Supporting Information

for

Evaluation of intensity drift correction strategies using MetaboDrift, a normalization tool for multi-batch metabolomics data

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Table S1. List of all 122 metabolites measured in the MMTT study; also includes adjusted p-values and designation of metabolites significantly different in non-obese subjects (n=16) between t=0 and t=60 min following consumption of MMTT. p-values were adjusted for multiple comparisons using Benjamini-Hochberg correction with FDR of 0.05. A= significantly different using QUAD-drift corrected data ($p<0.05$); B= significantly different using RAW data ($p<0.05$)

Metabolite	Benj Hoch adj. p-value (QUAD drift corrected)	Benj Hoch adj. p-value (RAW data)	Sig?	Metabolite	Benj Hoch adj. p-value (QUAD drift corrected)	Benj Hoch adj. p-value (RAW data)	Sig?
Palmitic acid	3.6553E-07	6.10741E-06	A,B	Acetoacetate	0.137401224	0.904483751	
Linoleic acid	4.67745E-07	6.10741E-06	A,B	Carnitine-L	0.150977561	0.231747482	
Oleic acid	5.54432E-07	6.10741E-06	A,B	Succinate	0.152619931	0.087794242	
Stearic acid	1.28802E-06	6.10741E-06	A,B	Ornithine	0.169659816	0.843596605	
Myristic acid	1.51476E-06	6.10741E-06	A,B	TG(54:1)	0.169659816	0.821761824	
Proline	1.51476E-06	0.000161712	A,B	Citrulline	0.179710914	0.881977334	
Alanine	3.76227E-05	0.000529608	A,B	DAG(36:1)	0.180360221	0.881977334	
Palmitoleic acid	3.76227E-05	0.000189576	A,B	PE(38:4)	0.181293783	0.74358905	
Tyrosine	3.76227E-05	0.003081065	A,B	LysoPC(18:0)	0.182664884	0.786713883	
Lysine	3.91925E-05	0.009581752	A,B	TG(52:4)	0.190455454	0.581298183	
(Iso)leucine	7.06099E-05	0.001584709	A,B	DAG(36:2)	0.200798824	0.762417211	
Methionine	0.000137403	0.004873003	A,B	PA(36:1)	0.201138951	0.562948726	
Valine	0.000137403	0.07838589	A	Carnitine-C18	0.218159831	0.762417211	
Phenylalanine	0.000153922	0.033869865	A,B	PE(38:6)	0.218159831	0.769716631	
Chenodeoxyglycocholic acid	0.000283313	0.004992161	A,B	PI(36:1)	0.218159831	0.897969275	
LysoPG(18:1)	0.000529572	0.009655643	A,B	TG(52:2)	0.218159831	0.645457836	
Histidine	0.000569032	0.01919214	A,B	LysoPE(16:0)	0.240725308	0.786713883	
Arachidic acid	0.000618785	0.000196553	A,B	Carnitine-C8:1	0.250910604	0.288796917	
Tryptophan	0.000678228	0.233085688	A	PE(34:1)	0.256264987	0.655581338	
Carnitine-C14:1	0.00128093	0.00824919	A,B	N-Acetylornithine	0.260424703	0.60422045	
Threonine	0.0013842	0.01325256	A,B	LysoPS(18:0)	0.261528057	0.390490028	
Arginine	0.001455225	0.011895367	A,B	TG(52:3)	0.261528057	0.538125356	
Serine	0.001455225	0.04254198	A,B	p-Aminobenzoate	0.34453598	0.74358905	
Carnitine-C12	0.002250728	0.008547364	A,B	DAG(34:1)	0.349618988	0.843596605	
Carnitine-C12:1	0.002250728	0.004992161	A,B	PC(34:1)	0.357378527	0.897969275	
Asparagine	0.003248218	0.059726535	A	TG(50:2)	0.359448083	0.74358905	
Cytosine	0.003830723	0.77191989	A	Homocysteic acid	0.364937368	0.762417211	
Carnitine-C14:2	0.003949826	0.007902628	A,B	PG(36:2)	0.395035948	0.900351008	
Citrate	0.004817971	0.589895492	A	PC(36:1)	0.406013872	0.904483751	
Malate	0.00615898	0.009581752	A,B	Creatinine	0.425922974	0.264731949	
Glycine	0.007518042	0.024767514	A,B	Glutamate	0.425922974	0.645457836	
LysoPC(18:1)	0.007706794	0.60477849	A	TG(50:1)	0.457026021	0.97653221	
Lactate	0.009091327	0.00741099	A,B	TG(54:8)	0.480416858	0.317487839	
Carnitine-C16:1	0.009298869	0.007843882	A,B	PC(34:2)	0.495318091	0.904483751	
Uridine	0.009775654	0.021572963	A,B	2,3-Dihydroxybenzoate	0.51311999	0.978033853	
Carnitine-C10	0.010078168	0.005851187	A,B	PG(34:1)	0.524830588	0.897969275	
Glutamine	0.010481249	0.113281265	A	PG(38:1)	0.524830588	0.786713883	
PE(36:2)	0.017685369	0.74358905	A	Docosanoic acid	0.549131881	0.74358905	
Acetyl-CoA	0.018543809	0.74358905	A	LysoPC(16:0)	0.553015109	0.897969275	
CoA	0.018567595	0.74358905	A	FAD	0.57283394	0.571441637	
Carnitine-C2	0.019608735	0.000161712	A,B	LysoPI(18:0)	0.57283394	0.843596605	
Carnitine-C5	0.01998981	0.904483751	A	TG(52:1)	0.57283394	0.544654114	
AKG	0.023597776	0.244809498	A	PG(36:1)	0.590676812	0.897969275	
Carnitine-C3	0.027289359	0.897969275	A	Carnitine-C4	0.600683526	0.03501179	B
LysoPE(18:1)	0.034042066	0.544654114	A	PA(34:1)	0.600683526	0.897969275	
Hypoxanthine	0.034646622	0.022551906	A,B	PS(40:6)	0.600683526	0.843596605	
Ketoisoleucine+leucine	0.043991338	0.632765275	A	PE(36:1)	0.618117012	0.778649036	
Allochenodeoxycholic acid*	0.04643009	0.091211922	A	TG(48:0)	0.618996535	0.904483751	
Carnitine-C10:1	0.046438313	0.00382414	A,B	PC(36:4)	0.62820389	0.904483751	
Carnitine-C8	0.048629561	0.00382414	A,B	PI(36:2)	0.63591752	0.897969275	
Carnitine-C14	0.064221902	0.008547364	B	PA(36:2)	0.67135358	0.904483751	
Carnitine-C18:1	0.068589324	0.008066292	B	Geranyl-PP	0.689182506	0.467341939	
TG(54:2)	0.072109001	0.904483751		PE(38:2)	0.772377421	0.652724906	
Hexose	0.074797639	0.598974795		LysoPE(20:2)	0.772841151	0.674249624	
LysoPE(20:0)	0.083824951	0.269954168		Aspartate	0.842411763	0.904483751	
MAG(18:0)	0.083824951	0.021572963	B	PS(38:4)	0.842411763	0.762417211	
PC(38:6)	0.113570262	0.897969275		Carnitine-C16	0.862243737	0.047838936	B
Carnitine-C18:2	0.121097528	0.011895367	B	Taurine	0.862243737	0.803215287	
LysoPE(18:0)	0.121097528	0.75495083		NAD+	0.891095732	0.904483751	
PS(36:2)	0.121097528	0.544654114		PI(34:1)	0.892810764	0.99054495	
PC(36:2)	0.124905112	0.762417211		PI(38:4)	0.922617571	0.904483751	

