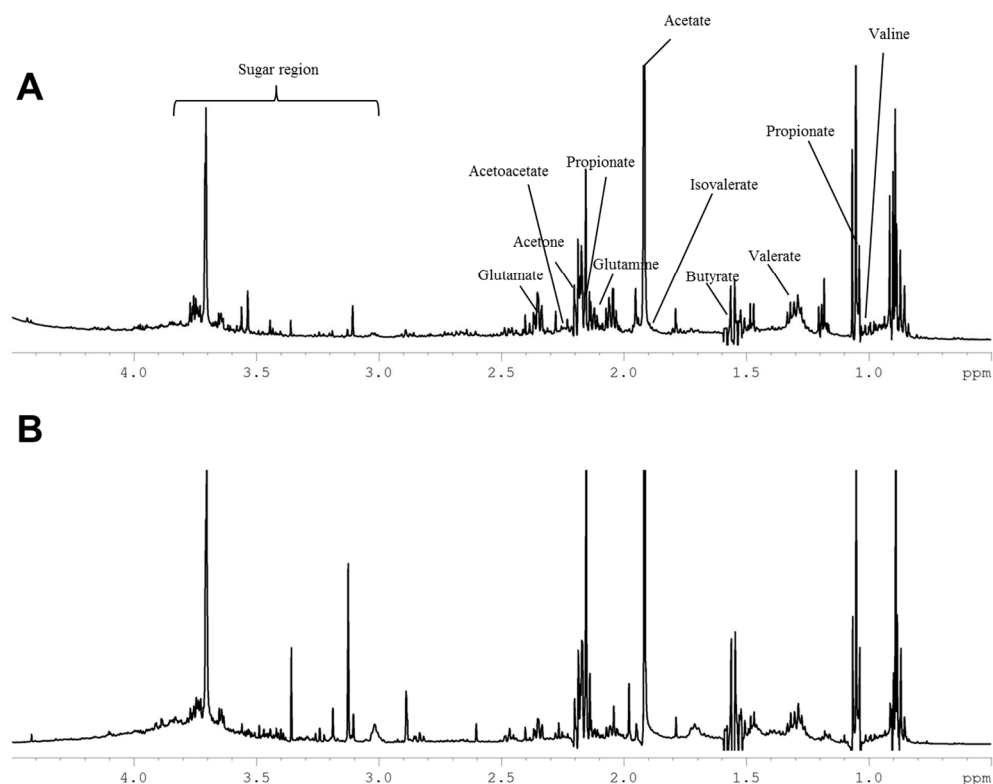
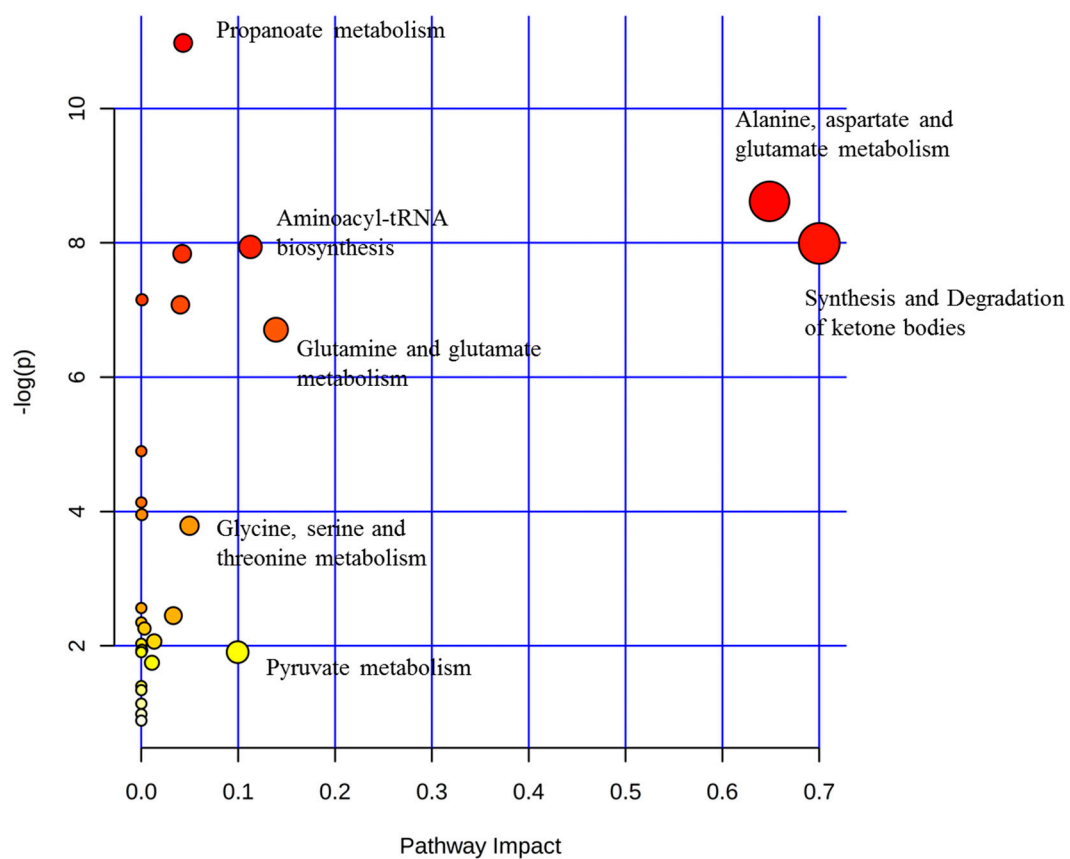


