This gap restricts our mechanistic knowledge on the role of lipid remodeling in cell dysfunction in metabolic diseases. Therefore, in the current study, we will aim to build a lipidomics-based memnmap of the membrane phospholipid remodeling statuses from patients with metabolic syndrome, discover the enzymatic drivers that are implicated in the cause of cell dysfunction, and establish correlation between lipidomic signatures, enzyme activity, and clinical severity. Such integrative profiling may unlock novel therapeutic targets against restoration of membrane homeostasis and mitigate metabolic injury.

Aim of the Study

The objective of this work is to create an extensive lipidomics-based map of membrane phospholipid remodeling in patients with metabolic syndrome (MetS) and identify the drivers of this process (enzymes) involved in cellular dysfunction.

2. Materials and Methods

2.1. Study Design and Population

A hospital-based, observational, case-case control study was done between October 2025 and January 2026 in the Department of Endocrinology and Clinical Biochemistry, Babylon Teaching Hospital. Enrollment of 100 adult participants (50 MetS patients and 50 age- and sex-matched healthy controls) was done. MetS was diagnosed based on the International Diabetes Federation (IDF, 2023) criteria of central obesity (waist circumference 94 or more cm for men or 80 or more cm for women) with two or more of the following: increased triglycerides 150 or more milligrams

per deciliter (mg/dL) of blood; decreased HDL-C (<40 mg/dL in men or <50 mg/dL in women); increased blood pressure 130/85 of Systolic blood pressure and/or Diastolic blood pressure of mm Hg; and fasting plasma glucose Participants were excluded if they had acute infection, failed liver/kidney, neoplasm, pregnancy, or lipid altering medications within 3 months.

2.2. Ethical Approval and Consent

Approvement to the study protocol was received from the Institutional Review Board (IRB) of the University of Babylon (Approval No. BB-2025-211). All participants gave written informed consent before enrollment. The research complied with principles of the Declaration of Helsinki (2013 revision) on research involving humans and data confidentiality.

2.3. Assessment of Clinical or Biochemical Profiles

Anthropometric (BMI, WH) and vital signs were documented. Venous blood samples were taken after fasting for 10-12 hours. Serum glucose, triglycerides, total cholesterol, HDL-C and LDL-C concentrations was measured by use of enzymatic colorimetric kits (Roche Diagnostics, Germany). High sensitivity C-reactive protein (hs-CRP) and fasting insulin were measured by an enzyme immunoassay (ELS, Abcam, UK). Insulin resistance was estimated using the formula HOMA-IR (fasting insulin * glucose / 22.5), (Matthews *et al*., 1985). Liver enzymes (ALT, AST) and creatinine were done to rule out organ dysfunction.

2.4. Sample Collection and Sample Prem exposed Lipidomics

Peripheral blood from the veins (10 mL) was collected into tubes containing Ethylene Diamine Tetraacetate salt solution and immediately centrifuged (1,500 x g, 10 min, 4^{o}C) to obtain plasma. Peripheral blood mononuclear cell (PBMCs) preparation was done with Ficoll-Paque Plus (GE Healthcare, USA) and washed with PBS. Plasma and PBMC pellets were aliquoted and kept at minus 80^{o}C until analysis. To avoid pre-analytical variation as much as possible, all samples were processed within 2 hours of the moment of collection, and the aliquots are not thawed more than once (Cajka & Fiehn, 2017).

2.5. Lipid Extraction with Internal Standards

Lipids were extracted with a modified Bligh-Dyer method with chloroform/methanol/water (2:2:1.8, v/v/v) (Bligh & Dyer, 1959). Deuterated internal standards spanning major lipid classes (SPLASH(R) Lipidomix, Avanti Polar Lipids, USA) were added to each of the samples prior to the extraction step in order to ensure quantitative reproducibility. The organic phase was collected, dried under the nitrogen and reconstituted in methanol--isopropanol (1:1 (v/v)), for the LC--MS analysis.

2.6. Lipidomics Analysis UHPLC-MS/MS

Comprehensive lipid profiling was done by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC--MS--MS) (Thermo Scientific Q Exactive Plus). Chromatographic separation was performed on C18 column (2.1*100 mm, 1.7 um) using water/acetonitrile (60:40 v/v, 0.1% formic acid+10mM of ammonium formate) and isopropanol/acetonitrile (90:10 v/v). The MS operated in positive and negative ion modes for range (m/z) = 200-2,000. Quality control (QC) samples were injected every 10 samples to monitor the drift in the analytical measurements (Quehenberger *et al*., 2023).

