Supplementary Material

Metabolic Evidence of Diminished Lipid Oxidation in Women With Polycystic Ovary Syndrome

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Supplementary Table 1. Mean and standard error of the mean (SEM) for normal and PCOS women before and after a 2-hour oral glucose tolerance test.

Average	2-Amino-butyrate	2-Hydroxy-butyrate	2-Hydroxy-isovalerate	2-Oxo-caproate	2-Oxo-isocaproate	3-Hydroxy-butyrate	3-Methyl-2-oxo-valerate	Acetate	Aceto-acetate	Acetone	Alanine	Asparagine	Betaine	Citrate	Creatine	Creatinine	DSS (Chemical Shape Indicator)	Dimethyl-amine	Formate	Fructose	Glucose	
Normal Fasted	0.009	0.020	0.004	0.002	0.014	0.050	0.010	0.066	0.005	0.001	0.217	0.050	0.044	0.007	0.030	0.051	1.000	0.004	0.068	0.267	2.125	
Normal 2hOGTT	0.012	0.015	0.004	0.003	0.009	0.007	0.006	0.069	0.003	0.001	0.233	0.043	0.048	0.009	0.032	0.047	1.001	0.003	0.074	0.243	1.932	
PCOS fasted	0.018	0.029	0.006	0.005	0.017	0.068	0.015	0.127	0.014	0.001	0.401	0.079	0.051	0.003	0.058	0.072	1.000	0.004	0.087	0.471	4.299	
PCOS 2hOGTT	0.014	0.017	0.006	0.004	0.011	0.010	0.010	0.108	0.009	0.001	0.370	0.060	0.056	0.003	0.058	0.072	1.000	0.003	0.086	0.534	4.713	
SEM																						
Normal Fasted	0.003	0.002	0.001	0.001	0.001	0.018	0.001	0.003	0.002	0.000	0.012	0.003	0.003	0.002	0.004	0.004	0.000	0.001	0.005	0.010	0.030	
Normal 2hOGTT	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.000	0.011	0.002	0.004	0.002	0.005	0.002	0.000	0.001	0.004	0.031	0.076	
PCOS fasted	0.003	0.008	0.002	0.003	0.003	0.019	0.003	0.027	0.004	0.000	0.084	0.015	0.009	0.002	0.015	0.013	0.000	0.001	0.008	0.097	1.071	
PCOS 2hOGTT	0.002	0.003	0.002	0.002	0.001	0.002	0.002	0.019	0.003	0.000	0.066	0.009	0.009	0.001	0.015	0.014	0.000	0.001	0.004	0.125	1.350	

Average	Glutamate	Glutamine	Glycerol	Homo-serine	Isobutyrate	Isoleucine	Lactate	Leucine	Lysine	Mannose	Methionine	Ornithine	Phenyl-alanine	Proline	Propionate	Pyruvate	Sarcosine	Taurine	Threonine	Tryptophan	Tyrosine	Valine
Normal Fasted	0.086	0.282	1.887	0.029	0.005	0.034	0.951	0.054	0.081	0.030	0.021	0.035	0.049	0.109	0.009	0.003	0.001	0.050	0.106	0.031	0.042	0.110
Normal 2hOGTT	0.077	0.245	1.961	0.021	0.004	0.020	1.274	0.034	0.069	0.019	0.015	0.028	0.040	0.097	0.005	0.006	0.001	0.052	0.085	0.026	0.030	0.086
PCOS fasted	0.125	0.353	1.027	0.111	0.008	0.055	1.803	0.080	0.130	0.070	0.031	0.052	0.062	0.200	0.015	0.010	0.002	0.092	0.202	0.045	0.077	0.161
PCOS 2hOGTT	0.103	0.320	1.235	0.044	0.007	0.031	1.681	0.052	0.111	0.044	0.021	0.051	0.048	0.191	0.011	0.009	0.001	0.069	0.156	0.040	0.057	0.127
SEM																						
Normal Fasted	0.006	0.014	0.498	0.014	0.001	0.002	0.061	0.002	0.003	0.004	0.001	0.004	0.002	0.007	0.002	0.001	0.000	0.010	0.006	0.001	0.003	0.006
Normal 2hOGTT	0.006	0.013	0.609	0.012	0.000	0.001	0.113	0.001	0.004	0.003	0.001	0.004	0.002	0.007	0.001	0.001	0.000	0.003	0.005	0.001	0.002	0.004
PCOS fasted	0.018	0.035	0.304	0.045	0.001	0.011	0.440	0.014	0.023	0.017	0.005	0.018	0.010	0.049	0.005	0.004	0.000	0.025	0.049	0.008	0.018	0.031
PCOS 2hOGTT	0.013	0.028	0.307	0.038	0.001	0.006	0.288	0.009	0.019	0.011	0.003	0.010	0.007	0.046	0.004	0.003	0.000	0.018	0.034	0.007	0.015	0.023