Table S2. Internal standards used for quantitation of targeted metabolites. NSK-B internal standard mix was purchased from Cambridge Isotope (Andover, MA). All other carbon-13 stable isotope internal standards were purchased from Sigma-Aldrich (St. Louis, MO).

Internal Standards	**Internal standard concentration in extraction solvent**
Algal amino acid mixture_^{13}C (Sigma 426199)	20 µg/mL
Alpha-ketoglutarate_$^{13}C_4$	4 µM
Citrate_$^{13}C_6$	20 µM
Lactate_$^{13}C_3$	400 µM
Malate_$^{13}C_4$	8 µM
NSK-B acylcarnitine internal standard mix	
$^{2}H_9$-Carnitine (L-carnitine)	510.33 nM
$^{2}H_3$-Acetylcarnitine (C2)	128.33 nM
$^{2}H_3$-Propionylcarnitine (C3)	25.33 nM
$^{2}H_3$-Butyrylcarnitine (C4)	25.33 nM
$^{2}H_9$-Isovalerylcarnitine (C5)	25.33 nM
$^{2}H_3$-Octanoylcarnitine (C8)	25.33 nM
$^{2}H_9$-Myristoylcarnitine (C14)	25.33 nM
$^{2}H_3$-Palmitoylcarnitine (C16)	50.67 nM
Oleic acid_$^{13}C_{18}$	5 µM
Palmitic acid_$^{13}C_{16}$	5 µM
Succinate_$^{13}C_4$	40 µM

