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EXPLORING THE IMPACT OF NANOMEMBRANE–BASED LOW VOLUME PLASMA EXCHANGE ON GUT BARRIER INTEGRITY IN METABOLIC SYNDROME: A PROSPECTIVE STUDY

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SUMMARY

A comprehensive understanding of the human intestine and its structural-functional unit, the "gut barrier," implies an intricate cross-talk between epithelial cells and the underlying immune system to coordinate the surveillance of intestinal luminal contents. Disruption of this barrier leads to an increased passage of antigens and macromolecules from the external environment into the host, triggering local or systemic inflammation and immune activation, commonly referred to as "leaky gut." In recent times, increased intestinal permeability has been recognized as a key factor or pivotal pathogenic component in chronic inflammatory diseases, including metabolic disorders. Metabolic syndrome (MetSy) encompasses a cluster of metabolic disorders associated with an elevated risk of cardiovascular diseases, despite lifestyle modifications and medications. Zonulin, among the non-invasive markers of intestinal permeability, stands out due to its sensitivity. Nanomembrane-based low-volume plasma exchange (LVPE) is an innovative approach to blood purification designed to remove toxic and inflammatory blood components. This safe and minimally invasive procedure involves a device that pumps and filters the patient's blood through nanopores in a multi-membrane layout. ***Objective.*** *This study aims to investigate the impact of nanomembrane-based LVPE on the intestinal barrier in individuals with MetSy, elucidating its potential therapeutic role in chronic inflammatory diseases.* ***Materials and methods.*** *In this prospective study, 48 outpatient participants (31.3% female, 68.7% male) with an average age of 50 years underwent four cycles of nanomembrane-based LVPE, conducted every other day. Each cycle involved the removal of 30% of circulating plasma, replaced with a saline solution. Serum samples were collected before the first and after the fourth LVPE cycle, measuring markers including Zonulin, C-reactive protein (CRP), high-sensitive CRP, Interleukin-6 (IL6), vitamin D3, and cardiometabolic parameters. Additionally, these markers were measured in plasma samples obtained after each LVPE cycle.* ***Results.*** *After four cycles of LVPE, there was a significant decrease in the concentrations of vitamin D3 ($p<0.001$), CRP ($p<0.02$), glucose ($p<0.0001$), total cholesterol ($p<0.0001$), triglycerides ($p<0.011$), and HDL-C ($p<0.006$). Before the first cycle, Zonulin was significantly associated with HDL-C ($\beta=1.406$; $p=0.002$), LDL-C ($\beta=-1.263$; $p=0.012$), and hsCRP ($\beta=0.302$; $p=0.046$). After the fourth cycle, significant associations were obtained for HbA1c ($\beta=0.342$; $p=0.025$) and total cholesterol ($\beta=0.570$; $p=0.001$).* ***Conclusion.*** *Our study advocates for the use of nanomembrane-based LVPE as a targeted method to enhance gut barrier permeability in individuals with MetSy. Through four LVPE cycles, our research validates the efficacy of this approach in correcting carbohydrate and lipid metabolism. Notably, our investigation reveals LVPE's potential immunomodulatory effect on inflammatory pathways.*

KEYWORDS: *gut barrier, metabolic syndrome, Zonulin, low-volume plasma exchange.*

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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ИЗУЧЕНИЕ ВЛИЯНИЯ НИЗКООБЪЕМНОГО ПЛАЗМООБМЕНА НА ОСНОВЕ НАНОМЕМБРАН НА ЦЕЛОСТНОСТЬ КИШЕЧНОГО БАРЬЕРА ПРИ МЕТАБОЛИЧЕСКОМ СИНДРОМЕ: ПРОСПЕКТИВНОЕ ИССЛЕДОВАНИЕ

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РЕЗЮМЕ

Всестороннее понимание работы кишечника человека и его структурно-функциональной единицы, «кишечного барьера», подразумевает сложную взаимосвязь между эпителиальными клетками и лежащей в их основе иммунной системой для координации контроля содержимого просвета кишечника. Нарушение этого барьера приводит к усиленному проникновению антигенов и макромолекул из внешней среды в организм хозяина, вызывая местное или системное воспаление и активацию иммунитета, обычно назы-

