

Supplementary file S1. Intergroup comparisons for SS samples (univariate and multivariate analyses; and MSEA)

Figure S1(A) shows the scores plot between the first two principal components (PCs) of sPLS-DA, while Figure S1(B) shows the loadings plot of component 1 from the sPLS-DA. Table S1 shows the univariate analysis through the normalized metabolite concentration of the stimulated saliva samples shown as the mean and standard deviation. The superscript (1) after the metabolite name indicates statistical difference in the univariate analysis, (2) indicates statistical difference in the multivariate analysis (according to the loadings factor from sPLS-DA).

The scores plot between the first two principal components (PCs) of sPLS-DA indicates mainly a difference of OP and NP groups from controls (OWP and NWP), but with no great distinction between them. According to the univariate analysis, OP and NP groups showed higher concentration of butyric acid, isovaleric acid, leucine, valine, isoleucine, propionic acid, acetic acid, trimethylamine, ornithine, and phenylalanine. Besides the higher levels of propionic acid, butyric acid, and acetic acid, the OP and NP groups showed lower concentrations of proline, *N*-Acetylglutamine acid, citric acid, pyruvic acid, lactate, *N*-Acetylneuraminic acid, and galactose, according to the multivariate analysis by loading factor of component 1 from sPLS-DA.

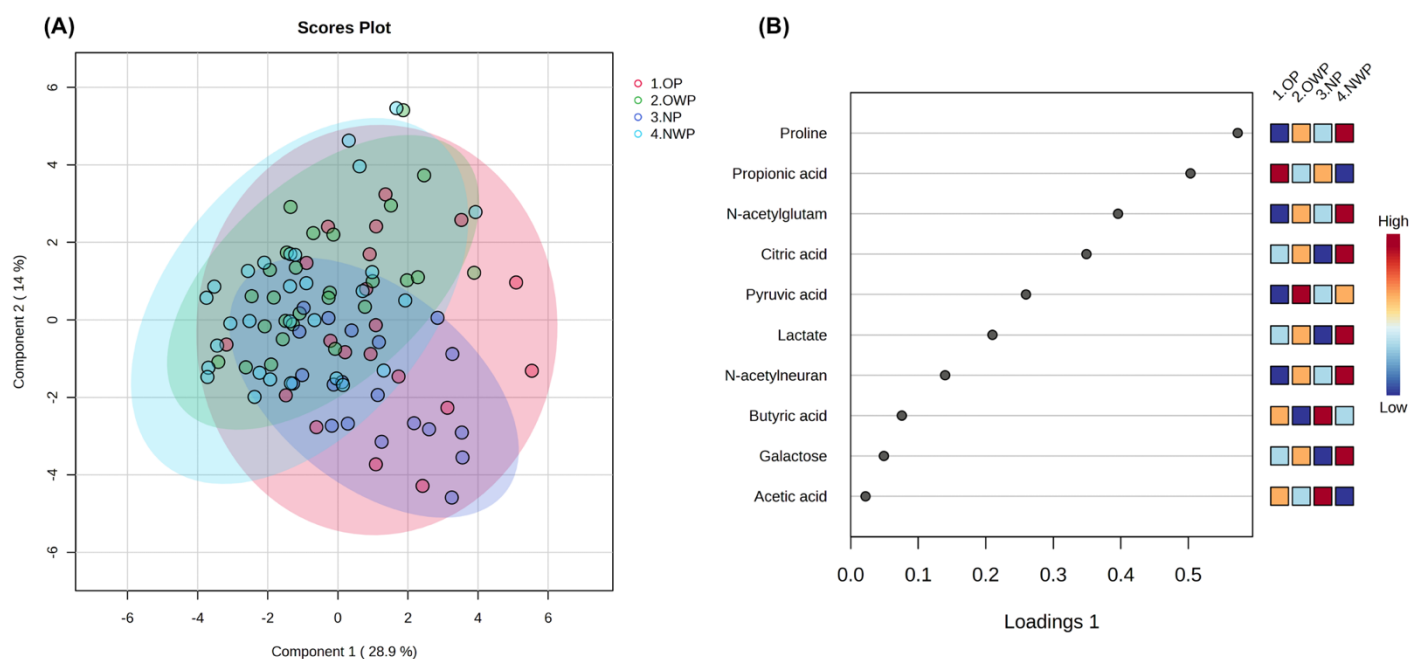


Figure S1. Scores plot between the first two principal components (PCs) of sPLS-DA (A), and loading plot of component 1 from the sPLS-DA (B).