Table S3. A) Pearsons correlation coefficients of 27 metabolites for five different normalization strategies compared to internal standard normalization B) % RSD of stable-isotope labeled IS compounds in non-QC samples from MMTT data

	A) Pearsons correlation coefficient for normalization methods vs. internal standard normalization						B) % RSD for IS compounds in non-QC samples					
	Raw	Quad	CUB-SPL	LOE-SS	Total Signal	Med. Signal	Raw	Quad	CUB-SPL	LOE-SS	Total Signal	Med. Signal
Alanine	0.455	0.797	0.807	0.798	0.565	0.561	42.9%	15.4%	15.3%	15.1%	41.0%	38.8%
Alpha-ketoglutarate	0.591	0.811	0.809	0.802	0.673	0.780	41.2%	12.6%	12.7%	12.0%	32.2%	28.0%
Aspartic acid	0.797	0.969	0.968	0.970	0.833	0.929	30.0%	6.9%	6.8%	6.8%	27.1%	28.0%
C14:0 carnitine	-0.179	0.577	0.642	0.652	-0.260	-0.009	109.1 %	25.0%	15.2%	17.3%	124.2 %	79.0%
C16:0 carnitine	0.596	0.297	0.348	0.324	0.462	0.546	68.4%	29.5%	28.0%	26.9%	78.6%	47.6%
C2 carnitine	0.683	0.763	0.804	0.763	0.520	0.680	44.9%	22.0%	18.4%	19.5%	50.1%	28.7%
C3 carnitine	0.516	0.580	0.605	0.558	0.554	0.182	84.9%	26.2%	22.3%	20.8%	69.0%	80.7%
C4 carnitine	0.656	0.810	0.852	0.859	0.550	0.821	54.2%	22.1%	19.1%	18.6%	60.5%	24.4%
C5 carnitine	0.555	0.887	0.953	0.923	0.530	0.831	48.8%	15.5%	10.2%	13.2%	56.5%	20.0%
C8 carnitine	0.592	0.872	0.967	0.953	0.487	0.816	72.8%	17.8%	10.6%	13.5%	83.8%	36.8%
Citrate	0.308	0.852	0.870	0.842	0.275	0.598	71.5%	17.5%	15.7%	18.5%	62.5%	34.2%
Glutamic acid	0.886	0.983	0.986	0.985	0.899	0.866	30.4%	8.1%	7.9%	7.9%	27.1%	30.3%
Glycine	0.158	0.597	0.589	0.610	0.353	0.520	36.2%	11.5%	12.4%	11.5%	40.7%	42.4%
Histidine	0.367	0.470	0.462	0.451	0.423	0.309	48.8%	15.7%	16.0%	15.7%	52.6%	58.7%
Lactate	0.730	0.919	0.923	0.921	0.794	0.759	34.5%	15.4%	15.5%	15.3%	30.7%	28.4%
L-Carnitine	0.425	0.421	0.474	0.427	0.265	0.380	50.1%	24.0%	19.2%	19.9%	61.9%	28.7%
Leucine+Isoleucine	0.601	0.923	0.934	0.925	0.531	0.436	29.1%	8.5%	8.0%	8.5%	28.1%	36.4%
Malate	0.486	0.910	0.918	0.906	0.714	0.567	41.2%	8.9%	8.6%	8.9%	32.3%	34.9%
Oleic acid	0.785	0.948	0.950	0.951	0.864	0.611	34.8%	18.5%	18.3%	18.5%	44.6%	46.2%
Palmitic acid	0.763	0.930	0.932	0.934	0.803	0.562	35.2%	19.0%	18.9%	19.1%	44.9%	46.3%
Phenylalanine	0.517	0.896	0.913	0.905	0.510	0.547	26.7%	9.2%	8.4%	9.0%	26.5%	34.2%
Proline	0.652	0.852	0.854	0.854	0.616	0.567	26.0%	10.1%	10.7%	9.9%	27.6%	34.6%
Serine	0.377	0.639	0.646	0.630	0.246	0.219	44.9%	22.7%	22.6%	22.8%	51.4%	66.5%
Succinate	0.638	0.935	0.935	0.931	0.702	0.284	30.9%	7.7%	7.4%	7.3%	29.0%	31.3%
Threonine	0.480	0.838	0.842	0.841	0.441	0.507	38.5%	9.9%	10.0%	10.1%	37.4%	37.7%
Tyrosine	0.675	0.898	0.913	0.903	0.568	0.583	24.5%	10.4%	9.8%	10.1%	32.3%	38.3%
Valine	0.465	0.828	0.848	0.820	0.415	0.335	32.2%	12.1%	11.4%	12.2%	28.0%	34.9%
AVERAGE	**0.540**	**0.785**	**0.805**	**0.794**	**0.531**	**0.548**	**45.7%**	**15.6%**	**14.1%**	**14.4%**	**47.4%**	**39.8%**