Supplementary Table 2. Metabolites with different responses to glucose perturbation. The mean concentrations of these metabolites in each group at fasting and 2hOGTT are shown along with their corresponding SEM. The fold changes columns show the direction of changes from the fasting to 2hOGTT; Equation 1 was used to calculate the fold changes. The fold changes in normal women indicate increasing concentration changes in these metabolites after the 2hOGTT challenge. In contrast, in PCOS women the fold changes after 2hOGTT challenge decrease, meaning the response in these metabolites to oral glucose is opposite in PCOS and normal subjects.

Metabolite	Concentration (mM) +/- SEM		Normal Fold Changes	Concentration +/- SEM		PCOS Fold Changes
	Normal (fasting)	Normal (2h)		PCOS (fasting)	PCOS (2h)	
2-Aminobutrate	0.01792 +/- 0.00473	0.02376 +/- 0.00354	0.3260	0.03614 +/- 0.00630	0.02732 +/- 0.00398	-0.2441
2-Oxocaporate	0.00434 +/- 0.00161	0.00595 +/- 0.00141	0.3712	0.01097 +/- 0.00533	0.00892 +/- 0.00452	-0.1870
Acetate	0.13160 +/- 0.00486	0.13886 +/- 0.00419	0.0551	0.25387 +/- 0.04985	0.21599 +/- 0.03470	-0.1492
Acetone	0.00185 +/- 0.00010	0.00202 +/- 0.00014	0.0938	0.00211 +/- 0.00067	0.00177 +/- 0.00015	-0.1620
Alanine	0.43414 +/- 0.02249	0.46583 +/- 0.02133	0.0729	0.80207 +/- 0.15560	0.74059 +/- 0.12156	-0.0766
Formate	0.13602 +/- 0.00943	0.14754 +/- 0.00695	0.0847	0.17317 +/- 0.01541	0.17122 +/- 0.00819	-0.0112
Fructose	0.53448 +/- 0.01883	0.48657 +/- 0.05708	-0.0896	0.94188 +/- 0.17946	1.06769 +/- 0.23161	0.1335
Glucose	4.24894 +/- 0.05585	3.85964 +/- 0.14193	-0.0916	8.59852 +/- 1.98379	9.42809 +/- 2.50166	0.0964
Lactate	1.90185 +/- 0.11360	2.54671 +/- 0.21269	0.3390	3.60643 +/- 0.81563	3.36272 +/- 0.53344	-0.0675
Pyruvate	0.00610 +/- 0.00176	0.01237 +/- 0.00167	1.0279	0.02052 +/- 0.00697	0.01863 +/- 0.00593	-0.0918
Sarcosine	0.00130 +/- 0.00030	0.00190 +/- 0.00020	0.4603	0.00329 +/- 0.00077	0.00197 +/- 0.00076	-0.4001
Taurine	0.09969 +/- 0.01804	0.10301 +/- 0.00526	0.0333	0.18446 +/- 0.04651	0.13862 +/- 0.03327	-0.2485

