Journal of Translational Medicine

RESEARCH

Open Access

Successful endodontic treatment improves glucose and lipid metabolism: a longitudinal metabolomic study

Yuchen Zhang[1], Adrien Le Guennec[2], Pirkko Pussinen[3,4], Gordon Proctor[5] and Sadia Ambreen Niazi[6*]

Abstract

Background Apical periodontitis (AP) is one of the most prevalent dental diseases. Its presence can increase systemic inflammatory burden and is associated with cardiometabolic disorders including higher cardiovascular risks and impaired glycaemic control. There is currently a paucity of knowledge showing whether successful endodontic treatment is associated with systemic metabolic improvements. This study aims to identify the potential impact of successful endodontic treatment on patients' serum metabolomic profiles, and to evaluate how these impacts are associated with the metabolic syndrome (MetS) indicators, inflammatory biomarkers, as well as blood and intracanal microbiomes.

Methods This self-controlled two-year longitudinal cohort study investigated the metabolic improvements associated with successful endodontic treatment in 65 AP patients using nuclear magnetic resonance (NMR) spectroscopy on serum samples. The samples were collected at 5 time points including pre-operative baseline and 3 month, 6 month, 1 year, and 2 year reviews.

Results Significant postoperative changes were observed in 24 (54.5%) metabolites. The results suggested improved glucose and lipid metabolism as well as reduced inflammatory burdens, as evidenced by a significant reduction in branched-chain amino acids at the 3 month review, a significant decrease in glucose and pyruvate at the 2 year review, a short-term reduction in cholesterol, choline, and fatty acid levels, and a progressive increase in tryptophan. In addition, strong associations were found between metabolic profile and clinical metabolic syndrome indicators, inflammatory markers, and pre-operative blood and intracanal microbiome, underscoring the systemic impact of AP and its treatment. A dynamic Bayesian model identified that metabolites associated with the tricarboxylic acid (TCA) cycle are the key regulators in the longitudinal alterations of the metabolic profiles.

Conclusions Successful endodontic treatment in AP patients is associated with improved glucose and lipid metabolic profiles, and a reduction in systemic inflammation, suggesting a potential role in mitigating cardiometabolic disease risk.

Keyword Apical periodontitis, Cardiovascular disease risk, Hyperglycemia, Dyslipidemia, Metabolomics

*Correspondence:
Sadia Ambreen Niazi
sadia.niazi@kcl.ac.uk
[1]Centre for Oral, Clinical & Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, UK
[2]Wolfson SPaRC, King's College London, London, UK
[3]Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland
[4]Institute of Dentistry, University of Eastern Finland, Kuopio, Finland
[5]Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, UK
[6]Department of Endodontics, Centre for Oral, Clinical & Translational Sciences, Faculty of Dentistry, Oral & Craniofacial Sciences, Guy's Dental Hospital, King's College London, London, UK

Introduction

Apical periodontitis (AP) is a chronic inflammatory condition affecting the peri-radicular tissue. It is initiated with the bacterial invasion into the pulp and root canal system consequent to caries or dental trauma. The interactions between microbial virulence factors and host immune response further leads to peri-apical bone resorption [1, 2]. In recent years, emerging evidence has implicated potential links between AP and cardiometabolic disorders [3, 4]. The establishment of AP lesions increases the systemic inflammatory burden and negatively impacts the general health [5, 6]. It is reported that worse periapical status correlates significantly with poorly controlled glycosylated haemoglobin (HbA1c) levels [7], and that the presence of type 2 diabetes mellitus may have a negative impact on the outcome of endodontic treatment [8]. The chronic systemic inflammation induced by AP can contribute to promoted insulin resistance and compromised glycaemic control [9]. There is also a potential link between endodontic infection and cardiovascular diseases (CVD), where subjects with apical radiolucency exhibit a 2.72 times higher risk of developing coronary artery disease [10, 11]. Recent study showed that common oral infections including endodontic conditions were associated with adverse signatures of circulating metabolites typical for systemic inflammation and CVD risk [12]. In short, the effects of AP are not limited to oral manifestations, it can also alter the inflammatory and metabolic burden thus effecting general health.

The association between AP and systemic conditions may also be attributable to the oral microbiome, potentially through AP-associated bacteraemia and the oral-gut axis [13, 14]. Bacterial translocation from root canal infections into the circulation may contribute to an increased systemic inflammatory burden [14], highlighting a broader impact of AP. Furthermore, it is documented that the oral microbiota plays a role in inducing dysbiosis of the gut microbiota and thereby increases the risks of associated systemic conditions [15].

Links to systemic metabolic dysregulations have been well documented for generalised periodontitis, another common, microbial-driven chronic inflammatory diseases in the oral cavity. Multiple trials and meta-analyses indicate that periodontal therapy can improve glycaemic control [16], endothelial function [17], and lipid profiles [18]. By contrast, evidence supporting the systemic benefits of endodontic treatment remains scarce.

Our earlier studies showed a significant elevation in inflammatory biomarkers of CVD risk in AP subjects when compared to healthy controls at baseline [19], and that these biomarkers correlated with blood and intracanal microbiome [20]. These CVD risk biomarkers then reduced significantly at 2 year following successful endodontic treatment [6], again revealing the systemic impact of AP while underlying the beneficial effects of successful endodontic treatment on the inflammatory burden. However, in terms of serum metabolic profile, it remains unclear how it alters during the healing process after endodontic treatment, and what are the potential factors that cause the alterations.

To narrow this gap of knowledge, we applied metabolomics analysis on serum samples from subjects with AP. In recent years, metabolomics has been widely applied in dental sciences and offers great potential to investigate host metabolism in oral diseases [21]. Nuclear magnetic resonance (NMR) is one of the common analytical approaches for metabolomics, which is well-suited for serum samples due to its precision and reproducibility [22]. In this longitudinal cohort study, we hypothesised that successful endodontic treatment would be associated with measurable improvements in systemic metabolic profiles and reductions in inflammatory burden, as reflected by alterations in specific metabolic biomarkers. To validate our hypothesis, we used NMR to investigate serum metabolites of AP subjects undergoing endodontic treatment at 5 time-points (pre-operative baseline, 3 month, 6 month, 1 year, and 2 year post-operative reviews). We aimed to identify the potential impact of successful endodontic treatment on serum metabolomic profiles. We also aimed to evaluate how these impacts are associated with the clinical metabolic syndrome (MetS) indicators, inflammatory biomarkers, as well as blood and intracanal microbiomes, to comprehensively understand the effect of AP and successful endodontic treatment on the risks for systemic conditions.

Materials and methods

Participant recruitment

Participants in this study were adult AP subjects referred to the endodontic consultation clinic at the Dental Institute of Guy's Hospital undergoing endodontic treatment (including non-surgical root canal retreatment and periapical surgery). All participants were over 18 years old, had no other systemic diseases such as diabetes or CVDs, and did not meet the NCEP ATP III criteria for MetS [23]. This was the same prospective cohort (N = 65) as stated in our previous study [6, 19, 20, 24] (Table S1). Exclusion criteria included smoking, pregnancy or lactation, uncontrolled periodontal condition with pockets probing deeper than 4 mm, subjects with systemic inflammatory condition (e.g. inflammatory bowel diseases, rheumatoid arthritis, ulcerative colitis, liver diseases, or autoimmune conditions), medications altering bone metabolism, unrestorable teeth, and antibiotics in the last 3 months. This study complied with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines (Fig. 1).

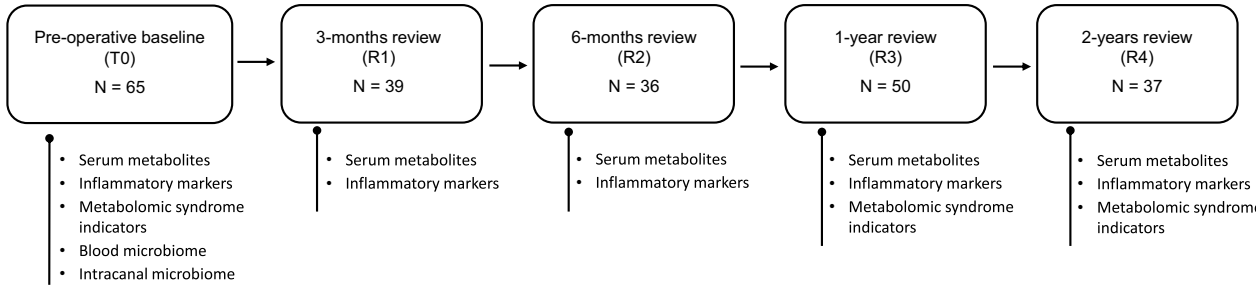

Fig. 1 Flowchart of the cohort showing the five time points and the data collected at each point. T0: preoperative baseline, R1: 3 month post-operative review, R2: 6 month post-operative review, R3: 1 year post-operative review, R4: 2 year post-operative review