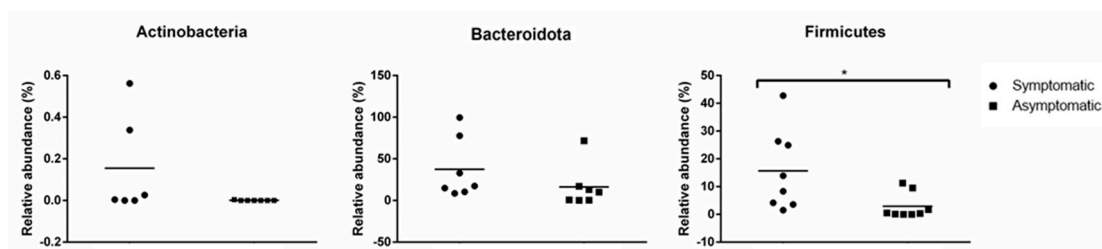
## SUPPLEMENTARY MATERIAL



**Figure S1.** Representative spectra of symptomatic (A) and asymptomatic (B) subjects, showing the NMR assignments for important metabolites.  $^1\text{H}$  1D spectra were obtained at 500 MHz in a Bruker Advance spectrometer at 25°C.



**Figure S2.** Metabolic pathways and their impact in the fecal metabolic profile changes, provided by Metaboanalyst 4.0.



**Figure S3.** Difference in relative abundance of the Actinobacteria, Bacteroidota and Firmicutes phyla between symptomatic and asymptomatic groups. Only the Firmicutes phylum presented a statistically significant difference between groups ( $p = 0.015$ ).

**Table S1.** Performances indexes of the classifications performed with PLS-DA methods for fecal metabolites from Asymptomatic x Symptomatic and High Vagal tone x Low Vagal tone groups.

<b>Model Performance</b>	<b>Asymptomatic x Symptomatic</b>	<b>High Vagal tone x Low Vagal tone</b>
ACC	0.68	0.66
R <sup>2</sup>	0.99	0.99
Q <sup>2</sup>	0.19	0.01
AUC	0.38	0.67
Sensitivity	0.63	1.00
Specificity	0.63	0.45

**Table S2.** Metabolite intensities shown as the mean (arbitrary units), confidence interval, bucket region (ppm) and statistical analysis from multivariate analysis using the VIP score and parametric t-test univariate analysis ( $p < 0.05$ ). NSD = No statistical difference in the t-test.

