Statistical Analysis Plan

Title: **Dietary Ketosis a Metabolic Sister to Calorie Restriction (CR): Fatty acids activate AMPK energy circuits modulating global methylation via the SAM/SAH axis**

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Statistical Analyses Plan (SAP)

Aaron Rendahl, PhD

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All analyses performed using R version 3.5.2 (2018-12-20). Standard citation is:

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Analysis Plan

To assess differences between the control and experimental groups, a mixed model will be fit for each variable separately using both baseline (week 0) and post program (week 12) values for each subject. Models will include week, group, and their interaction as fixed effects, gender as a covariate, and subject as a random effect. To assess significant differences, we will use the difference at week 12 adjusted for the baseline difference, and will report p-values, least squares means, and 95% confidence intervals. Further analysis of treatment effects over time will be examined by comparing the within-group differences over time.

Variables that will be modeled are HgA1c, fasting insulin, fasting glucose, NMR particle size testing, SAM/SAH ratio test and whole blood histamine from the methylation profile, and the cognitive measures, the Ray Auditory Verbal Learning Task, BVMT-R, and MoCA. Additionally, blood ketones and fasting triglycerides.

NOTE: For blood ketones and fasting triglycerides, we planned to use all measured weeks (0, 3, 6, 9, and 12) but so far we are only using weeks 0 and 12.

Variables will be checked for normality using the Box-Cox method and by viewing qqplots on the residuals. Variables that are more normally distributed after a log transformation will be transformed and ratios will be reported instead of raw differences.

After checking the Box-Cox results, all should be log-transformed except MoCA and LP-IR and SAH.

For consistency, LP-IR is log-transformed too, it’s not that much better on the original scale.

