Supplemental information

Rapid LA-REIMS and Comprehensive UHPLC-HRMS Metabolic Phenotyping of Feces

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Supplemental Figure 1

Supplemental Figure 1. MS² spectrum (A) of the key metabolite marker (p -value < 0.0005), whereby the established fragmentation tree (B) suggested the identity of metformin. The Tier 1 identification level was reached using an authentic reference standard, analyzed under identical analytical conditions.

47 Supplemental Figure 2

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49 **Supplemental Figure 2:** Marker metabolites that were identified as in-source fragments of
 50 metformin, presented below the metformin panel and organized according to the metformin
 51 fragmentation tree (Supplemental Figure 1). Marker metabolite that was identified as an
 52 alternative ionization adduct of metformin (*i.e.* addition of acetonitrile (ACN)), presented above
 53 the metformin panel. Fragments are indicated in red when corresponding to the metformin
 54 fragmentation pattern.

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56 Supplemental Figure 3

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58 **Supplemental Figure 3:** Marker metabolites that were identified as products of metformin
 59 metabolization, as being mediated by the host or microbial community. Fragments were
 60 indicated in red when corresponding to the fragmentation pattern of metformin and in orange
 61 when considered supportive towards the identification process.

Supplemental Figure 4

Supplemental Figure 4: MS² spectrum that was obtained for the authentic reference standard of cholesterol. Fragments were indicated in red when corresponding to the fragmentation pattern that was obtained for the metformin-cholesterol condensation product.

Supplemental Table 1. Evaluation of BMI, gender, age, and metformin as potential confounding factors within the established clinical cohort and glycemic index (HbA1c)-based classes. To this end, restructuring of the datasets and evaluation of the validation parameters of the associated OPLS-DA models (excluding specific confounding factors) was performed.

OPLS-DA model description	Q ²	p-value
<u>Impact of metformin treatment</u>		
1) Lipidomics LC-MS, after removal of metformin-related metabolites; normal glycemic state (n = 21) vs. type 2 diabetes (n = 21)	0.619	0.001
2) Polar LC-MS, after removal of metformin-related metabolites; normal glycemic state (n = 21) vs. type 2 diabetes (n = 21)	0.697	1.05 e ⁻⁶
3) LA-REIMS, after removal of metformin-related metabolites; normal glycemic state (n = 36) vs. type 2 diabetes (n = 38)	0.524	4.04 e ⁻⁹
<u>Impact of BMI (also after removal of the metformin-related fraction)</u>		
1) Lipidomics LC-MS, after BMI-matching; normal glycemic state (n = 12, BMI 26.7 ± 2.5 kg/m ²) vs. type 2 diabetes (n = 12, BMI 28.6 ± 2.2 kg/m ²)	0.349	0.070
2) Polar LC-MS, after BMI-matching; normal glycemic state (n = 12, BMI 26.7 ± 2.5 kg/m ²) vs. type 2 diabetes (n = 12, BMI 28.6 ± 2.2 kg/m ²)	0.692	0.008
3) LA-REIMS, after BMI-matching; normal glycemic state (n = 24, BMI 25.5 ± 2.7 kg/m ²) vs. type 2 diabetes (n = 24, BMI 28.8 ± 3.1 kg/m ²)	0.480	9.3 e ⁻⁵
<u>Impact of gender (also after removal of the metformin-related fraction)</u>		
1) Lipidomics LC-MS, considering only male participants; normal glycemic state (n = 8) vs. type 2 diabetes (n = 18)	0.514	0.020
2) Lipidomics LC-MS, considering only female participants; normal glycemic state (n = 12) vs. type 2 diabetes (n = 4)	0.498	0.001
3) Polar LC-MS, considering only male participants; normal glycemic state (n = 8) vs. type 2 diabetes (n = 18)	0.666	0.005
4) Polar LC-MS, considering only female participants; normal glycemic state (n = 8) vs. type 2 diabetes (n = 18)	0.656	0.013
5) LA-REIMS LC-MS, considering only male participants; normal glycemic state (n = 13) vs. type 2 diabetes (n = 25)	0.511	0.001
6) LA-REIMS LC-MS, considering only female participants; normal glycemic state (n = 23) vs. type 2 diabetes (n = 12)	0.324	0.280
<u>Impact of BMI (also after removal of the metformin-related fraction)</u>		
1) Lipidomics LC-MS, after age-matching; normal glycemic state (n = 12, 51.9 ± 4.8 years) vs. type 2 diabetes (n = 12, age 57.0 ± 4.8 years)	0.350	0.070
2) Polar LC-MS, after age-matching; normal glycemic state (n = 12, 51.9 ± 4.8 years) vs. type 2 diabetes (n = 12, age 57.0 ± 4.8 years)	0.698	0.007
3) LA-REIMS, after age-matching; normal glycemic state (n = 28, 54.2 ± 5.2 years) vs. type 2 diabetes (n = 28, age 57.1 ± 5.2 years)	0.505	3.10 e ⁻⁶

88 **Supplemental Table 2.** Chemical information of UHPLC-HRMS markers that were revealed
 89 upon discriminative fingerprinting by lipidomics or polar metabolomics.