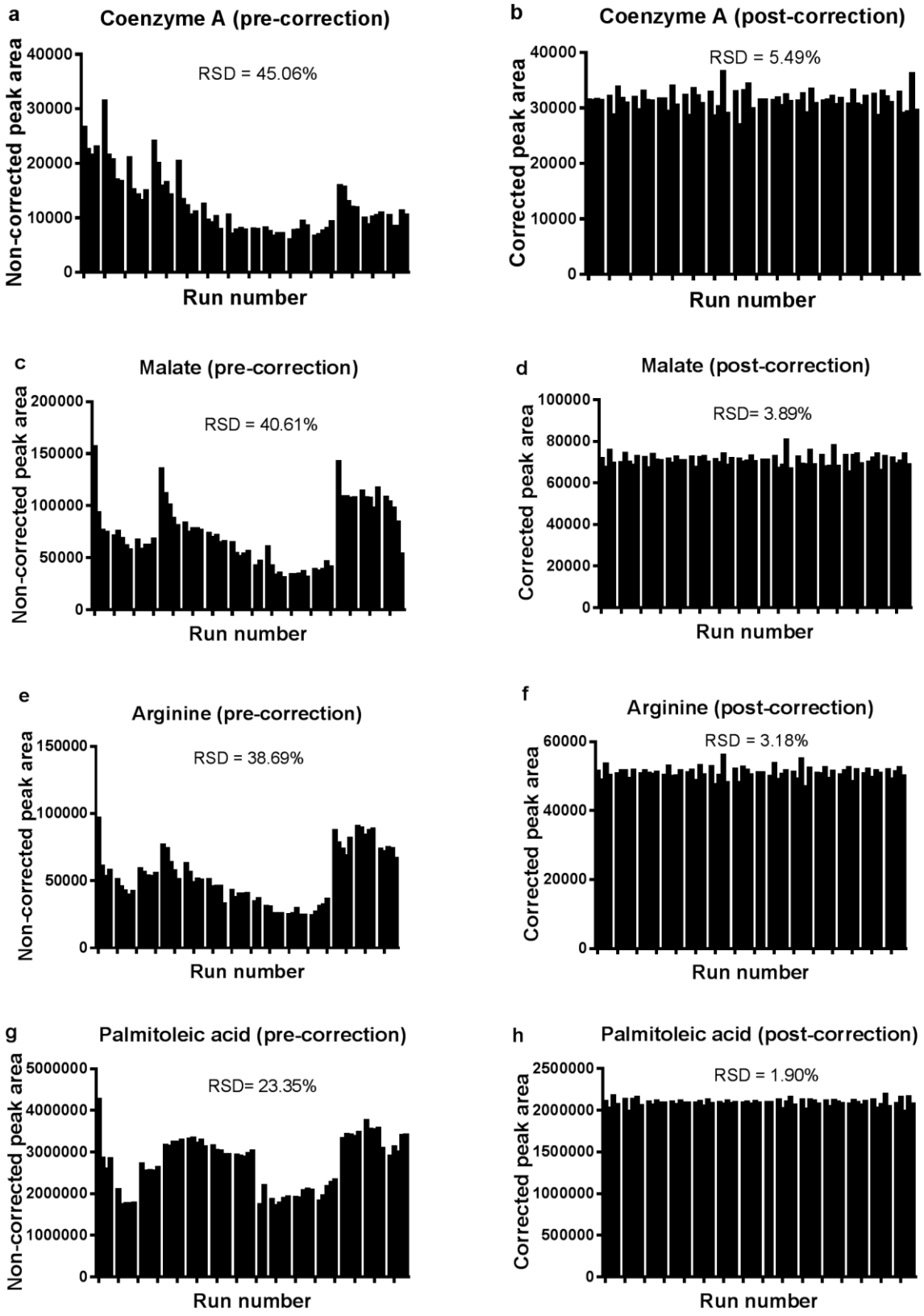

Figure S1. Effect of QC drift correction on metabolites from MMTT dataset. QC-sample peak areas for select metabolites before (a,c,e,g) and after (b,d,f,h) quadratic drift correction, performed as described in section 2.4.

