

Metabolomic profiling of peri-implant crevicular fluid after conventional or laser-assisted non-surgical treatment of peri-implantitis: a randomized controlled trial

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Abstract

Objectives

To evaluate metabolomic alterations in peri-implant crevicular fluid (PICF) following non-surgical peri-implantitis therapy and to explore whether different treatment approaches and clinical treatment success were associated with distinct metabolic changes.

Materials and Methods

In this randomized controlled clinical trial, 45 subjects with peri-implantitis received either conventional ultrasonic mechanical debridement (control, n = 24) or Er:YAG laser therapy combined with photobiomodulation (test, n = 20). PICF samples were collected at baseline and 3 months. Untargeted metabolomic profiling was performed using gas chromatography–mass spectrometry. Within-group, between-group, and exploratory subgroup analyses based on treatment success were conducted.

Results

Clinical outcomes were assessed in all participants, while metabolomic analyses included 44 participants. Thirty-eight metabolites were identified. Twelve metabolites changed significantly after therapy following false discovery rate adjustment, involving mainly lipid, carbohydrate, organic acid, and nitrogen metabolism. Increases in lipid-related metabolites and decreases in glucose and xylose were observed after treatment. Some differences in metabolite changes were observed in the test group and among subjects achieving treatment success. However, given the exploratory nature of the metabolomic analyses, these findings should be interpreted cautiously.

Conclusions

Non-surgical peri-implantitis therapy was associated with metabolic changes in the peri-implant microenvironment. PICF metabolomics may provide insights into treatment-associated biochemical responses, although further validation is needed.

Clinical Relevance

Metabolomic profiling of PICF may contribute to understanding biological responses following peri-implantitis treatment and may support future research on treatment monitoring.

Trial Registration

<https://clinicaltrials.gov/study/NCT06890117>.

Introduction

Peri-implantitis is a biofilm-mediated inflammatory disease that can lead to progressive bone loss and implant failure. Non-surgical therapy is the first-line approach, but clinical outcomes are often limited by persistent microbial biofilms and complex implant surface characteristics [1]. Adjunctive interventions, such as erbium-doped yttrium–aluminum–garnet (Er:YAG) laser therapy and photobiomodulation (PBM), have shown potential to enhance biofilm disruption, modulate inflammation, and promote tissue repair [2,3].

Metabolomics, the comprehensive analysis of low-molecular-weight metabolites, provides a dynamic snapshot of tissue physiology and host–microbial interactions [4]. In dentistry, metabolomic profiling of saliva, gingival crevicular fluid (GCF), and peri-implant crevicular fluid (PICF) has revealed metabolites associated with inflammation, tissue breakdown, oxidative stress, and microbial activity [5]. Because metabolomics reflects the combined metabolic activity of host tissues and microbial communities, it offers a functional readout of the biological processes occurring within the peri-implant environment.

Despite increasing evidence from periodontal disease research, metabolomic profiling remains relatively underexplored in peri-implant therapy, particularly in the context of therapeutic interventions. Previous studies have primarily focused on metabolomic differences between healthy and diseased peri-implant tissues, while little is known about metabolic changes occurring after peri-implantitis treatment or their relationship with clinical outcomes [6]. Furthermore, the potential metabolic effects of laser-assisted therapy remain largely unexplored.

Within this context, the present randomized controlled clinical trial (RCT) was designed to explore the metabolic response to non-surgical peri-implantitis therapy. The primary hypothesis-driven objective was to evaluate whether treatment may be associated with changes in the PICF metabolomic profile. Secondary analyses examining differences between treatment modalities and associations with clinical treatment success were considered exploratory and hypothesis-generating.

Methods and Materials

Study Design

This study represents a predefined metabolomic analysis of a prospective RCT evaluating non-surgical peri-implantitis therapy (Photobiomodulation, Photomedicine, and Laser Surgery Journal; in press). The trial was conducted at the Department of Periodontology and Implant Biology, School of Dentistry, Aristotle University of Thessaloniki, Greece. The study was registered prior to patient enrolment at ClinicalTrials.gov (January 2025; <https://clinicaltrials.gov/study/NCT06890117>). Ethical approval was obtained from the Ethics Committee of the School of Dentistry, Aristotle University of Thessaloniki (Protocol No. 614/13-11-2025). All study procedures were performed in accordance with the Declaration of Helsinki.

Clinical, microbiological, and patient-reported outcome measures (PROMs) have been reported in a separate publication, while the present manuscript focuses exclusively on untargeted metabolomic outcomes.

Participants

Adults (≥ 18 years) with at least one implant diagnosed with peri-implantitis according to the 2017 World Workshop criteria [7], with maximum probing depth (PD) ≤ 10 mm, were included. Diagnosis criteria were bleeding/suppuration on probing (BoP/SUP) with increased PD and radiographic bone loss, or, if baseline data were unavailable, BoP/SUP with PD ≥ 6 mm and bone loss ≥ 3 mm.

Participants were periodontally healthy or previously treated and stable under supportive periodontal care (SPC), with good oral hygiene (OH), non-smokers, and no uncontrolled systemic conditions. Exclusion criteria included untreated periodontitis, antibiotic or peri-implantitis therapy within the previous 3 months, pregnancy or lactation, medications affecting healing, and prosthetic restorations preventing access to the peri-implant sulcus when temporary removal was not feasible.

Written informed consent was obtained from all participants.

Sample Size Calculation

Sample size calculation was performed to ensure 80% power ($\beta=0.2$) at $\alpha=0.05$. The statistical unit of analysis was the patient, with only one implant per participant included in the analysis.

The cadaverine/lysine ratio was selected as the reference parameter based on previously data demonstrating its association with peri-implant inflammatory status and microbial activity [8]. The selection of this metabolite ratio was justified by its relevance as a biomarker reflecting proteolytic activity and tissue breakdown within the peri-implant environment. Assuming a mean value of 0.57 (standard deviation [SD] 0.975) for the test group and -0.3 (SD 0.9) for the control group, a minimum of 20 participants per group was required to detect a statistically significant difference.