ваемое «дырявым кишечником». В последнее время повышенная проницаемость кишечника была признана ключевым фактором или основным патогенетическим компонентом хронических воспалительных заболеваний, включая нарушения обмена веществ. Метаболический синдром (MetSy) включает в себя группу метаболических нарушений, связанных с повышенным риском сердечно-сосудистых заболеваний, несмотря на изменение образа жизни и медикаментозное лечение. Зонулин, один из неинвазивных маркеров проницаемости кишечника, выделяется своей чувствительностью. Малообъемный плазмообмен на основе наномембран (МОПН) – это инновационный подход к очистке крови, предназначенный для удаления токсичных и воспалительных компонентов крови. Эта безопасная и малоинвазивная процедура включает в себя устройство, которое прокачивает и фильтрует кровь пациента через нанопоры в виде мультимембраны. ***Цель.*** *Это исследование направлено на изучение влияния LVPE на основе наномембран на кишечный барьер у людей с MetSy, выяснение его потенциальной терапевтической роли при хронических воспалительных заболеваниях.* ***Материалы и методы.*** *В этом проспективном исследовании 48 амбулаторных участников (31,3% женщин, 68,7% мужчин), средний возраст которых составлял 50 лет, прошли четыре цикла МОПН на основе наномембран, которые проводились через день. Каждый цикл включал удаление 30% циркулирующей плазмы, которая заменялась физиологическим раствором. Образцы сыворотки были взяты до первого и после четвертого цикла МОПН, для определения таких маркеров, как зонулин, С-реактивный белок (СРБ), высокочувствительный СРБ, интерлейкин-6 (IL6), витамин D3 и кардиометаболические параметры. Кроме того, эти маркеры были измерены в образцах плазмы, полученных после каждого цикла МОПН.* ***Результаты.*** *После четырех циклов МОПН наблюдалось значительное снижение концентраций витамина D3 (p < 0,001), СРБ (p < 0,02), глюкозы (p < 0,0001), общего холестерина (p < 0,0001), триглицеридов (p < 0,011) и хс-ЛПВП (p<0,011).0,006). Перед первым циклом зонулин был достоверно связан с уровнем Хс-ЛПВП (β = 1,406; p=0,002), ХС-ЛПНП (β = -1,263; p=0,012) и hsCRP (β=0,302; p=0,046). После четвертого цикла были получены значимые ассоциации для уровня HbA1c (β=0,342; p=0,025) и общего холестерина (β=0,570; p=0,001).* ***Выводы.*** *В нашем исследовании мы выступаем за использование МОПН на основе наномембран в качестве целенаправленного метода повышения проницаемости кишечного барьера у людей с MetSy. Проведя четыре цикла МОПН, мы подтвердили эффективность этого подхода в коррекции углеводного и липидного обмена. Примечательно, что наше исследование выявило потенциальный иммуномодулирующий эффект МОПН на воспалительные процессы.*

КЛЮЧЕВЫЕ СЛОВА: кишечный барьер, метаболический синдром, зонулин, малообъемный плазмообмен.

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Introduction

Twenty-five hundred years ago, Hippocrates stated that "All diseases begin in the gut" [1]. Today, we recognize the human intestine as the largest and most exposed biological interface, spanning an area of 200 m², serving not only for nutrient absorption and fluid exchange but also playing a crucial role in allowing various environmental antigens from the intestinal lumen into the body [2, 3]. This structural-functional unit, known as the "Gut Barrier" (GB), encompasses the epithelial/intestinal mucosal barrier, the gut microbiota, the intestinal mucus layer, and the complex immune system associated with the gut mucosa, including the intestinal vascular/lymphatic system and the intestinal endocrine and neuroenteric system [4]. The intricate arrangement of the GB suggests an intimate cross talk between epithelial cells and the underlying immune system for coordinated surveillance of intestinal luminal contents [5]. Disruption of the GB results in increased passage of antigens and macromolecules from the external environment into the host, triggering local or systemic inflammation and immune activation, known as "leaky gut," significantly affecting tolerance and immunity [6].