Sample collection and processing

Serum samples were taken at 5 time points (pre-operative baseline, 3 month, 6 month, 1 year, and 2 year post-operative reviews) for NMR metabolomic analysis. To investigate the associations of the metabolites with serum inflammatory biomarkers, MetS indicators, as well as blood and intracanal data from our previous studies were used for the correlation analysis [6, 19, 20, 24]. Serum inflammatory markers, including IL-1β, IL-6, IL-8, MMP-2, MMP-8, MMP-9, hs-CRP, pentraxin-3, TNF-α, E-selectin, VCAM-1, ICAM-1, and FGF-23, were quantified using Magnetic Human Premixed Multi-analyte Assay Kit (R&D systems, Bio-techne, Minnesota, USA), along with ADMA and C3 quantified using enzyme-linked immunosorbent assay at each time point. These inflammatory markers were selected as they have been repeatedly linked to AP in previous meta-analyses, and on metabolic aspects demonstrated robust epidemiological links to cardiometabolic risk [25, 26]. MetS indicators including HbA1c level, lipid profile, waist circumference (WC) and body mass index (BMI) were also obtained, among which HbA1c and lipid profile were measured from whole blood using A1cNow and CardioChek PA (BHR Pharmaceuticals, Warwickshire, UK), respectively. Intracanal samples were collected by a trained clinician following a strict protocol [24]. Bacterial DNA was extracted from intracanal and whole blood samples and subjected to targeted sequencing of the V1-V2 hypervariable region of bacterial 16S rRNA gene, performed by Eurofins Genomics (Eurofins Genomics, Constance, Germany) with Illumina MiSeq 300. Detailed procedures for sample collection and processing are described in the supplementary material.

Clinical outcome assessment

Following standard endodontic procedures for root canal treatment, root canal re-treatment, or periapical surgery, the success of the treatment was defined in accordance with the ESE 2006 guidelines [27]. Periapical healing was assessed by periapical radiographs and cone beam computed tomography (CBCT) scans (see supplementary material). The assessment was performed by two calibrated endodontic specialists and the radiographic outcomes were classified according to the six-point system described by Patel et al. (2012) [28].

Nuclear magnetic resonance

A total of 44 metabolites were analysed from the serum samples collected at 5 time points using NMR metabolomics analysis (Table S2). These metabolites were selected due to their repeatedly associations with insulin resistance, incident type 2 diabetes, and cardiovascular risk as reported in previous studies [29, 30]. For ^{1}H-NMR spectroscopy analysis, all spectra were acquired on a Bruker Avance NEO 600 MHz equipped with a TCI Cryoprobe Prodigy (Bruker Biospin, Karlsruhe, Germany), operating at a proton frequency of 600.2 MHz at 298 K. The PROJECT1 pulse sequence was used, with a spectral window of 20.8 ppm, a total spin-echo time of 77 ms, a relaxation time of 4 s, an acquisition of 2.62 s and 64 scans. Additionally, a diffusion-ordered spectrum was applied to look at lipoproteins, using a gradient of 80% for the diffusion, a spectral width of 29.75 ppm, a relaxation time of 4 s, an acquisition time of 1.84 s, 64 scans. TSP peak was used as internal reference. The spectra were then analysed in TopSpin (Bruker Biospin, Karlsruhe, Germany). A 0.3 Hz exponential line broadening function was applied before Fourier transformation and automatic phase correction. The data were normalized to arbitrary units using probabilistic quotient normalization.

Statistical analysis

To assess the associations between metabolite abundance and clinical MetS indicators while adjusting for potential confounders, generalized linear models were applied, which included multiple covariates (e.g., BMI, blood pressure, age, gender, HbA1c, and TC/HDL ratio) as fixed effects and subject ID as a random effect to account for repeated measures in the longitudinal design. The association strength was assessed using β coefficients from the regression model. The p-values were corrected for multiple testing using the Benjamini–Hochberg False Discovery Rate (FDR) method.

For comparisons between the levels of serum metabolites across different time points, mixed-effects analyses with Geisser-Greenhouse corrections were performed with Tukey's post-hoc using GraphPad Prism (version 10.0.2). Correlation tests were performed using *psych* package in R (version 4.3.2) based on Spearman correlation coefficient. Pairwise deletion was utilized to manage missing values. Principal component analyses (PCA) on the metabolic profile were performed using GraphPad Prism. The correlation networks were constructed using Gephi (version 0.10.1) by keeping significant correlations ($p < 0.05$). Heatmap showing how serum metabolites associated with other indicators were plotted using *pheatmap* and *ggplot2* packages in R.

Dynamic Bayesian modeling

To investigate the temporal shifts in the metabolomic profiles following endodontic treatment, we employed dynamic Bayesian network modeling, which enables the inference of the probabilistic dependencies and directed relationships among metabolites over time. After normalizing the data and addressing the missing values using forward-filling, metabolite abundances were processed using *bnlearn* and *dbnR* packages in R. The network structure was learned using Hill-Climbing algorithm. Temporal constraints were enforced by prohibiting reverse edges from later to earlier time points.

The learned network structure was then exported to Cytoscape (version 3.10.3) for visualisation, where the topological properties of each metabolite was analysed. This approach provides insights into the key regulating metabolites during post-treatment recovery, as well as the metabolic pathways involved in periapical healing. The regulatory role of each metabolite was assessed based on its betweenness centrality, which in short quantifies how frequently a metabolite appears on the shortest link between others.

Results

Demographic and clinical assessment

The age, gender, clinical symptom, and size of periapical radiolucency (SOR) of the cohort are summarised in Table S1. The MetS indicators and the genus-resolution abundance in the intracanal and blood microbiome were summarised in Table S3–S5. A total of 44 serum metabolites were analysed (Table S2). Among these, 24 metabolites demonstrated significantly altered levels during follow-up when compared to the baseline. These metabolites were primarily involved in amino acids, glucose, and lipid metabolism.

The potential influence of these demographic and clinical factors on the longitudinal changes of the analysed metabolites was assessed based on generalised linear models (Fig. S1a). The results showed that the TC/HDL ratio was positively associated with serum fatty acids levels ($p = 0.0053$, Fig. S1b). Other demographic and clinical factors, including age, BMI, blood pressure (systolic and diastolic), waist circumference (WC), SOR, and HbA1c, did not show significant associations with metabolite profiles after multiple testing correction ($p > 0.05$), suggesting that they do not act as confounding factors in our dataset.

Based on our radiographic assessment, 4 participants demonstrated failure at 1 year review. The remaining cases demonstrated healing or healed periapical radiolucency. Whilst at 2 year review, treatments for all participants were considered radiographically successful, with reduced or fully resolved periapical lesions.

Alterations in amino acids profile

The amino acid profile demonstrated robustness postoperatively (Fig. 2a–c). Although the overall proportion of each amino acid remained stable across baseline and reviews, the levels of leucine, valine, glutamic acid, asparagine, ornithine, glycine, histidine, and tryptophan had significant changes throughout the follow-up compared to their baseline levels (mixed-effects analysis with Greenhouse–Geisser correction, $p < 0.05$). Their levels, except for tryptophan, showed significant decreases at one or more time points during the follow-up period when compared to baseline levels (Fig. 2d). On the contrary, tryptophan level demonstrated a progressive and extensive increase at the review appointments compared to the baseline ($p < 0.001$; $p < 0.0001$; $p < 0.0001$; $p < 0.001$ respectively), underlining its unique role in the healing process of periapical lesions. It should also be noted that the branched chain amino acids (BCAAs) including valine, leucine, and isoleucine made a higher contribution to the compositional dissimilarity in the amino acid profile (Fig. 2c).

Alterations in glucose metabolism and energy production

Some metabolites involved in glucose metabolism and energy production via glycolysis, gluconeogenesis, and the tricarboxylic acid cycle (TCA cycle) were also analysed. These included the BCAAs, citric acid, glutamic acid, glucose, and pyruvate. The shared pathways of these metabolites are summarised in Fig. 3a. Our results suggested significant changes in the serum levels of these metabolites (Fig. 3b). The BCAAs, more specifically leucine and valine, reduced significantly at 3 month ($p < 0.0001$ and $p = 0.0296$, respectively) while the citric acid level increased at 3 month and 6 month reviews ($p = 0.0006$ and $p = 0.0273$, respectively) when compared to their pre-treatment levels. At the 1-year review, the serum level of glutamic acid showed a significant decrease ($p < 0.0001$).