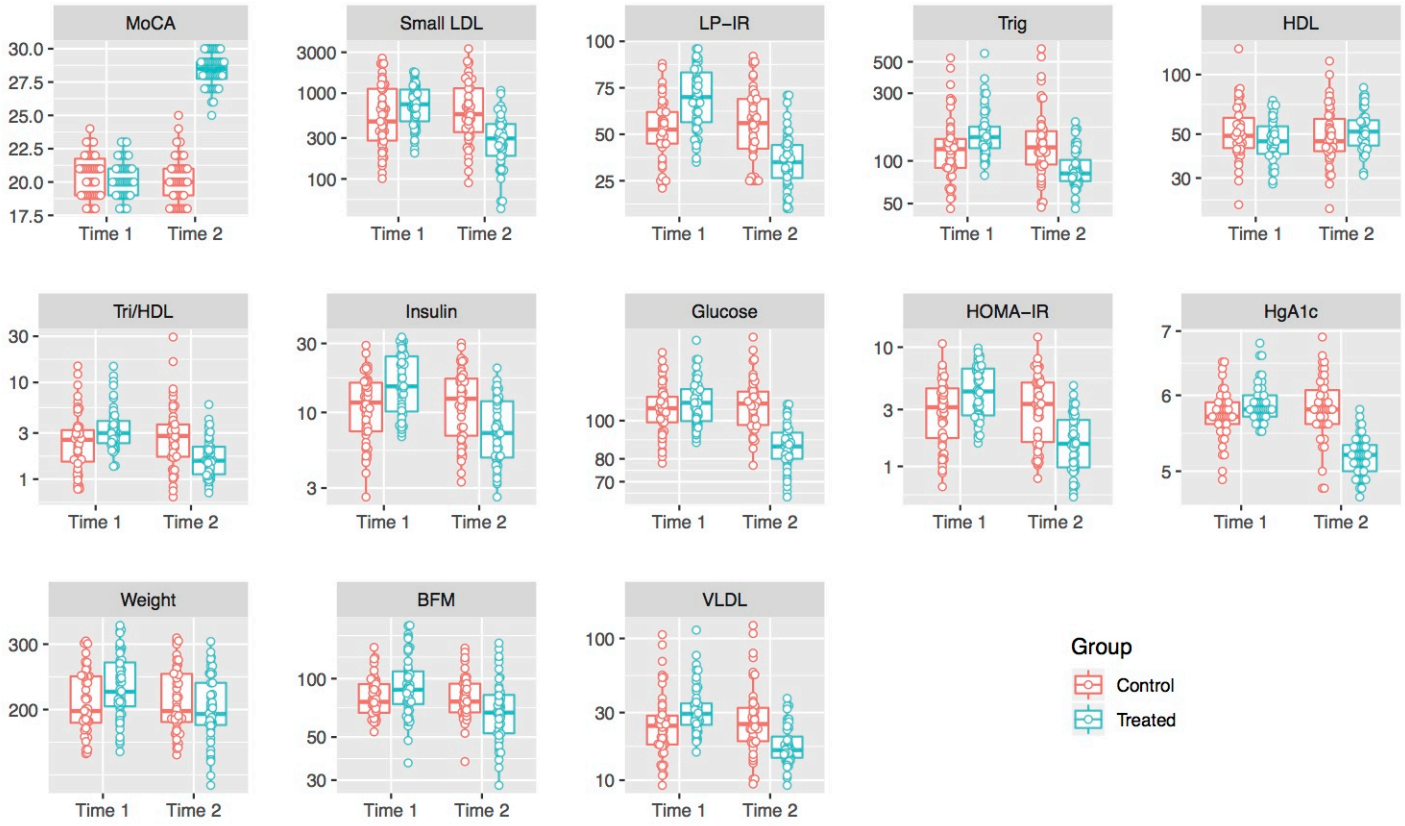

Figure 1: Boxplots for each variable, separated by timepoint and treatment group, with jittered points showing the individual data points. Variables that were log-transformed for analysis are plotted on the log-scale.

Results

The first table shows the difference (as either a ratio or a raw difference) of week 12 compared to week 1, between the two groups, along with 95% confidence intervals and p-values. That is, for a ratio, it is (week 12 / week 1 for Treatment) / (week 12 / week 1 for Control).

The second table shows the estimated marginal means (also known as least square means) for each combination, as back-transformed from the model with a log transformation was used, along with 95% confidence intervals.

Finally, for evaluation of how this affects individuals, the mean and standard deviation, as well as the median, quantiles, and ranges for the differences (or ratios) are reported for each group.

These tables are also included as csv files (named diffs, estimates, and groups).

Table 1: Estimated differences in the change from day 0 to day 12 between treatment and control groups, as either an absolute difference or a ratio (if the variable was log-transformed). A negative change (or ratio less than 1 1) means the treatment group decreased relative to the control group. Also included are 95% confidence intervals and p-values. All values are estimated from the linear mixed models.

var	measure	estimate	SE	df	lower.CL	upper.CL	t.ratio	p.value
MoCA	diff	8.241	0.229	96	7.786	8.696	35.947	< 0.0001
Small LDL	ratio	0.348	0.021	96	0.308	0.393	-17.242	< 0.0001
LP-IR	ratio	0.461	0.027	96	0.410	0.518	-13.114	< 0.0001
Trig	ratio	0.514	0.024	96	0.468	0.564	-14.062	< 0.0001
HDL	ratio	1.165	0.024	96	1.119	1.213	7.547	< 0.0001
Tri/HDL	ratio	0.441	0.024	96	0.395	0.491	-14.967	< 0.0001
Insulin	ratio	0.444	0.022	96	0.402	0.491	-16.039	< 0.0001
Glucose	ratio	0.747	0.015	96	0.719	0.777	-14.776	< 0.0001
HOMA-IR	ratio	0.332	0.020	96	0.296	0.373	-18.750	< 0.0001
HgA1c	ratio	0.867	0.007	96	0.853	0.881	-17.742	< 0.0001
Weight	ratio	0.865	0.007	96	0.850	0.879	-17.240	< 0.0001
BFM	ratio	0.752	0.022	96	0.709	0.798	-9.578	< 0.0001
VLDL	ratio	0.514	0.024	96	0.468	0.564	-14.062	< 0.0001
SAM	ratio	1.037	0.006	80	1.025	1.049	6.302	< 0.0001
SAH	ratio	0.925	0.008	80	0.909	0.942	-8.730	< 0.0001
SAM/SAH	ratio	1.130	0.013	80	1.104	1.156	10.412	< 0.0001

var	measure	estimate	SE	df	lower.CL	upper.CL	t.ratio	p.value
Adenosine	ratio	1.122	0.014	80	1.096	1.149	9.556	< 0.0001

Table 2: Estimated average values and 95% confidence intervals for each variable for day 0 and day 12 for both the treatment and control groups, as estimated by the linear mixed models, and back-transformed for variables that were first log transformed.