2.7. Quantification of Enzyme activities

Activities of cytosolic PLA2, LPCAT and ACSL4 were quantified in PBMC lysates by colorimetric and fluorometric kits (Abcam, UK). Enzymatic reactions were conducted following to the manufacturer's protocols and normalized for the total content of proteins (BCA assay, Thermo Scientific). Results were expressed in nmol substrate hydrolyzed/acyl-CoA formed/mg protein/ min. Every assay was repeated thrice times so that we can experience reproducibility (Inoue *et al*., 2024).

2.8. Processing and Statistical Analysis of Data

Raw MS data were processed with LipidSearch 5.0 (Thermo) to detect peaks, align peaks and identify peaks. Lipid species with > 30% missing values were excluded. Intensities were log2 transformed and Pareto Scaled. Multivariate analysis (principal component analysis [PCA], partial least squares-discriminant analysis [PLS-DA]) and univariate statistics (t-test, FDR corrected $p < 0.05$) were carried out using MetaboAnalyst 5.0. Correlations between the enzyme activity and lipid indices (LPC/PC ratio, saturation index) were studied by Spearman's correlation. Multiple linear

regression was used to determine independent predictors of metabolic dysfunction. Data were analyzed and graphs were created with GraphPad Prism 10 (GraphPad Software, USA).

2.9. Quality Control and Their Reproducibility

All analyses were to follow the Lipidomics Standards Initiative (LSI, 2020) guidelines for minimal reporting. Batch effects were corrected by using QC-based robust LOEss (Van der Kloet *et al*., 2019) Duplicate extraction, and analytical runs were done for 10% of samples to determine intra and inter assay variability (CV < 15%). Analytical blanks and pooled quality control (QC) samples were used to demonstrate negligible carryover and instrument stability over the course of the study.

3. Results

MetS participants exhibited significantly higher adiposity and blood pressure, confirming the clinical phenotype of metabolic risk. No significant age or sex differences were observed, indicating matched cohort selection.

Table 1 Baseline demographic and clinical characteristics

Variable	Controls (n=50)	MetS (n=50)	Test	*p* value
Age (years)	46.2 ± 8.4	47.8 ± 7.9	t = 0.95	0.34
Sex (M/F)	26/24	28/22	$\chi^2 = 0.16$	0.69
BMI (kg/m^2)	24.8 ± 2.9	32.6 ± 3.8	t = 11.3	< 0.001
Waist circumference (cm)	83.2 ± 6.1	101.5 ± 7.4	t = 13.5	< 0.001
Systolic BP (mmHg)	116 ± 10	138 ± 12	t = 9.4	< 0.001
Diastolic BP (mmHg)	74 ± 7	88 ± 8	t = 9.1	< 0.001

MetS subjects showed extreme insulin resistance, dyslipidemia and raised levels of hs-CRP which points towards the presence of chronic metabolic-inflammatory stress; ideal to investigate the mechanisms of lipid remodeling.

Table 2 Biochemical and inflammatory profile after fasting

Variable	Controls	MetS	*p* value
Fasting glucose (mg/dL)	92 ± 8	118 ± 14	< 0.001
Fasting insulin (μIU/mL)	7.4 ± 2.6	18.2 ± 5.1	< 0.001
HOMA-IR	1.69 ± 0.52	5.31 ± 1.72	< 0.001
Triglycerides (mg/dL)	118 ± 25	212 ± 44	< 0.001
HDL-C (mg/dL)	53 ± 9	36 ± 8	< 0.001
LDL-C (mg/dL)	116 ± 21	145 ± 25	< 0.001
hs-CRP (mg/L)	1.4 ± 0.6	4.8 ± 1.9	< 0.001
ALT (U/L)	23 ± 8	39 ± 12	< 0.001
AST (U/L)	22 ± 7	33 ± 10	< 0.001

MetS patients showed a depletion of structural phospholipids (PC, PE) and accumulation of lysophospholipids, which is one of the hallmarks of increased phospholipase activity and impaired reacylation.

Table 3 Concentrations of plasma lipids class

Lipid class	Controls (µmol/L)	MetS (µmol/L)	Fold change	FDR-q
Total PC	195.3 ± 28.4	173.1 ± 24.7	0.89	0.018
Total PE	118.6 ± 21.3	97.8 ± 19.1	0.82	0.010
Total PI	45.7 ± 10.6	42.1 ± 9.7	0.92	0.081
Total PS	34.8 ± 7.2	30.2 ± 6.8	0.87	0.025
Total LPC	11.5 ± 2.3	21.8 ± 3.4	1.90	< 0.001
Total LPE	6.2 ± 1.4	10.4 ± 2.1	1.68	< 0.001

Indices verified remodeling shift towards increase in saturation and reduction in incorporation of PUFA which was related to altered flux by Lands' cycle, as well as slowdown in ACSL4/LPCAT3 function in MetS.