Considering the largest sample size requirement among all trial's evaluated endpoints, as well as an anticipated dropout rate of 12%, the final sample size was set at 45 participants. This ensured adequate statistical power for all analyses.

Treatment Timeline

Baseline Visit

Medical and dental histories were recorded and full-mouth periodontal examinations performed (PD, clinical attachment level [CAL], BoP, full mouth plaque score [FMPS]). Peri-implant parameters (PD, BoP, CAL, plaque index [PI], mucosal recession [REC], SUP) were recorded at six sites per implant. Radiographs and intraoral photographs were obtained and OH instructions provided (mechanical plaque removal in the area twice daily with soft toothbrush and personalized selected interdental brushes and chlorhexidine mouthrinse 0.12% twice a day for 15 days).

Treatment Visit

The treatment visit occurred at least 24 hours after baseline to avoid probing-induced trauma or bleeding affecting sample integrity from the previously clinical measurements. Procedures included PICF metabolomic sampling from the second-deepest peri-implant pocket using three sterile paper points (10 seconds), and microbiological sampling from the deepest one. Sampling was performed between 09:00 and 12:00 to minimize circadian variability. Samples were immediately stored at -80°C until analysis.

Following treatment, participants were provided with a questionnaire to be completed at home regarding PROMs.

Randomization and Allocation Concealment

Participants were randomly allocated to treatment groups in a 1:1 ratio using a computer-generated randomization sequence (www.randomizer.org). Allocation concealment was ensured through sealed, opaque, sequentially numbered envelopes, which were opened immediately prior to treatment. In cases where participants presented with more than one eligible implant, an additional randomization procedure was performed to select the study implant.

Blinding

Due to the nature of the interventions, blinding of the operator was not feasible. However, outcome assessment, metabolomic and statistical analyses were performed under blinded conditions, with examiners and laboratory personnel unaware of group allocation to minimize detection and measurement bias.

Interventions

After sampling the participants received control or test interventions, starting without anesthesia, which was provided only when needed.

Control group

Non-surgical peri-implant mechanical debridement (MD) and sterile saline irrigation for plaque and biofilm removal using an implant-specific polyether ether ketone (PEEK) fiber-coated, ultrasonic piezo tip (Instrument PI, EMS, Nyon, Switzerland) until the clinician deemed the area properly cleaned.

Test group

Er:YAG laser treatment (2940 nm; Fotona LightWalker AT, Ljubljana, Slovenia) delivered in two stages:

1. Biofilm disruption and granulation tissue removal: Pulse Energy: 40 mJ; Pulse Repetition Rate: 15 Hz; Pulse Duration 50µs (SSP mode); Varian 500/14 tip (cylindrical, quartz, Ø0.5 mm, length 14 mm); H14-N handpiece, water/air 6/0.
2. Surface debridement and decontamination: Pulse Energy: 100 mJ; Pulse Repetition Rate: 20 Hz; Pulse Duration 50µs (SSP mode); Varian 500/14 tip (cylindrical, quartz, Ø0.5 mm, length 14 mm); H14-N handpiece, water/air 6/4.

Following MD, PBM was applied using an 808 nm diode laser (Assy Sol Laser, Den-Mat Holdings, LLC, Lompoc, CA, USA): Mode of Operation: CW; Output Power: 1 W; delivered at a distance of 1 cm from the mucosa for 1 minute per buccal and lingual/palatal surface, using a 400 µm optical fiber covering ~1 cm² per site.

All procedures were performed by the same clinician under standardized protocols.

Follow-Up Visit

At 3 months, clinical measurements and intraoral photographs were repeated. PICF and microbiological samples were collected from the same sites before probing.

Treatment success was defined according to the clinical endpoints for non-surgical peri-implantitis therapy as proposed by the European Federation of Periodontology (EFP) guideline [1], including PD ≤5 mm, ≤1 site with BoP, and absence of SUP.

Patients not meeting success criteria were evaluated for further therapy.

Metabolomic Analysis

Chemicals and Reagents

All reagents were of liquid chromatography–mass spectrometry (LC–MS) grade. Methanol (MeOH) and chloroform (CHCl₃) were obtained from CHEM-LAB NV (Zedelgem, Belgium). Derivatization reagents, including methoxyamine hydrochloride (MeOX), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS), and anhydrous pyridine, as well as myristic acid-d7 (internal standard) and pentadecane (injection standard), were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). All reagents were handled and stored according to manufacturer specifications.

Sample Preparation

PICF samples were thawed at room temperature immediately prior to processing. Each sample was transferred to a sterile microcentrifuge tube and mixed with 10 µL of myristic acid-d7 (internal standard), followed by 490 µL of MeOH:H₂O:CHCl₃ (2.5:1:1, v/v/v). Samples were vortex-mixed for 2 min, sonicated for 20 min, and centrifuged at 10,000 rpm at 4 °C for 15 min. A volume of 350 µL of the resulting supernatant was evaporated to dryness under vacuum. Methoximation was performed with 25 µL MeOX (40 mg/mL) at 70 °C for 120 min, followed by silylation with 50 µL MSTFA + 1% TMCS at 70 °C for 60 min. Ten microliters of pentadecane (100 µg/mL) were added as injection standard.

Normalization

Metabolite abundances were normalized to the internal standard (myristic acid-d7) and to the PICF sample volume to correct for variations in sample collection and extraction efficiency, ensuring accurate quantitative comparison across samples.

