Metabolic Profile of Supragingival Plaque Exposed to Arginine and Fluoride

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Appendix

MATERIALS AND METHODS

Study Population. The selection process excluded individuals who presented: less than 20 teeth, removable or fixed dental appliance, advanced periodontal disease, bleeding disorders or under anticoagulant therapy, immune compromised disorders, any systemic disease or were taking any medication known to cause xerostomia, had been treated with antibiotics within the past 3 months, have participated in another clinical study one week prior to the start of the washout period or during the study period, and those who use tobacco. Informed consent was obtained from all subjects under a protocol approved by the Institutional Review Board of the UF Health Science Center (IRB #201600297). The recruitment period was from 04/14/2014 to 08/05/2014, and the study visits were performed between 05/13/2014 and 11/07/2014.

Caries Diagnosis. Lesion progression was assessed and recorded over the study period as “active”, “inactive”, “progressed” (to a higher ICDAS score) or “treated” (by endodontic therapy, restorative treatment or extraction). Surface hardness (and not color) was the main diagnostic characteristic used to distinguish active from inactive lesions. Active enamel lesions (ICDAS 1-3) presented loss of luster and ‘chalky’ appearance, rough surface upon gently probing, and in some cases, localized shallow defects or microcavitations. Inactive enamel lesions presented shiny and smooth surfaces upon gentle probing (in spite of the presence of microcavities). Active cavitated lesions (ICDAS 5-6) presented surface breakdown with exposed soft or leathery dentinal carious tissues. Inactive cavitated lesions were shiny and felt hard upon on gentle probing. ICDAS 4 lesions (showing no distinct cavitation but underlying dentin shadow) were characterized as either active or treated.

Plaque acidogenicity. Plaque samples were centrifuged and resuspended in 50 µl of 50 mM KCl and 1 mM $MgCl_2$. Samples were then incubated at 37°C for 30 minutes with either 55 mM glucose (LPG) or an equal volume of deionized water (LPE). Next, 10 µl of the suspensions were incubated in duplicate with the L-lactate assay reagent solution at 37°C for 30 minutes using 96 well microtiter-plates. To stop the reaction, 50 µl of 0.5 M acetic acid was added to the wells and the absorbance read at 490nm.

Bacterial Community Profiles. The Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS; http://homings.forsyth.org) was used to survey the bacterial profiles of 150 site-specific plaque samples collected at baseline and 12-weeks after treatment with Arg and F as described elsewhere (Richards et al. 2017). Plaque DNA was purified using a protocol that includes overnight incubation in the presence of Ready-Lyse™ Lysozyme Solution (Epicentre, WI, USA) and the MasterPure DNA Purification Kit (Epicentre, WI, USA). Purified DNA was sequenced using the Illumina

MiSeq platform (Illumina, San Diego, CA). Sequencing of the 16S rRNA V3-V4 region using primers described elsewhere (Belstrom et al., 2016) was performed at the HOMINGS Core facility at the Forsyth Institute (Boston, MA, USA). The HOMINGS approach assigns taxonomy using a customized BLAST program called ProbeSeq, which contains sequences of species and genus specific 16S rRNA probes based on the Human Oral Microbiome Database (HOMD). Each probe represents a distinct Operational Taxonomic Unit (OTU). Bacterial identification is based on the use of 638 oligonucleotide probes (17 to 40 bases) targeting individual oral bacterial species and/or a few closely-related species and 129 genus-specific probes that identify closely-related species within the same genus. Supplemental Material 1 shows the description of the HOMINGS taxa, species and genus probes. Python scripts within the software package QIIME version 1.9.0 (57) were used to analyze HOMINGS Operational Taxonomic Unit (OTU) counts. Alpha diversity rarefaction plots using the choa1 richness factor were generated for all samples (Chao, 1984). Read counts for each sample were normalized by random sub-sampling of each sample. Normalized counts were used to calculate beta (Bray-Curtis) diversity measures (Bray J.R. 1957). A distance-based redundancy analysis (db-RDA) was performed and significant differences in beta-diversity among the study groups was determined using a PERMANOVA test. The frequency of OTUs among the groups was tested for significant difference using a Kruskal Wallis test. P values were generated using 10,000 permutations and corrected for multiple testing using FDR.

Global metabolomics. The metabolomic analyses were performed at the University of Florida Southeast Center for Integrated Metabolomics (SECIM) using a Thermo Q-Exactive high resolution mass spectrometer with a Dionex UHPLC and autosampler. The instrument was run in both positive and negative ion modes with heated electrospray ionization. Mass resolution was 35,000 at m/z 200 with mass accuracy of less than 5 ppm in positive mode and less than 10 ppm in negative mode. Exact chromatography and additional details of the methodology are available elsewhere (Ulmer et al., 2015). The sum total of all features represented an initial QC of the data, and the data was normalized to the sum of metabolites for each sample, log transformed and autoscaled. MZmine was used to identify features, deisotope, align features and perform gap filling (Pluskal et al., 2010). All adducts and complexes were identified and removed from the data set. Metabolite identification was performed first by searching the internal retention time deposited at the SECIM metabolite library. For unknown compounds, a combination of high mass accuracy, tandem mass spectrometry and database searching (e.g. www.hmdb.ca) were used to identify potential metabolites. For statistical analysis, missing values had to be present in 80% of the samples in order to be replaced with half the minimum value. All metabolomic data from this study has been deposit at metabolomicsworkbench.org.

Appendix Table 1. Demographic characteristics and DMFT scores of the study population.