The transport mechanism through the epithelial/intestinal barrier (EB) involves two pathways: the transcellular and the paracellular pathways. Roughly, 90% of antigens use the selective transcellular pathway, involving lysosomal degradation into small, non-immunogenic peptides. The remaining 10% cross the EB through the less selective paracellular pathway as intact proteins or partially digested peptides, crucial for antigenic tolerance [7, 3]. The EB comprises a single layer of specialized epithelial cells called enterocytes. These cells' junctions are regulated by adherens junctions (AJs) and tight junctions (TJs), forming a physical barrier impermeable to bacteria or other substances [8]. TJs, the main apical junctional multiprotein complex, consist of over 150 proteins, including occludin, claudins, junctional adhesion molecules, and tricellulin connecting neighboring epithelial and endothelial cells [9, 10, 11, 12]. These transmembrane proteins' intracellular domains interact with cytosolic scaffold proteins like zonula occludens (ZO) proteins, anchoring them to the actin cytoskeleton [13]. The interplay between TJs and the actin cytoskeleton is fundamental in maintaining TJ structure, regulating the paracellular pathway, and maintaining barrier homeostasis, crucial for both transcellular and paracellular transport [5, 14, 15]. The discovery of Zonulin, an analog to Vibrio cholerae zonula occludens toxin, is considered the only known physiological intestinal modulator, affecting small intestinal permeability through reversible actin polymerization [16, 17]. Zon, a 47 kDa protein in the haptoglobin family, serves as a precursor of haptoglobin-2 (HP2), involved in various protective and modulatory activities [18, 19]. Recent studies propose increased intestinal permeability as a key factor in chronic inflammatory diseases (CID), including metabolic diseases [20, 15].

Impaired GB is now considered a pivotal pathogenic component in several CIDs [21, 22]. MetSy encompasses a cluster of metabolic disorders associated with an increased risk of cardiovascular diseases, despite lifestyle modifications and medications [23, 24].

Innovative methods like nanomembrane-based therapeutic plasma exchange, known as low volume plasma exchange (LVPE), offer a non-selective approach to blood purification by removing toxic and inflammatory blood components through nanopores [25, 26]. This minimally invasive procedure is utilized in treating over 75 diseases and syndromes, including MetSy [27], replacing plasma with solutions like albumin, saline, or artificial plasma [28]. Nanomembrane-based LVPE efficiently removes small molecules like cytokines and toxins while preserving essential blood components [25, 26]. Despite its promising role in CID management, its effects on GB and EB remain unexplored.

This study aims to investigate the impact of nanomembrane-based LVPE on the intestinal barrier in MetSy individuals, elucidating its potential therapeutic role in CID.

Materials and Methods

Participants:

A prospective study enrolled 48 participants who met the criteria for MetSy. Inclusion criteria required the presence of three out of the five risk factors defined by the National Cholesterol Education Programs Adult Treatment Panel III (NCEP: ATP III) [29]. Exclusion criteria included recent acute infection, injury, surgical treatment, individuals under 18 years old, and pregnant women. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The study received approval from the Ethical Committee of the Faculty of Medicine, University of Montenegro (No. 778/3/2020).

Protocol of LVPE

The LVPE protocol consisted of four cycles of LVPE performed every other day using the Hemofenix device (Trackpore Technology Company, Russia). Approved by the American Society for Apheresis (ASFA) in 2013 [30], the minimally invasive and safe procedure employed the following specifications:

- Utilization of a one-needle procedure with small catheters in the peripheral vein in the arm.
- Application of a small volume (65–70 ml) for filling the extracorporeal contour to maintain cardiovascular system stability and circulating blood volume.
- Use of a nanotech membrane (PFM 500 filter; ZAO Plasmafilter, Russia), requiring only 15–20 ml of blood.
- Incorporation of a pump for extracorporeal circulation functioning on the systole-diastole principle.
- Infusion of sodium citrate (ACD-A, Fresenius Kabi, Germany) as an anticoagulant for the extracorporeal circulation.
- Removal of 30% of circulating plasma or 1% of body weight, replaced with a usual isotonic sodium chloride solution via constant infusion for four cycles, removing up to 1.5 times the circulating plasma volume.

Clinical Assessments

Before the initiation of the first cycle of LVPE, a comprehensive set of clinical assessments were conducted to gather baseline data on the participants.

- *Anthropometric Measurements*

Participants were instructed to wear light clothes and be barefoot during anthropometric measurements, which included assessments of body height, weight, body mass index (BMI), waist circumference (Wc), and hip circumference (Hc). The measurements were performed using the Vaga Seka SE 711 equipment from Germany, ensuring accuracy and standardization.

