Integrative metabonomics as potential method for diagnosis of thyroid malignancy

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Supplementary Files

Table S1. NMR assignment for the metabolites found in thyroid tissues

no.	metabolites	moieties	δ ^{1}H (ppm) and multiplicity[a]	δ ^{13}C (ppm)
1	lipid	$CH_3(CH_2)_n$	0.86(t)	15.0
		$CH_3CH_2CH_2C=$	0.88(t)	15.0
		$(CH_2)_n$	1.27(m)	32.0
		CH_2CH_2CO	1.57(m)	27.5
		$CH_2C=C$	2.01(m)	29.5
		CH_2CO	2.23(m)	36.0
		$C=CCH_2C=C$	2.76(m)	28.5
		-CH=CH-	5.30(m)	130.0
2	isoleucine	δCH_3	0.94(t)	14.2
		γCH_3	1.02(d)	17.2
		γCH_2	1.26(m)	27.5
		$\gamma' CH_2$	1.48(m)	27.5
		βCH	1.98(m)	37.7
		αCH	3.67(d)	62.4
3	leucine	δCH_3	0.96(d)	24.5

		δCH_3	0.97(d)	24.2
		γCH	1.72(m)	27.0
		βCH_2	1.72(m)	42.6
		αCH	3.74(t)	57.2
4	valine	γCH_3	0.99(d)	19.6
		γCH_3	1.04(d)	20.8
		βCH	2.27(m)	32.6
		αCH	3.62(d)	63.3
5	lactate	CH_3	1.33(d)	22.9
		CH	4.11(q)	71.3
6	threonine	γCH_3	1.33(d)	22.5
		αCH	3.59(d)	63.1
		βCH	4.26(m)	69.0
7	alanine	βCH_3	1.48(d)	19.0
		αCH	3.79(q)	53.4
8	lysine	γCH_2	1.48(m)	24.1
		δCH_2	1.72(m)	29.2
		βCH_2	1.90(m)	33.0
		εCH_2	3.03(t)	42.2
		αCH	3.76(t)	57.6
9	arginine	γCH_2	1.73(m)	27.0
		βCH_2	1.93(m)	30.6
		δCH_2	3.23(t)	43.4
		αCH	3.75(t)	57.6
		C=NH		159.6
10	acetate	CH_3	1.92(s)	26.2
11	glutamate	βCH_2	2.09(m)	29.9
		$\beta' CH_2$	2.10(m)	29.9
		γCH_2	2.36(m)	36.4
		αCH	3.77(m)	57.2
12	methionine	δCH_3	2.14(s)	16.8
		βCH_2	2.16(m)	33.0
		γCH_2	2.65(t)	31.7
		αCH	3.86(m)	56.9
13	glutamine	βCH_2	2.10(m)	30.0
		γCH_2	2.46(m)	33.8
		αCH	3.77(m)	57.5
14	aspartate	βCH_2	2.68(m)	39.5
		$\beta' CH_2$	2.82(m)	39.8
		αCH	3.91(m)	55.3
15	glutathione(GSH)	$3CH_2$	2.17(m)	#
		$4CH_2$	2.56(m)	#
		$12CH_2$	2.94(m)	#
		$12'CH_2$	2.97(m)	#

		2CH	3.79(t)	#
		7CH	4.56(t)	#
16	choline	$N(CH_3)_3$	3.21(s)	56.8
		NCH_2	3.52(m)	74.1
		OCH_2	4.07(m)	54.1
17	phosphocholine(PC)	$N(CH_3)_3$	3.23(s)	57.0
		NCH_2	3.61(m)	69.3
		OCH_2	4.17(m)	58.0
18	glycerophosphocholine(GPC)	$N(CH_3)_3$	3.23(s)	57.0
		NCH_2	3.69(m)	69.0
		OCH_2	4.34(m)	62.7
19	taurine	CH_2SO_3	3.28(t)	50.7
		NCH_2	3.43(t)	38.2
20	*scyllo*-inositol	CH_3	3.35(s)	76.6
21	*myo*-inositol	5CH	3.27(t)	77.2
		1CH,3CH	3.54(m)	74.2
		4CH,6CH	3.63(m)	75.3
		2CH	4.07(m)	75.1
22	glycine	CH_2	3.56(s)	44.3
23	phosphoethanolamine(PE)	CH_2NH_3	3.23(t)	43.8
		CH_2OH	3.99(m)	63.1
24	inosine	CH_2	3.85(dd)	63.8
		$'CH_2$	3.92(dd)	63.8
		5H'	4.28(q)	88.6
		4H'	4.44(t)	73.4
		2H'	6.11(d)	91.1
		8H	8.25(s)	149.8
		2H	8.36(s)	143.2
25	tyrosine	βCH_2	3.06(dd)	38.3
		$\beta'CH_2$	3.15(dd)	38.3
		αCH	3.94(dd)	59.2
		3 or 5CH	6.91(d)	118.9
		2 or 6CH	7.20(d)	133.8
26	phenylalanine	βCH_2	3.13(dd)	38.4
		$\beta'CH_2$	3.29(dd)	38.4
		αCH	3.98(dd)	59.3
		2 or 6CH	7.33(m)	132.1
		4CH	7.38(m)	131.2
		3 or 5CH	7.43(m)	132.2
27	histidine	5CH	7.09(s)	120.1
		3CH	7.83(s)	138.3
28	fumarate	CH	6.52(s)	138.5
29	uracil	CH	5.81(d)	100.9
		CH	7.54(d)	146.5