Caries-Status		CF	CA	Total
DMFT		0	17.1 (±6.6)	--
Age		25.2 (±8.8)	37.8 (±14.3)	32.1 (±13.6)
Gender	**Male**	14 (42)	19 (58)	33 (100)
	Female	24 (48)	26 (52)	50 (100)
Ethnicity	**Hispanic**	9 (90)	1 (10)	10 (100)
	Not Hispanic	29 (40)	44 (60)	73 (100)
Race	**White**	27 (63)	16 (37)	43 (100)
	Black	6 (24)	19 (76)	25 (100)
	Asian	2 (33)	4 (67)	6 (100)
	Other	3 (33)	6 (67)	9 (100)

CF: caries-free and CA: caries-active. DMFT: number of decayed, missing and filled teeth due to dental caries. Other race: Pacific Islander, Native American, Indian, Alaska Native, Native Hawaiian and others. Age is shown in years. DMFT and age are shown as [Mean, (±SD)]; SD: standard deviation; DMFT: number of decayed, missing and filled teeth. Gender, ethnicity and race are shown as [N (%)]. Percentages are within rows for each demographic characteristic.

Appendix Table 2: Initial and final pH values after the incubation of plaque samples with glucose.

Caries Group	Plaque Group	Toothpaste	Baseline		12 weeks	
			Initial pH	Final pH	Initial pH	Final pH
CF+CA	**PF+PE+PD**	**Arg**	6.62 (±0.30)	5.25 (±0.48)	6.68 (±0.21)	5.32 (±0.60)*
CF+CA	**PF+PE+PD**	**F**	6.63 (±0.17)	5.07 (±0.45)	6.68 (±0.14)	4.99 (±0.40)*
CA	**PF+PE+PD**	**Arg**	6.60 (±0.29)	5.31 (±0.38)	6.86 (±0.20)	5.21 (±0.42)
CA	**PF+PE+PD**	**F**	6.60 (±0.16)	5.35 (±0.56)	6.67 (±0.13)	4.99 (±0.57)
CA	**PF**	**Arg**	6.57 (±0.33)	5.09 (±0.46)	6.90 (±0.21)	5.16 (±0.64)
CA	**PF**	**F**	6.64 (±0.20)	4.94 (±0.41)	6.70 (±0.16)	4.94 (±0.45)
CF	**PF**	**Arg**	6.70 (±0.34)	5.23 (±0.52)*	6.86 (±0.25)	5.36 (±0.66)*
CF	**PF**	**F**	6.71 (±0.19)	5.00 (±0.39)*	6.71 (±0.15)	4.99 (±0.34)*
CF+CA	**PF**	**Arg**	6.65 (±0.34)	5.20 (±0.43)	6.87 (±0.23)	5.19 (±0.53)
CF+CA	**PF**	**F**	6.70 (±0.19)	5.09 (±0.50)	6.71 (±0.16)	4.96 (±0.49)
CA	**PE**	**Arg**	6.64 (±0.31)	5.08 (±0.49)	6.75 (±0.18)	5.21 (±0.59)
CA	**PE**	**F**	6.52 (±0.90)	4.87 (±0.36)	6.64 (±0.92)	4.90 (±0.28)
CA	**PD**	**Arg**	6.59 (±0.21)	5.54 (±0.55)	6.93 (±0.17)	5.74 (±0.66)*
CA	**PD**	**F**	6.61 (±0.12)	5.13 (±0.38)	6.64 (±0.11)	5.09 (±0.19)*

The pH values are shown as [Mean ±SD (N)]; SD: standard deviation; N: number of samples; CA: caries-active subjects; CF: caries-free subjects; PF: plaque from carious-lesion free tooth surfaces; PE: plaque from active, enamel carious lesion; PD: plaque from active, dentinal carious lesions; Arg: 1.5% arginine toothpaste; F: 1100 ppm F as NaF. *: significant p-values ($p<0.05$) for comparisons of final pH between toothpaste Arg and F within the same study visit (baseline and 12-weeks samples).

Appendix Table 3: The top 50 taxa detected when the CF-PF and CA-PD groups were compared before and after treatment with Arg or F.