- *Medical Examination*

A qualified Endocrinologist conducted a thorough medical examination of each participant. This examination aimed to assess overall health, identify any pre-existing conditions, and ensure participants were fit for the LVPE procedure. Before each cycle of LVPE, blood pressure (BP) and heart rate (HR) were measured to monitor cardiovascular parameters. An automatic digital blood pressure monitor (M6 Comfort, Omron Healthcare Co, Japan) was used for accurate and efficient readings. This allowed for the continuous evaluation of cardiovascular stability throughout the LVPE cycles.

Hematological and Biochemical Measurements

Hematological parameters were evaluated with a complete blood count (CBC) analyzer (Celltak α, Nihon-Kohden, Japan). Sedimentation rate (SE) was measured both before the first cycle and after the fourth cycle.

Serum concentrations of various biomarkers were determined before the first cycle and after the fourth cycle using an automatic biochemistry analyzer (A15, Biosystems, Spain) for glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), high-sensitive CRP (hsCRP), glycosylated hemoglobin (HbA1c), Interleukin-6 (IL-6) (Cobas e 801, Switzerland), and vitamin D3 (Vit D3) (Alinity, Abbott, USA). Plasma samples, totaling four (one after each cycle of LVPE), were analyzed for the same set of biochemical parameters.

Biomarker of Intestinal Permeability

Zonulin concentrations were assessed using the Human Zonulin ELISA kit (Immunodiagnostik AG, Germany) and enzyme-linked immunosorbent assay techniques (Rayto 2100-C, ELISA reader, China). The kit has a sensitivity limit of 34 ng/ml and a detection range of 3.03–40.25 ng/ml. Measurements were conducted in serum samples obtained immediately before the first cycle and after the fourth cycle. Additionally, Zonulin concentrations were measured in plasma samples collected after each LVPE cycle, totaling four plasma samples.

Statistical analyses were conducted using IBM SPSS Statistics, version 26. Descriptive statistics were employed for quantitative variables with a normal distribution, reporting mean values and standard deviations. Frequencies were calculated for qualitative variables. Student's t-tests were utilized to compare paired samples of quantitative variables. One-way ANOVA was employed to compare means across one or more variables based on repeated observations. Mul-

tiple linear regression models were used to assess the interaction between applied LVPE and analyzed biomarkers. Interactional models were applied to evaluate the relationship between Zonulin concentration and various parameters. The statistical significance threshold was set at $p < 0.05$.

Results

Participants and Anthropometric Characteristics

The study involved 48 participants diagnosed with MetSy, comprising 68.7% men and 31.3% women, with an average age of 50 years. The research included the calculation of blood and plasma volumes (BV; PV) for the subjects, considering that 7% of body mass constitutes blood volume (Nadler 1962). Anthropometric characteristics were analyzed to compare physical attributes and blood volumes between male and female participants (Table 1).

Table 1
Anthropometric Characteristics of Participants with MetSy by sex (mean±SD)

Variables	Total (n=48)	Men (n=33)	Women (n=15)	p Value
Age (years)	50.38±9.41	51.27±9.87	48.40±8.27	0.332
Height (cm)	91.55±16.82	98.85±12.31	75.48±14.16	<0.001
Weight (kg)	182.38±8.96	186.55±5.96	173.20±7.57	<0.001
BMI (kg/m^2)	27.44±4.04	28.43±3.30	25.26±4.74	<0.010
Wc (cm)	99.13±13.28	104.39±10.86	87.53±10.62	<0.001
Hc (cm)	105.42±10.09	106.30±10.19	103.47±9.94	0.373
BV (ml)	6409.17±1177.30	6920.79±861.44	5283.60±991.22	<0.001
PV (ml)	2884.44±528.15	3113.64±387.88	2380.20±443.08	<0.001

BMI – body mass index, Wc – waist circuference, Hc – hip circuference, BV – blood volume, PV – plasma volume.

Men demonstrated significant differences in physical attributes compared to women, indicating distinctive physical disparities between genders in the MetSy group.

LVPE Cycle Parameters

The LVPE cycle protocol assessed its impact on cardiovascular and circulatory measures, monitoring various parameters (Table 2).