Metabolites and IDs	ppm region	A Asymptomatic	B Symptomatic	C Low Vagal Tone	D High Vagal Tone	p-value
Acetate <sup>1</sup> (HMDB000042)	1.91	21.0 x 10 <sup>-2</sup> (8.2 x 10 <sup>-2</sup> – 36.5 x 10 <sup>-2</sup> )	19.1 x 10 <sup>-2</sup> (14.3 x 10 <sup>-2</sup> – 30.0 x 10 <sup>-2</sup> )	18.4 x 10 <sup>-2</sup> (11.4 x 10 <sup>-2</sup> – 30.0 x 10 <sup>-2</sup> )	24.2 x 10 <sup>-2</sup> (8.2 x 10 <sup>-2</sup> – 36.5 x 10 <sup>-2</sup> )	NSD
Acetoacetate <sup>1,2</sup> (HMDB0304256)	2.27	0.4 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.5 x 10 <sup>-2</sup> )	0.6 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.8 x 10 <sup>-2</sup> )	0.5 x 10 <sup>-2</sup> (0.3 x 10 <sup>-2</sup> – 0.8 x 10 <sup>-2</sup> )	0.3 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.5 x 10 <sup>-2</sup> )	0.02 <sup>AB</sup> 0.006 <sup>CD</sup>
Acetone <sup>1,2</sup> (HMDB01659)	2.21	0.5 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.9 x 10 <sup>-2</sup> )	0.8 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 1.3 x 10 <sup>-2</sup> )	0.8 x 10 <sup>-2</sup> (0.4 x 10 <sup>-2</sup> – 1.3 x 10 <sup>-2</sup> )	0.4 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.8 x 10 <sup>-2</sup> )	0.005 <sup>CD</sup>
Aspartate <sup>2</sup> (HMDB00191)	2.69	0.2 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.3 x 10 <sup>-2</sup> )	0.3 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.4 x 10 <sup>-2</sup> )	0.2 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.4 x 10 <sup>-2</sup> )	0.2 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.2 x 10 <sup>-2</sup> )	0.02 <sup>AB</sup> 0.04 <sup>CD</sup>
Butyrate II <sup>1</sup> (HMDB0000039)	1.55			0.6 x 10 <sup>-2</sup> (0.0 x 10 <sup>-2</sup> – 1.0 x 10 <sup>-2</sup> )	0.1 x 10 <sup>-2</sup> (0.0 x 10 <sup>-2</sup> – 0.4 x 10 <sup>-2</sup> )	NSD
Glutamine <sup>1</sup> (HMDB0003423)	2.12	1.1 x 10 <sup>-2</sup> (0.8 x 10 <sup>-2</sup> – 1.5 x 10 <sup>-2</sup> )	1.1 x 10 <sup>-2</sup> (0.9 x 10 <sup>-2</sup> – 2.3 x 10 <sup>-2</sup> )			NSD
Glutamate I <sup>1,2</sup> (HMDB00148)	2.33	1.2 x 10 <sup>-2</sup> (0.7 x 10 <sup>-2</sup> – 2.2 x 10 <sup>-2</sup> )	1.6 x 10 <sup>-2</sup> (0.9 x 10 <sup>-2</sup> – 2.6 x 10 <sup>-2</sup> )	1.5 x 10 <sup>-2</sup> (0.8 x 10 <sup>-2</sup> – 2.6 x 10 <sup>-2</sup> )	0.9 x 10 <sup>-2</sup> (0.7 x 10 <sup>-2</sup> – 1.4 x 10 <sup>-2</sup> )	0.04 <sup>CD</sup>
Glutamate II <sup>1,2</sup> (HMDB00148)	2.36	0.6 x 10 <sup>-2</sup> (0.4 x 10 <sup>-2</sup> – 1.0 x 10 <sup>-2</sup> )	0.8 x 10 <sup>-2</sup> (0.4 x 10 <sup>-2</sup> – 1.2 x 10 <sup>-2</sup> )	0.8 x 10 <sup>-2</sup> (0.4 x 10 <sup>-2</sup> – 1.2 x 10 <sup>-2</sup> )	0.5 x 10 <sup>-2</sup> (0.4 x 10 <sup>-2</sup> – 0.6 x 10 <sup>-2</sup> )	0.01 <sup>CD</sup>
Isovalerate <sup>1,2</sup> (HMDB0000718)	1.97			0.7 x 10 <sup>-2</sup> (0.3 x 10 <sup>-2</sup> – 1.0 x 10 <sup>-2</sup> )	0.4 x 10 <sup>-2</sup> (0.3 x 10 <sup>-2</sup> – 0.7 x 10 <sup>-2</sup> )	0.01 <sup>CD</sup>
Malate <sup>2</sup> (HMDB0031518)	2.66	0.3 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.4 x 10 <sup>-2</sup> )	0.4 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.5 x 10 <sup>-2</sup> )	0.4 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.5 x 10 <sup>-2</sup> )	0.2 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.3 x 10 <sup>-2</sup> )	0.04 <sup>AB</sup> 0.02 <sup>CD</sup>
Methionine <sup>2</sup> (HMDB00696)	2.63			0.4 x 10 <sup>-2</sup> (0.1 x 10 <sup>-2</sup> – 0.5 x 10 <sup>-2</sup> )	0.2 x 10 <sup>-2</sup> (0.2 x 10 <sup>-2</sup> – 0.3 x 10 <sup>-2</sup> )	0.01 <sup>CD</sup>
Propionate I <sup>1</sup> (HMDB00237)	1.04			4.0 x 10 <sup>-2</sup> (1.8 x 10 <sup>-2</sup> – 8.1 x 10 <sup>-2</sup> )	4.8 x 10 <sup>-2</sup> (3.4 x 10 <sup>-2</sup> – 6.1 x 10 <sup>-2</sup> )	NSD
Propionate II <sup>1</sup> (HMDB00237)	2.15			3.4 x 10 <sup>-2</sup> (1.3 x 10 <sup>-2</sup> – 5.6 x 10 <sup>-2</sup> )	4.2 x 10 <sup>-2</sup> (2.3 x 10 <sup>-2</sup> – 6.0 x 10 <sup>-2</sup> )	NSD