30	guanosine	CH	8.01(s)	#
31	hypoxanthine	2CH	8.20(s)	148.6
		7CH	8.22(s)	145.0
32	xanthine	CH	7.94(s)	139.1
33	formate	CH	8.45(s)	#
34	acetamide	CH_3	2.02(s)	24.7
35	succinate	CH_2	2.41(s)	37.0
36	citrate	CH_2	2.54(d)	46.5
		'CH_2	2.66(d)	46.5
37	uridine	'CH_2	3.92(d)	64.3
		4H'	4.14(dd)	86.6
		3H'	4.24(dd)	73.1
		2H'	4.36(dd)	78.0
		5H	5.91(d)	95.0
		6H	5.92(d)	90.5
		1H'	7.89(d)	144.0
38	U1		8.19(s)	#

Table S2. Summary of OPLS-DA models[a].

	intact thyroid tissue				tissue aqueous extracts			
	A vs. (B+C)	A vs. B	B vs. C	A vs. C	A vs. (B+C)	A vs. B	B vs. C	A vs. C
R^2X	0.28	0.33	0.16	0.35	0.78	0.82	0.77	0.73
Q^2	0.75	0.81	0.77	0.77	0.33	0.31	0.44	0.49
p	3.9×10^{-25}	1.5×10^{-22}	4.7×10^{-14}	1.1×10^{-20}	8.1×10^{-6}	1.0×10^{-3}	1.3×10^{-4}	7.3×10^{-7}

[a]A: healthy adjacent thyroid tissue, B: benign thyroid lesions, C: malignant thyroid lesions.

Table S3. Fatty acid compositions in thyroid tissues.