Table 4 Remodeling indices (cycle signatures of Lands).

Remodeling index	Controls	MetS	*p* value
LPC/PC ratio	0.059 ± 0.013	0.126 ± 0.022	< 0.001
LPE/PE ratio	0.053 ± 0.012	0.107 ± 0.019	< 0.001
Saturation index (PC)	0.72 ± 0.11	0.94 ± 0.15	< 0.001
Saturation index (PE)	0.69 ± 0.10	0.91 ± 0.13	< 0.001
AA-enrichment index	0.36 ± 0.07	0.24 ± 0.06	< 0.001
DHA-enrichment index	0.18 ± 0.05	0.11 ± 0.04	< 0.001

The lipidomic fingerprint showed that it was enriched for the saturated lysophospholipids and depleted for the PUFA-rich species. This pattern changes is analogous to impaired reacylation of arachidonoyl and docosahexaenoyl chains (which are important for membrane flexibility and relay of signals).

Table 5 Distinct phospholipid species (top discriminants).

Lipid species	Controls (µmol/L)	MetS (µmol/L)	Fold change	FDR-q
PC (16:0/18:1)	14.2 ± 3.1	20.7 ± 3.6	1.46	0.002
PC (16:0/20:4)	10.5 ± 2.7	6.8 ± 2.0	0.65	0.004
PE (18:0/20:4)	7.3 ± 1.9	4.5 ± 1.4	0.62	0.006
PI (18:0/20:4)	5.4 ± 1.6	3.8 ± 1.3	0.70	0.015
LPC (16:0)	2.3 ± 0.5	4.8 ± 1.0	2.09	< 0.001
LPC (18:0)	1.8 ± 0.4	3.6 ± 0.8	2.00	< 0.001
LPE (18:1)	0.9 ± 0.2	1.7 ± 0.4	1.89	0.003

Increased activity of phospholipase (PLA2) accompanied by decreased activity of acyltransferase and acyl-CoA synthetase was observed in subjects with metS. These enzyme imbalances provide a mechanistic explanation for the high elevation of the lysophospholipids and loss of the PUFA-enriched phospholipids.

Table 6 Enzyme Activity in PBMC Lysate

Enzyme	Controls (nmol/min/mg protein)	MetS	*p* value
PLA_2	2.15 ± 0.46	3.91 ± 0.71	< 0.001
LPCAT	1.87 ± 0.42	1.21 ± 0.34	< 0.001
ACSL4	1.64 ± 0.38	1.05 ± 0.29	< 0.001
MBOAT7	1.21 ± 0.31	0.82 ± 0.27	0.002

Strong positive correlations were found to correlate phospholipase activation with lysophospholipid accumulation and insulin resistance and PUFA depletion with systemic inflammation. These relationships indicate enzymatic remodeling as a mechanistic linker between the lipid composition and the metabolic dysfunction.

Table 7 Correlation analysis between indices of remodeling, enzymes and metabolic markers

Association	Spearman's ρ	*p* value
PLA_2 vs LPC/PC	+0.72	< 0.001
LPCAT vs LPC/PC	−0.61	< 0.001
ACSL4 vs AA-enrichment	+0.66	< 0.001
LPC/PC vs HOMA-IR	+0.59	< 0.001
Saturation index vs hs-CRP	+0.51	0.002
PUFA-enrichment vs TG	−0.48	0.004

Multivariable modelling found LPC/PC ratio and PLA2 activity were the best independent predictors of insulin resistance and ACSL 4 activity was an inverse predictor of inflammation. Together, these parameters accounted for more than half the variance in MetS-related cellular dysfunction, confirming the enzymatic-lipidomic (proposed in the study hypothesis) relationship in the study's hypothesis.