Table 2
Protocol Parameters and Hemodynamic Measures During LVPE Cycles (mean±SD)

Variables	I cycle	II cycle	III cycle	IV cycle	p Values
SBP (mmHg)	131.60±19.60	126.90±16.94	125.52±15.53	122.69±15.65	<0.0001
DBP (mmHg)	86.65±10.34	82.19±9.96	81.08±10.10	80.81±10.75	<0.0001
HR (/min)	79.04±12.07	77.65±11.96	77.52±11.43	78.15±12.32	0.816
CL (min)	76.23±27.90	70.38±24.14	76.48±25.04	75.23±31.97	0.179
BV (ml)	3724.17±1216.56	3873.13±1282.59	3716.46±1056.12	3800.83±1174.50	0.673
PV (ml)	897.71±166.26	932.29±174.43	916.46±169.02	919.79±179.53	0.246
ACD-A	228.33±86.23	306.46±100.35	251.25±73.62	263.54±91.89	<0.0001
0.9%NaCl (ml)	903.13±168.51	912.92±184.53	902.71±183.21	869.38±199.93	0.341

SBP – systolic blood pressure, DBP – diastolic blood pressure, CL – cycle lenght, BV – blood volume, PV – plasma volume, ACD – Anticoagulant Citrate Dextrose Solution A, 0.9%NaCl – saline solution.

The LVPE cycles resulted in a significant reduction in both SBP ($F = 6.648$; $p < 0.0001$) and DBP ($F = 8.599$; $p < 0.0001$), indicating effective blood pressure regulation. However, HR, processed BV, and removed PV did not show significant changes. The consumption of ACD-A increased significantly across cycles ($F = 8.376$; $p < 0.0001$), with no significant change in the consumption of the saline solution.

Hematological and Biochemical Measurements

Hematological Measurements

The study assessed the impact of four LVPE cycles on hematological parameters, revealing significant changes in specific measurements (Table 3).

Table 3
Hematological Parameters: CBC and SE before I and after IV LVPE cycle (mean±SD)

Variables	Before I cycle	After IV cycle	p Values
SE	7.81±5.87	4.69±3.28	<0.0001
Leucocytes	7.23±1.64	7.45±1.89	0.256
Erythrocytes	4.75±0.44	4.76±0.51	0.863
Hemoglobin	142.77±16.29	142.75±17.52	0.985
Hematocrit	42.56±4.56	42.57±5.12	0.326
MCV	89.52±4.52	89.48±4.64	0.622
MCH	30.02±1.79	30.01±1.83	0.877
MCHC	335.19±6.29	334.52±6.90	0.501
Platelates	232.02±53.08	217.92±53.50	<0.002
Lymphocytes	36.47±8.77	38.87±9.45	0.077
Monocytes	3.59±1.16	3.29±1.52	0.223
Granulocytes	59.90±9.29	57.83±10.30	0.145
RDW	14.66±0.81	14.55±0.87	0.215
PCT	0.17±0.04	0.17±0.04	0.375
MPV	7.55±0.70	7.80±0.75	<0.001
PDW	17.11±0.71	17.02±0.84	0.498

SE – sedimentation rate, MCV – Mean corpuscular volume, MCH – Mean corpuscular hemoglobin, MCHC – Mean corpuscular hemoglobin concentration, RDW – Red cell distribution width, PCT – plateletcrit, MPV – Mean platelate volume, PDW – Platelate cell distribution width

Following the four LVPE cycles, significant decreases were observed in SE ($t = 5.678$; $p < 0.0001$) and platelet count ($t = 3.312$; $p < 0.002$), along with a significant increase in MPV ($t = 3.700$; $p < 0.001$). Other complete blood count parameters remained unchanged.

Cardiometabolic Biomarkers in Serum Samples

The study explored the impact of LVPE cycles on cardiometabolic biomarkers in serum samples (Table 4).

Table 4
Cardiometabolic Markers in Serum Samples before I and after IV cycle (mean±SD)

Variables	Before I cycle	After IV cycle	p Values
Glucose (mg/L)	5.58±1.64	4.79±1.08	<0.0001
Cholesterol (mmol/L)	5.53±1.36	4.84±0.99	<0.0001
Triglycerides (mmol/L)	1.94±1.48	1.34±1.35	<0.011
HDL-C (mmol/L)	1.40±0.38	1.28±0.35	<0.006
LDL-C (mmol/L)	3.09±0.95	2.96±0.89	0.260
CHD index	3.98±1.13	3.92±1.28	0.265
AI index	2.28±0.80	2.42±0.98	0.342
HbA1c (%)	5.67±0.81	5.70±0.78	0.729

HDL-C – high-density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, CHD – index for coronary heart diseases, AI – atherosclerosis index, HbA1c – glycolisate hemoglobin.

