Supporting Information

Global Metabolomics Profiling of Glucuronides in Human Plasma, Fecal, and Cerebrospinal Fluid Samples

Ioanna Tsiara [a], Mario S.P. Correia [a], Fan Yang [a], Weiming Zeng [a], Pauline Seeburger [a], Belén Hervás Povo [a], Iben Lundgaard [b,c], Manuel Menéndez-González [d,e], Miroslav Vujasinovic [f,g], J.-Matthias Löhr [g,h], and Daniel Globisch [a,*]

[a] Department of Chemistry - BMC, Science for Life Laboratory, Uppsala University, Box 576, SE-75124, Uppsala, Sweden

[*] Corresponding author: Daniel.globisch@kemi.uu.se

[b] Department of Experimental Medical Science, Lund University, 22362 Lund, Sweden

[c] Wallenberg Centre for Molecular Medicine, Lund University, 22362 Lund, Sweden

[d] Universidad de Oviedo, Department of Medicine, Oviedo, Asturias, 33006, Spain

[e] Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, 33011, Spain

[f] Department of Medicine Huddinge, Karolinska Institute, Stockholm, Sweden

[g] Department for Upper Abdominal Diseases, Karolinska University Hospital, Stockholm, Sweden

[h] Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institute, Stockholm, Sweden

Determination of glucuronidase activity

Glucuronidase activity was tested according to the protocol described by Sigma Aldrich (S9626). To calculate the activity of glucuronidase in solution, 65 µL of H2O were mixed with 50 µL of 75 mM potassium phosphate buffer with 1% (w/v) bovine serum albumin, pH 6.8, 25 µL of 3 mM of phenolphthalein-glucuronide and 10 µL of enzyme test solution. A negative control was also tested, in which no enzyme was added. To stop the reaction, 500 µL of 200 mM glycine buffer, pH 10.4 were added. The resulting solution was transferred to a 96-well plate and the absorbance at 540 nM was measured to monitor the production of phenolphthalein. At the same time, a phenolphthalein standard curve was prepared, with a ranging quantity of 1-5 µg. The amount of phenolphthalein was plotted against the A540 value and test results were based on the measured absorbance.

The units in solution were calculated using the following equation:

$$Units/mL = \frac{(\mu g \text{ of phenolphthalein released}) \times \text{df}}{V_E \, x \, t}$$

Details:

t – Time factor correction (Unit definition for 1 hour)

df – Protein dilution factor

V_E – Volume (in mL) of purified glucuronidase used

Figures

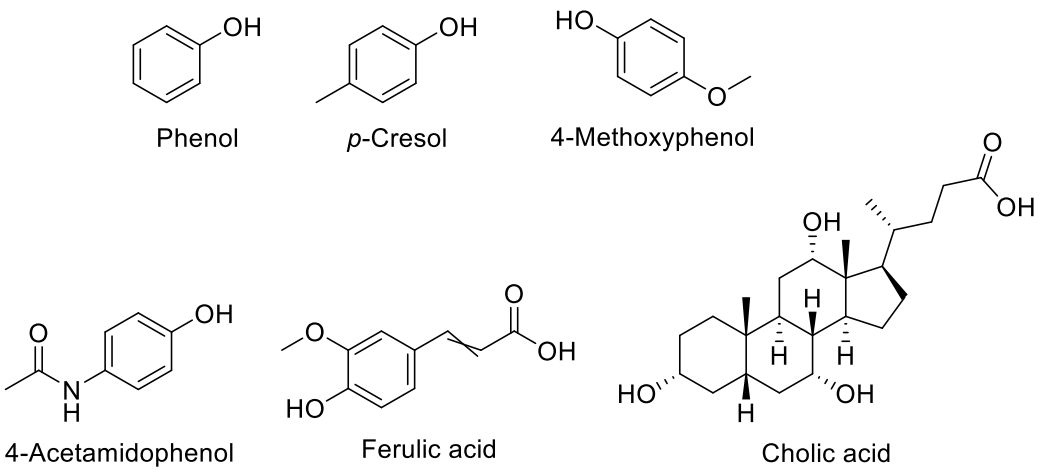

Figure S1 Selected compounds used for the enzymatic synthesis of their corresponding glucuronides, utilizing three UGT enzymes (UGT1A1, UGT2B7 and UGT2B15).