Lipidomics			Polar metabolomics		
<i>m/z</i> -value (Da)	Ion	R _t (min)	<i>m/z</i> -value (Da)	Ion	R _t (min)
85.0515 ^a	[M+H] ⁺	1.38	60.0564 ^a	[M+H] ⁺	1.09
88.0875 ^a	[M+H] ⁺	1.38	71.0610 ^a	[M+H] ⁺	1.07
130.1089 ^a	[M+H] ⁺	1.38	83.0347	[M-H] ⁻	1.07
263.1066	[M+H] ⁺	1.66	85.0514 ^a	[M+H] ⁺	1.07
271.1516	[M+H] ⁺	1.38	86.0589	[M+H] ⁺	1.09
280.2347	[M+H] ⁺	1.82	88.0874 ^a	[M+H] ⁺	1.07
280.2347	[M+H] ⁺	2.65	111.0661	[M-H] ⁻	1.07
280.2350	[M+H] ⁺	2.26	113.0820 ^a	[M+H] ⁺	1.07
283.1150	[M+H] ⁺	1.40	114.0855	[M+H] ⁺	1.09
299.1467	[M+H] ⁺	1.38	126.0220	[M+H] ⁺	1.00
317.2087	[M+H] ⁺	2.33	130.1086 ^a	[M+H] ⁺	1.05
323.1470	[M+H] ⁺	1.43	158.1400	[M+H] ⁺	4.49
323.2191	[M+H] ⁺	3.89	160.1191 ^b	[M+H] ⁺	1.51
327.1776	[M+H] ⁺	1.44	171.1350 ^a	[M+ACN+H] ⁺	1.09
327.1778	[M+H] ⁺	1.71	179.0337	[M+H] ⁺	3.11
341.2057	[M+H] ⁺	2.28	181.0523	[M-H] ⁻	6.09
341.2060	[M+H] ⁺	2.68	184.0827	[M+H] ⁺	2.31
372.3110	[M+H] ⁺	3.90	184.1191 ^b	[M+H] ⁺	1.73
378.3589	[M+H] ⁺	5.99	185.1223	[M+H] ⁺	1.69
387.2885	[M+H] ⁺	6.65	202.1296	[M+H] ⁺	3.72
398.3990	[M+H] ⁺	8.28	214.1295	[M+H] ⁺	1.42
411.2867	[M+H] ⁺	5.79	228.1452	[M+H] ⁺	2.48
411.2869	[M+H] ⁺	6.24	244.1399 ^b	[M+H] ⁺	1.43
414.3212	[M+H] ⁺	3.62	247.1873 ^b	[M+H] ⁺	1.18
416.3368	[M+H] ⁺	4.19	253.1541	[M+H] ⁺	0.94
424.3421	[M+H] ⁺	5.09	258.1557	[M+H] ⁺	1.54
426.3578	[M+H] ⁺	5.26	259.2094 ^b	[M+H] ⁺	1.03
440.3732	[M+H] ⁺	5.60	261.2027 ^b	[M+H] ⁺	0.99
442.3527	[M+H] ⁺	4.75	262.2061	[M+H] ⁺	1.06
444.3682	[M+H] ⁺	5.05	267.1559	[M+H] ⁺	1.04
448.3408	[M+H] ⁺	5.24	273.2028	[M+H] ⁺	1.12
452.3734	[M+H] ⁺	5.62	274.0681	[M+H] ⁺	1.20
464.3194	[M+H] ⁺	6.19	274.1503 ^b	[M+H] ⁺	1.18
468.3688	[M+H] ⁺	5.18	274.2060	[M+H] ⁺	1.04
470.2868	[M+H] ⁺	1.64	289.2342	[M+H] ⁺	1.58
470.3840	[M+H] ⁺	5.57	291.2133 ^b	[M+H] ⁺	1.05
483.2133	[M-H] ⁻	1.43	295.1860 ^b	[M+H] ⁺	1.09
496.3997	[M+H] ⁺	5.83	297.1831	[M+H] ⁺	1.05
498.4530 ^b	[M+H] ⁺	7.60	308.1446	[M+H] ⁺	1.39
534.5237	[M+H] ⁺	8.02	339.1923	[M-H] ⁻	7.95
599.3795	[M+H] ⁺	1.94	341.2064	[M+H] ⁺	7.97
600.5079	[M+H] ⁺	14.81	369.2376	[M+H] ⁺	9.37
615.5401	[M-H] ⁻	8.18	396.1507	[M+H] ⁺	5.8
646.5135	[M-H] ⁻	7.25	434.2867	[M+H] ⁺	9.29
667.6411	[M+H] ⁺	9.76	462.2402	[M-H] ⁻	2.34

685.5773	[M+H] ⁺	11.18
694.5765	[M+H] ⁺	9.00
694.5768	[M+H] ⁺	10.14
696.5917	[M+H] ⁺	9.10
696.5920	[M+H] ⁺	9.85
703.5048	[M+H] ⁺	6.30
719.5356	[M+H] ⁺	6.95
824.7695	[M+H] ⁺	12.77
912.7606	[M+H] ⁺	12.98

90 ^a Metformin or related features, *i.e.* in-source fragments

91 ^b Metformin metabolization products