Sarcosine <sup>2</sup> (HMDB00271)	2.72	$0.2 \times 10^{-2}$ ( $0.1 \times 10^{-2} - 0.3 \times 10^{-2}$ )	$0.3 \times 10^{-2}$ ( $0.1 \times 10^{-2} - 0.4 \times 10^{-2}$ )			0.04 <sup>AB</sup>
Sugar region I <sup>1</sup>	3.38	$0.5 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 1.8 \times 10^{-2}$ )	$0.2 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 0.3 \times 10^{-2}$ )			NSD
Sugar region II <sup>1</sup>	3.41	$0.7 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 2.4 \times 10^{-2}$ )	$0.3 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 0.4 \times 10^{-2}$ )			NSD
Sugar region III <sup>1</sup>	3.47	$0.6 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 1.7 \times 10^{-2}$ )	$0.3 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 0.5 \times 10^{-2}$ )	$3.2 \times 10^{-2}$ ( $1.0 \times 10^{-2} - 11.2 \times 10^{-12}$ )	$4.2 \times 10^{-2}$ ( $0.7 \times 10^{-2} - 15.5 \times 10^{-2}$ )	NSD
Sugar region IV <sup>1</sup>	3.68	$4.5 \times 10^{-2}$ ( $1.1 \times 10^{-2} - 6.2 \times 10^{-2}$ )	$3.4 \times 10^{-2}$ ( $1.0 \times 10^{-2} - 11.2 \times 10^{-2}$ )	$2.1 \times 10^{-2}$ ( $1.0 \times 10^{-2} - 3.2 \times 10^{-2}$ )	$1.6 \times 10^{-2}$ ( $1.4 \times 10^{-2} - 1.7 \times 10^{-2}$ )	NSD
Unassigned I <sup>2</sup>	1.07	$0.1 \times 10^{-2}$ ( $0.0 \times 10^{-2} - 0.2 \times 10^{-2}$ )	$0.3 \times 10^{-2}$ ( $0.1 \times 10^{-2} - 0.9 \times 10^{-2}$ )			0.005 <sup>AB</sup>
Unassigned II <sup>1,2</sup>	1.94	$1.0 \times 10^{-2}$ ( $0.5 \times 10^{-2} - 1.5 \times 10^{-2}$ )	$1.3 \times 10^{-2}$ ( $1.0 \times 10^{-2} - 1.8 \times 10^{-2}$ )	$1.3 \times 10^{-2}$ ( $0.6 \times 10^{-2} - 1.8 \times 10^{-2}$ )	$0.9 \times 10^{-2}$ ( $0.5 \times 10^{-2} - 1.3 \times 10^{-2}$ )	0.03 <sup>AB</sup> 0.04 <sup>CD</sup>
Unassigned III <sup>1,2</sup>	2.24			$0.7 \times 10^{-2}$ ( $0.4 \times 10^{-2} - 1.0 \times 10^{-2}$ )	$0.5 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 0.9 \times 10^{-2}$ )	0.01 <sup>CD</sup>
Unassigned IV <sup>1,2</sup>	2.30			$0.4 \times 10^{-2}$ ( $0.3 \times 10^{-2} - 0.6 \times 10^{-2}$ )	$0.3 \times 10^{-2}$ ( $0.2 \times 10^{-2} - 0.4 \times 10^{-2}$ )	0.002 <sup>CD</sup>
Valerate I <sup>1</sup> (HMDB00892)	0.89			$3.3 \times 10^{-2}$ ( $1.5 \times 10^{-2} - 7.0 \times 10^{-2}$ )	$4.3 \times 10^{-2}$ ( $2.5 \times 10^{-2} - 6.6 \times 10^{-2}$ )	NSD
Valerate II <sup>1</sup> (HMDB00892)	1.31	$1.2 \times 10^{-2}$ ( $0.8 \times 10^{-2} - 1.5 \times 10^{-2}$ )	$1.5 \times 10^{-2}$ ( $0.9 \times 10^{-2} - 2.0 \times 10^{-2}$ )			NSD
Valerate III <sup>1,2</sup> (HMDB00892)	1.34	$1.0 \times 10^{-2}$ ( $0.8 \times 10^{-2} - 1.3 \times 10^{-2}$ )	$1.3 \times 10^{-2}$ ( $0.9 \times 10^{-2} - 1.6 \times 10^{-2}$ )			0.04 <sup>AB</sup>
Valine <sup>1</sup> (HMDB0000883)	1.01	$0.4 \times 10^{-2}$ ( $0.0 \times 10^{-2} - 0.8 \times 10^{-2}$ )	$0.7 \times 10^{-2}$ ( $0.4 \times 10^{-2} - 1.9 \times 10^{-2}$ )			NSD