Significant decreases were recorded in glucose ($t = 3.899$; $p < 0.0001$), total cholesterol ($t = 4.746$; $p < 0.0001$), triglycerides ($t = 2.660$; $p < 0.011$), and HDL-C ($t = 2.899$; $p < 0.006$). However, there were no significant changes in LDL-C or HbA1c. Calculated indices for CHD and AI did not exhibit significant alteration.

Cardiometabolic biomarkers in removed plasma

The study also investigated cardiometabolic biomarkers in removed plasma after each cycle of LVPE (Table 5).

Table 5

Cardiometabolic biomarkers in removed plasma after each cycle (mean±SD)

Variables	I cycle	II cycle	III cycle	IV cycle	p Values
Glucose (mg/L)	27.89±4.99	28.21±5.57	27.43±5.18	27.95±5.56	0.802
Cholesterol (mmol/L)	2.01±0.95	1.92±0.96	1.99±0.91	1.88±0.76	0.806
Tryglicerides (mmol/L)	0.72±0.81	0.72±0.63	0.70±0.63	0.66±0.66	0.780
HDL-C (mmol/L)	0.51±0.30	0.49±0.31	0.49±0.35	0.45±0.25	0.672
LDL-C (mmol/L)	1.15±0.69	1.07±0.73	1.10±0.68	1.11±0.60	0.904

HDL-C – high-density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol

No significant differences were observed in glucose, cholesterol, triglycerides, HDL-C, and LDL-C in the removed plasma samples after each individual LVPE cycle.

The regression model indicated overall significance for the analyzed biomarkers in both serum and removed plasma samples, with varying degrees of correlation (R-squared values) for each biomarker: glucose ($F = 4.639$; $p < 0.002$, $R^2 = 0.361$), cholesterol ($F = 7.277$; $p < 0.001$, $R^2 = 0.464$), triglycerides ($F = 41.050$; $p < 0.001$, $R^2 = 0.830$), HDL-C ($F = 13.742$; $p < 0.001$, $R^2 = 0.644$), and LDL-C (F 6.794; $p < 0.001$, $R^2 = 0.485$).

Parameters of intestinal permeability, inflammation, and low-grade inflammation

Table 6

Serum concentrations of Zonulin, IL-6, Vitamin D3, CRP, and hsCRP before I and after IV LVPE cycle (mean±SD)

Variables	Before I cycle	After IV cycle	p Values
Zonulin (ng/ml)	16.37±10.94	16.62±8.41	0.800
IL-6 (pg/ml)	3.16±3.03	3.06±3.90	0.801
VitD3 (nmol/L)	89.59±49.85	75.98±52.46	<0.001
CRP (mg/L)	5.04±3.04	3.54±3.44	<0.02
hsCRP (mg/L)	2.31±2.33	1.78±1.70	0.060

IL-6 – interleukin 6, VitD3 – vitamin D3, CRP – C-reactive protein, hsCRP – high sensitive CRP

Significant decreases were observed in the serum concentrations of Vitamin D3 ($t = 5.633$; $p < 0.001$) and CRP ($t = 2.409$; $p < 0.02$) after the fourth LVPE cycle. Other parameters did not exhibit significant alterations (Table 7).

Table 7

Concentrations of Zonulin, IL-6, Vitamin D3, CRP, and hsCRP in removed plasma samples after each cycle of LVPE (mean±SD)

Variables	I cycle	II cycle	III cycle	IV cycle	p Values
Zonulin (ng/ml)	9.67±5.65	9.37±5.46	10.43±6.32	9.86±5.78	0.656
IL-6 (pg/ml)	1.80±0.94	1.98±1.15	2.09±1.46	2.04±1.22	0.119
VitD3 (nmol/L)	30.56±17.62	28.16±14.20	29.76±13.45	29.21±16.46	0.685
CRP (mg/L)	3.78±6.01	2.66±2.32	2.76±2.73	3.29±4.04	0.321
hsCRP (mg/L)	0.86±0.78	0.84±0.89	0.76±0.67	0.97±0.88	0.423

IL-6 – interleukin 6, VitD3 – vitamin D3, CRP – C-reactive protein, hsCRP – high sensitive CRP.