GC–MS Analysis

Derivatized samples were analyzed using an Agilent 8860 GC coupled with a 5977 MSD (Agilent Technologies, Santa Clara, CA, USA) on a 30 m HP-5ms UI column (0.25 mm i.d., 0.25 µm film thickness). The oven was programmed from 60 °C (1 min hold) to 325 °C at 10 °C/min, held for 10 min, total runtime 37.5 min. Helium (99.999%) was used as carrier gas at 1.3 mL/min. One microliter was injected in split mode (1:10) at 250 °C. The mass spectrometer operated in electron impact ionization mode (70 eV) with source and transfer line temperatures at 230 °C and 290 °C, respectively, acquiring full-scan spectra.

Quality Control and Data Handling

Pooled QC samples and QC–FAMES samples were injected at the beginning of each batch and every 10 samples to monitor analytical stability and retention index calibration. Data preprocessing, including peak picking, deconvolution, and spectral matching, was performed using MS-DIAL v5.5. Retention indices were calibrated using FAME standards and NIST library reference values. Metabolites were annotated according to MSI level 2 criteria based on spectral similarity and retention index agreement.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics (version 29). Within-group comparisons were performed using the Wilcoxon signed-rank test. Between-group comparisons of metabolite changes were analyzed using the Mann–Whitney U test. Subgroup analyses were conducted according to clinical treatment success. To account for multiple comparisons in metabolomic data, false discovery rate correction using the Benjamini–Hochberg procedure was applied, with a significance threshold of $q < 0.05$. All tests were two-sided. Additional data visualization and multivariate analyses were performed using R version 4.3.1 with standard metabolomics packages. The analysis was conducted on per-protocol basis including only participants with paired baseline and 3-month metabolomic data.

Results

A total of 45 participants were enrolled and randomized. Clinical outcomes were analyzed in all randomized participants ($n = 45$). Metabolomic analyses were conducted on participants with available paired samples. The final metabolomic cohort included 44 participants (control group: $n = 24$; test group: $n = 20$), as one randomly selected sample was used for method testing and excluded from the final analysis. (Figure 1). A statistically significant difference was observed for plaque index at baseline ($p = 0.041$), while all other variables were comparable between groups (all $p > 0.05$) (Table 1).

Table 1: Baseline characteristics of the study participants

Baseline characteristics	Test group (n = 21)	Control group (n = 24)	p-value
Age (mean ± sd)	60.81 ± 9.48	64.38 ± 13.59	0.202 [§]
Gender, N (%)			
Male	7 (33.3)	11 (45.8)	0.393 [§]
Female	14 (66.7)	13 (54.2)	
Health status, N (%)			
Healthy	9 (42.9)	8 (33.3)	0.511 [§]
Compromised	12 (57.1)	16 (66.7)	
Whole-mouth periodontal parameters (mean ± SD)			
Probing depth (mm)	2.68 ± 0.29	2.53 ± 0.31	0.569 [§]
Clinical attachment level (mm)	3.10 ± 0.32	3.07 ± 0.33	0.578 [§]
Bleeding on probing (%)	9.7 ± 3.9	8.6 ± 4.2	0.658 [§]
Full mouth plaque score (%)	9.2 ± 5.2	8.9 ± 5.3	0.598 [§]
Peri-implant clinical parameters (baseline, mean ± SD)			
Probing depth (mm)	5.74 ± 0.85	6.03 ± 1.35	0.715 [§]
Bleeding on probing (%)	89.68 ± 17.85	79.17 ± 31.18	0.328 [§]
Clinical attachment level (mm)	5.90 ± 0.91	6.37 ± 1.51	0.374 [§]
Plaque index (%)	9.52 ± 30.08	29.17 ± 43.48	0.041 ^{*§}
Recession (mm)	0.21 ± 0.49	0.33 ± 0.68	0.700 [§]
Suppuration (%)	0.79 ± 3.63	2.78 ± 10.61	0.617 [§]
§ Independent-Samples Mann–Whitney U Test § Pearson Chi-Square Test § Fisher's exact test § Asymptotic significance is displayed * Statistically significant difference at 0.05 level			

Peri-implant clinical parameters

At 3 months, both groups demonstrated significant improvements in PD, BoP, and CAL. However, the laser-treated group exhibited significantly greater reductions in PD (−2.30 vs −1.45 mm; $p < 0.001$), BoP (−73.0% vs −42.4%; $p = 0.004$), and CAL (−2.56 vs −1.47 mm; $p = 0.001$) compared with the control group. No significant intergroup differences were observed for PI, REC, or SUP. Treatment success was achieved in 81% of patients in the test group compared with 42% in the control group ($p = 0.007$) (Table 2).

Table 2: Clinical peri-implant outcomes at baseline and 3 months

Clinical parameters	Test group (n = 21) (mean ± SD)	Control group (n = 24) (mean ± SD)	Inter-group p-value
Probing depth (mm)			
Baseline	5.74 ± 0.85	6.03 ± 1.35	0.715 [§]
3 months	3.44 ± 1.03	4.58 ± 1.41	0.002 ^{*§}
Difference (3 months – baseline)	–2.30 ± 0.65	–1.45 ± 0.52	<0.001 ^{*§}
Intragroup p-value	<0.001 ^{*§}	<0.001 ^{*§}	
Bleeding on probing (%)			
Baseline	89.68 ± 17.85	79.17 ± 31.18	0.328 [§]
3 months	16.67 ± 21.08	36.81 ± 35.77	0.035 ^{*§}
Difference (3 months – baseline)	–73.02 ± 25.54	–42.36 ± 36.44	0.004 ^{*§}
Intragroup p-value	<0.001 ^{*§}	<0.001 ^{*§}	
Clinical attachment level (mm)			
Baseline	5.90 ± 0.91	6.37 ± 1.51	0.374 [§]
3 months	3.33 ± 1.46	4.90 ± 1.50	0.002 ^{*§}
Difference (3 months – baseline)	–2.56 ± 1.21	–1.47 ± 0.78	0.001 ^{*§}
Intragroup p-value	<0.001 ^{*§}	<0.001 ^{*§}	
Plaque index (%)			
Baseline	9.52 ± 30.08	29.17 ± 43.48	0.041 ^{*§}
3 months	0 ± 0	4.17 ± 12.29	0.097 [§]
Difference (3 months – baseline)	–0.10 ± 0.30	–0.25 ± 0.38	0.051 [§]
Intragroup p-value	0.157 [§]	0.007 ^{*§}	
Recession (mm)			
Baseline	0.21 ± 0.49	0.33 ± 0.68	0.700 [§]
3 months	0.25 ± 0.56	0.31 ± 0.59	0.557 [§]
Difference (3 months – baseline)	0.03 ± 0.19	–0.02 ± 0.36	0.464 [§]
Intragroup p-value	0.496 [§]	0.719 [§]	
Suppuration (%)			
Baseline	0.79 ± 3.63	2.78 ± 10.61	0.617 [§]
3 months	1.59 ± 7.27	2.08 ± 7.47	0.655 [§]
Difference (3 months – baseline)	0 ± 0.08	0 ± 0.10	0.661 [§]
Intragroup p-value	0.655 [§]	0.785 [§]	
Treatment success (%) (3 months)	81% (17/21)	42% (10/24)	0.007 ^{*§}
[§] Independent-Samples Mann–Whitney U Test [§] Related-Samples Wilcoxon Signed Rank Test [§] Pearson Chi-Square Test [§] Asymptotic significance is displayed [*] Statistically significant difference at 0.05 level			