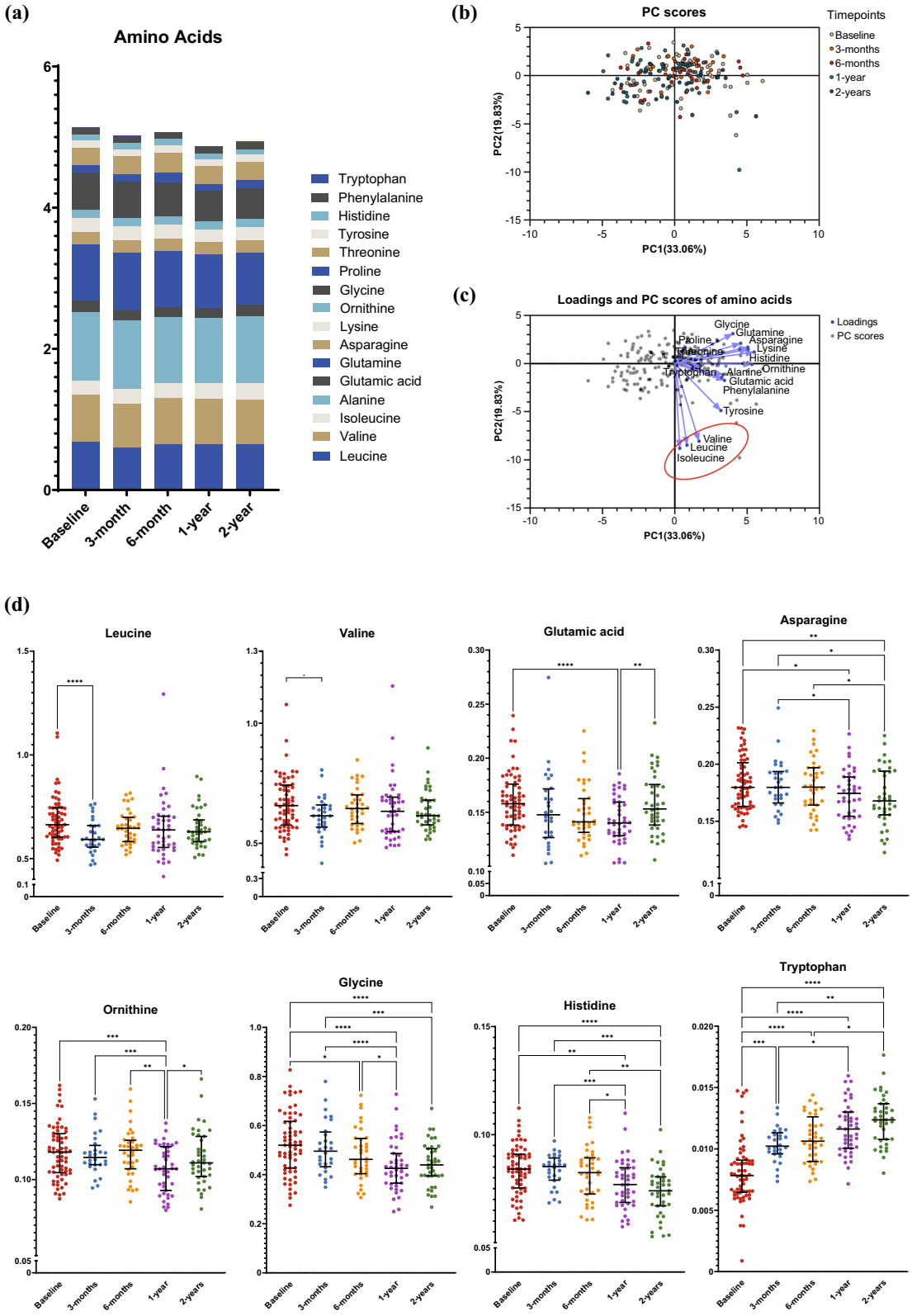

Fig. 2 **a** Stacked bar plot showing the proportion of the analysed amino acids at different time points. **b** PCA showing the compositional differences of amino acids. Both plots indicated a stable amino profile throughout the review process. **c** The loadings of each amino acids are shown in blue vectors. A longer vector indicated a higher contribution to the compositional dissimilarity among groups in terms of amino acids. **d** Scatter plots showing the amino acids that showed significant changes during the review process when compared to baseline * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, mixed-effects analysis with Greenhouse–Geisser correction. Levels of the metabolites are presented in normalized arbitrary units

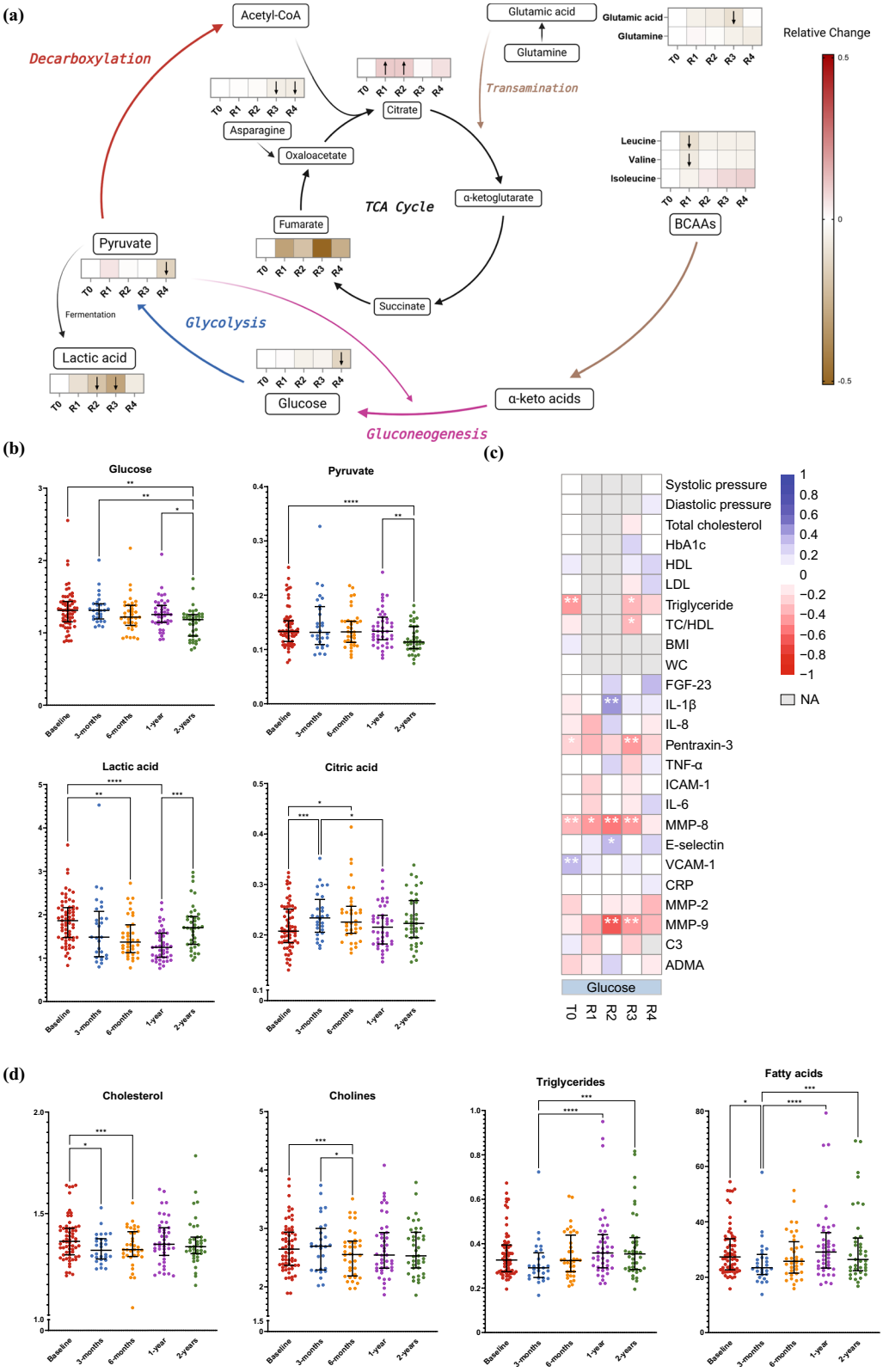

Fig. 3 (See legend on next page.)

(See figure on previous page.)

Fig. 3 **a** A schematic diagram showing the pathways associated with energy production and glucose metabolism involving the analysed metabolites. For each analysed metabolite, a heatmap shows relative change in its levels. Arrows in the heatmap represented significant increase (↑) or decrease (↓) in levels compared to baseline. (Figure created with BioRender). **b** Scatter plots showing the changes in glucose, pyruvate, lactic acid, and citric acid levels after treatment (mixed-effects analysis with Greenhouse–Geisser correction). **c** Heatmap showing the Spearman correlation coefficient between serum glucose level and inflammatory markers, MetS indicators, and lipid profiles. **d** Scatter plots showing the changes in the levels of lipid-associated metabolites at the baseline and post treatment reviews (mixed-effects analysis with Greenhouse–Geisser correction). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. T0: baseline, R1: 3 month, R2: 6 month, R3: 1 year, R4: 2 year. Levels of the metabolites are presented in normalized arbitrary units

Furthermore, when compared to baseline levels, glucose and pyruvate decreased significantly at the 2 year review ($p = 0.0079$ and $p < 0.0001$, respectively) and lactic acid decreased significantly at 6 month and 1 year reviews ($p = 0.0020$ and $p < 0.0001$, respectively). These alterations suggested improved glucose tolerance and long-term benefits for glucose metabolism following successful endodontic treatment. We also computed the correlations between serum glucose levels and the inflammatory biomarkers as well as MetS indicators (Fig. 3c). Unlike other time points, at the 2 year review when glucose significantly decreased, the serum glucose level was not associated with any inflammatory markers or MetS indicators. This was indicative that improved glucose metabolism was associated with reduced inflammatory burden after endodontic treatment.