var	time	Group	emmean	SE	df	lower.CL	upper.CL
MoCA	1	Control	20.437	0.216	124.653	20.009	20.864
MoCA	2	Control	20.217	0.216	124.653	19.789	20.644
MoCA	1	Treated	20.358	0.221	124.653	19.921	20.794
MoCA	2	Treated	28.378	0.221	124.653	27.942	28.815
Small LDL	1	Control	522.881	54.254	103.438	425.634	642.346
Small LDL	2	Control	588.076	61.019	103.438	478.704	722.436
Small LDL	1	Treated	705.506	74.713	103.438	571.862	870.382
Small LDL	2	Treated	276.244	29.254	103.438	223.915	340.802
LP-IR	1	Control	48.608	2.575	128.164	43.771	53.979
LP-IR	2	Control	50.663	2.684	128.164	45.622	56.261
LP-IR	1	Treated	66.939	3.619	128.164	60.148	74.497
LP-IR	2	Treated	32.135	1.737	128.164	28.875	35.763

var	time	Group	emmean	SE	df	lower.CL	upper.CL
Trig	1	Control	121.520	7.818	108.453	106.972	138.046
Trig	2	Control	131.728	8.474	108.453	115.958	149.643
Trig	1	Treated	156.660	10.286	108.453	137.543	178.434
Trig	2	Treated	87.229	5.727	108.453	76.585	99.353
HDL	1	Control	51.364	1.836	102.794	47.848	55.138
HDL	2	Control	48.900	1.748	102.794	45.553	52.493
HDL	1	Treated	46.953	1.713	102.794	43.675	50.476
HDL	2	Treated	52.097	1.901	102.794	48.461	56.007
Tri/HDL	1	Control	2.366	0.200	105.245	2.001	2.798
Tri/HDL	2	Control	2.694	0.228	105.245	2.278	3.186
Tri/HDL	1	Treated	3.337	0.288	105.245	2.812	3.959
Tri/HDL	2	Treated	1.674	0.145	105.245	1.411	1.987
Insulin	1	Control	10.595	0.733	108.241	9.237	12.152
Insulin	2	Control	11.252	0.778	108.241	9.810	12.905
Insulin	1	Treated	15.518	1.096	108.241	13.491	17.849
Insulin	2	Treated	7.323	0.517	108.241	6.367	8.424

var	time	Group	emmean	SE	df	lower.CL	upper.CL
Glucose	1	Control	106.208	2.036	122.629	102.252	110.316
Glucose	2	Control	109.606	2.102	122.629	105.523	113.846
Glucose	1	Treated	111.368	2.179	122.629	107.136	115.767
Glucose	2	Treated	85.903	1.681	122.629	82.639	89.296
HOMA-IR	1	Control	2.778	0.222	108.485	2.372	3.254
HOMA-IR	2	Control	3.045	0.243	108.485	2.600	3.566
HOMA-IR	1	Treated	4.267	0.347	108.485	3.632	5.014
HOMA-IR	2	Treated	1.553	0.126	108.485	1.322	1.825
HgA1c	1	Control	5.730	0.046	121.498	5.640	5.821
HgA1c	2	Control	5.820	0.047	121.498	5.728	5.912
HgA1c	1	Treated	5.897	0.048	121.498	5.802	5.993
HgA1c	2	Treated	5.191	0.042	121.498	5.108	5.276
Weight	1	Control	209.187	5.712	97.248	198.151	220.836
Weight	2	Control	210.595	5.751	97.248	199.486	222.323
Weight	1	Treated	230.042	6.411	97.248	217.663	243.125
Weight	2	Treated	200.232	5.581	97.248	189.457	211.620

var	time	Group	emmean	SE	df	lower.CL	upper.CL
BFM	1	Control	79.578	3.470	106.408	72.988	86.763
BFM	2	Control	79.353	3.460	106.408	72.782	86.517
BFM	1	Treated	90.562	4.030	106.408	82.915	98.915
BFM	2	Treated	67.947	3.024	106.408	62.209	74.214
VLDL	1	Control	24.304	1.564	108.453	21.394	27.609
VLDL	2	Control	26.346	1.695	108.453	23.192	29.929
VLDL	1	Treated	31.332	2.057	108.453	27.509	35.687
VLDL	2	Treated	17.446	1.145	108.453	15.317	19.871
SAM	1	Control	89.365	2.646	79.783	84.252	94.789
SAM	2	Control	88.177	2.611	79.783	83.131	93.529
SAM	1	Treated	87.723	2.535	79.783	82.821	92.915
SAM	2	Treated	89.784	2.594	79.783	84.767	95.099
SAH	1	Control	19.448	0.319	85.209	18.825	20.093
SAH	2	Control	19.566	0.321	85.209	18.938	20.214
SAH	1	Treated	19.616	0.314	85.209	19.002	20.250
SAH	2	Treated	18.257	0.292	85.209	17.685	18.847

var	time	Group	emmean	SE	df	lower.CL	upper.CL
SAM/SAH	1	Control	4.595	0.200	80.482	4.214	5.010
SAM/SAH	2	Control	4.491	0.195	80.482	4.119	4.896
SAM/SAH	1	Treated	4.472	0.190	80.482	4.110	4.866
SAM/SAH	2	Treated	4.938	0.209	80.482	4.538	5.372
Adenosine	1	Control	22.524	0.482	85.700	21.587	23.503
Adenosine	2	Control	21.475	0.459	85.700	20.581	22.407
Adenosine	1	Treated	21.837	0.456	85.700	20.950	22.762
Adenosine	2	Treated	23.363	0.488	85.700	22.413	24.352