CF-PF at baseline and 12-weeks after Arg

OTU	Test-Statistic	P-value	FDR_P	Bonferroni_P	baseline_mean	12-weeks _mean	total_mean
Streptococcus_Genus_probe_4_GP-081	0.67	0.41	1.00	1.00	7330.50	8417.61	15748.11
Streptococcus_sanguinis_ST-20	0.17	0.68	1.00	1.00	1235.77	1422.48	2658.25
Streptococcus_Genus_probe_1_GP-126	1.19	0.28	1.00	1.00	1253.09	1064.87	2317.96
Veillonella_dispar_VE-03	0.87	0.35	1.00	1.00	1110.45	1106.00	2216.45
Rothia_dentocariosa_RO-02	0.38	0.54	1.00	1.00	1366.09	748.48	2114.57
Haemophilus_parainfluenzae_HA-05	1.21	0.27	1.00	1.00	463.50	1140.57	1604.07
Gemella_morbillorum_GE-03	2.35	0.13	1.00	1.00	928.41	245.43	1173.84
Veillonella_Genus_probe_2_GP-089	2.38	0.12	1.00	1.00	682.18	474.74	1156.92
Granulicatella_Genus_probe_GP-110	2.35	0.13	1.00	1.00	593.27	416.52	1009.79
Rothia_mucilaginosa_RO-03	1.77	0.18	1.00	1.00	467.23	374.65	841.88
Gemella_haemolysans_GE-02	0.69	0.41	1.00	1.00	521.41	301.48	822.89
Rothia_Genus_probe_GP-073	0.71	0.40	1.00	1.00	528.95	253.26	782.22
Neisseria_Genus_probe_2_GP-060	0.16	0.69	1.00	1.00	425.95	348.48	774.43
Corynebacterium_matruchotii_CO-03	0.43	0.51	1.00	1.00	324.41	448.30	772.71
Actinomyces_Genus_probe_3_GP-003	1.95	0.16	1.00	1.00	288.95	475.26	764.22
Fusobacterium_periodonticum_FU-10	2.42	0.12	1.00	1.00	465.77	181.74	647.51
Gemella_Genus_probe_GP-039	3.34	0.07	1.00	1.00	366.82	259.26	626.08
Rothia_aeria_RO-01	0.10	0.75	1.00	1.00	355.00	260.17	615.17
Porphyromonas_sp_oral_taxon_279_PO-09	0.34	0.56	1.00	1.00	229.50	384.48	613.98
Fusobacterium_Genus_probe_4_GP-038	0.12	0.72	1.00	1.00	300.32	311.04	611.36
Prevotella_melaninogenica_PR-14	0.10	0.75	1.00	1.00	207.59	374.87	582.46
Lautropia_mirabilis_LA-29	0.13	0.72	1.00	1.00	270.18	293.00	563.18
Leptotrichia_wadei_LE-22	2.87	0.09	1.00	1.00	46.73	451.91	498.64
Streptococcus_anginosus_ST-09	0.08	0.78	1.00	1.00	392.55	62.00	454.55
Abiotrophia_defectiva_AB-01	0.50	0.48	1.00	1.00	306.73	135.87	442.60
Bergeyella_sp_oral_taxon_322_BE-02	0.16	0.69	1.00	1.00	264.50	174.43	438.93
Corynebacterium_durum_CO-02	0.24	0.63	1.00	1.00	146.32	291.39	437.71
Veillonella_parvula_VE-06	2.11	0.15	1.00	1.00	267.50	127.78	395.28
Gemella_morbillorum_GE-05	0.51	0.47	1.00	1.00	195.82	172.09	367.91
Aggregatibacter_Genus_probe_2_GP-100	0.18	0.67	1.00	1.00	131.27	223.96	355.23
Leptotrichia_sp_oral_taxon_212_LE-08	0.07	0.79	1.00	1.00	242.45	109.26	351.72
Actinobaculum_sp_oral_taxon_183_AC-03	0.00	0.95	1.00	1.00	165.45	185.83	351.28
Leptotrichia_hongkongensis_LE-06	0.23	0.63	1.00	1.00	153.05	160.57	313.61
Leptotrichia_Genus_probe_4_GP-050	1.37	0.24	1.00	1.00	189.82	102.26	292.08
Porphyromonas_Genus_probe_2_GP-067	0.51	0.47	1.00	1.00	152.32	125.78	278.10
TM7[G-1]_sp_oral_taxon_349_TM-04	0.62	0.43	1.00	1.00	146.82	130.52	277.34
TM7_Genus_probe_GP-083	0.21	0.65	1.00	1.00	97.95	142.13	240.08
Neisseria_elongata_NE-02	0.05	0.83	1.00	1.00	93.82	128.52	222.34
Neisseria_pharyngis_NE-08	0.00	0.96	1.00	1.00	85.91	106.17	192.08
Campylobacter_gracilis_CA-04	0.06	0.81	1.00	1.00	103.23	75.78	179.01
TM7[G-1]_sp_oral_taxon_346_TM-01	0.19	0.66	1.00	1.00	77.14	82.30	159.44
Peptostreptococcus_stomatis_PE-21	0.00	1.00	1.00	1.00	98.41	58.57	156.97
Corynebacterium_Genus_probe_GP-025	0.65	0.42	1.00	1.00	55.95	97.96	153.91
TM7[G-5]_sp_oral_taxon_437_TM-12	0.13	0.72	1.00	1.00	41.73	110.43	152.16
Parvimonas_Genus_probe_GP-063	0.19	0.67	1.00	1.00	87.41	59.78	147.19
TM7[G-1]_sp_oral_taxon_347_TM-02	1.74	0.19	1.00	1.00	90.05	51.17	141.22
Granulicatella_elegans_GR-02	0.02	0.89	1.00	1.00	58.82	80.65	139.47
Actinomyces_naeslundii_AC-36	0.22	0.64	1.00	1.00	78.86	60.39	139.25
Parvimonas_micra_PA-03	1.45	0.23	1.00	1.00	56.41	77.43	133.84
Fusobacterium_Genus_probe_3_GP-096	0.71	0.40	1.00	1.00	58.36	72.91	131.28