Table S1. Univariate analysis of the Stimulated Saliva samples. Normalized concentration of metabolites shown as the mean and standard deviation for each group (ANOVA and Tukey test as post-hoc).

HMDB Card	Metabolites	CS (ppm)	OP (n = 20) Mean ± SD	OWP (n = 27) Mean ± SD	NP (n = 20) Mean ± SD	NWP (n = 29) Mean ± SD	p
HMDB0000491	3-Methyl-2-ketovaleric acid	0.85	0.41 ± 0.21	0.36 ± 0.17	0.49 ± 0.21	0.37 ± 0.18	0.152
HMDB0000039	Butyric acid ^{1,2}	0.89	0.32 ± 0.25	0.18 ± 0.14	0.40 ± 0.23	0.18 ± 0.14	<0.001
			AB	A	B	A	

HMDB0000718	Isovaleric acid ¹	0.91	0.27 ± 0.23 AB	0.15 ± 0.10 A	0.33 ± 0.20 B	0.17 ± 0.09 A	<0.001
HMDB0000687	Leucine ¹	0.97	0.29 ± 0.27 A	0.16 ± 0.09 B	0.27 ± 0.13 AB	0.17 ± 0.10 AB	0.006
HMDB0000883	Valine ¹	0.99	0.24 ± 0.26 A	0.10 ± 0.07 B	0.23 ± 0.15 A	0.10 ± 0.07 B	0.001
HMDB0000172	Isoleucine ¹	1.01	0.25 ± 0.27 A	0.10 ± 0.07 B	0.21 ± 0.14 AB	0.11 ± 0.09 B	0.002
HMDB0000237	Propionic acid ^{1,2}	1.06	0.30 ± 0.23 A	0.17 ± 0.15 AB	0.30 ± 0.20 A	0.15 ± 0.14 B	0.004
HMDB0003156	2,3-Butanediol	1.14	0.23 ± 0.19	0.18 ± 0.18	0.16 ± 0.06	0.17 ± 0.19	0.606
HMDB0000108	Ethanol	1.18	0.22 ± 0.22	0.20 ± 0.14	0.19 ± 0.11	0.15 ± 0.11	0.376
HMDB0000174	Fucose	1.25	0.36 ± 0.25	0.31 ± 0.21	0.35 ± 0.22	0.35 ± 0.20	0.817
HMDB0003243	Acetoin	1.38	0.27 ± 0.22	0.22 ± 0.14	0.28 ± 0.11	0.18 ± 0.10	0.077
HMDB0000161	Alanine	1.48	0.33 ± 0.25	0.26 ± 0.16	0.38 ± 0.20	0.32 ± 0.16	0.224
HMDB0001414	Putrescine	1.76	0.27 ± 0.25	0.17 ± 0.11	0.26 ± 0.20	0.20 ± 0.12	0.146
HMDB0000042	Acetic acid ^{1,2}	1.92	0.31 ± 0.22 A	0.20 ± 0.17 AB	0.32 ± 0.18 A	0.17 ± 0.11 B	0.008
HMDB0000230	N-Acetylneuraminic acid ²	2.21	0.38 ± 0.27	0.31 ± 0.19	0.43 ± 0.18	0.35 ± 0.21	0.317
HMDB0003355	5-Aminopentoate	2.24	0.28 ± 0.24	0.21 ± 0.14	0.26 ± 0.17	0.21 ± 0.13	0.423
HMDB0006029	N-Acetylglutamine ²	2.33	0.30 ± 0.21	0.32 ± 0.20	0.34 ± 0.19	0.39 ± 0.23	0.408
HMDB0000243	Pyruvic acid ²	2.37	0.27 ± 0.21	0.29 ± 0.17	0.28 ± 0.13	0.29 ± 0.15	0.959
HMDB0000254	Succinic acid	2.41	0.35 ± 0.26	0.30 ± 0.19	0.34 ± 0.25	0.32 ± 0.20	0.869
HMDB0000094	Citric acid ²	2.53	0.29 ± 0.21	0.28 ± 0.14	0.28 ± 0.13	0.36 ± 0.22	0.288
HMDB0000164	Methylamine	2.60	0.24 ± 0.24	0.15 ± 0.08	0.22 ± 0.10	0.17 ± 0.07	0.059