Table 3: Descriptive statistics on the changes for each variable from day 0 to day 12 for each group, including mean, standard deviation, median, quartiles, and ranges. Changes were first computed for each individual as an absolute difference, or a ratio for variables that were log-transformed.

var	Group	measure	mean	sd	min	Q1	median	Q3	max
MoCA	Control	diff	-0.220	0.954	-3.000	-1.000	0.000	0.000	2.000
MoCA	Treated	diff	8.021	1.296	5.000	7.000	8.000	9.000	11.000
Small LDL	Control	ratio	1.142	0.230	0.891	1.040	1.058	1.147	2.037
Small LDL	Treated	ratio	0.422	0.164	0.134	0.318	0.398	0.524	1.000
LP-IR	Control	ratio	1.055	0.174	0.703	1.028	1.063	1.094	1.935

var	Group	measure	mean	sd	min	Q1	median	Q3	max
LP-IR	Treated	ratio	0.513	0.172	0.164	0.394	0.517	0.613	0.931
Trig	Control	ratio	1.105	0.257	0.767	1.031	1.048	1.108	2.306
Trig	Treated	ratio	0.577	0.145	0.291	0.501	0.579	0.683	0.893
HDL	Control	ratio	0.955	0.076	0.596	0.932	0.959	0.983	1.190
HDL	Treated	ratio	1.117	0.131	0.833	1.050	1.101	1.150	1.587
Tri/HDL	Control	ratio	1.169	0.317	0.787	1.039	1.100	1.178	2.416
Tri/HDL	Treated	ratio	0.526	0.157	0.232	0.415	0.520	0.626	0.986
Insulin	Control	ratio	1.068	0.116	0.806	1.016	1.061	1.128	1.342
Insulin	Treated	ratio	0.498	0.163	0.198	0.387	0.484	0.576	0.928
Glucose	Control	ratio	1.034	0.059	0.898	0.991	1.044	1.063	1.176
Glucose	Treated	ratio	0.777	0.088	0.438	0.735	0.788	0.839	0.913
HOMA-IR	Control	ratio	1.105	0.139	0.859	1.033	1.129	1.181	1.553
HOMA-IR	Treated	ratio	0.391	0.143	0.101	0.303	0.374	0.469	0.839
HgA1c	Control	ratio	1.016	0.037	0.943	0.983	1.017	1.036	1.131
HgA1c	Treated	ratio	0.881	0.037	0.742	0.869	0.882	0.899	0.947
Weight	Control	ratio	1.007	0.018	0.949	0.994	1.011	1.018	1.040

var	Group	measure	mean	sd	min	Q1	median	Q3	max
Weight	Treated	ratio	0.872	0.049	0.743	0.841	0.868	0.903	1.003
BFM	Control	ratio	1.000	0.068	0.548	0.996	1.009	1.024	1.065
BFM	Treated	ratio	0.762	0.124	0.378	0.711	0.780	0.843	1.010
VLDL	Control	ratio	1.105	0.257	0.767	1.031	1.048	1.108	2.306
VLDL	Treated	ratio	0.577	0.145	0.291	0.501	0.579	0.683	0.893
SAM	Control	ratio	0.987	0.031	0.876	0.975	0.987	1.000	1.054
SAM	Treated	ratio	1.024	0.020	0.977	1.011	1.025	1.035	1.068
SAH	Control	ratio	1.007	0.040	0.975	0.990	1.000	1.011	1.230
SAH	Treated	ratio	0.932	0.041	0.860	0.899	0.927	0.963	1.011
SAM/SAH	Control	ratio	0.979	0.051	0.780	0.966	0.982	1.006	1.057
SAM/SAH	Treated	ratio	1.105	0.058	1.004	1.068	1.099	1.137	1.296
Adenosine	Control	ratio	0.954	0.046	0.870	0.916	0.953	0.973	1.056
Adenosine	Treated	ratio	1.072	0.066	0.947	1.040	1.053	1.100	1.263