CF-PF at baseline and 12-weeks after F

OTU	Test-Statistic	P-value	FDR_P	Bonferroni_P	baseline_mean	12-weeks _mean	total_mean
Streptococcus_Genus_probe_4_GP-081	1.32	0.25	1.00	1.00	6294.18	7371.23	13665.41
Streptococcus_sanguinis_ST-20	0.67	0.41	1.00	1.00	1223.55	1613.09	2836.64
Streptococcus_Genus_probe_1_GP-126	0.62	0.43	1.00	1.00	1304.00	1495.68	2799.68
Veillonella_dispar_VE-03	0.50	0.48	1.00	1.00	1278.91	1225.41	2504.32
Rothia_dentocariosa_RO-02	0.69	0.40	1.00	1.00	1326.45	738.55	2065.00
Porphyromonas_sp_oral_taxon_279_PO-09	1.89	0.17	1.00	1.00	549.82	1037.36	1587.18
Haemophilus_parainfluenzae_HA-05	1.17	0.28	1.00	1.00	749.14	781.64	1530.77
Gemella_Genus_probe_GP-039	0.05	0.82	1.00	1.00	820.18	628.64	1448.82
Veillonella_Genus_probe_2_GP-089	0.21	0.65	1.00	1.00	693.91	537.45	1231.36
Granulicatella_Genus_probe_GP-110	1.12	0.29	1.00	1.00	599.14	448.64	1047.77
Gemella_morbillorum_GE-03	0.07	0.80	1.00	1.00	504.50	470.64	975.14
Leptotrichia_sp_oral_taxon_417_LE-16	0.76	0.38	1.00	1.00	699.82	214.27	914.09
Corynebacterium_matruchotii_CO-03	0.09	0.76	1.00	1.00	418.64	404.73	823.36
Gemella_morbillorum_GE-05	0.00	0.94	1.00	1.00	469.68	330.32	800.00
Rothia_Genus_probe_GP-073	0.84	0.36	1.00	1.00	549.50	247.59	797.09
Rothia_aeria_RO-01	0.01	0.91	1.00	1.00	390.68	342.55	733.23
Leptotrichia_hongkongensis_LE-06	0.39	0.53	1.00	1.00	357.77	314.73	672.50
Gemella_haemolysans_GE-02	0.17	0.68	1.00	1.00	315.82	349.23	665.05
Fusobacterium_periodonticum_FU-10	2.74	0.10	1.00	1.00	439.00	175.95	614.95
Bergeyella_sp_oral_taxon_322_BE-02	0.00	0.96	1.00	1.00	289.41	322.95	612.36
Actinobaculum_sp_oral_taxon_183_AC-03	0.25	0.61	1.00	1.00	434.09	171.95	606.05
Fusobacterium_Genus_probe_4_GP-038	1.67	0.20	1.00	1.00	358.64	179.55	538.18
Neisseria_Genus_probe_2_GP-060	3.94	0.05	1.00	1.00	226.64	301.91	528.55
Porphyromonas_Genus_probe_2_GP-067	1.03	0.31	1.00	1.00	152.86	368.18	521.05
Lautropia_mirabilis_LA-29	1.17	0.28	1.00	1.00	183.73	332.14	515.86
Actinomyces_Genus_probe_3_GP-003	0.17	0.68	1.00	1.00	204.86	272.45	477.32
Rothia_mucilaginosa_RO-03	0.18	0.67	1.00	1.00	287.50	140.36	427.86
Corynebacterium_durum_CO-02	3.27	0.07	1.00	1.00	106.64	319.41	426.05
Prevotella_melaninogenica_PR-14	2.02	0.16	1.00	1.00	78.91	338.73	417.64
Abiotrophia_defectiva_AB-01	0.55	0.46	1.00	1.00	204.55	170.95	375.50
Leptotrichia_wadei_LE-22	1.14	0.29	1.00	1.00	168.32	179.18	347.50
TM7_Genus_probe_GP-083	0.01	0.92	1.00	1.00	195.14	143.14	338.27
Neisseria_pharyngis_NE-08	0.32	0.57	1.00	1.00	145.86	142.50	288.36
Aggregatibacter_Genus_probe_2_GP-100	0.00	0.95	1.00	1.00	122.86	155.50	278.36
Leptotrichia_Genus_probe_4_GP-050	1.85	0.17	1.00	1.00	170.55	102.32	272.86
Streptococcus_mutans_ST-15	0.32	0.57	1.00	1.00	151.95	120.82	272.77
Porphyromonas_sp_oral_taxon_279_PO-24	1.16	0.28	1.00	1.00	124.73	144.27	269.00
Leptotrichia_sp_oral_taxon_212_LE-08	0.28	0.60	1.00	1.00	133.59	124.68	258.27
Veillonella_parvula_VE-06	0.07	0.79	1.00	1.00	145.95	88.95	234.91
TM7[G-1]_sp_oral_taxon_347_TM-02	0.57	0.45	1.00	1.00	141.36	73.45	214.82
Campylobacter_gracilis_CA-04	0.03	0.87	1.00	1.00	139.64	52.64	192.27
Streptococcus_anginosus_ST-09	0.16	0.69	1.00	1.00	133.05	55.27	188.32
Fusobacterium_Genus_probe_3_GP-096	0.56	0.45	1.00	1.00	98.50	78.41	176.91
Leptotrichia_Genus_probe_3_GP-049	0.32	0.57	1.00	1.00	112.82	62.55	175.36
TM7[G-1]_sp_oral_taxon_348_TM-13	0.09	0.77	1.00	1.00	84.82	72.95	157.77
Streptococcus_intermedius_ST-14	0.21	0.65	1.00	1.00	107.14	46.00	153.14
Kingella_oralis_KI-03	0.46	0.50	1.00	1.00	60.86	88.50	149.36
Cardiobacterium_hominis_CA-31	0.62	0.43	1.00	1.00	79.59	66.68	146.27
Veillonella_parvula_VE-05	0.47	0.49	1.00	1.00	84.95	61.18	146.14
TM7[G-1]_sp_oral_taxon_346_TM-01	0.23	0.63	1.00	1.00	79.32	66.05	145.36