HMDB0000906	Trimethylamine ¹	2.89	0.14 ± 0.14 AB	0.06 ± 0.03 A	0.21 ± 0.20 B	0.07 ± 0.03 A	<0.001
HMDB0000214	Ornithine	3.06	0.30 ± 0.25 A	0.15 ± 0.09 B	0.25 ± 0.19 AB	0.19 ± 0.11 AB	0.021
HMDB0000097	Choline	3.20	0.24 ± 0.26	0.18 ± 0.14	0.29 ± 0.15	0.19 ± 0.11	0.134
HMDB0000904	Citrulline	3.14	0.13 ± 0.21	0.07 ± 0.04	0.10 ± 0.03	0.07 ± 0.03	0.119
HMDB0004983	Dimethylsulfone	3.15	0.30 ± 0.26	0.23 ± 0.17	0.28 ± 0.12	0.23 ± 0.11	0.391
HMDB0001875	Methanol ¹	3.36	0.28 ± 0.23 AB	0.16 ± 0.11 A	0.23 ± 0.13 AB	0.28 ± 0.18 B	0.038
HMDB0000251	Taurine	3.43	0.25 ± 0.22	0.16 ± 0.09	0.22 ± 0.08	0.18 ± 0.09	0.088
HMDB0000123	Glycine	3.56	0.28 ± 0.23	0.21 ± 0.14	0.21 ± 0.13	0.23 ± 0.15	0.521
HMDB0000190	Lactate ²	4.12	0.20 ± 0.25	0.19 ± 0.12	0.16 ± 0.08	0.19 ± 0.11	0.794
HMDB0000162	Proline ²	4.14	0.30 ± 0.23	0.30 ± 0.17	0.30 ± 0.12	0.35 ± 0.19	0.710
HMDB0000122	Glucose	5.24	0.16 ± 0.26	0.09 ± 0.12	0.11 ± 0.13	0.11 ± 0.15	0.655
HMDB0000143	Galactose ²	5.27	0.34 ± 0.21	0.32 ± 0.23	0.27 ± 0.14	0.34 ± 0.21	0.683
HMDB0000163	Maltose	5.41	0.09 ± 0.22	0.08 ± 0.17	0.04 ± 0.04	0.07 ± 0.12	0.787
HMDB0000294	Urea	5.78	0.32 ± 0.22	0.33 ± 0.17	0.33 ± 0.23	0.33 ± 0.17	0.998
HMDB0000296	Uridine	5.91	0.30 ± 0.23	0.30 ± 0.20	0.29 ± 0.22	0.26 ± 0.12	0.888
HMDB0000134	Fumaric acid	6.52	0.50 ± 0.19	0.48 ± 0.12	0.46 ± 0.13	0.49 ± 0.13	0.879
HMDB0000020	para-Hydroxyphenylacetic acid	6.87	0.34 ± 0.21	0.29 ± 0.19	0.38 ± 0.17	0.28 ± 0.19	0.370
HMDB0000158	Tyrosine	6.90	0.29 ± 0.24	0.20 ± 0.13	0.26 ± 0.08	0.21 ± 0.14	0.165
HMDB0000177	Histidine	7.07	0.30 ± 0.21	0.23 ± 0.16	0.26 ± 0.11	0.24 ± 0.16	0.584
HMDB0000159	Phenylalanine ¹	7.43	0.31 ± 0.25 A	0.16 ± 0.10 B	0.27 ± 0.11 AB	0.18 ± 0.13 B	0.004
HMDB0000142	Formic acid	8.45	0.16 ± 0.15	0.18 ± 0.23	0.13 ± 0.11	0.10 ± 0.15	0.418

HMDB, human metabolome database; CS, chemical shift, OP, obesity and periodontitis; OWP, obesity without periodontitis; NP, normal BMI with periodontitis; NWP, normal BMI without periodontitis; SD, standard deviation; *p*, significance level; ¹ univariate analysis (ANOVA with post-hoc Tukey); ² multivariate analysis using the loading factor of component 1 from sPLS-DA. Different bold letters mean statistically significant differences between groups. Bold values, statistical significance lower than 5%.