Alterations in lipid metabolism

We also observed significant alterations in the metabolites associated with lipid metabolism, including cholesterol, choline, and fatty acids (Fig. 3d). After treatment, cholesterol levels were significantly lower at 3 and 6 month reviews when compared to baseline level ($p = 0.0314$ and $p = 0.0007$, respectively). Choline levels also reduced significantly at 6 month review ($p = 0.0004$). In addition, the levels of fatty acids ($p = 0.0180$) reduced significantly at the 3 month review and then recovered at a later period. Triglyceride, although not significantly reduced, demonstrated a tendency to decrease at 3 month review and then increased at 1 year and 2 year reviews. The same tendency was also observed in fatty acids levels. Together, these findings highlighted a link between successful endodontic treatment and a short-term benefit on lipid metabolism.

Correlations between serum metabolites and the other parameters

Based on Spearman correlation coefficients, we investigated how serum metabolites were associated with levels of MetS indicators (at baseline, 1 year, and 2 year reviews) and inflammatory biomarkers (at baseline, 3 month, 6 month, 1 year, and 2 year reviews), as well as the abundance of blood and intracanal microbes at baseline. The rho and p values of all correlations are presented in Table S6. Baseline correlations were visualised as a network (Fig. 4). In general, levels of matrix metalloproteinase-8 (MMP-8), complement component 3 (C3), and triglyceride, the abundance of *Mycobacterium* and *Acinetobacter* in blood, as well as the abundance of *Afipia*, *Bacillus*, and *Flavitalea* in root canal had the most correlations with the metabolomic profile. Significant correlations with $|\text{rho}| > 0.5$ are summarised in Fig. 5a. The longitudinal changes in the rho values for each metabolite and MetS indicator/inflammatory marker pair are presented in Fig. 5b and c.

Serum metabolites and metabolic syndrome indicators

Triglyceride was highly correlated with the metabolomic profile at baseline, 1 year, and 2 year reviews (Fig. 5b). Throughout the review process, it was positively associated with the levels of acetone, mannose, cholesterol, and fatty acids, and was negatively associated with the levels of acetic acid, asparagine, citric acid, creatine phosphate, formic acid, glutamine, glycine, histidine, lysine, methanol, ornithine, and proline. In addition, HDL and total cholesterol were positively correlated with the level of choline. TC/HDL was positively correlated with the level of mannose.

Stronger correlations emerged at the 2 year review including the positive correlations between BMI and isoleucine; HbA1c and leucine; LDL and choline; WC and isoleucine; and WC and leucine, as well as the negative correlations between LDL and proline; TC/HDL and acetic acid; TC/HDL and glutamine; and triglyceride and hypoxanthine (Fig. 5b).

Serum metabolites and inflammatory markers

The correlations between serum metabolites and inflammatory markers were less robust than those between metabolites and MetS indicators, exhibiting significant correlations only at certain time points (Fig. 5c). The most robust correlation was identified between MMP-8 and glucose, which were significantly negatively correlated at baseline, 3 month, 6 month, and 1 year reviews. IL-6 and glutamine were significantly negatively correlated at 3 month, 6 month, and 1 year reviews. However, at the 2 year review, the correlation became positive. Strong significant positive correlations were present between IL-6 and glutamine (2 year) and mannose (6 month, 1 year); IL-8 and alanine (2 year) and lactic acid (3 month and 2 year); and MMP-8 and glutamic acid (3 month), lactic acid (3 month, 1 year), and phenylalanine (3 month). Strong negative correlations were present between FGF-23 and citric acid (6 month,

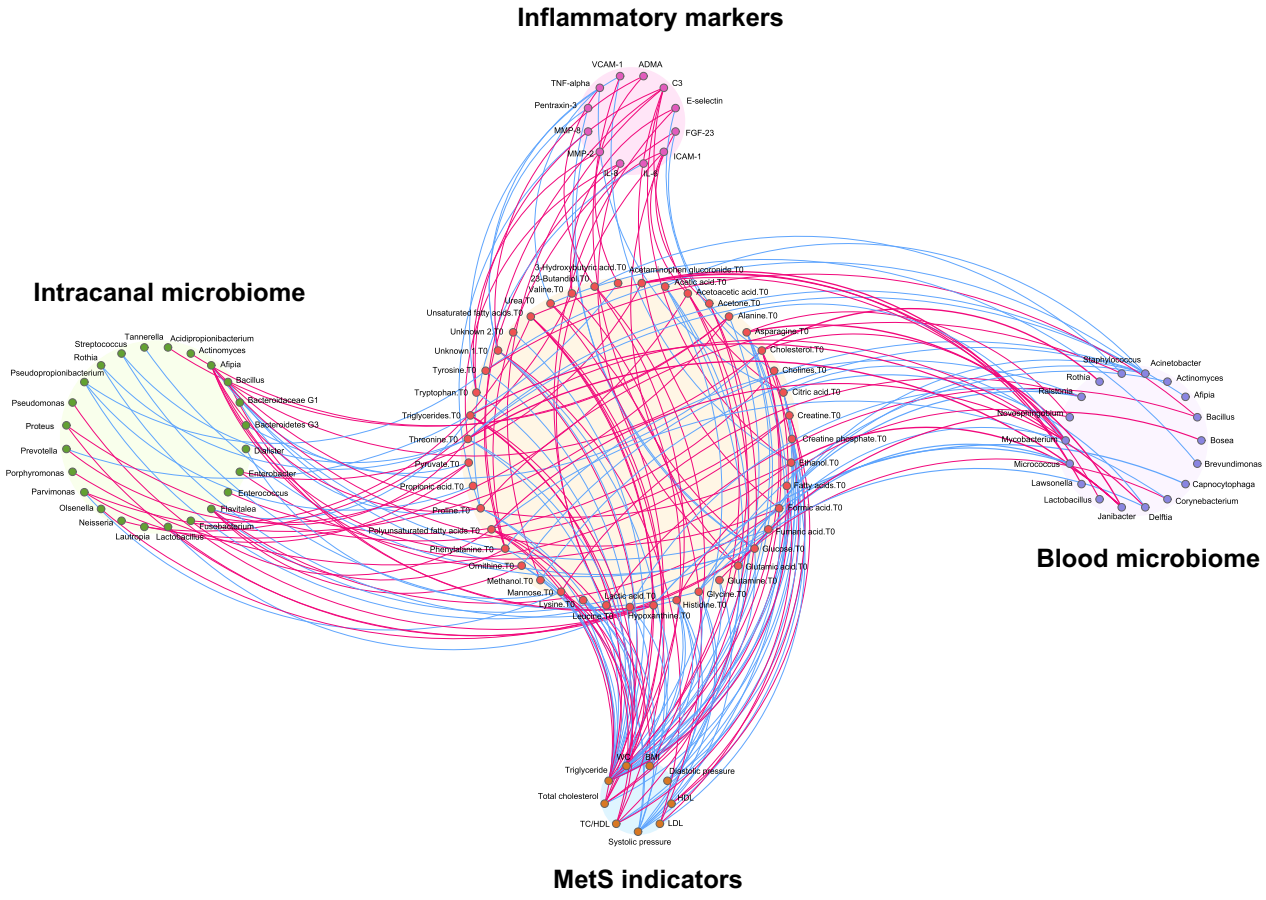

Fig. 4 Network showing the correlations between serum metabolites (middle circle) with inflammatory biomarkers, MetS indicators, blood and intracanal microbiome. Blue lines indicated negative correlations while red lines indicated positive ones

1 year); ICAM-1 and hypoxanthine (3 month); IL-6 and glycine (1 year) and histidine (6 month, 1 year); IL-8 and glutamine (3 month); MMP-2 and glycine (1 year), methanol (1 year), and threonine (6 month); MMP-8 and glutamine (3 month, 1 year); MMP-9 and acetic acid (1 year), glucose (6 month, 1 year), glutamine (3 month, 1 year), and mannose (3 month), and also between TNF-alpha and glycine (6 month, 1 year) (Fig. 5c and Table S6).

Serum metabolites and blood/intracanal microbiome

The correlations between metabolites and the preoperative blood microbiome were found to be very strong (Table S6). The strongest correlations were those between *Rothia* and cholesterol (rho = 1, $p < 0.0001$), *Janibacter* and ethanol (rho = 1, $p < 0.0001$), asparagine (rho = 1, $p < 0.0001$), as well as threonine (rho = 1, $p < 0.0001$). Amongst other strong correlations, *Acinetobacter*, *Ralstonia*, *Delfia*, *Micrococcus*, and *Mycobacterium* had the most associations with serum metabolites (Fig. 4). Specifically, *Acinetobacter* was negatively associated with acetic acid (rho = −0.62, $p = 0.02$), glutamic acid (rho = −0.64, $p = 0.01$), ornithine (rho = −0.66, $p = 0.01$), methanol (rho = −0.62, $p = 0.02$), threonine (rho = −0.56, $p = 0.04$), and tyrosine (rho = −0.57, $p = 0.03$). *Ralstonia* was negatively associated with glutamic acid (rho = −0.88, $p < 0.001$), lactic acid (rho = −0.72, $p = 0.03$), and phenylalanine (rho = −0.72, $p = 0.03$), and was positively associated with mannose (rho = 0.72, $p = 0.03$). *Delfia* was negatively associated with citric acid (rho = −0.83, $p = 0.04$) and glycine (rho = −0.83, $p = 0.04$), and was positively associated with triglyceride (rho = 0.89, $p = 0.02$). *Micrococcus* was negatively associated with glycine (rho = −0.89, $p = 0.02$) and glucose (rho = −0.89, $p = 0.02$), and was positively associated with acetaminophen glucuronide (rho = 0.94, $p < 0.001$). *Mycobacterium* was negatively associated with alanine (rho = −0.75, $p = 0.02$), citric acid (rho = −0.67, $p = 0.05$), and glucose (rho = −0.67, $p = 0.05$), and was positively associated with threonine (rho = 0.72, $p = 0.03$), cholesterol (rho = 0.78, $p = 0.01$), polyunsaturated fatty acids (rho = 0.82, $p = 0.01$), and unsaturated fatty acids (rho = 0.77, $p = 0.02$).