CA-PD at baseline and 12-weeks after Arg

OTU	Test-Statistic	P-value	FDR_P	Bonferroni_P	baseline_mean	12-weeks _mean	total_mean
Streptococcus_Genus_probe_4_GP-081	0.09	0.76	1.00	1.00	8769.40	8389.00	17158.40
Streptococcus_Genus_probe_1_GP-126	0.00	1.00	1.00	1.00	1389.30	979.00	2368.30
Veillonella_dispar_VE-03	2.06	0.15	1.00	1.00	837.20	549.80	1387.00
Rothia_dentocariosa_RO-02	0.01	0.91	1.00	1.00	627.00	494.60	1121.60
Gemella_morbillorum_GE-03	2.06	0.15	1.00	1.00	653.20	397.60	1050.80
Gemella_morbillorum_GE-05	0.21	0.65	1.00	1.00	366.20	652.40	1018.60
Veillonella_Genus_probe_2_GP-089	0.82	0.36	1.00	1.00	537.50	425.90	963.40
Actinobaculum_sp_oral_taxon_183_AC-03	1.66	0.20	1.00	1.00	589.80	355.90	945.70
Streptococcus_sanguinis_ST-20	0.02	0.88	1.00	1.00	544.60	389.40	934.00
TM7[G-1]_sp_oral_taxon_349_TM-04	0.02	0.88	1.00	1.00	287.10	576.20	863.30
Gemella_Genus_probe_GP-039	0.69	0.41	1.00	1.00	519.10	337.90	857.00
Granulicatella_Genus_probe_GP-110	0.97	0.33	1.00	1.00	500.60	317.00	817.60
Corynebacterium_matruchotii_CO-03	0.28	0.60	1.00	1.00	433.30	347.00	780.30
Haemophilus_parainfluenzae_HA-05	0.41	0.52	1.00	1.00	99.90	644.50	744.40
Rothia_mucilaginosa_RO-03	0.69	0.41	1.00	1.00	607.70	122.00	729.70
Porphyromonas_sp_oral_taxon_279_PO-09	0.02	0.88	1.00	1.00	359.90	360.50	720.40
Leptotrichia_wadei_LE-22	1.37	0.24	1.00	1.00	339.30	379.00	718.30
Prevotella_melaninogenica_PR-14	0.28	0.60	1.00	1.00	534.90	164.70	699.60
Leptotrichia_Genus_probe_3_GP-049	1.12	0.29	1.00	1.00	266.50	395.10	661.60
Neisseria_Genus_probe_2_GP-060	0.01	0.91	1.00	1.00	223.40	400.50	623.90
Rothia_aeria_RO-01	0.00	0.97	1.00	1.00	143.70	383.80	527.50
TM7_Genus_probe_GP-083	0.05	0.82	1.00	1.00	186.20	323.50	509.70
TM7[G-1]_sp_oral_taxon_346_TM-01	0.04	0.85	1.00	1.00	284.70	204.30	489.00
Streptococcus_anginosus_ST-09	0.07	0.79	1.00	1.00	221.90	256.70	478.60
Veillonella_parvula_VE-06	0.57	0.45	1.00	1.00	303.30	170.40	473.70
Lautropia_mirabilis_LA-29	0.00	1.00	1.00	1.00	123.80	348.00	471.80
Leptotrichia_sp_oral_taxon_417_LE-16	2.06	0.15	1.00	1.00	206.10	265.30	471.40
Parvimonas_Genus_probe_GP-063	0.00	0.97	1.00	1.00	37.00	410.40	447.40
Neisseria_pharyngis_NE-08	0.76	0.38	1.00	1.00	192.90	253.60	446.50
Fusobacterium_Genus_probe_4_GP-038	0.37	0.55	1.00	1.00	156.30	287.40	443.70
Rothia_Genus_probe_GP-073	0.69	0.41	1.00	1.00	234.30	208.20	442.50
Gemella_haemolysans_GE-02	0.14	0.71	1.00	1.00	227.90	132.00	359.90
Leptotrichia_shahii_LE-07	1.76	0.18	1.00	1.00	95.60	258.70	354.30
Streptococcus_mutans_ST-15	0.70	0.40	1.00	1.00	33.40	279.90	313.30
Fusobacterium_periodonticum_FU-10	0.05	0.82	1.00	1.00	83.40	227.30	310.70
Leptotrichia_hongkongensis_LE-06	0.28	0.60	1.00	1.00	130.50	176.10	306.60
Actinomyces_Genus_probe_3_GP-003	0.01	0.91	1.00	1.00	111.60	176.60	288.20
Campylobacter_gracilis_CA-04	0.37	0.54	1.00	1.00	117.80	151.80	269.60
Corynebacterium_durum_CO-02	0.01	0.94	1.00	1.00	49.50	199.10	248.60
Lachnospiraceae[G-2]_sp_oral_taxon_096_	0.21	0.65	1.00	1.00	200.80	47.70	248.50
Leptotrichia_Genus_probe_4_GP-050	0.05	0.82	1.00	1.00	115.10	118.10	233.20
TM7[G-1]_sp_oral_taxon_348_TM-03	0.09	0.76	1.00	1.00	71.60	156.10	227.70
Bergeyella_sp_oral_taxon_322_BE-02	0.17	0.68	1.00	1.00	97.50	126.40	223.90
Stomatobaculum_longum_ST-04	0.21	0.65	1.00	1.00	140.20	69.00	209.20
Abiotrophia_defectiva_AB-01	0.28	0.60	1.00	1.00	110.20	93.20	203.40
Neisseria_flavescens_NE-03	0.16	0.69	1.00	1.00	150.40	49.70	200.10
Porphyromonas_sp_oral_taxon_275_PO-19	0.06	0.80	1.00	1.00	62.60	130.20	192.80
Parvimonas_micra_PA-03	0.01	0.91	1.00	1.00	73.50	118.20	191.70
Neisseria_elongata_NE-02	0.37	0.54	1.00	1.00	80.50	100.50	181.00
Leptotrichia_sp_oral_taxon_215_LE-26	1.65	0.20	1.00	1.00	32.00	145.70	177.70