Metabolite set enrichment analysis (MSEA) was conducted to identify patterns of metabolite concentration changes considering the metabolite pathway and subclasses of metabolites. Thus, we described the MSEA for visualization and functional analysis of metabolite data. Metabolites that were in higher concentrations according to the univariate and multivariate analyses in the intergroup comparisons of SS samples (butyric acid, isovaleric acid, leucine, valine, isoleucine, propionic acid, acetic acid, trimethylamine, ornithine, and phenylalanine) were included (Figure S2). The three top pathways were: Valine, leucine and isoleucine degradation; Fatty acid biosynthesis; and Propanoate metabolism (Figure S2-A). The main metabolic subclasses were: Amino acids; Saturated fatty acids; and Straight chain fatty acid (Figure S2-B). In contrast, metabolites that were in lower concentrations according to the multivariate analysis in the intergroup comparisons of SS samples (Proline, *N*-Acetylglutamine acid, citric acid, pyruvic acid, lactate, *N*-Acetylneuraminic acid, and galactose) were also included in the MSEA (Figure S3). The five top pathways were: Warburg effect; Transfer of acetyl groups into mitochondria; Citric acid cycle; Amino sugar metabolism; and Gluconeogenesis (Figure S3-A). The main metabolic subclasses were: Amino acids; *N*-Acetylneuraminic acids; and Short-chain acids and derivatives (Figure S3-B).