Metabolites changed significantly after treatment (all subjects)

A total of 38 metabolites were identified in PICF and classified into distinct biochemical groups. Organic acids represented the most abundant class and included acetic acid, glycolic acid, oxalic acid, succinic acid, glyceric acid, malic acid, and 5-oxoproline. Amino acids comprised valine, leucine/isoleucine, proline, glycine, threonine, aspartic acid, glutamic acid, and tyrosine. Carbohydrates and sugar alcohols included glucose, galactose, mannose, xylose, lactose, ribitol, erythronic acid, and myo-inositol. Lipids and fatty acids were represented by palmitic acid, linoleic acid, oleic acid, stearic acid, and cholesterol. FAMES included dodecanoic acid methyl ester, methyl tetradecanoate, methyl stearate, and docosanoic acid methyl ester. Glycerides and monoacylglycerols comprised 2-palmitoylglycerol, 1-monopalmitin, and glycerol monoesterate, while nitrogen-containing and other metabolites included urea, ethanolamine, and glycerol.

Among these, 12 metabolites exhibited statistically significant changes at 3 months post-treatment (Table 3). The most pronounced alterations were observed among lipid-related metabolites, with marked increases in monoacylglycerols, including 1-monopalmitin, glycerol monoesterate, and 2-palmitoylglycerol, which also demonstrated the largest effect sizes. In parallel, palmitic acid showed a substantial increase. Within fatty acid derivatives, dodecanoic acid methyl ester and docosanoic acid methyl ester increased significantly, whereas methyl tetradecanoate decreased.

In contrast, carbohydrate-related metabolites, specifically glucose and xylose, exhibited significant decreases at 3 months, although with smaller absolute magnitudes of change compared to lipid-associated metabolites. Similarly, the organic acid succinic acid decreased, while lactic acid showed a significant increase. Additionally, urea levels increased significantly.

Table 3: Significantly altered metabolites at 3 months post-treatment (all subjects n=44).

Metabolite	Mean Difference (3 months – Baseline)	p-value	Direction of Change
Monoacylglycerols / Glycerides			
1-Monopalmitin	4.48×10^6	<0.001 * ^a	↑ Increased
Glycerol monoesterate	4.57×10^6	<0.001 * ^a	↑ Increased
2-Palmitoylglycerol	1.96×10^5	<0.001 * ^a	↑ Increased
Saturated Fatty Acids			
Palmitic Acid	1.02×10^6	0.001 * ^a	↑ Increased
Fatty Acid Methyl Esters (FAMES) / Fatty acid derivatives			
Dodecanoic acid methyl ester	2.31×10^4	0.009 * ^a	↑ Increased
Docosanoic acid methyl ester	9.14×10^4	0.018 * ^a	↑ Increased
Methyl tetradecanoate	1.02×10^4	0.002 * ^a	↓ Decreased
Carbohydrates / Sugars			
Glucose	8.38×10^3	<0.001 * ^a	↓ Decreased
Xylose	6.49×10^5	<0.001 * ^a	↓ Decreased
Organic Acids			
Lactic Acid	3.06×10^6	0.001 * ^a	↑ Increased
Succinic Acid	2.26×10^5	0.024 * ^a	↓ Decreased
Nitrogen-containing metabolites			
Urea	6.98×10^5	0.003 * ^a	↑ Increased

a. Related-Samples Wilcoxon Signed Rank Test

* Statistically significant difference at 0.05 level

Treatment-dependent metabolic changes between groups

Comparative analysis of metabolite changes between the control and laser-treated groups revealed both shared and treatment-dependent metabolic responses. All the statistically significant alterations in both groups and between groups are presented at Table 4.

Several metabolites exhibited significant within-group changes in both groups without significant intergroup differences, including xylose and glucose, which decreased significantly in both groups, and 2-palmitoylglycerol, 1-monopalmitin, and glycerol monoesterate, which increased significantly over time. In contrast, certain metabolites demonstrated group-specific changes. The laser-treated group showed significant increases in palmitic acid, cholesterol, lactic acid, tyrosine, and urea, whereas these metabolites did not change significantly in the control group. For these metabolites, significant intergroup differences were observed, too. Additionally, docosanoic acid methyl ester increased significantly only in the laser group, while dodecanoic acid methyl ester decreased significantly only in the control group; however, no significant intergroup differences were detected for these metabolites. Stearic acid, valine, proline, and 5-oxoproline increased significantly only in the laser group, though intergroup comparisons did not reach statistical significance. Methyl tetradecanoate decreased significantly in the laser group only. Collectively, these findings suggest differences in metabolic responses following laser-assisted therapy compared with conventional treatment; however, this interpretation is based on a limited number of metabolites showing intergroup differences and should be considered exploratory.