CA-PD at baseline and 12-weeks after F

OTU	Test-Statistic	P-value	FDR_P	Bonferroni_P	baseline_mean	12-weeks _mean	total_mean
Streptococcus_Genus_probe_4_GP-081	2.39	0.12	1.00	1.00	6883.22	8374.67	15257.89
Streptococcus_Genus_probe_1_GP-126	0.56	0.45	1.00	1.00	1419.11	1049.22	2468.33
Leptotrichia_sp_oral_taxon_417_LE-16	0.86	0.35	1.00	1.00	1281.22	759.67	2040.89
Veillonella_dispar_VE-03	0.10	0.76	1.00	1.00	939.78	747.33	1687.11
Veillonella_Genus_probe_2_GP-089	0.44	0.51	1.00	1.00	831.00	784.78	1615.78
Streptococcus_mutans_ST-15	0.02	0.89	1.00	1.00	854.22	565.56	1419.78
Leptotrichia_wadei_LE-22	0.86	0.35	1.00	1.00	521.11	879.78	1400.89
Streptococcus_sanguinis_ST-20	0.02	0.89	1.00	1.00	663.22	684.67	1347.89
Actinobaculum_sp_oral_taxon_183_AC-03	0.24	0.63	1.00	1.00	787.11	460.78	1247.89
Granulicatella_Genus_probe_GP-110	0.16	0.69	1.00	1.00	523.56	573.00	1096.56
Haemophilus_parainfluenzae_HA-05	2.12	0.15	1.00	1.00	178.44	814.44	992.89
Veillonella_parvula_VE-06	0.10	0.76	1.00	1.00	590.89	272.22	863.11
Gemella_Genus_probe_GP-039	0.24	0.63	1.00	1.00	583.67	273.44	857.11
Neisseria_pharyngis_NE-08	0.40	0.53	1.00	1.00	777.11	75.00	852.11
Corynebacterium_matruchotii_CO-03	5.07	0.02	1.00	1.00	210.56	622.33	832.89
Porphyromonas_sp_oral_taxon_279_PO-09	0.24	0.63	1.00	1.00	337.22	487.56	824.78
Prevotella_melaninogenica_PR-14	2.12	0.15	1.00	1.00	262.33	506.33	768.67
Lautropia_mirabilis_LA-29	0.28	0.60	1.00	1.00	160.78	467.44	628.22
Rothia_dentocariosa_RO-02	0.44	0.51	1.00	1.00	287.00	333.00	620.00
Atopobium_rimae_AT-03	2.85	0.09	1.00	1.00	549.78	42.78	592.56
Neisseria_Genus_probe_2_GP-060	0.02	0.89	1.00	1.00	68.22	444.56	512.78
Leptotrichia_Genus_probe_3_GP-049	0.70	0.40	1.00	1.00	222.78	264.67	487.44
Scardovia_wiggsiae_SC-02	0.97	0.33	1.00	1.00	415.89	44.89	460.78
Abiotrophia_defectiva_AB-01	0.10	0.76	1.00	1.00	234.89	207.56	442.44
TM7[G-1]_sp_oral_taxon_346_TM-01	0.24	0.62	1.00	1.00	263.56	174.00	437.56
Gemella_haemolysans_GE-02	0.00	0.96	1.00	1.00	185.89	232.44	418.33
Leptotrichia_hongkongensis_LE-06	0.24	0.63	1.00	1.00	146.11	221.89	368.00
Campylobacter_gracilis_CA-04	0.10	0.76	1.00	1.00	173.33	156.22	329.56
Veillonella_parvula_VE-05	0.28	0.59	1.00	1.00	142.11	167.44	309.56
Rothia_Genus_probe_GP-073	0.00	0.96	1.00	1.00	139.89	142.22	282.11
Actinomyces_Genus_probe_3_GP-003	5.07	0.02	1.00	1.00	73.00	202.00	275.00
Gemella_morbillorum_GE-05	1.88	0.17	1.00	1.00	205.78	58.89	264.67
Fusobacterium_Genus_probe_4_GP-038	1.03	0.31	1.00	1.00	126.33	128.78	255.11
Rothia_aeria_RO-01	0.24	0.63	1.00	1.00	129.11	103.33	232.44
Bergeyella_sp_oral_taxon_322_BE-02	0.03	0.86	1.00	1.00	133.89	93.00	226.89
Leptotrichia_shahii_LE-07	0.16	0.69	1.00	1.00	99.11	118.56	217.67
Prevotella_denticola_PR-06	0.33	0.56	1.00	1.00	68.78	139.89	208.67
Tannerella_sp_oral_taxon_286_TA-02	0.02	0.89	1.00	1.00	149.11	47.44	196.56
Leptotrichia_sp_oral_taxon_498_LE-19	0.10	0.75	1.00	1.00	91.56	104.44	196.00
Leptotrichia_Genus_probe_4_GP-050	0.01	0.93	1.00	1.00	108.89	85.56	194.44
Porphyromonas_Genus_probe_2_GP-067	0.01	0.93	1.00	1.00	135.89	47.78	183.67
Streptococcus_anginosus_ST-09	3.62	0.06	1.00	1.00	138.78	44.22	183.00
Parvimonas_Genus_probe_GP-063	1.04	0.31	1.00	1.00	105.00	69.78	174.78
Atopobium_parvulum_AT-02	2.98	0.08	1.00	1.00	141.22	33.22	174.44
Actinomyces_sp_oral_taxon_448_AC-27	0.53	0.47	1.00	1.00	96.56	76.56	173.11
Streptococcus_intermedius_ST-14	0.01	0.93	1.00	1.00	78.11	87.56	165.67
Granulicatella_elegans_GR-02	0.33	0.57	1.00	1.00	52.44	113.00	165.44
Parvimonas_micra_PA-03	2.42	0.12	1.00	1.00	57.78	105.11	162.89
Prevotella_Genus_probe_2_GP-069	0.20	0.66	1.00	1.00	78.67	82.67	161.33
Dialister_invisus_DI-01	0.86	0.35	1.00	1.00	93.78	61.89	155.67

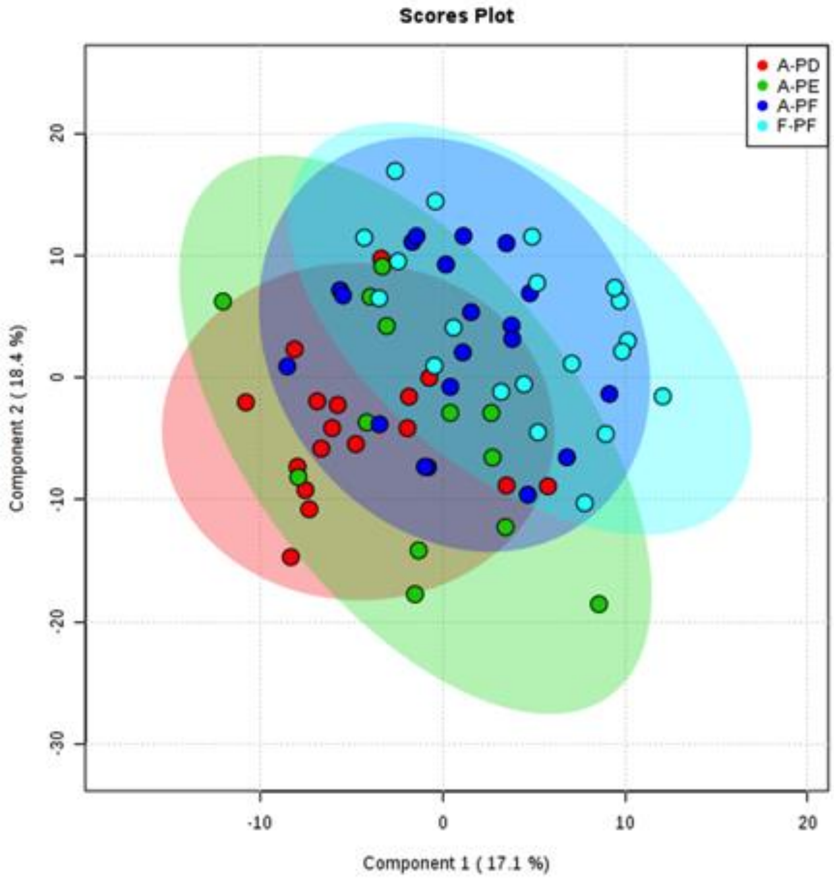
Appendix Table 4: List of known metabolites with significant difference in the detection levels between CF-PF and CA-PD at baseline.