In terms of the intracanal microbiome, significant correlations with specific metabolites were also present. The significant positive correlations were between acetaminophen glucuronide and *Enterobacter* (rho = 0.71, $p = 0.010$); hypoxanthine and *Bacillus* (rho = 0.68, $p = 0.001$); and

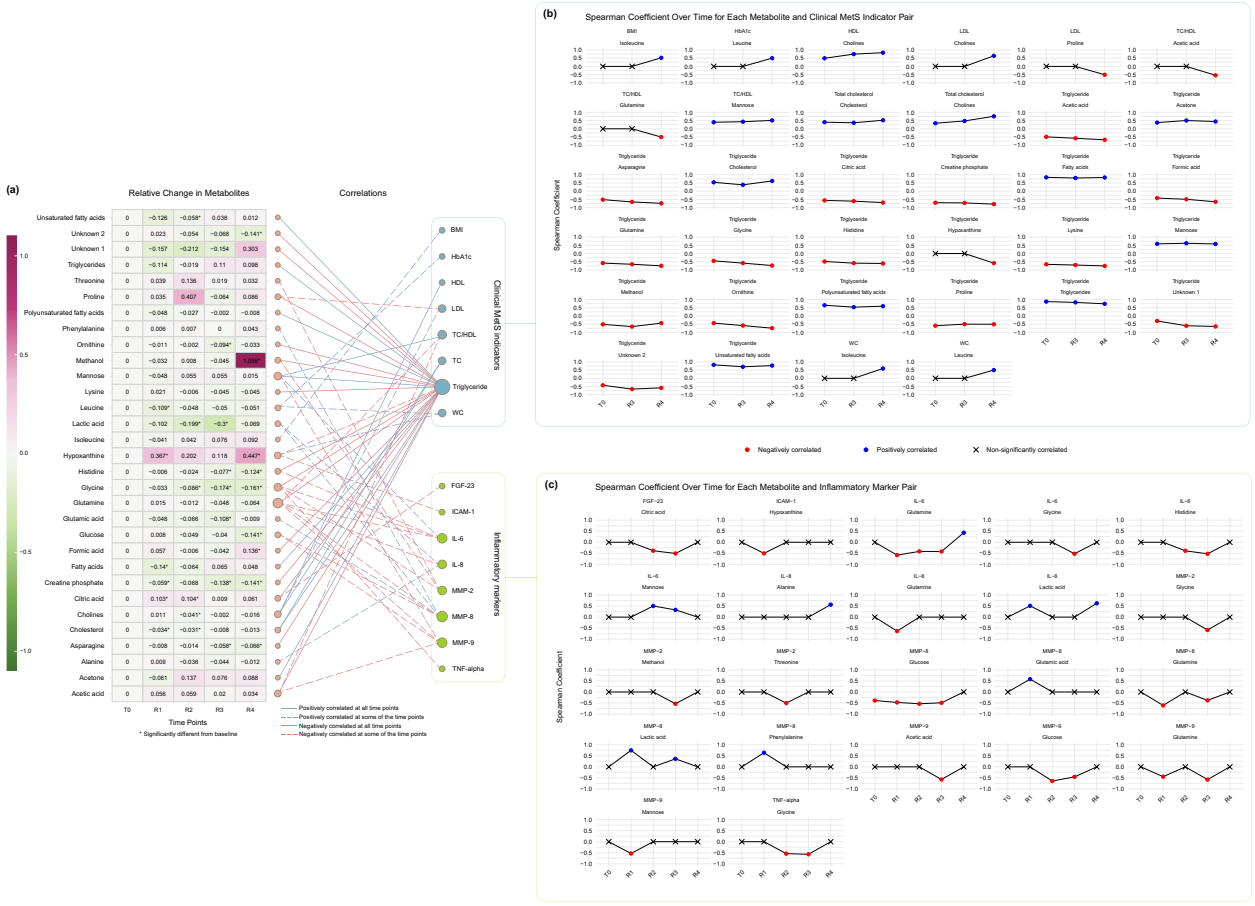

Fig. 5 **a** Correlations between serum metabolites and MetS indicators and inflammatory markers are shown in blue (significantly positive) and red (significantly negative) lines. The relative changes in levels of the related metabolites are shown in the heatmap. **b** Longitudinal changes in Spearman coefficients for each metabolite and MetS indicator pair, with rho values greater than 0.5 or less than − 0.5 at one or more time points. **c** Longitudinal changes in Spearman coefficients for each metabolite and inflammatory marker pair, with rho values greater than 0.5 or less than − 0.5 at one or more time points

ethanol and *Enterobacter* (rho = 0.62, $p = 0.031$), whereas significant negative correlations were between proline and *Bacteroidaceae G1* (rho = − 0.76, $p = 0.011$); glucose and *Bacillus* (rho = − 0.62, $p = 0.005$) (Fig. 4).

Dynamic Bayesian modeling

A dynamic Bayesian network was constructed and analysed to identify key regulatory metabolites throughout the review process (Fig. 6a). The regulatory influence of each metabolite was assessed using betweenness centrality. Based on the dynamic Bayesian analysis, the top 10 metabolites with the highest betweenness centrality included acetaminophen glucuronide (1 year), valine (6 month), glutamine (6 month), lysine (3 month, 1 year, and 2 year), methanol (6 month), alanine (1 year), ornithine (6 month), and threonine (6 month). Although the abundances of these key regulators were not significantly different when compared to their baseline levels (Fig. 6b), the topological analysis revealed that they exhibited either high outdegree or high indegree values (Fig. 6c), indicating their strong involvement in the modulation of other metabolites. This highlighted their pivotal roles in shaping the temporal dependencies among serum metabolites.

An essential metabolic pathway shared by these regulators is the TCA cycle (Fig. 6d), which plays a central role in cellular energy production. Valine can be metabolized into succinyl-CoA, which directly enters the TCA cycle to replenish intermediates and enhance ATP production. Glutamine and ornithine undergo a series of metabolic transformations to generate α-ketoglutarate (α-KG), a critical intermediate that fuels energy metabolism through TCA cycle. Similarly, alanine and threonine contribute to TCA cycle replenishment by converting into pyruvate, which is further metabolized into succinyl-CoA. Lysine can be degraded into acetyl-CoA, contributing to citrate synthesis in the TCA cycle, supporting energy production.