Overall linear regression models highlighted the significance of these parameters, indicating varying degrees of correlation for each biomarker: Zonulin ($F = 23.421$; $p < 0.001$, $R^2 = 0.741$), Vitamin D3 ($F = 90.227$; $p < 0.001$, $R^2 = 0.917$), IL-6 ($F = 11.598$; $p < 0.001$, $R^2 = 0.580$), CRP ($F = 9.639$; $p < 0.001$, $R^2 = 0.534$), and hsCRP ($F = 23.937$; $p < 0.001$, $R^2 = 0.740$).

Summary of the interaction models with β-coefficient and 95% confidence interval

Association Between Zonulin Concentration and Anthropometric Measurements

Before the initial LVPE cycle, a significant association was observed between Zonulin concentration and sex ($\beta = 0.790$; $p = 0.023$). No significant associations were found with other anthropometric parameters.

Association between Zonulin concentration and cardiometabolic biomarkers

Before the first LVPE cycle, significant associations were found between Zonulin concentration and HDL-C ($\beta = 1.406$; $p = 0.002$), LDL-C ($\beta = -1.263$; $p = 0.012$), and IA ($\beta = 1.648$; $p = 0.047$). After the fourth LVPE cycle, significant associations were identified between Zonulin concentration and HbA1c ($\beta = 0.342$; $p = 0.025$), total cholesterol ($\beta = 0.570$; $p = 0.001$), and CHD ($\beta = 0.803$; $p = 0.039$).

Association between Zonulin concentration and markers of inflammation and low-grade inflammation

Before the first LVPE cycle, Zonulin concentration showed a significant association with hsCRP ($\beta = 0.302$; $p = 0.046$). However, after completing the LVPE protocol, no significant associations were observed with markers of inflammation and low-grade inflammation.

Discussion

The current study investigates the potential of nanomembrane-based LVPE as an innovative method to improve gut barrier permeability in individuals affected by MetSy, a global public health concern. The prevalence of MetSy ranges from 13 to 36% in the European population [31] and one-fourth to one-fifth in the Mediterranean region [32].

Significant differences in anthropometric measures were noted, highlighting distinct physical variations between male and female participants diagnosed with MetSy. These observed differences align with established literature on sexual dimorphism and physical disparities [33, 34].

Nanomembrane-based LVPE introduces an innovative method for plasma filtration and purification, leveraging nanotechnology and specially engineered microscopic Lavsan film membranes. These membranes, created through argon particle irradiation in a collider [35, 36, 37], feature pores ranging from 30 to 50 nanometers in diameter. They demonstrate the capability to filter molecules weighing less than 40 kilodaltons and can accommodate a filling volume of up to 70 milliliters [38, 39].

The LVPE process involves the exchange of components within the plasma, utilizing a minimal volume of replacement fluid. In our study, we embraced a low-volume approach, extracting up to 30% of the plasma and substituting it exclusively with a saline solution. This method ensures the preservation of plasma quality while effectively achieving our purification objectives. We conducted four cycles of LVPE every other day, removing up to 1–1.5 times the volume of circulating plasma [40].

According to our findings, four cycles of LVPE successfully regulated BP, leading to a significant reduction in both SBP and DBP. Parameters such as HR, CL, processed BV, and removed PV did not show significant changes. The increased consumption of ACD-A highlights its importance in stabilizing blood during the procedure, while saline solution consumption remained consistent. These results provide valuable insights into the physiological effects of LVPE cycles.

These filters, owing to nanotechnology, effectively preserve blood cells through the size of the pores and their rounded edges [35, 27, 41]. Nevertheless, observed changes in hematological parameters in our study, including SE, platelet count, and MPV, suggest that LVPE may influence inflammatory and coagulation pathways.