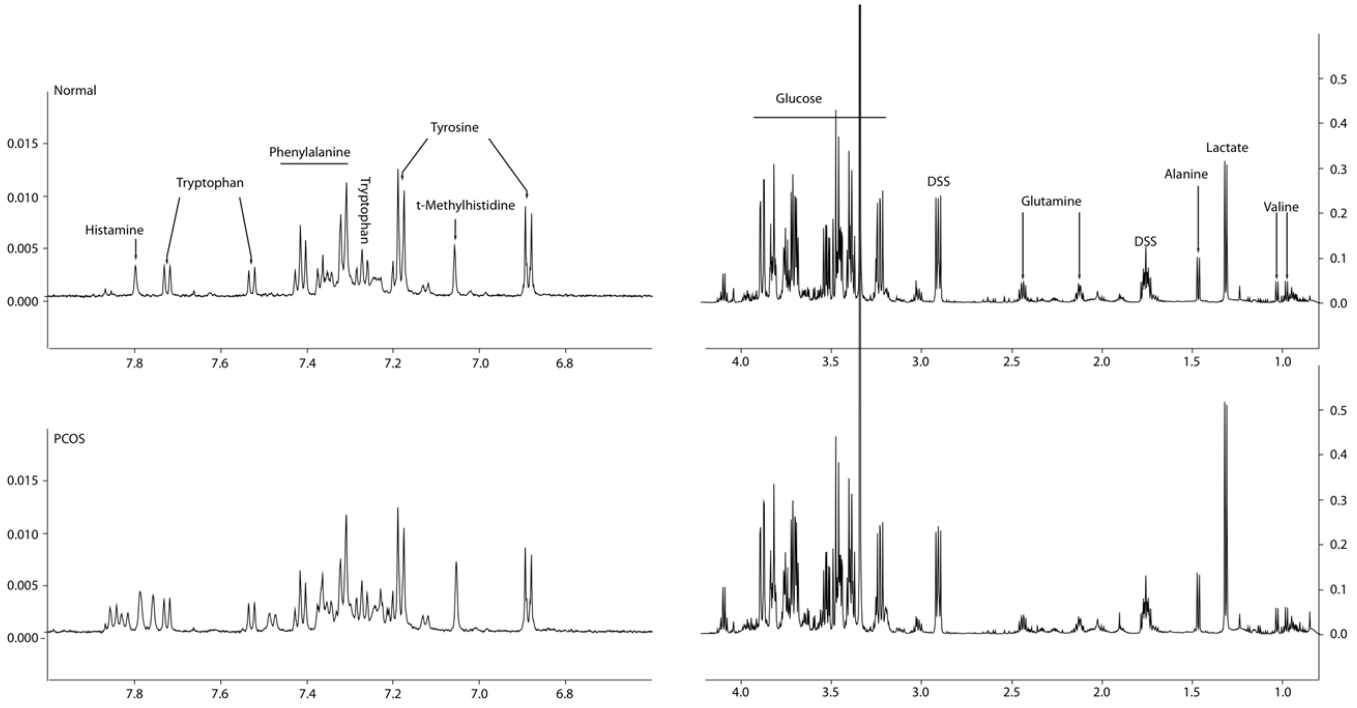
Supplementary Table 3. Mean (± SEM) urinary N excretion.