Table 8 Multiple linear models of the predictors of metabolic dysfunction

Outcome variable	Independent predictors	β (95% CI)	*p* value	Adjusted R^2
HOMA-IR	LPC/PC ratio	0.64 (0.48–0.79)	< 0.001	0.58
	PLA_2 activity	0.53 (0.35–0.71)	< 0.001	
hs-CRP	Saturation index	0.41 (0.21–0.61)	0.001	0.47
	ACSL4 activity	−0.38 (−0.59 to −0.17)	0.002	
Triglycerides	PUFA-enrichment index	−0.44 (−0.67 to −0.21)	0.001	0.42

4. Discussion

This study offers global information on the lipidomic and enzymatic aspects of the remodeling of membrane phospholipids in patients with metabolic syndrome (MetS). Using a combined approach of untargeted lipidomics and enzymatic profiling, we demonstrated that MetS is characterized by (1) increased lysophospholipids, (2) reduction of polyunsaturated phospholipid species and (3) alterations of the remodeling enzymes including PLA2, LPCAT, as well as ACSL4. Collectively, these results supply structural information on a biochemistry of remodeling (phospholipase overactivation/defection reacylation) that is associated with insulin resistance and systemic inflammation.

The observed elevation in lysophospholipid species especially LPC (16:0) and LPC (18:0) is consistent with other studies that indicate accumulation of lysophospholipids appears to accompany metabolic stress and obesity (Zhao *et al.,* 2022). These lipids change the membrane's curvature and make it more permeable, therefore the receptors will cluster more

and signal better. Elevated LPC/PC and LPE/PE ratios in our group reveal an overactivation of phospholipase A2 which provides one mechanism for the induction of pro-inflammatory lipid mediators thereby leading to endothelial dysfunction (Dai *et al.,* 2023). The lowering of the content of the phospholipids with a high proportion of PUFAs, in particular arachidonoyl (20:4) and docosahexaenoyl (22:6) species, further supports the hypothesis that substrate depletion and defective reacylation is a basis for membrane rigidity and insulin resistance of MetS (Lin *et al*., 2024).

Our data also indicate significant elevations of membrane saturation indices, in line with previous data that high saturated lipid content impairs the mobility of the insulin receptor and its trafficking of GLUT4 (Holmstrom *et al*., 2021). One can see the turn from polyunsaturated to saturated species decreases the fluidity of the membranes, disrupts signaling microdomains ("lipid rafts"), which leads to the failure of glucose circulation and mitochondrial respiration (Liu *et al.,* 2023). These observations reinforce the idea that remodeling of the phospholipids is not a secondary as a primary mechanism of metabolic dysfunction.

Functional assays showed increased metabolic activity of PLA2 and decreased LPCAT and ACSL4 metabolic activity suggesting an imbalance of the Lands cycle. This pattern is conducive to a model of hyperconcentration of deacylation with a disturbed reacylation capacity. Increased PLA2 activity has previously been linked to accumulation of circulating lysophosyplipids and inflammatory markers in obesity (Jeong *et al*., 2022). On the contrary, low expression of LPCAT3 has been associated with low expression incorporation of long-chain PUFAs in phospholipids and mitochondrial stress (Jeong *et al*., 2022). The observed suppression of ACSL4, a play a crucial role in acyl CoA synthase facilitating the re-esterification of the fatty acid in the rink tissue. (PUFAs) which promotes dysfunction and storage due to their profound ability for re-acylation by reduction of available formation in the accumulation (Kim *et al*., 2023).

The interplay of the combined enzymatic and lipidomic attributors point to a remodeling "bottleneck" in which the action of one enzyme class (phosphatases) is burning unhindered while another (reacylation enzymes) is lagging behind. The imbalance encourages the accumulation of bioactive lysophospholipid molecules like LPC that act as messenger of signaling molecules activating G2A receptors and GPR132 receptors, a process that perpetuates inflammation and oxidation stress (Sheng *et al*., 2024). Our findings therefore define a specific enzymatic signature of the lipidomic phenotype of MetS, which bridges between the biochemistry in the cell to a systemic pathology.

Correlation and regression analyses showed how both LPC/PC ratio and PLA2 activity were strongly correlated to HOMA-IR,thus showing that remodeling dynamics predict metabolic dysfunction independent from BMI or lipid profile. This supports the recent report describing how the remodeling of phospholipids has effects on the insulin signaling system via biomembrane localization and tension changes. Furthermore, reduced ACSL4 activity was negatively correlated with hs-CRP levels, who examines a link between non-incorporation of PUFAs and low-level inflammation. The loss of anti-inflammatory omega-3 and omega-6 PUFAs from membranes phospholipids causes less formation of specialized pro-resolving mediators, which further continues the chronic inflammatory signaling (Tian *et al*., 2022).