Recent studies propose that LVPE can positively influence carbohydrate and lipid metabolism in patients with diabetes mellitus (DM) by reducing levels of cholesterol, triglycerides, fibrinogen, as well as LDL-C [42]. In our study, LVPE demonstrated a favorable impact on several key cardiometabolic markers, specifically leading to reduced levels of glucose, total cholesterol, triglycerides, and HDL-C. The stability observed in LDL-C and HbA1c suggests that these parameters may not be significantly affected by LVPE treatment. Furthermore, there seem to be no significant differences in the levels of cardiometabolic biomarkers (glucose, cholesterol, triglycerides, HDL-C, and LDL-C) in the removed plasma samples after each individual LVPE cycle. The absence of significant differences in individual cycles implies a consistent response of cardiometabolic biomarkers to LVPE. The overall significance in the regression model indicates that the LVPE procedure has a measurable impact on these biomarkers collectively. The varying degrees of correlation among biomarkers suggest that each biomarker responds differently to LVPE, with triglycerides showing the highest correlation. Additionally, the stability of glucose, cholesterol, triglycerides, HDL-C, and LDL-C levels in removed plasma after LVPE cycles may indicate the procedure's safety in terms of maintaining these cardiometabolic parameters.

The study aimed to evaluate parameters related to intestinal permeability, inflammation, and low-grade inflammation before the first and after the fourth cycle of LVPE. Serum concentrations of Zonulin, IL-6, Vitamin D3, CRP, and hsCRP were examined to understand the impact of LVPE on these biomarkers. According to the literature, circulating IL-6 has been suggested to act as a promoter of the Zonulin gene, increasing its circulating levels [43]. Although no significant alterations were observed in Zonulin and IL-6 serum concentrations before and after LVPE cycles, these results suggest the stability of these markers in response to the cycles. MetSy has been reported to be associated with inflammatory status, with subjects showing high concentrations of hsCRP [44]. Additionally, CRP, produced in response to IL-6, can induce chronic inflammation at higher levels [45]. Onthe other hand, Vitamin D3 has a wide range of impacts, including influencing gene expression, modulating immune response, inflammation, oxidative stress, and the gut microbiota signature [46]. A significant decrease was noted in serum Vitamin D3 levels, accompanied by a significant reduction in CRP after the fourth LVPE cycle, as well as a decreasing trend in hsCRP. The decline in Vitamin D3 may implicate altered immune responses, while the reduction in CRP and hsCRP indicates an anti-inflammatory effect post-LVPE. Furthermore, the decrease in Vitamin D3, CRP, and hsCRP levels post-LVPE suggests potential immunomodulatory effects. Overall linear regression models emphasized the significance of the parameters, revealing varying degrees of correlation. Strong correlations were found for Zonulin, Vitamin D3, IL-6, CRP, and hsCRP, indicating interrelated dynamics among these biomarkers.

In our study, we aim to explore the relationship between Zonulin concentration and anthropometric measurements, cardiometabolic biomarkers, and markers of inflammation. Existing literature suggests that higher Zonulin levels are linked to increased waist circumference (Wc), diastolic blood pressure (DBP), fasting glucose levels, and an elevated risk of overweight, obesity, and hyperlipidemia [47]. This implies that serum Zonulin levels may be more dependent on metabolic conditions. Our results also highlight the role of Zonulin in metabolic and cardiovascular health, as evidenced by its significant association with cardiometabolic biomarkers both before the first and after the fourth cycle of LVPE. Moreover, the noteworthy association with hsCRP before the first cycle of LVPE suggests a potential involvement of Zonulin in inflammation. Additionally, a significant association with gender indicates that Zonulin concentration may be influenced by sex differences. Further research is warranted to comprehensively understand the underlying mechanisms and causality behind the observed associations.

Conclusion

In conclusion, our study advocates for the use of nanomembrane-based LVPE as a targeted method to enhance gut barrier permeability in individuals with MetSy. By integrating nanotechnology into LVPE, we achieve a breakthrough in precision plasma filtration, maintaining blood cell integrity. Through four LVPE cycles, our research validates the efficacy of this approach in correcting carbohydrate and lipid metabolism. Notably, our investigation reveals LVPE's potential immunomodulatory effect on inflammatory pathways.

The association between Zonulin concentration – a marker of impaired gut barrier function – and anthropometric measurements, cardiometabolic biomarkers, and markers of inflammation provides a nuanced understanding of its role in metabolic and cardiovascular health. Gender and biomarker associations underscore the complexity, prompting further research to unveil underlying mechanisms.

In essence, our study contributes to refining therapeutic interventions for MetSy. The innovative application of nanomembrane-based LVPE, coupled with observed physiological impacts, positions it as a promising avenue for future research and clinical applications, offering new possibilities for enhancing treatment strategies and improving health outcomes.

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