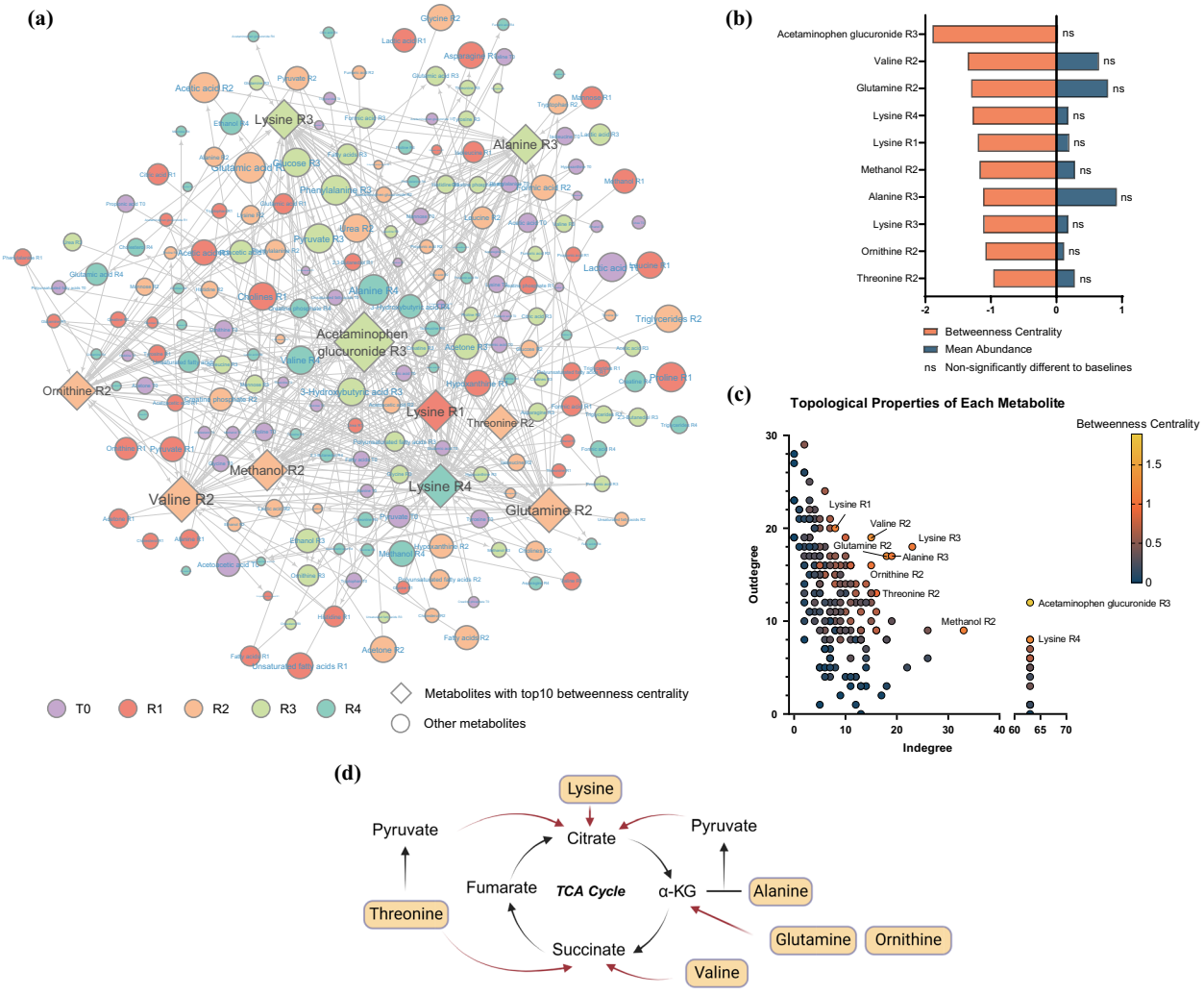

Fig. 6 **a** A dynamic Bayesian network showing the temporal dependencies of the metabolites across the review period. Nodes are presented as diamonds (metabolites with top 10 betweenness centrality, namely key regulators) and circles (other metabolites). A larger node implicated higher betweenness centrality. Different colours represent different time points. Only the edges linked to the key regulators are presented. **b** The betweenness centrality and mean abundance of the key regulators. **c** Topological properties of each metabolite. **d** A schematic diagram showing that some of the key regulators are closely linked to the TCA cycle

Discussion

Previous studies showed that the presence of AP contributed to bacteraemia and elevated CVD-related inflammatory markers, and that successful endodontic treatment was beneficial for long-term reduction in these inflammatory markers [6, 19, 24]. In this study, we further analysed 44 serum metabolites from AP subjects through the same 2 year longitudinal cohort. The results supported our hypothesis that endodontic treatment was also associated with exceptional improvements in amino acids, glucose, and lipid metabolism. These improvements were further linked with energy production and bone remodeling possibly via the TCA cycle.

The BCAAs were the most contributing amino acids to the dissimilarity in the amino acids profile across baseline and reviews (Fig. 2c). Human epidemiological studies suggested positive associations between circulating BCAA levels and the development of cardiometabolic disorders such as diabetes, hypertension, and atherosclerotic cardiovascular disease. Increased BCAA levels and upregulated BCAA turnover are linked with chronic systemic inflammation [31, 32]. Previous studies on generalised periodontitis reported reduction in BCAAs after periodontal therapies [33]. This is in line with our results where circulating BCAA levels reduced significantly at 3 months (Fig. 2d), which was due to shifted dynamics of BCAA metabolism possibly consequent to periapical healing and remodeling after endodontic treatment. Previous studies suggested that increased circulating BCAAs are associated with insulin resistance and are linked with

higher triglyceride and lower HDL [34–36]. Accordingly, the post-treatment decrease in BCAAs may be indicative of improved glucose tolerance and lipoprotein metabolism.

In the periodontal field, randomized trials and meta-analyses has shown that periodontal therapy can improve systemic metabolic indices, including short-term reductions in glycaemic measures and favourable changes in lipid profiles [16–18]. Similarly for AP, our longitudinal data indicate that successful endodontic treatment is associated with improvements in glucose and lipid metabolism.

Improvements in glucose metabolism were reflected in the changes of glucose and pyruvate levels. The associations between circulating glucose level and various cardiometabolic disorders have been well documented [37]. During states of inflammation, elevated inflammatory cytokines can cause insulin resistance and subsequently increase the circulating glucose level [38]. Elevated glucose can be converted into vascular deleterious agents by pro-inflammatory stimuli, which in turn exacerbates the pro-inflammatory pathways [39]. In short, there is a mutually reinforcing relationship between hyperglycaemia and systemic inflammation. Our results showed a significant progressive reduction in glucose and pyruvate after endodontic treatment, especially at the 2 year review (Fig. 3b), highlighting that successful endodontic treatment is associated with on glucose tolerance and reduced systemic inflammatory burden.

Alterations in lipid metabolism were evidenced by the significant reduction in cholesterol, choline, and fatty acids at 3 months or 6 months review (Fig. 3d) and indicated a potential link between short-term benefit on lipid metabolism and endodontic treatment. However, we were unable to further subdivide fatty acids and cholesterol based on our NMR strategy, which precluded us from drawing explicit conclusions in terms of improvements in lipid metabolism. Nonetheless, although not statistically significant, a trend of reduction was observed in serum triglyceride level at 3 month review, which was also a sign of improved lipid metabolism. This may arise from the clearance of bacterial infection with reduced endotoxemia and pro-inflammatory mediators, inducers of de novo fatty acid and triglyceride synthesis [40]. Indeed, baseline triglyceride level was strongly positively correlated with *Delftia* abundance in blood (rho = 0.89. $p = 0.019$) and had a clear tendency to correlate positively with blood *Ralstonia* and *Sphingomonas* (rho = 0.58 and 0.53, $p < 0.1$) as well as intracanal *Dialister* (rho = 0.40, $p < 0.1$) (Table S6).

Tryptophan is involved in various biological processes such as via kynurenine pathway, where tryptophan is degraded into kynurenine metabolites, which have pivotal roles in CVDs [41, 42]. Studies showed that serum levels of tryptophan are inversely associated with mortality and fatal CVDs while its downstream kynurenine metabolites were positively correlated [43]. Increased serum kynurenine-to-tryptophan ratio under proinflammatory condition is associated with systemic conditions such as MetS, atherosclerosis, and acute myocardial infarction [44]. It is therefore considered a predictive biomarker for CVDs. In our study, lower tryptophan levels at baseline were probably due to its catabolism caused by the inflammatory burden of AP. However, after successful endodontic treatment, when inflammation decreased, a constant and significant increase in serum tryptophan level was observed, with a 2 year serum tryptophan level almost twice that at the baseline (Fig. 2d), implying reductions in both systemic inflammation and CVD risks. Overall, we suggest that serum tryptophan can serve as an indicator predicting the prognosis of AP and AP-related risks for developing CVDs.

According to our correlation tests, triglyceride demonstrated the most and strongest correlations with subjects' metabolic profile, especially cholesterol and fatty acids, positioning it as a potential predictor for AP prognosis after treatment. In our earlier study [12], the AP-associated increase of triglycerides was due to TG-enriched very-large VLDL particles and small- and medium-sized HDL particles. This suggests that the decreased lipoprotein lipase and cholesterol ester transfer protein activity during inflammation maintains high TG-enriched particle concentrations [40, 45]. TG-enriched lipoproteins are causally associated with both low-grade inflammation and CVD risk. Further studies are needed to investigate the details of the molecular mechanisms how triglycerides, their fatty acid composition, and saturation degree are affected by AP treatment.

In our study, bacteria in blood were detected even before starting endodontic treatment and Bakhsh et al. (2025) confirmed endodontic source of this bacteraemia [24]. This AP-associated bacteraemia may contribute to a chronic, systemic effects. This was highlighted in our study as both intracanal and blood microbiome genera were strongly correlated with several serum metabolites, showing their potential impact on systemic metabolic profile. In addition to this oral-blood-axis, oral bacteria may associate with lipid measures through other pathways. These could be the oral-gut axis affecting the major role of the gut microbiome in the regulation of metabolism and immunity, or even the gut-brain-axis putatively modulating the eating behaviours [46]. Recent studies showed that *Rothia* species, which were recovered in the present study from both intracanal and blood samples, are relevant to infective endocarditis by building destructive biofilms in the heart valve [47]. Similarly, nontuberculous mycobacterium is also associated with cardiac abnormalities and infective endocarditis [48, 49]. In the

present study, the strong positive correlations between blood *Mycobacterium* abundance and lipid parameters suggests that lipid metabolism improvement associated with successful endodontic treatment might also be beneficial in reducing *Mycobacterium*-related CVD risks. However, the complex lipid-immune interactions including active lipid mediators and immunomodulatory properties of lipids are poorly studied concerning the oral infections. Thus, they warrant for further research since oral infections may accelerate progression of chronic cardiometabolic diseases and threat general health in the form of acute infections [50].