	Normal (g)	PCOS (g)
Day	5.86 ± 0.75	7.16 ± 1.07
Night	1.98 ± 0.21	2.54 ± 0.30

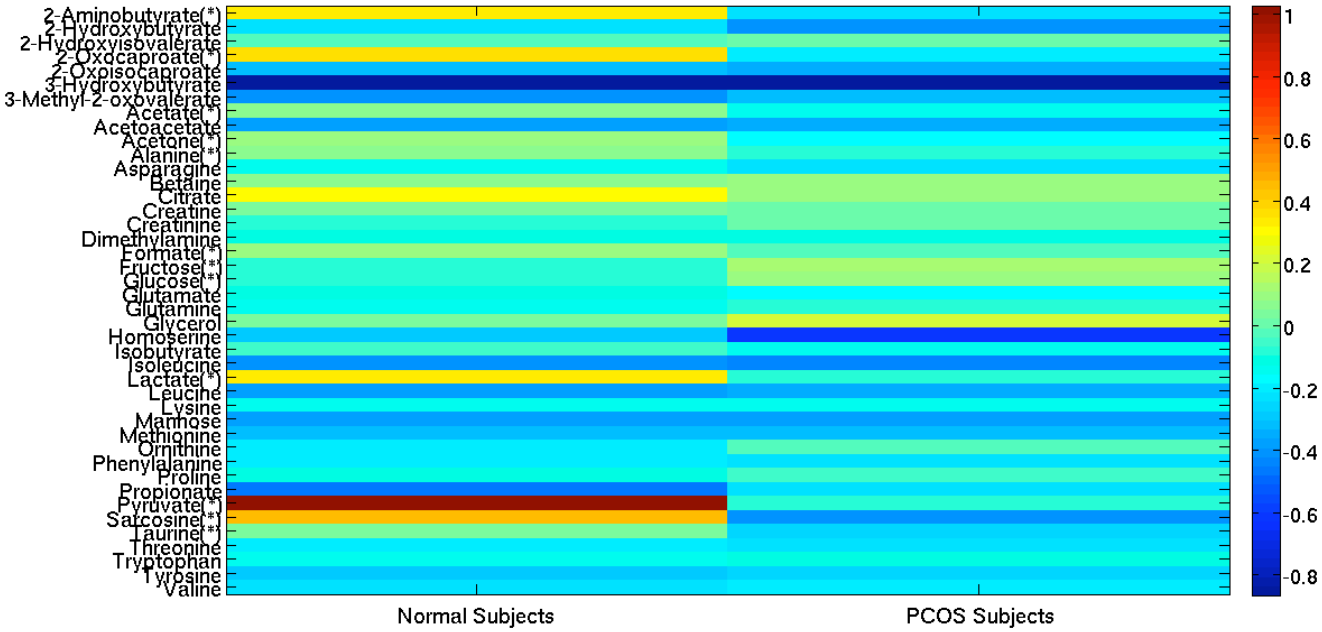
Supplementary Table 4. Mean (± SEM)* breath $\delta^{13}CO_2$..

	Control (δ, ‰)	PCOS (δ, ‰)
06:00 (WAKE-UP)	-23.1 ± 0.3	-21.6 ± 0.3
07:40 (PRE-BREAKFAST)	-22.6 ± 0.3	-21.9 ± 0.3
POST-BREAKFAST	-22.6 ± 0.3	-21.9 ± 0.3
9:40	-21.9 ± 0.3	-21.4 ± 0.3
12:00 (PRE-LUNCH)	-20.9 ± 0.3	-20.8 ± 0.3
POST-LUNCH	-21.1 ± 0.3	-21.1 ± 0.3
14:00	-20.9 ± 0.3	-21.1 ± 0.3
15:00	-20.6 ± 0.3	-20.8 ± 0.3
18:00 (PRE-DINNER)	-21.7 ± 0.3	-21.8 ± 0.3
POST-DINNER	-22.0 ± 0.3	-22.0 ± 0.3
20:00	-21.7 ± 0.3	-21.7 ± 0.3

*Least square means from repeated measures ANOVA.



Supplementary Fig. (1). Representative 1D ^{1}H-NMR spectra of fasting serum from a normal (upper panels) and a PCOS (lower panels) subject: right panels show the 0.85−4.15 ppm chemical shift region; left panels show the 6.60−8.00 ppm chemical shift region. Serum samples were collected from subjects after waking during an inpatient stay. Spectra were collected as 512 scans on Varian 800 MHz NMR spectrometer equipped with a cold probe. Chenomx NMR suite 6.1 was used to identify the metabolites.



Supplementary Fig. (2). Heat map showing the fold changes between the fasting and 2hOGTT conditions for the normal and PCOS subjects. Metabolites labeled with (*) are ones that change in opposite directions in normal and PCOS subjects; with exception of glucose and fructose, these metabolites increase from fasting to 2hOGTT in the normal subjects and decrease from fasting to 2hOGTT in the PCOS subjects. The color index at the right of the figure provides a key to the fold change. Those metabolites that show trends toward significant increase are: acetoacetate (P = 0.079), acetate (P = 0.06), 2-amino butyrate (P = 0.055), and sarcosine (P = 0.06).