	Healthy[a] (n = 25)	**Benign** (n = 25)	**Malignant** (n = 15)	***p* values** (healthy *vs* benign)	***p* values** (healthy *vs* malignant)	***p* values** (benign *vs* malignant)
SFA[b]	23.343 ±8.423	30.412 ±4.751	35.377 ±9.423	1.63E-2	2.02E-2	3.08E-1
C14:0	0.443 ±0.191	0.247 ±0.049	0.462 ±0.127	4.48E-3	8.43E-1	1.82E-2
C16:0	13.005 ±4.115	15.193 ±2.558	19.159 ±4.081	4.94E-2	1.30E-2	6.93E-3
C18:0	6.681 ±1.029	15.083 ±2.020	15.550 ±6.164	1.21E-12	4.89E-3	8.75E-1
C20:0	0.133 ±0.039	0.146 ±0.031	0.207 ±0.078	2.51E-1	1.00E-1	1.59E-1
UFA[b]	26.445 ±10.818	17.739 ±6.759	26.651 ±17.083	3.66E-3	9.76E-1	3.11E-1
MUFA[b]	13.415 ±6.655	5.913 ±2.558	8.210 ±4.780	2.45E-3	1.74E-1	4.11E-1
C16:1n7	0.665 ±0.452	0.075 ±0.026	0.187 ±0.200	8.61E-4	6.35E-2	3.46E-1
C18:1n7	1.186 ±0.411	1.112 ±0.385	1.599 ±1.031	6.15E-1	4.31E-1	3.53E-1
C18:1n9	11.031 ±5.679	4.024 ±1.698	6.568 ±3.960	1.28E-3	1.71E-1	2.90E-1
C20:1n9	0.291 ±0.071	0.184 ±0.056	0.320 ±0.152	1.26E-5	6.00E-1	1.73E-1
PUFA[b]	13.030 ±4.360	11.827 ±4.709	14.240 ±6.921	4.54E-1	6.66E-1	3.33E-1
n6 [b]	11.979 ±4.145	10.832 ±4.449	12.834 ±6.647	4.51E-1	7.49E-1	3.96E-1
C18:2n6	8.967 ±4.651	6.372 ±2.654	6.621 ±3.226	9.15E-2	3.70E-1	8.65E-1
C20:2n6	0.180 ±0.052	0.182 ±0.090	0.239 ±0.143	9.49E-1	4.75E-1	2.84E-1
C20:3n6	0.361 ±0.075	0.642 ±0.339	0.436 ±0.086	2.97E-4	1.23E-1	1.57E-2
C20:4n6	2.446 ±0.721	3.267 ±1.106	2.575 ±0.930	2.61E-2	7.60E-1	2.04E-1
n3[b]	1.051 ±0.306	0.995 ±0.366	1.406 ±0.402	6.43E-1	6.52E-2	2.97E-2
C18:3n3	0.719 ±0.295	0.486 ±0.070	0.865 ±0.267	2.01E-2	3.54E-1	3.32E-2
C22:6n3	0.269 ±0.034	0.539 ±0.312	0.540 ±0.299	1.04E-4	1.12E-1	9.94E-1
ToFA[b]	62.029 ±12.976	47.414 ±10.629	49.788 ±14.968	1.20E-2	4.63E-2	5.48E-1
n6/n3[b]	11.663 ±3.017	9.874 ±0.630	7.689 ±2.052	6.66E-1	2.94E-2	1.22E-1
PUFA/UFA[b]	0.509 ±0.070	0.690 ±0.020	0.579 ±0.028	1.47E-6	1.22E-1	1.35E-8
MUFA/UFA[b]	0.491 ±0.070	0.310 ±0.020	0.421 ±0.028	1.47E-6	1.22E-1	1.35E-8
PUFA/MUFA[b]	0.904 ±0.109	2.214 ±0.246	1.244 ±0.306	3.84E-19	1.08E-2	2.20E-07
PUFA/ToFA[b]	0.262 ±0.047	0.252 ±0.051	0.221 ±0.066	5.70E-1	1.66E-1	2.46E-1
MUFA/ToFA[b]	0.264 ±0.089	0.109 ±0.019	0.136 ±0.058	7.90E-5	1.86E-2	4.28E-1
SFA/ToFA[b]	0.473 ±0.127	0.628 ±0.068	0.665 ±0.107	1.41E-3	1.72E-2	3.56E-1
UFA/ToFA[b]	0.527 ±0.127	0.372 ±0.068	0.335 ±0.107	1.41E-3	1.72E-2	3.56E-1
SFA/UFA[b]	1.020 ±0.559	1.687 ±0.506	1.351 ±0.683	8.26E-4	3.47E-1	2.46E-1
SCD16[c]	0.053 ±0.035	0.005 ±0.001	0.009 ±0.009	5.13E-4	1.26E-3	3.69E-1
SCD18[c]	1.459 ±1.033	0.255 ±0.090	0.372 ±0.203	1.96E-3	4.29E-3	3.34E-1
D5D[c]	5.817 ±0.834	5.466 ±1.193	4.835 ±0.865	4.04E-1	5.33E-2	2.75E-1
D6D[c]	0.058 ±0.048	0.098 ±0.034	0.081 ±0.045	2.00E-3	2.78E-1	3.28E-1

[a] data are presented as mean ± SD (μmol/g)

[b] SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6: n6 PUFA ; n3: n3 PUFA ;ToFA: total fatty acids; n6/n3: n6-to-n3 ratio; PUFA/UFA:

PUFA-to-UFA ratio; MUFA/UFA: MUFA-to-UFA ratio; PUFA/MUFA: PUFA-to-MUFA ratio; PUFA/ToFA: PUFA-to-ToFA ratio; MUFA/ToFA: MUFA-to-ToFA ratio; SFA/ToFA: SFA-to-ToFA ratio; UFA/ToFA: UFA-to-ToFA ratio; SFA/UFA: SFA-to-UFA ratio; SFA = C14:0+C16:0+C18:0+C20:0; UFA = MUFA+PUFA; MUFA = C16:1n7+C18:1n7+C18:1n9+C20:1n9; PUFA = n6+n3; n6 = C18:2n6+C18:3n6+C20:2n6+C20:3n6+C20:4n6 ; n3 = C18:3n3+C20:5n3+C22:6n3

[c] SCD16: stearoyl-CoA desaturase-16; SCD18: stearoyl-CoA desaturase-18; D5D: Δ5-desaturase; D6D: Δ6-desaturase. Hepatic desaturase were calculated by product-precursor ratio. SCD16 = C16:1n7/C16:0; SCD18 = C18:1n9/C18:0 ; D5D = C20:4n6/C20:3n6 ; D6D = C20:3n6/C18:2n6