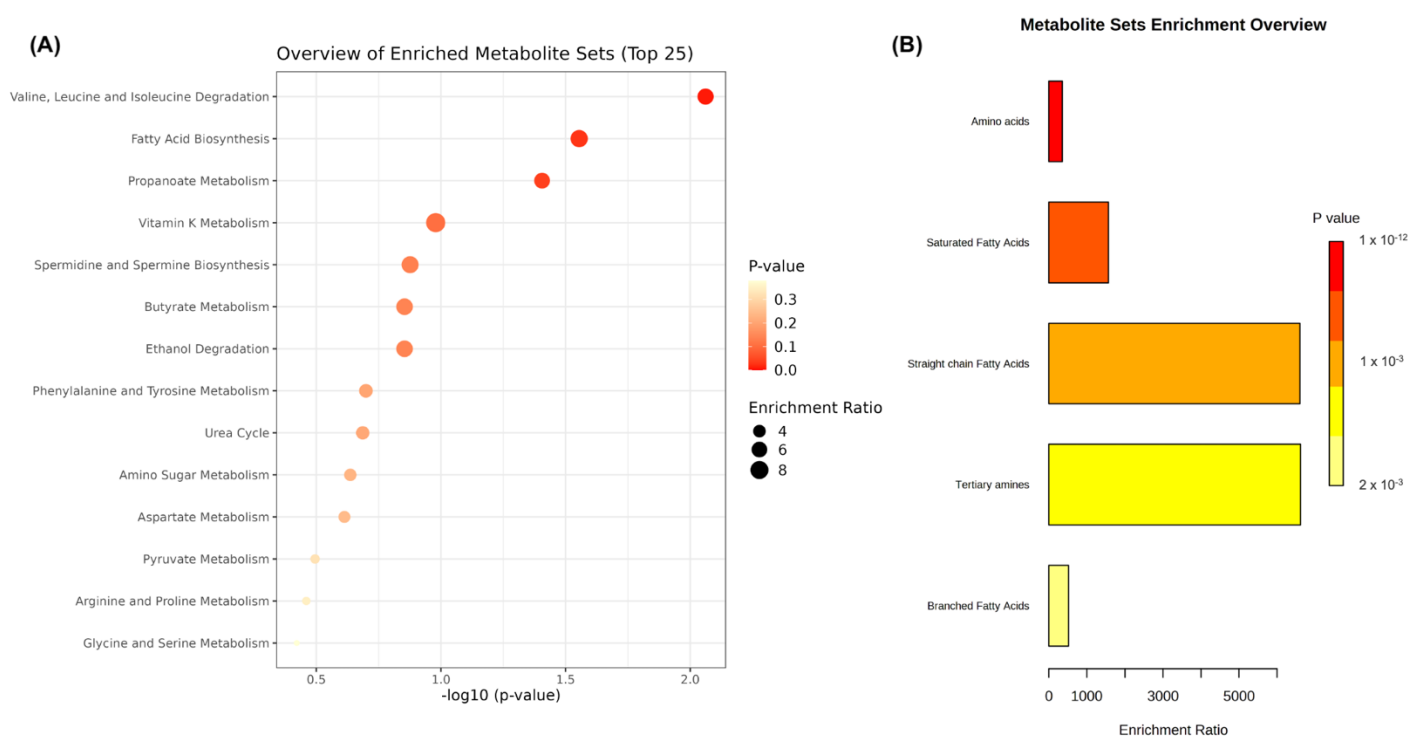


Figure S2. Main pathways (A) and subclasses (B) of higher concentrated metabolites in stimulated saliva samples from OP and NP groups assigned by MSEA.

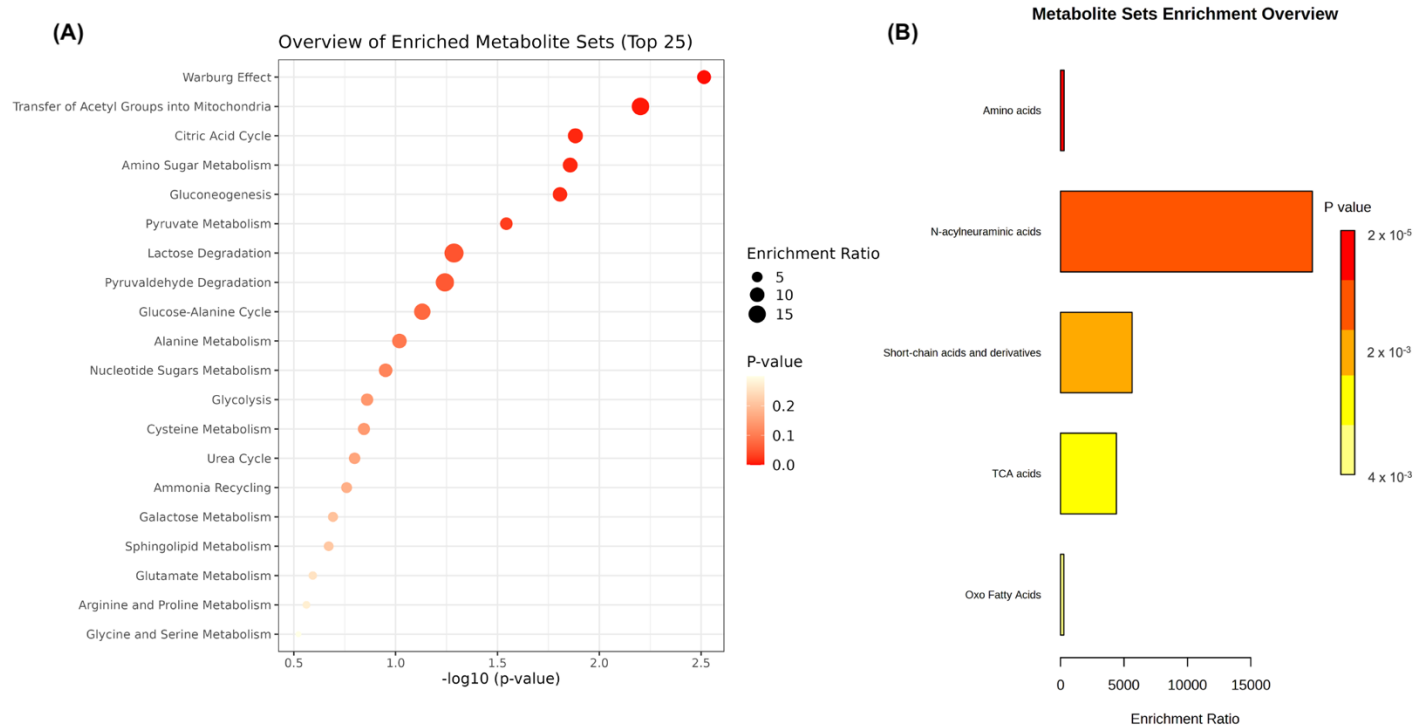


Figure S3. Main pathways (A) and subclasses (B) of lower concentrated metabolites in stimulated saliva samples from OP and NP groups assigned by MSEA.