

Supplementary Information

Zebrafish Lipidomics via HPLC-MS and Chemometrics: Diet and Exercise-Associated Lipid Alterations

Almir C. Batista Junior,^a Jussara V. Roque,^a Lanaia Ítala L. Maciel,^a Moises S. A. Martins,^b William F. Carneiro,^b André R. C. B. Vianna,^c Luis David S. Murgas,^b Ricardo A. Bernardo^{,d} and Andréa R. Chaves^{*,a}*

^a*Centro de Excelência em Estudos Moleculares, Energia e Petróleo (CEMEP),
Universidade Federal de Goiás, 74690-900 Goiânia-GO, Brazil*

^b*Departamento de Medicina Veterinária, Universidade Federal de Lavras,
37200-000 Lavras-MG, Brazil*

^c*Departamento de Biociências, Universidade Federal do Paraná, 85950-000 Curitiba-PR, Brazil*

^d*Departamento de Química, Universidade Federal do Paraná, 81531-980 Curitiba-PR, Brazil*

*e-mail: andrea_chaves@ufg.br; ricardo.bernardo@ufpr.br

Almir Custodio Batista Junior: <https://orcid.org/0000-0003-2977-1004>

Jussara Valente Roque: <https://orcid.org/0000-0002-5220-959X>

Moises Silvestre de Azevedo Martins: <https://orcid.org/0000-0001-7116-0579>

William Franco Carneiro: <https://orcid.org/0000-0002-5163-4615>

André Rodrigues da Cunha Barreto Vianna: <https://orcid.org/0000-0001-8354-3163>

Luis David Solis Murgas: <https://orcid.org/0000-0002-0066-7505>

Ricardo Alves Bernardo: <https://orcid.org/0000-0002-8244-2544>

Andréa Rodrigues Chaves: <https://orcid.org/0000-0002-1600-1660>

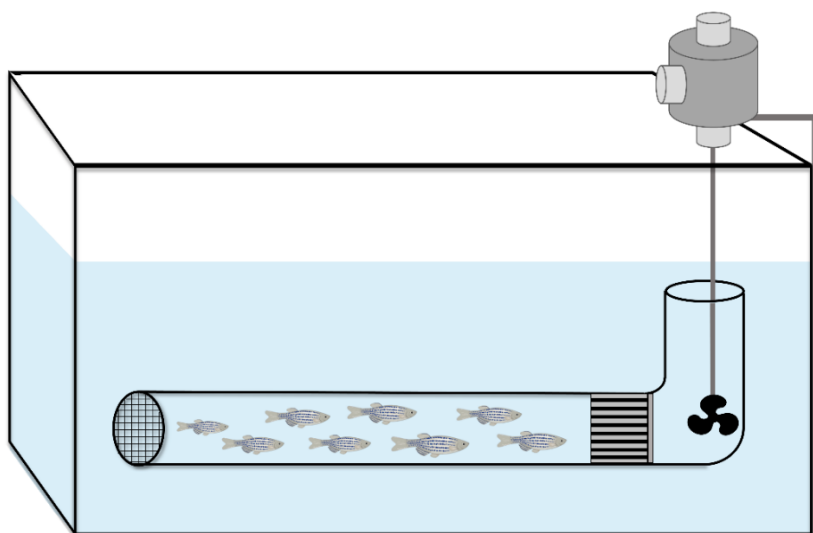


Figure S1. Scheme of the physical exercise model of the zebrafish.

Table S1. Ingredients and composition of the normal-fat and high-fat diet diets of the zebrafish

Ingredient / %	Diet	
	NFD	HFD
Egg yolk	10.40	28.57
Albumin	31.12	23.61
Corn starch	29.95	21.28
Fish meal	10.00	10.00
Gelatin	6.00	6.00
Soybean oil	2.00	5.00
Bicalcium phosphate	3.00	3.00
Cellulose	6.00	1.00
Vitamin premix ^a	1.00	1.00
Salt	0.50	0.50
BHT	0.02	0.02
Centesimal composition ^b		
Gross energy / (kcal kg ⁻¹)	4.630	4.889
Ether extract / %	8.32	19.77
Protein / %	40.02	39.31
Ash / %	6.65	6.20
Moisture / %	5.44	6.03
Dry matter / %	94.56	93.97

^aGuaranteed levels of vitamin and mineral supplementation *per* kg of product: vitamin A = 1,200,000 IU; vitamin D3 = 200,000 IU; vitamin E = 12,000 mg; vitamin K3 = 2400 mg; vitamin B1 = 4,800 mg; vitamin B2 = 4800 mg; vitamin B6 = 4,000 mg; vitamin B12 = 4,800 mg; folic acid = 1,200 mg; calcium pantothenate = 12,000 mg; vitamin C = 48,000 mg; biotin = 48 mg; choline = 65,000 mg; niacin = 24,000 mg; Fe = 10,000 mg; Cu = 6000 mg; Mn = 4,000 mg; Zn = 6,000 mg; I = 20 mg; Co = 2 mg; Se = 20 mg. ^bThe diets were analyzed by the Animal Research Laboratory (ARL) from Faculty of Animal Science and Veterinary Medicine, Universidade Federal de Lavras, Lavras-MG, Brazil. NFD: normal-fat diet; HFD: high-fat diet; BHT: butyl hydroxy toluene;

Table S2. Classification parameters of models PLS-DA with and without selected variables by OPSDA for the prediction of zebrafish sample groups NFD, NFD-E, HFD, and HFD-E in negative and positive ion mode