Table 4: Treatment-dependent metabolic changes between groups.

Metabolite Class	Metabolite	Mean Change – Control Group (n = 24)	Mean Change – Laser Group (n = 20)	Intergroup p- value	Summary of Changes
Monoacylglycerols	2-Palmitoylglycerol	1.34×10^5 ($p = 0.022$ ^a)	2.70×10^5 ($p = 0.001$ ^a)	0.090 ^{b,c}	Significant increase in both groups
	1-Monopalmitin	3.35×10^6 ($p = 0.006$ ^a)	5.82×10^6 ($p < 0.001$ ^a)	0.157 ^{b,c}	Significant increase in both groups
	Glycerol monoesterate	3.41×10^6 ($p = 0.014$ ^a)	5.96×10^6 ($p < 0.001$ ^a)	0.187 ^{b,c}	Significant increase in both groups
Saturated fatty acids	Palmitic Acid	4.57×10^5 ($p = 0.137$ ^{b,c})	1.67×10^6 ($p = 0.002$ ^a)	0.045 ^a	Significant increase in laser group; intergroup difference
	Stearic Acid	-3.36×10^5 ($p = 0.932$ ^{b,c})	6.78×10^5 ($p = 0.009$ ^a)	0.053 ^{b,c}	Significant increase only in laser group
Sterols	Cholesterol	-3.83×10^5 ($p = 0.052$ ^{b,c})	9.00×10^5 ($p = 0.332$ ^{b,c})	0.034 ^a	Significant intergroup difference without intragroup significance
Fatty Acid Methyl Esters (FAMES)	Dodecanoic acid methyl ester	-3.03×10^3 ($p = 0.024$ ^a)	-1.54×10^3 ($p = 0.145$ ^{b,c})	0.437 ^{b,c}	Significant decrease only in control group
	Docosanoic acid methyl ester	5.09×10^3 ($p = 0.458$ ^{b,c})	1.38×10^4 ($p = 0.006$ ^a)	0.172 ^{b,c}	Significant increase only in laser group
	Methyl tetradecanoate	-7.13×10^3 ($p = 0.123$ ^{b,c})	-1.38×10^4 ($p = 0.007$ ^a)	0.383 ^{b,c}	Significant decrease only in laser group
Carbohydrates	Glucose	-8.42×10^3 ($p = 0.001$ ^a)	-8.38×10^3 ($p = 0.006$ ^a)	0.981 ^{b,c}	Significant decrease in both groups; no intergroup difference
	Xylose	-6.62×10^3 ($p = 0.003$ ^a)	-6.39×10^5 ($p = 0.003$ ^a)	0.540 ^{b,c}	Significant decrease in both groups; no intergroup difference
Amino Acids	Tyrosine	6.52×10^3 ($p = 0.088$ ^{b,c})	1.79×10^4 ($p = 0.002$ ^a , ^{b,c})	0.006 ^a , ^{b,c}	Significant increase in laser group; intergroup difference
	Valine	-2.91×10^4 ($p = 0.909$ ^{b,c})	1.01×10^5 ($p = 0.009$ ^a , ^{b,c})	0.099 ^{b,c}	Significant increase only in laser group
	Proline	-1.43×10^4 ($p = 0.753$ ^{b,c})	4.29×10^4 ($p = 0.019$ ^a , ^{b,c})	0.172 ^{b,c}	Significant increase only in laser group
Organic Acids	Lactic Acid	6.34×10^5 ($p = 0.511$ ^{b,c})	5.92×10^6 ($p < 0.001$ ^a , ^{b,c})	0.001 ^a , ^{b,c}	Significant increase only in laser group; intergroup difference
	5-Oxoproline	-2.18×10^5 ($p = 0.775$ ^{b,c})	2.99×10^5 ($p = 0.006$ ^a , ^{b,c})	0.229 ^{b,c}	Significant increase only in laser group
Nitrogen-containing metabolites	Urea	1. $\times 10^5$ 2. ($p = 0.304$ ^{b,c})	1.22×10^6 ($p = 0.001$ ^a , ^{b,c})	0.025 ^a , ^{b,c}	Significant increase in laser group; intergroup difference

a. Related-Samples Wilcoxon Signed Rank Test

b. Independent-Samples Mann-Whitney U Test

c. Asymptotic significance is displayed

* Statistically significant difference at 0.05 level

Metabolites associated with clinical treatment success

To investigate the biological relevance of treatment-associated metabolic alterations subgroup analyses were conducted based on clinical outcome (treatment success: n = 26; 16 laser, 10 control; no success: n = 18; 4 laser, 14 control) at three months post-treatment (Table 5).

In subjects who achieved clinical treatment success, several metabolites exhibited significant increases over time, including lipid-related compounds such as 1-monopalmitin, glycerol monoesterate, 2-palmitoylglycerol, palmitic acid, stearic acid, oleic acid, and docosanoic acid methyl ester. In addition, elevations in lactic acid and urea were observed. In contrast, methyl tetradecanoate, glucose, and xylose showed significant decreases in this group.

Among subjects who did not reach a stable peri-implant condition at 3 months, fewer metabolites demonstrated significant within-group changes. Specifically, dodecanoic acid methyl ester, glucose, and xylose decreased, whereas increases were detected in 1-monopalmitin and glutamic acid.

Between-group comparison of metabolite changes identified a limited subset of compounds that differed significantly according to clinical outcome, primarily lipid-related metabolites, including 1-monopalmitin (p = 0.040), glycerol monoesterate (p = 0.045), palmitic acid (p = 0.030), and stearic acid (p = 0.005), all showing greater increases in the treatment success group.

Table 5: Metabolites associated with clinical treatment success.