Metabolite	FC	log2(FC)	raw.pval	negLOG10(p)
PHENETHYLAMINE	0.26714	-1.9044	8.01E-09	8.0964
AGMATINE	0.36924	-1.4374	0.001128	2.9478
n_L-CYSTEINE	0.33528	-1.5766	0.00127	2.8963
N-ALPHA-ACETYL-L-LYSINE-189.123_1.25	0.43969	-1.1854	0.004073	2.3901
n_NALPHA-ACETYL-L-LYSINE-187.1088-1.26	0.44193	-1.1781	0.004285	2.3681
n_Pantetheine-277.1223-7.88	0.41357	-1.2738	0.007954	2.0994
Stachydrine-144.1018_1.05	0.32097	-1.6395	0.008985	2.0465
DL-5-HYDROXYLYSINE-163.1075_0.63	3.4606	1.791	0.009479	2.0232
n_Pantetheine	0.41323	-1.275	0.014523	1.8379
n_INDOLE-3-ACETATE-174.0566-9.16	2.0453	1.0323	0.023239	1.6338
1,3-DIAMINOPROPANE-75.0918_0.59	0.49921	-1.0023	0.032035	1.4944
5'-DEOXYADENOSINE-252.1086_6.07	2.9613	1.5662	0.045596	1.3411

FC: fold change; raw pval: not corrected p value; neglog10(pval): negative log to the base 10 of the p value.

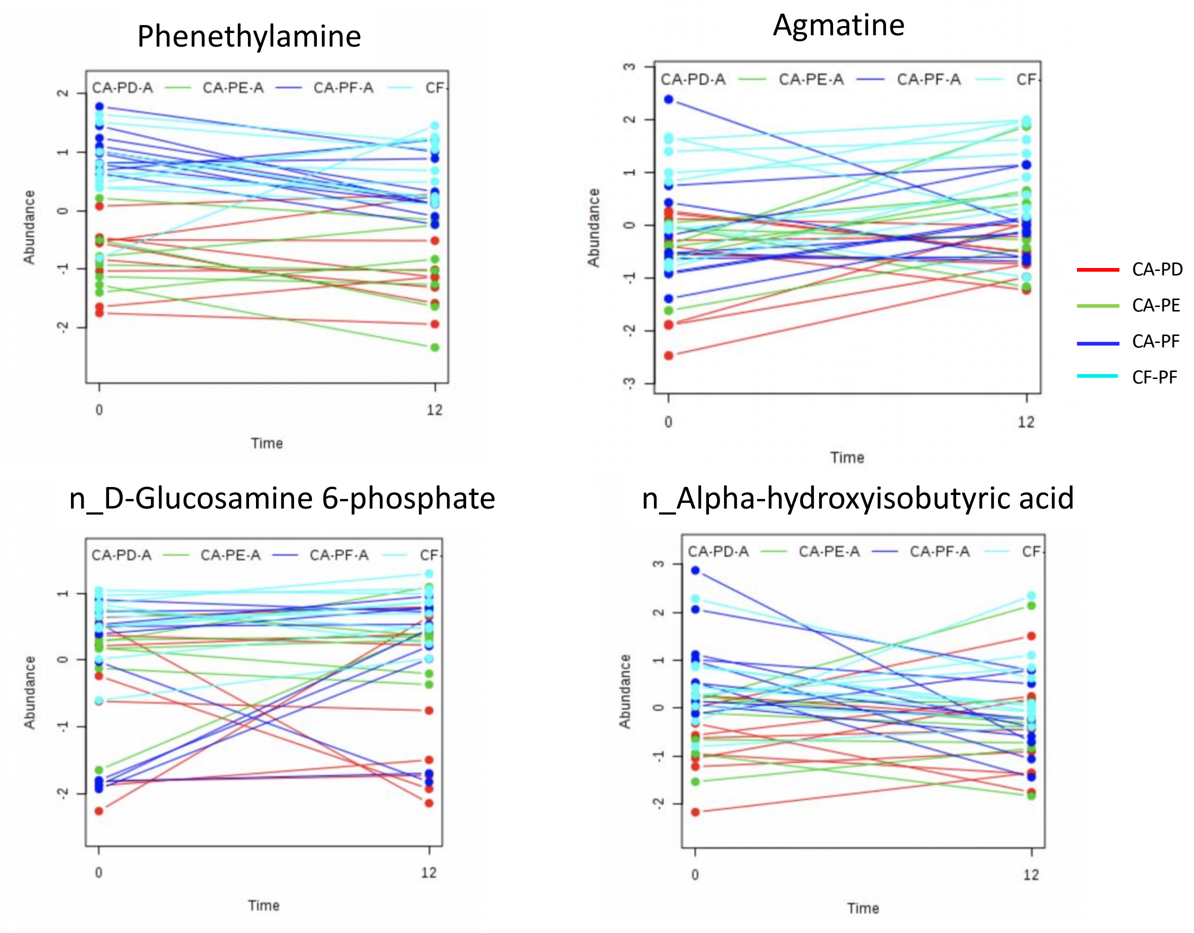
Appendix Table 5: Detection levels of certain metabolites from the EMP, pentose- phosphate, and TCA cycle pathways.