In this study, the metabolomic profiles exhibited time-series and multi-dimensional characteristics, necessitating a dynamic probabilistic model to infer temporal dependencies amongst all metabolites at different time points [51, 52]. To achieve this, we employed dynamic Bayesian modeling to highlight the influential metabolites throughout the review period. Notably, most of the key regulatory metabolites identified were closely linked to the TCA cycle, a central hub of energy metabolism that oxidizes nutrients to produce ATP and biosynthetic precursors [53]. The TCA cycle is intricately connected to inflammation through the accumulation of its intermediates [54]. Within an inflammatory microenvironment, macrophages undergo metabolic rewiring to adapt to altered energy demands [55], shifting from high oxidative phosphorylation to aerobic glycolysis and disrupting TCA cycle [56]. This impairment results in the accumulation of intermediates such as fumarates [57, 58], which have been implicated in immune regulation and disease progression. Furthermore, the impaired TCA cycle also affects bone metabolism, potentially through mammalian target of rapamycin (mTOR) signaling pathway. The mTOR pathway regulates various energy- and nutrient-intensive cellular processes [59], including osteoblast activation and differentiation [60], and is highly sensitive to cellular levels of ATP [61]. Consequently, the impaired TCA cycle leads to ATP depletion, inhibiting mTOR signaling and subsequently reducing bone remodeling.

Through dynamic Bayesian analyses, we identified valine, lysine, ornithine, threonine, alanine, and glutamine as key regulators in the longitudinal alterations in the metabolomic profiles. These amino acids exhibit a strong association with the TCA cycle (Fig. 6a). Along with alterations in the abundance of other TCA cycle-related metabolites (Fig. 3a), we hypothesize that successful endodontic treatment may restore impaired TCA cycle function by improving glucose and lipid metabolism. The restoration of TCA cycle activity may, in turn, promote periapical bone regeneration via the mTOR signaling pathway. However, further studies are required to elucidate the underlying mechanisms. Interestingly, we also found that acetaminophen glucuronide and methanol, which are typically considered exogenous metabolites, also exert extensive regulatory effects on the metabolic network. However, their specific regulatory roles and mechanisms in the healing of periapical inflammatory lesions remain to be further explored.

Several cautions are warranted when interpreting our findings. Firstly, the sample size was modest (n = 65). Although we applied strict inclusion criteria and conservative statistical procedures to capture subtle metabolomic shifts associated with endodontic treatment, larger studies in broader populations are needed to validate these associations. Another major limitation is that the absence of control groups—either individuals without AP or cases with unsuccessful treatment—precluded us from assessing whether baseline metabolomic profiles in AP differ from those of healthy individuals or whether adverse metabolic states persist or even worsen in unsuccessful cases. Nonetheless, whilst longitudinally comparing the serum metabolites, we also compared their levels at 1 year review between healing/healed cases (n = 46) and failed cases (n = 4) within the cohort (Fig. S2). The differences were not statistically significant, likely due to limited subgroup sizes, with only 4 failed cases at 1 year. Future studies with appropriate control groups, including healthy individuals and cases of unsuccessful treatment, are warranted to better elucidate the baseline metabolic disparities and their potential trajectories following different treatment outcomes. Thirdly, despite rigorous recruitment criteria and evaluation of selected confounders (Figure S1), residual confounding factors (e.g., dietary habits) may have influences on metabolomic profiles or periapical healing. These factors should be systematically recorded and adjusted for in future work.

Taken together, our data support an association between successful endodontic treatment and improvements in glucose and lipid metabolism; however, they do not establish a causal effect of endodontic treatment on systemic metabolism. Definitive conclusions on the causative role of successful endodontic treatment will require well-powered, controlled epidemiological studies and complementary mechanistic experiments in animal models.

Conclusion

In summary, our findings suggest that successful endodontic treatment in AP patients is associated with short-term benefits in lipid profile and long-term improvements in glucose tolerance, and a reduction in systemic inflammation. These metabolic improvements are potentially associated with energy production and bone remodeling in the post-treatment healing process, possibly by restoring the TCA cycle function. Notably, serum tryptophan, triglyceride, and glucose levels emerge as potential AP prognostic biomarkers for monitoring

post-treatment healing and assessing AP-related cardiometabolic disease risks. Taken together, this study highlights the broader systemic benefits of successful endodontic treatment beyond oral health, suggesting a potential role in mitigating cardiometabolic disease risk and the clinical significance of metabolic monitoring in AP patients undergoing endodontic treatment.

Abbreviations

AP	Apical periodontitis
HbA1c	Glycosylated haemoglobin
CVD	Cardiovascular diseases
NMR	Nuclear magnetic resonance
MetS	Metabolic syndrome
IL	Interleukin
MMP	Matrix metalloproteinase
hs-CRP	High-sensitivity C-reactive protein
TNF	Tumor necrosis factor
VCAM	Vascular cell adhesion molecule
ICAM	Intercellular adhesion molecule
ADMA	Asymmetric dimethylarginine
C3	Complement component 3
WC	Waist circumference
BMI	Body mass index
CBCT	Cone beam computed tomography
FDR	False discovery rate
PCA	Principal component analyses
SOR	Size of periapical radiolucency
TC	Total cholesterol
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
BCAA	Branched chain amino acid
mTOR	Mammalian target of rapamycin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-07110-0.

Additional file1

Additional file2

Acknowledgements

We thank the Centre for Biomolecular Spectroscopy funded by the Wellcome Trust and British Heart Foundation (ref. nos. 202767/Z/16/Z and IG/16/2/32273) for their efforts in conducting NMR analysis. We thank the clinicians and nurses at the post-graduate endodontic clinic at Guy's Hospital for their efforts in sample collection.

Author contributions

YZ contributed to the acquisition, analysis, and interpretation of the data, drafted and substantively revised the manuscript. AG contributed to the acquisition and analysis of the data. PP contributed to interpretation of the data and substantively revised the manuscript. GP contributed to conception and design of the study and substantively revised the manuscript. SN contributed to conception and design of the study, interpretation of the data, drafted and substantively revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was funded by the Royal College of Surgeons of England—FDS Pump Priming Grant 2022. Sadia Ambreen Niazi is the principal grant holder of these grants.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

This study was approved by the London-Hampstead Research Ethics Committee (IRAS project ID 207795). Written consent was obtained from each participant in accordance with the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

Received: 9 June 2025 / Accepted: 8 September 2025
Published online: 18 November 2025

References

1. Sundqvist, G. Bacteriological studies of necrotic dental pulps, Umea University (1976).
2. Abbott PV. Classification, diagnosis and clinical manifestations of apical periodontitis. Endodontic Top. 2004;8:36–54.
3. Niazi SA, Bakhsh A. Association between endodontic infection, its treatment and systemic health: a narrative review. Medicina (Kaunas). 2022;58:931.
4. Braz-Silva PH, et al. Inflammatory profile of chronic apical periodontitis: a literature review. Acta Odontol Scand. 2019;77:173–80. https://doi.org/10.1080/00016357.2018.1521005.
5. Nagendrababu V, Segura-Egea JJ, Fouad AF, Pulikkotil SJ, Dummer PMH. Association between diabetes and the outcome of root canal treatment in adults: an umbrella review. Int Endod J. 2020;53:455–66. https://doi.org/10.1111/iej.13253.
6. Al-Abdulla N, et al. Successful endodontic treatment reduces serum levels of cardiovascular disease risk biomarkers-high-sensitivity C-reactive protein, asymmetric dimethylarginine, and matrix metalloprotease-2. Int Endod J. 2023;56:1499–516. https://doi.org/10.1111/iej.13979.
7. Sánchez-Domínguez B, et al. Glycated hemoglobin levels and prevalence of apical periodontitis in type 2 diabetic patients. J Endod. 2015;41:601–6.
8. Arya S, et al. Healing of apical periodontitis after nonsurgical treatment in patients with type 2 diabetes. J Endod. 2017;43:1623–7. https://doi.org/10.1016/j.joen.2017.05.013.
9. Perez-Losada FD, Estrugo-Devesa A, Castellanos-Cosano L, Segura-Egea JJ, López-López J, Velasco-Ortega E. Apical Periodontitis and Diabetes Mellitus Type 2: a systematic review and meta-analysis. J Clinic Med. 2020;9(2):540.
10. Chauhan N, Mittal S, Tewari S, Sen J, Laller K. Association of apical periodontitis with cardiovascular disease via noninvasive assessment of endothelial function and subclinical atherosclerosis. J Endod. 2019;45:681–90. https://doi.org/10.1016/j.joen.2019.03.003.
11. Liljestrand JM, et al. Association of endodontic lesions with coronary artery disease. J Dent Res. 2016;95:1358–65. https://doi.org/10.1177/0022034516660509.
12. Salminen A, et al. Systemic metabolic signatures of oral diseases. J Dent Res. 2024;103:13–21. https://doi.org/10.1177/00220345231203562.
13. Ye L, et al. Interaction between apical periodontitis and systemic disease (review). Int J Mol Med. 2023;52:60. https://doi.org/10.3892/ijmm.2023.5263.
14. Savarrio L, Mackenzie D, Riggio M, Saunders WP, Bagg J. Detection of bacteraemias during non-surgicalroot canal treatment. J Dent. 2005;33:293–303. https://doi.org/10.1016/j.jdent.2004.09.008.
15. Yamazaki K. Oral-gut axis as a novel biological mechanism linking periodontal disease and systemic diseases: a review. Jpn Dent Sci Rev. 2023;59:273–80. https://doi.org/10.1016/j.jdsr.2023.08.003.
16. Simpson TC, et al. Treatment of periodontitis for glycaemic control in people with diabetes mellitus. Cochrane Database Syst Rev. 2022. https://doi.org/10.1002/14651858.CD004714.pub4.
17. Tonetti MS, et al. Treatment of periodontitis and endothelial function. N Engl J Med. 2007;356:911–20. https://doi.org/10.1056/NEJMoa063186.
18. Ma W, et al. Exploring the bi-directional relationship between periodontitis and dyslipidemia: a comprehensive systematic review and meta-analysis. BMC Oral Health. 2024;24:508. https://doi.org/10.1186/s12903-023-03668-7.
19. Bakhsh A, Moyes D, Proctor G, Mannocci F, Niazi SA. The impact of apical periodontitis, non-surgical root canal retreatment and periapical surgery on serum inflammatory biomarkers. Int Endod J. 2022;55:923–37. https://doi.org/10.1111/iej.13786.