Metabolite Class	Metabolite	No Treatment Success (n = 18)	p-value (within group)	Direction	Treatment Success (n = 26)	p-value (within group)	Direction	Between-Group p-value
Monoacylglycerols	1-Monopalmitin	2.73×10^6	0.039 ^{a,c}	↑ Increase	5.67×10^6	<0.001 ^{a,c}	↑ Increase	0.040 ^{a,b,c}
	Glycerol Monoesterate	2.59×10^6	0.078 ^{a,c}	↑ Increase	5.91×10^6	<0.001 ^{a,c}	↑ Increase	0.045 ^{a,b,c}
	2-Palmitoylglycerol	1.02×10^5	0.145 ^{a,c}	↑ Increase	2.60×10^5	<0.001 ^{a,c}	↑ Increase	0.053 ^{b,c}
Saturated fatty acids	Palmitic Acid	3.91×10^5	0.372 ^{a,c}	↑ Increase	1.44×10^6	0.002 ^{a,c}	↑ Increase	0.030 ^{a,b,c}
	Stearic Acid	4.30×10^5	0.372 ^{a,c}	↓ Decrease	5.19×10^5	0.007 ^{a,c}	↑ Increase	0.005 ^{a,b,c}
Unsaturated fatty acids	Oleic Acid	8.77×10^1	0.711 ^{a,c}	↑ Increase	8.37×10^3	0.038 ^{a,c}	↑ Increase	0.152 ^{b,c}
Fatty Acid Methyl Esters (FAMES)	Dodecanoic acid methyl ester	-3.08×10^3	0.016 ^{a,c}	↓ Decrease	1.80×10^4	0.137 ^{a,c}	↓ Decrease	0.445 ^{b,c}
	Methyl tetradecanoate	-8.12×10^3	0.071 ^{a,c}	↓ Decrease	-1.16×10^4	0.009 ^{a,c}	↓ Decrease	0.811 ^{b,c}
	Docosanoic acid methyl ester	7.80×10^2	0.948 ^{a,c}	↑ Increase	1.50×10^4	0.003 ^{a,c}	↑ Increase	0.053 ^{b,c}
Carbohydrates	D-Glucose	-1.19×10^4	0.004 ^{a,c}	↓ Decrease	-6.00×10^4	0.003 ^{a,c}	↓ Decrease	0.262 ^{b,c}
	Xylose	-7.45×10^5	0.014 ^{a,c}	↓ Decrease	-5.85×10^5	0.001 ^{a,c}	↓ Decrease	0.519 ^{b,c}
Organic Acids	Lactic Acid	1.34×10^6	0.145 ^{a,c}	↑ Increase	4.22×10^6	0.002 ^{a,c}	↑ Increase	0.100 ^{b,c}
Nitrogen-containing metabolites	Urea	2.19×10^5	0.267 ^{a,c}	↑ Increase	10.2×10^6	0.002 ^{a,c}	↑ Increase	0.181 ^{b,c}
Amino Acids	Glutamic Acid	6.78×10^3	0.035 ^{a,c}	↑ Increase	1.66×10^4	0.694 ^{a,c}	↑ Increase	0.145 ^{b,c}

a. Related-Samples Wilcoxon Signed Rank Test

b. Independent-Samples Mann-Whitney U Test

c. Asymptotic significance is displayed

* Statistically significant difference at 0.05 level

Discussion

This RCT evaluated the metabolic profile associated with non-surgical peri-implantitis therapy using Er:YAG laser and PBM compared with conventional ultrasonic MD. Treatment induced significant metabolic remodeling of PICF, involving coordinated changes in lipid, carbohydrate, amino acid, organic acid, and nitrogen metabolism. These alterations were observed in both groups, with some differences noted in the laser-treated group.

Overall, the metabolomic profile at three months suggests that clinical improvement is accompanied by active metabolic remodeling of the peri-implant microenvironment, reflecting inflammation resolution, tissue repair, and shifts in host–microbial interactions.

A baseline difference in plaque index was observed between groups and should be acknowledged as a potential source of confounding. Although absolute plaque levels were low and no baseline differences were detected in other clinical parameters, plaque accumulation may influence the peri-implant microenvironment and, consequently, metabolomic composition. It is therefore possible that part of the observed metabolic changes may reflect, in part, differences in baseline plaque levels and/or differences in the magnitude of response following debridement, rather than treatment-specific effects alone. This limitation should be considered when interpreting intergroup metabolomic differences.

Lipids and lipid-derived compounds

Across all subjects, the most pronounced post-treatment changes involved lipid-related metabolites, particularly monoacylglycerols and fatty acids, suggesting that lipid metabolism may play a central role in peri-implant tissue response rather than being merely a consequence of inflammation resolution. As key components of cell membranes and signaling molecules, lipids are essential for membrane remodeling, extracellular matrix synthesis, and tissue reorganization during wound healing and peri-implant repair. Increased lipid turnover may also reflect coordinated immune regulation and cellular migration [9,10], consistent with lipid-centered metabolic patterns reported in periodontal and peri-implant healing [11].

Monoacylglycerols, including 1-monopalmitin, glycerol monoesterate, and 2-palmitoylglycerol, increased markedly, particularly in the laser-treated group and in subjects with clinical treatment success, suggesting enhanced lipolysis and phospholipid turnover. Beyond their role as intermediates of triglyceride metabolism, monoacylglycerols act as bioactive signaling molecules [12] involved in immune activation, membrane dynamics, and inflammation resolution [13,14]. Their increase, especially in clinically successful cases, indicates that regulated lipid remodeling may be a feature of effective peri-implant healing [15].

Free fatty acids such as palmitic and stearic acid increased significantly only in the laser-treated group and were associated with treatment success. These fatty acids are precursors of membrane lipids and bioactive mediators with immunomodulatory and antimicrobial properties, supporting their role in restoring peri-implant homeostasis [16]. Their greater increase in clinically successful cases further supports the role of lipid metabolic activation in tissue recovery.