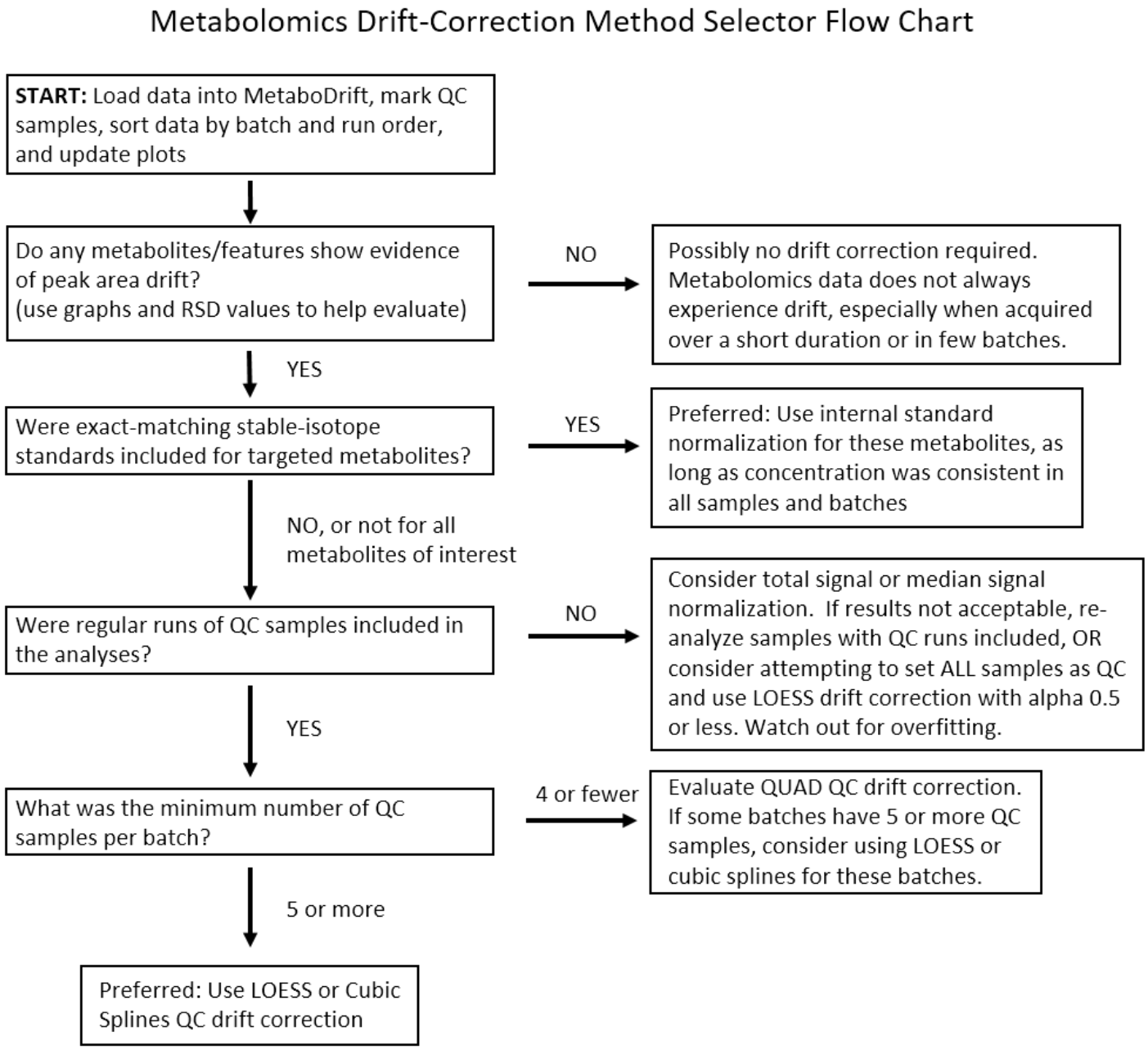

Figure S2. Flowchart to aid in selection of normalization or drift-correction strategy for metabolomics data.