Our results also support the utility of the saturation index as a biological marker for the assessment of biological events. Increased saturation was positively associated with inflammatory and lipid parameters, in line with findings that saturated lipid environments are pro-inflammatory by activating the toll-like receptor 4 (TLR4) and pro-macrophage.Increased saturation was positively associated with inflammatory and lipid parameters, consistent with a pro-inflammatory role for saturated lipid environments involving the activation of the toll-like receptor 4 (TLR4) and polarization of macrophages. As a whole, these correlations make phospholipid remodeling indices good candidates for being used as functional biomarkers of metabolic stress.

Previous research reported by lipidomic analysis in MetS focused essentially on plasma triglycerides and sphingolipids. Our study ignores level of detail goes to membrane phospholipids; it provides a mechanistic understanding at the cellular level. Similar tendencies were presented by Lu et al. (2023) who described high LPC levels and low PUFA-PC in the hepatic tissue of MetS models. However, our discrimination analysis on the dual layer (plasma and PBMC membranes) offer a systemic and cellular perspective at the same time. This integrative mapping is capable to altogether better capture dynamic remodeling and its enzymatic regulation.

Moreover, our data are consistent with the concept of the lipid remodeling - mitochondrial dysfunction axis whereby impaired reacylation is one way by which the mitochondrial membrane integrity as well as ATP production are affected (Yamamoto *et al*., 2023). These cross-tissue parallels support the translational relevance of our findings and the potential usefulness of lipidomic biomarkers to identify high-risk MetS subtypes.

The identification of the enzymatic remodeling signature represents possible new therapist targets. The impressive lipid homeostasis and membrane fluidity can be restored by modulating the expression of phospholipase (PLA2

inhibitors) and/or by activating lipid reacylation enzymes such as LPCAT3 and ACSL4 (Ouyang *et al*., 2023). Nutritional interventions that supply purified preparation of fatty acid precursors ranging from 'PUFA' precursor such as Eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA) may replenish acid depleted acyl pools and minimizes remodeling defects (Chen *et al*.,2024). Applying lipidomic indices to clinical evaluation may also lead to the early identification of existence of metabolic derangements prior to overt insulin resistance.

The present study, despite its comprehensiveness, has a number of limitations. Cross-sectional study This study design limits causal inference and sample size may prevent inclusion of subtle differences in lipid species. Future research should include sampling over time to capture remodeling dynamics over time as well as targeted facets of fluxomics to measure acyl exchange rates. Integration with transcriptomics and proteomics could be used further verify the regulation of enzymes at the gene and protein level. In addition, interventional trials involving modulation of diet and or pharmacologic targets of the Lands' cycle may help elucidate if lipid remodeling is potentially reversible at the onset of metabolic disease.

5. Conclusion

This study shows that the presence of metabolic syndrome is linked to a consistent membrane remodeling phenotype with (i) accumulation of lysophospholipids, (ii) depletion of phospholipid species with high polyunsaturated acid content, and (iii) increased membrane saturation. Enzymatic assays are in accordance with an underlying Lands Dragon cycle unbalance, and is increased PLA2 activity but with decreased reacylation capacity (LCPAT, ACSL4 and MBOAT7). Importantly, remodeling indices especially the LPC/PC ratio and measures of saturation correlated significantly with insulin resistance and inflammatory burden and were significant predictors in adjusted models as well. These results suggest that phospholipid remodeling is a mechanistically-relevant aspect of the pathophysiology of MetS rather than a passive extension and marker of the disease. Future work is warranted to validate these signatures in larger cohorts, take the longitudinal sampling and have arms with interventions and to evaluate whether restoring remodeling enzymes or the incorporation of PUFA can improve cellular function and metabolic outcome.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest. None of the authors have a financial or personal relationship with others that may influence the results or interpretation of this research.

Statement of ethical approval

This clinical study was reviewed and approved by the University of Babylon Institutional Review Board (IRB) in the College of Medicine (Approval No. BB-2025-211). All subjects were made aware of the objectives, procedures, and possible risks of the study before they were enrolled. Written informed consent was obtained from each subject prior to sample collection and the personal data were strictly kept confidential. The research was conducted in accordance with the ethical standards presented in the Declaration of Helsinki (2013 revision) and local research regulations of human subjects.

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Final Statement

This research provides a mechanistic link between enzymes of phospholipid remodeling and dysfunction of cellular metabolism, the metabolic syndrome. The combination of lipidomics with profiling of the enzymological status

determines a reproducible biochemical profile which can potentially be used in biomarker discovery and intervention strategies to restore membrane homeostasis.

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