20. Bakhsh A, et al. Apical periodontitis microbiome association with salivary and serum inflammatory burden. Int Endod J. 2025. https://doi.org/10.1111/iej.14184.
21. Takahashi N. Oral Microbiome Metabolism: From "Who Are They?" to "What Are They Doing?" J Dent Res. 2015;94:1628–37. https://doi.org/10.1177/0022034515606045.
22. Debik J, Sangermani M, Wang F, Madssen TS, Giskeodegard GF. Multivariate analysis of NMR-based metabolomic data. NMR Biomed. 2022;35:e4638. https://doi.org/10.1002/nbm.4638.
23. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009;2:231–7. https://doi.org/10.1242/dmm.001180.
24. Bakhsh A, Mannocci F, Proctor G, Moyes D, Niazi S. Links between nosocomial endodontic infections and Bacteraemia associated with apical periodontitis and endodontic treatment. J Endod. 2024. https://doi.org/10.1016/j.joen.2024.11.009.
25. Georgiou AC, Crielaard W, Armenis I, de Vries R, van der Waal SV. Apical periodontitis is associated with elevated concentrations of inflammatory mediators in peripheral blood: a systematic review and meta-analysis. J Endod. 2019;45:1279–95. https://doi.org/10.1016/j.joen.2019.07.017.
26. Gomes MS, et al. Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. J Endod. 2013;39:1205–17. https://doi.org/10.1016/j.joen.2013.06.014.
27. European Society of Endodontology. Quality guidelines for endodontic treatment: consensus report of the European society of Endodontology. Int Endod J. 2006;39(12):921–30.
28. Patel S, Wilson R, Dawood A, Foschi F, Mannocci F. The detection of periapical pathosis using digital periapical radiography and cone beam computed tomography–part 2: a 1-year post-treatment follow-up. Int Endod J. 2012;45:711–23.
29. Ramzan I, et al. The association between circulating branched chain amino acids and the temporal risk of developing type 2 diabetes mellitus: a systematic review & meta-analysis. Nutrients. 2022;14:4411. https://doi.org/10.3390/nu14204411.
30. Morze J, et al. Metabolomics and type 2 diabetes risk: an updated systematic review and meta-analysis of prospective cohort studies. Diabetes Care. 2022;45:1013–24. https://doi.org/10.2337/dc21-1705.
31. Fine KS, Wilkins JT, Sawicki KT. Circulating branched chain amino acids and cardiometabolic disease. J Am Heart Assoc. 2024;13:e031617. https://doi.org/10.1161/JAHA.123.031617.
32. Kittithaworn AA, et al. Enhanced chronic inflammation and increased branched-chain amino acids in adrenal disorders: a cross-sectional study. J Clin Endo Metab. 2024. https://doi.org/10.1210/clinem/dgae204.
33. Citterio F, et al. Changes in the salivary metabolic profile of generalized periodontitis patients after non-surgical periodontal therapy: a metabolomic analysis using nuclear magnetic resonance spectroscopy. J Clin Med. 2020;9:3977.
34. Guasch-Ferré M, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care. 2016;39:833–46.
35. Fukushima K, et al. Association between dyslipidemia and plasma levels of branched-chain amino acids in the Japanese population without diabetes mellitus. J Clinic Lipidol. 2019;13:932–9.
36. Mook-Kanamori D, et al. Increased amino acids levels and the risk of developing of hypertriglyceridemia in a 7-year follow-up. J Endocrinol Invest. 2014;37:369–74.
37. Xu X, et al. Association between systemic immune inflammation level and poor prognosis across different glucose metabolism status in coronary artery disease patients. J Inflamm Res. 2023;16:4031–42. https://doi.org/10.2147/JIR.S425189.
38. Yan Y, et al. Temporal relationship between inflammation and insulin resistance and their joint effect on hyperglycemia: the Bogalusa Heart Study. Cardiovasc Diabetol. 2019;18:109. https://doi.org/10.1186/s12933-019-0913-2.
39. Peiro C, et al. Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. Cardiovasc Diabetol. 2016;15:82. https://doi.org/10.1186/s12933-016-0397-2.
40. Khovidhunkit W, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res. 2004;45:1169–96. https://doi.org/10.1194/jlr.R300019-JLR200.
41. Friedman M. Analysis, nutrition, and health benefits of tryptophan. Int J Trypotophan Res. 2018;11:12. https://doi.org/10.1177/1178646918802282.
42. Basson C, Serem JC, Hlophe YN, Bipath P. The tryptophan-kynurenine pathway in immunomodulation and cancer metastasis. Cancer Med. 2023;12:18691–701. https://doi.org/10.1002/cam4.6484.
43. Teunis CJ, et al. Tryptophan metabolites and incident cardiovascular disease: the EPIC-Norfolk prospective population study. Atherosclerosis. 2023. https://doi.org/10.1016/j.atherosclerosis.2023.117344.
44. Melhem NJ, Taleb S. Tryptophan: from diet to cardiovascular diseases. Int J Mol Sci. 2021;22:9904.
45. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease. Circ Res. 2016;118:547–63. https://doi.org/10.1161/CIRCRESAHA.115.306249.
46. Schamarek I, Anders L, Chakaroun RM, Kovacs P, Rohde-Zimmermann K. The role of the oral microbiome in obesity and metabolic disease: potential systemic implications and effects on taste perception. Nutr J. 2023;22:28. https://doi.org/10.1186/s12937-023-00856-7.
47. Greve D, et al. *Rothia aeria* and *Rothia dentocariosa* as biofilm builders in infective endocarditis. Int J Med Microbiol. 2021;311:151478. https://doi.org/10.1016/j.ijmm.2021.151478.
48. Headley CA, et al. Nontuberculous mycobacterium *M. avium* infection predisposes aged mice to cardiac abnormalities and inflammation. Aging Cell. 2019;18:e12926. https://doi.org/10.1111/acel.12926.
49. Bouchiat C, et al. Nontuberculous mycobacteria: an underestimated cause of bioprosthetic valve infective endocarditis. Open Forum Infect Dis. 2015;2:ofv047. https://doi.org/10.1093/ofid/ofv047.
50. Garcia C, Andersen CJ, Blesso CN. The role of lipids in the regulation of immune responses. Nutrients. 2023;15:3899.
51. Nyamundanda G, Gormley IC, Brennan L. A dynamic probabilistic principal components model for the analysis of longitudinal metabolomics data. J R Stat Soc Ser C Appl Stat. 2014;63:763–82. https://doi.org/10.1111/rssc.12060.
52. Sarkar A, et al. Bayesian semiparametric inference in longitudinal metabolomics data. Sci Rep. 2024;14:31336. https://doi.org/10.1038/s41598-024-82718-8.
53. Arnold PK, Finley LWS. Regulation and function of the mammalian tricarboxylic acid cycle. J Biol Chem. 2023;299:102838. https://doi.org/10.1016/j.jbc.2022.102838.
54. Harber KJ, et al. Succinate is an inflammation-induced immunoregulatory metabolite in macrophages. Metabolites. 2020;10:372.
55. Galván-Peña S, O'Neill LA. Metabolic reprograming in macrophage polarization. Front Immunol. 2014;5:420.
56. O'Neill LA. A broken krebs cycle in macrophages. Immunity. 2015;42:393–4.
57. Murphy MP, O'Neill LA. Krebs cycle reimagined: the emerging roles of succinate and itaconate as signal transducers. Cell. 2018;174:780–4.
58. Ryan DG, et al. Coupling Krebs cycle metabolites to signalling in immunity and cancer. Nat Metab. 2019;1:16–33.
59. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149:274–93. https://doi.org/10.1016/j.cell.2012.03.017.
60. Zhao J, et al. Kaempferol promotes bone formation in part via the mTOR signaling pathway. Mol Med Rep. 2019;20:5197–207. https://doi.org/10.3892/mmr.2019.10747.
61. Dennis PB, et al. Mammalian TOR: a homeostatic ATP sensor. Science. 2001;294:1102–5.

Publisher's Note