Negative ion mode												
	PLS-DA				PLS-DA OPSDA				PLS-DA OPSDA cross-validation			
	NFD	NFD-E	HFD	HFD-E	NFD	NFD-E	HFD	HFD-E	NFD	NFD-E	HFD	HFD-E
Sensitivity ^a / %	44	25	44	22	100	100	100	100	94	85	84	92
Specificity ^b / %	80	70	77	84	100	100	100	100	81	90	77	85
Accuracy / %	71	60	68	68	100	100	100	100	88	88	81	88
Classification error / %	28	20	40	33	0	0	0	0	12	12	19	12
Positive ion mode												
	PLS-DA				PLS-DA OPSDA				PLS-DA OPSDA cross-validation			
	NFD	NFD-E	HFD	HFD-E	NFD	NFD-E	HFD	HFD-E	NFD	NFD-E	HFD	HFD-E
Sensitivity ^a / %	44	37	33	44	100	100	100	100	92	98	83	94
Specificity ^b / %	61	92	84	80	100	100	100	100	69	81	85	77
Accuracy / %	57	80	71	72	100	100	100	100	81	89	84	86
Classification error / %	42	20	28	28	0	0	0	0	19	11	16	14

NFD: normal-fat diet; NFD-E: normal-fat diet with exercise; HFD: high-fat diet; HFD-E: high-fat diet with exercise; PLS-DA OPSDA: partial least squares discriminant analysis with ordered predictors selection for discriminant analysis. ^aPercentage of samples of each class accepted by the class model; ^bpercentage of samples of the other class correctly rejected by the class model.

Table S3. REG and VIP scores from the ions annotated in Table 2 for zebrafish sample groups NFD, NFD-E, HFD, and HFD-E in negative ion mode

Lipid	<i>m/z</i>	NFD	NFD-E	HFD	HFD-E
FA(16:1)	253.216	−0.157	0.118	0.214	−0.127
FA(16:0)	255.231	−0.197	0.083	−0.008	0.132
FA(18:2)	279.232	0.731	0.719	0.009	−1.524
FA(18:1)	281.248	−0.173	1.857	−0.096	−1.634
FA(18:0)	283.262	−0.067	0.002	0.076	0.009
FA(20:4)	303.231	0.088	−0.144	0.359	−0.240
FA(20:3)	305.246	−0.169	−0.233	0.413	0.082
ST(20:5;O3)	311.167	−0.397	0.187	0.048	0.193
ST(21:5;O3)	325.182	0.020	0.202	−0.096	−0.149
FA(22:6)	327.232	−0.202	−0.027	0.246	0.043
FA(22:4)	331.262	−0.190	0.079	−0.157	0.250
ST(22:5;O3)	339.198	−0.114	−0.008	0.084	0.061
ST(26:1;O;S)	451.288	0.010	0.070	−0.001	−0.081
ST(30:6;O4)	465.304	−0.026	−0.136	0.232	−0.022
ST(24:0;O2)	537.341	−0.072	0.018	0.224	−0.124
CE(22:4)	699.607	0.114	−0.188	0.077	0.008
CerPE(42:0;O4)	762.598	−0.047	0.020	0.105	−0.055
CerPE(40:1;O4)	775.605	−0.157	0.118	0.214	−0.127
CerPE(42:0;O5)	778.596	−0.197	0.083	−0.008	0.132

REG: regression vector; VIP: variable importance on projection; NFD: normal-fat diet; NFD-E: normal-fat diet submitted to exercise; HFD: high-fat diet; HFD-E: high-fat diet submitted to exercise.

Table S4. REG and VIP scores from the ions annotated in Table 2 for zebrafish sample groups NFD, NFD-E, HFD, and HFD-E in positive ion mode

Lipid	<i>m/z</i>	NFD	NFD-E	HFD	HFD-E
DG(O-24:3;O)	253.216	−0.090	−0.121	−0.223	0.385
Cer(28:5;O)	255.231	0.018	−0.131	0.259	−0.106
Cer(28:3;O3)	279.232	−0.157	−0.073	0.656	−0.305
LPC(O-14:1)	281.248	−0.038	0.273	−0.077	−0.153
LPC(O-18:4)	283.262	−0.156	−0.035	0.295	−0.048
DG(36:5;O2)	303.231	−0.068	0.463	0.110	−0.450
CerPE(32:2;O4)	305.246	−0.292	0.084	0.354	−0.069
LPE(O-34:6)	311.167	−0.391	−0.073	−0.057	0.512
ACer(46:5;O5)	325.182	−0.379	0.277	0.274	−0.094
LPT(34:0)	327.232	−0.135	−0.003	0.049	0.100
ACer(54:1;O3)	331.262	0.380	1.504	−0.005	−1.779
CerP(52:0;O2)	339.198	0.264	−0.305	0.146	−0.104
Cer(54:8;O4)	451.288	0.065	−0.140	−0.186	0.214
ACer(56:1;O3)	465.304	−0.354	−0.387	0.262	0.507
DG(O-56:7)	537.341	−0.532	−0.017	−0.193	0.714
ACer(54:0;O6)	699.607	0.735	0.210	−0.325	−0.679
ACer(58:2;O3)	762.598	0.409	0.418	−0.089	−0.732

REG: regression vector; VIP: variable importance on projection; NFD: normal-fat diet; NFD-E: normal-fat diet submitted to exercise; HFD: high-fat diet; HFD-E: high-fat diet submitted to exercise.