Selective changes in FAMES, including increases in dodecanoic and docosanoic acid methyl esters and a decrease in methyl tetradecanoate, further suggest regulated lipid metabolism and potential shifts in microbial composition during peri-implant healing. These findings are in agreement with previous metabolomic studies in periodontal disease that have highlighted lipid metabolism as a key component of tissue response and healing [17–19].

Carbohydrates

Carbohydrates are primary energy sources for both host cells and oral microorganisms, and elevated levels in crevicular fluid are commonly associated with inflammation, microvascular changes, and microbial metabolism [20]. In contrast to lipid metabolites, glucose and xylose decreased significantly three months after treatment across all subjects, regardless of treatment modality or clinical outcome.

This reduction likely reflects decreased inflammatory exudation and microbial activity [21] following therapy, consistent with observations after successful periodontal treatment [22]. Lower carbohydrate availability in PICF may also limit bacterial growth and biofilm maturation, while normalization of vascular permeability during inflammation resolution may reduce diffusion of serum-derived sugars into the peri-implant sulcus. Similar reductions in carbohydrate metabolites have been reported following successful periodontal therapy, supporting their role as markers of inflammation resolution [23].

Organic Acids

Organic acids are key intermediates of cellular metabolism and may also derive from anaerobic bacterial activity [24,25]. Following treatment, lactic acid and 5-oxoproline increased, particularly in the laser-treated group and in subjects with clinical success, whereas succinic acid decreased across all subjects.

The reduction in succinic acid likely reflects decreased anaerobic bacterial activity, as succinate is a metabolic end product of several periodontal pathogens and a recognized pro-inflammatory metabolite associated with periodontal disease. In contrast, increased lactic acid may indicate enhanced host glycolytic activity during tissue repair. Lactate has been linked to angiogenesis, fibroblast activation, collagen synthesis, and wound healing, supporting its association with tissue regeneration rather than persistent inflammation.

The increase in 5-oxoproline, an intermediate of the γ -glutamyl cycle, further suggests restoration of redox balance and glutathione metabolism during healing.

Activated immune cells also undergo a metabolic shift toward glycolysis, similar to the Warburg effect, to support rapid energy production and effector functions [14]. Accordingly, the observed decrease in glucose alongside increased lactate may reflect host immune and reparative activity rather than

bacterial fermentation. Similar shifts in organic acid profiles have been reported after non-surgical periodontal therapy, supporting their role as indicators of metabolic resolution [26].

Amino Acids

Amino acids are essential for protein synthesis and play important roles in immune regulation, oxidative stress control, and extracellular matrix remodeling [27]. Changes in their concentrations in crevicular fluid may therefore reflect both tissue breakdown and repair processes.

In the present study, tyrosine, valine, and proline increased significantly only in the laser-treated group, with tyrosine also showing a significant intergroup difference. These increases likely reflect enhanced protein turnover and extracellular matrix remodeling during peri-implant healing. Proline, a major component of collagen, is critical for connective tissue integrity, and its elevation may indicate active tissue repair [28]. Tyrosine and branched-chain amino acids such as valine are involved in cellular signaling and immune modulation, suggesting increased metabolic activity in successfully treated sites [29].

In contrast, glutamic acid increased primarily in subjects without clinical treatment success, potentially reflecting persistent inflammation or tissue degradation, as glutamic acid has been linked to cellular stress responses and inflammatory signaling pathways [30].

Experimental studies also indicate that valine may inhibit *P. gingivalis* biofilm formation and alter bacterial polysaccharide production [31], although other reports associate valine with peri-implantitis [8,32], suggesting context-dependent effects.

Proline may also be released during inflammation through the activity of matrix metalloproteinases and peptidases [33]. Elevated extracellular proline has been shown to stimulate collagen synthesis and may contribute to peri-implant tissue repair and antimicrobial activity [32,34–36]. Together, these findings suggest that amino acid metabolism reflects active peri-implant tissue remodeling and treatment response.

Nitrogen-Containing Metabolites

Urea, a product of amino acid catabolism and nitrogen metabolism, may reflect protein turnover, microbial urease activity, or changes in inflammatory status [37,38].

In this study, urea levels increased significantly in the laser-treated group and in subjects achieving clinical treatment success, with significant intergroup differences. This increase may indicate enhanced protein metabolism and cellular turnover during peri-implant healing. The association between elevated urea levels and clinical success suggests that this change reflects physiological tissue remodeling rather than persistent inflammation.

Integrated interpretation and clinical relevance

The present findings indicate that successful peri-implant healing is a metabolically dynamic process characterized by coordinated lipid mobilization, membrane biosynthesis, and protein turnover. In contrast, the attenuated metabolic response observed in unsuccessful cases may reflect persistent inflammation and impaired tissue remodeling, consistent with patterns described in chronic periodontal disease [39]. Collectively, these results highlight the potential of PICF metabolomics as a sensitive approach for monitoring peri-implant tissue responses and identify lipid-related metabolites as promising indicators of treatment efficacy [39].

Notably, the metabolomic profile associated with laser therapy largely overlapped with that observed in subjects who achieved clinical treatment success. Laser treatment has been reported to modulate inflammatory responses, enhance microcirculation, and stimulate cellular metabolic activity [2,3,40]. However, the extent to which the metabolic alterations observed in the present study can be directly attributed to laser-specific effects remains unclear. Although laser-treated sites exhibited broader and more pronounced changes, particularly in lipid, amino acid, and nitrogen metabolism, these findings should be interpreted with caution, as the study design does not allow for causal inference.

Reconciliation with conflicting literature

Previous studies have reported elevated levels of lipids and amino acids as markers of persistent periodontal or peri-implant inflammation [22,23,39,41]. However, these investigations have largely focused on untreated or chronically inflamed conditions. In contrast, the present study evaluated metabolomic changes following active peri-implantitis therapy at a three-month follow-up, a time point that likely captures both the resolution of inflammation and ongoing tissue remodeling processes. Variations in study design, sample type, analytical platforms, and follow-up duration may therefore account for the discrepancies observed across studies.