Caries Group	Plaque Group	Toothpaste	Malate		D-glucosamine 6-phosphate		Glucose/Fructose 6-phosphate		Ribulose 5-Phosphate	
			Baseline	**12-weeks**	**Baseline**	**12-weeks**	**Baseline**	**12-weeks**	**Baseline**	**12-weeks**
CF	**PF**	**A**	139470	0	115198.7	135835.6	2298242	3078330	213510.7	167746.1
		F	0	153060	111788.7	168910.4	4210439	4339846	277259.6	284977.3
CA	**PF**	**A**	0	0	78770.74	119935.4	2674644	3262047	191876.9	227459
		F	0	0	110397.7	132453.4	3037108	2961259	181016.4	194007.1
CA	**PE**	**A**	90442	162505	87731.98	103423.9	2477434	3030009	188827.2	200513.1
		F	285278	176988	152617.5	171048.7	4880925	4100869	144642.1	217347.8
CA	**PD**	**A**	231560	236190	78287.19	78726.19	2977922	1933806	224280	136153.3
		F	226133	147593	78552.57	80341.86	2079417	1988902	150534.4	153679.6



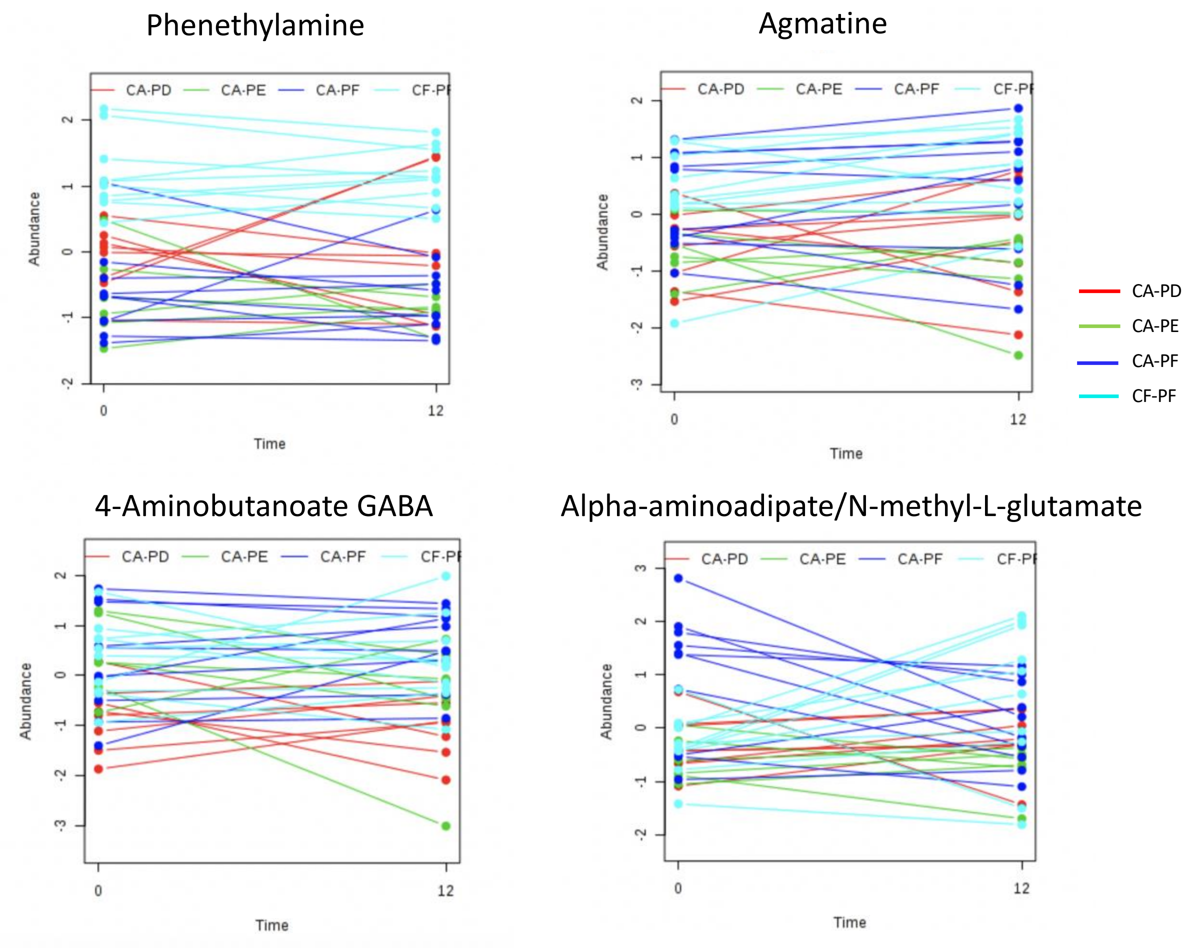
Appendix Figure 1: PLS-DA of known metabolites differently expressed at baseline.

CA: caries-active subjects; CF: caries-free subjects; PF: plaque from carious-lesion free tooth surfaces; PE: plaque from active, enamel carious lesion; PD: plaque from active, dentinal carious lesions



Appendix Figure 2: Responses of top four known metabolites to arginine treatment using Multivariate Bayesian Time Series Analysis (MEBA).

CA: caries-active subjects; CF: caries-free subjects; PF: plaque from carious-lesion free tooth surfaces; PE: plaque from active, enamel carious lesion; PD: plaque from active, dentinal carious lesions.



Phenethylamine

Agmatine

4-Aminobutanoate GABA

Alpha-aminoadipate/N-methyl-L-glutamate

Appendix Figure 3: Responses of top four known metabolites to fluoride treatment using Multivariate Bayesian Time Series Analysis (MEBA).

CA: caries-active subjects; CF: caries-free subjects; PF: plaque from carious-lesion free tooth surfaces; PE: plaque from active, enamel carious lesion; PD: plaque from active, dentinal carious lesions