<sup>1</sup> Metabolites presenting a statistical difference in VIP score; <sup>2</sup> Metabolites presenting a statistical difference in parametric t-test univariate analysis.

**Table S3.** Linear regression among parameters and metabolites. Bold letters indicate statistical differences ( $p < 0.05$ ).

	<b>RMSSD</b>	<b>BDI-II</b>	<b>STAI-T</b>	<b>HF</b>
RMSSD		0.05	0.08	<b>0.74</b>
BDI-II	0.05		<b>0.67</b>	0.00
STAI-T	0.008	<b>0.67</b>		0.03
HF	<b>0.74</b>	0.00	0.03	
Butyrate	0.002	0.08	0.10	0.001
Acetate	0.02	0.002	0.07	0.001
Propionate	0.06	0.01	0.02	0.009
Glutamate	0.20	0.01	0.06	0.06
Glutamine	0.008	0.00	0.04	0.00
Acetoacetate	<b>0.29</b>	0.19	<b>0.33</b>	0.20
Valerate	0.09	0.06	0.001	0.04
Aspartate	0.13	0.18	0.15	0.10
Valine	0.04	<b>0.28</b>	0.16	0.00
Isovalerate	0.14	0.00	0.04	0.17
Methionine	<b>0.33</b>	0.05	0.03	0.21
Malate	0.22	0.21	0.10	0.14
Sarcosine	0.01	0.10	0.16	0.01
Acetone	<b>0.34</b>	0.18	0.13	<b>0.26</b>