Laser-assisted therapy may further contribute to distinct metabolic responses compared with conventional treatment alone. Experimental and clinical evidence suggests that laser irradiation can enhance cellular bioenergetics, increase mitochondrial activity, and promote wound healing [2,40], which may partially explain the broader metabolic shifts observed in the present study.

Interestingly, metabolites previously proposed as discriminators between healthy and diseased peri-implant tissues, such as cadaverine, lysine, and α -ketoglutarate [6], were not significantly altered in this study. This finding may be attributed to the relatively short follow-up period, indicating that longer-term or longitudinal assessments may be necessary to capture their diagnostic relevance. Additionally, the use of GC–MS inherently limits detection to a subset of the metabolome, raising the possibility that other relevant metabolite classes were not captured. Together, these considerations suggest that a combination of biomarkers, assessed over time, may be required for a more comprehensive characterization of peri-implant health and treatment response [8].

Strengths and Limitations of the Metabolomic Analysis

A strength of this study is the application of untargeted metabolomic profiling of PICF within a randomized clinical trial, providing exploratory insight into metabolic changes associated with peri-implantitis treatment. The use of PICF is relevant because it reflects the local peri-implant microenvironment, and the longitudinal design, with sampling before and after therapy, enabled assessment of treatment-associated metabolic shifts.

However, several limitations should be considered. First, the relatively small sample size, combined with multiple metabolite comparisons and exploratory subgroup analyses, limits statistical power and increases the risk of both false-positive and false-negative findings. Accordingly, the metabolomic findings should be interpreted as exploratory and hypothesis-generating. Second, the three-month follow-up and single post-treatment sampling point limit assessment of longer-term metabolic remodeling. Third, the test intervention combined Er:YAG laser therapy with PBM, precluding separation of the individual effects of each intervention. Fourth, a baseline difference in plaque index between groups should be acknowledged as a potential source of confounding, as plaque levels may influence the peri-implant microenvironment and metabolomic composition. Fifth, only one implant per patient was included, which may not fully capture intra-individual variability, and exclusion of smokers may limit generalizability.

Additional limitations include the absence of a healthy peri-implant control group, the lack of external validation, and limitations inherent to the GC–MS platform, which captures only a subset of primarily volatile and semi-volatile metabolites and does not reflect the full metabolome. Further validation using targeted metabolomic approaches and larger cohorts is therefore needed.

Conclusion

This randomized controlled metabolomic analysis suggests that non-surgical peri-implantitis therapy may be associated with metabolic changes in the peri-implant microenvironment. Alterations involving lipid-, carbohydrate-, and amino acid-related metabolites were observed, although these findings should be interpreted cautiously given the exploratory nature of the analyses. Some differences in metabolic profiles were observed between treatment groups; however, these were limited to a subset of metabolites and should be interpreted with caution.

Overall, these findings provide preliminary insight into biochemical responses associated with peri-implantitis treatment. However, the results should be considered hypothesis-generating, and further studies with larger sample sizes, longer follow-up, appropriate multivariable modeling, and targeted validation are required before drawing conclusions regarding treatment-specific metabolic effects or potential biomarkers of treatment response.

Declarations

Trial Registration: <https://clinicaltrials.gov/study/NCT06890117>.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Chariklia Neophytou: Concept and design; Data analyses and collection; Data interpretation; Drafting article; Final approval; Ioannis Vouros: Critical revision of article; Supervision; Final approval; Dimitra Sakellari: Concept and design; Critical revision of article; Final approval; Athina Chatzigianni: Data interpretation; Final approval; Dimitris Strakas: Laser education; Final approval; Konstantinos Papadimitriou: Concept and design; Data interpretation; Critical revision of article; Final approval.

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Figures

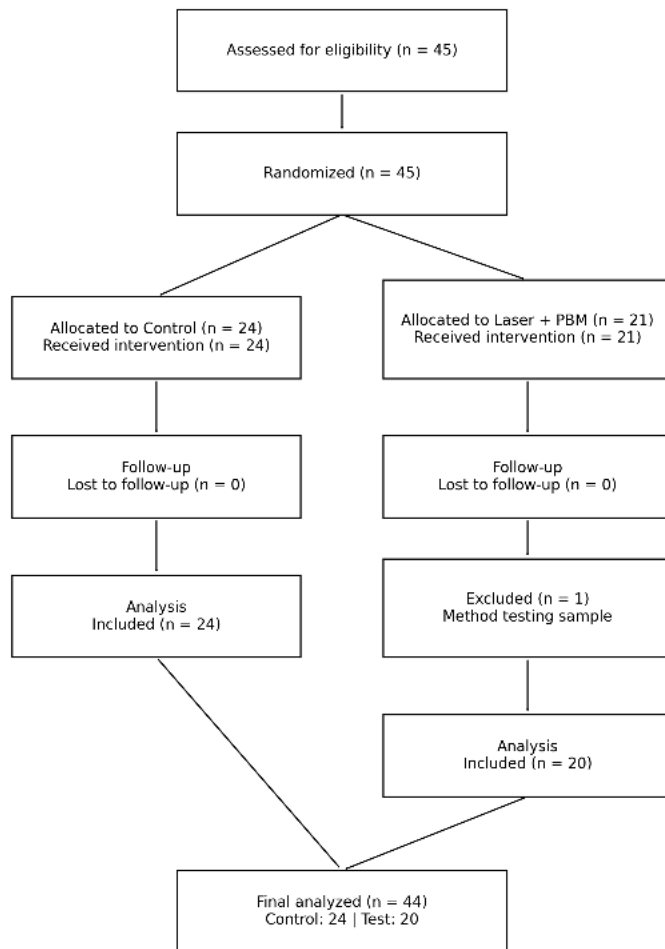


Figure 1

CONSORT diagram

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