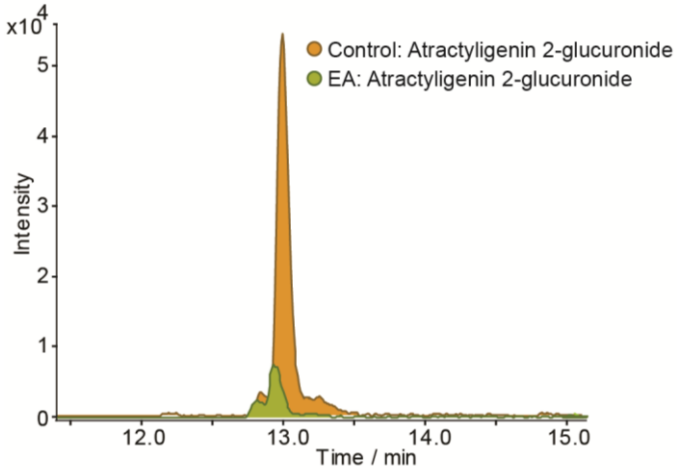

Figure S2 Extracted Ion Chromatogram (EIC) of atractyligenin 2-glucuronide (*m/z* 497.2390) in the control group and in the enzymatic treatment group in ESI^+.

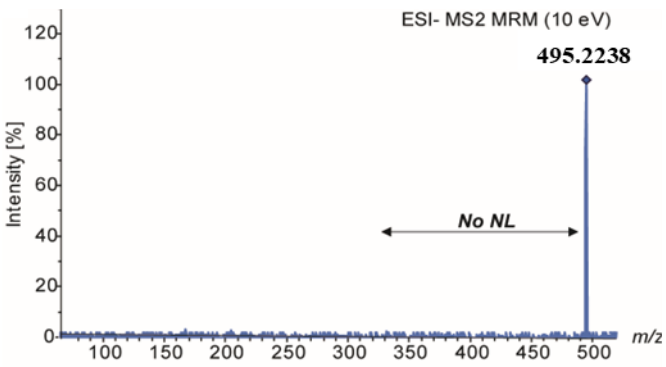

Figure S3 MS/MS fragmentation of atractyligenin 2-glucuronide (*m/z* 495.2238) in MRM mode using 10 eV in ESI$^-$.

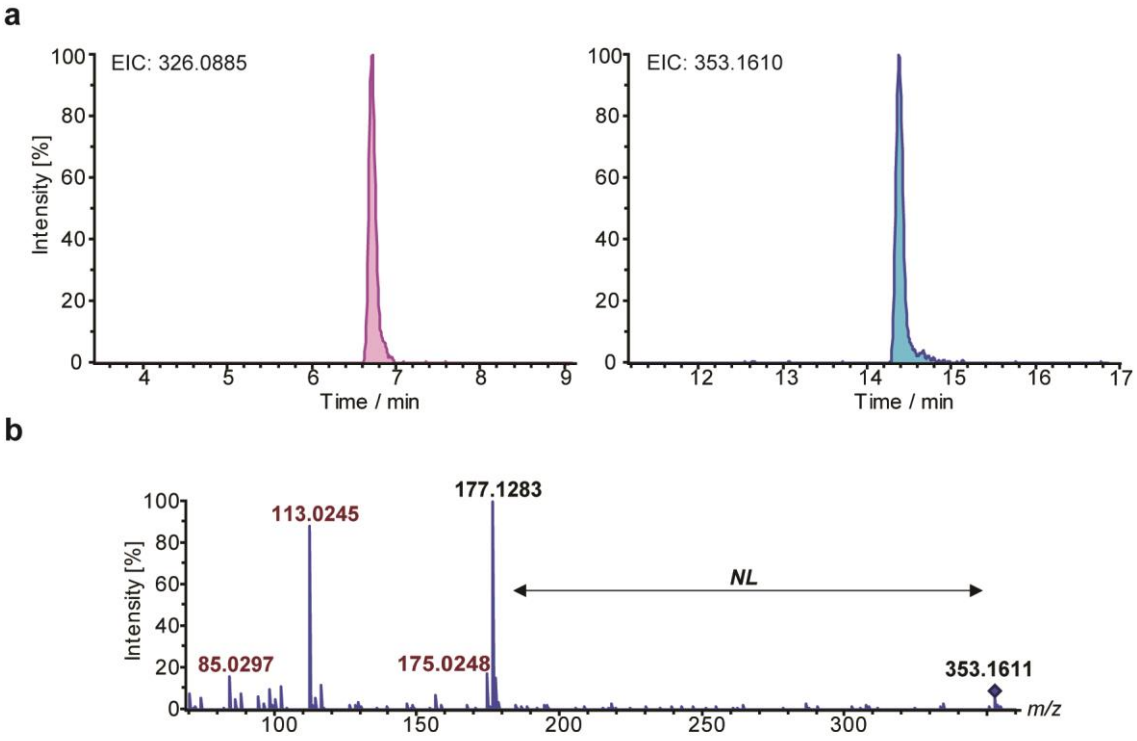

Figure S4 a EICs of acetaminophen glucuronide (*m/z* 326.0885) and propofol glucuronide (*m/z* 353.1610); **b** MS/MS fragmentation of propofol glucuronide validated in the CSF samples in ESI$^-$.

Tables

Table S1. The six compounds tested for enzymatic synthesis of the corresponding glucuronide utilizing three UGT enzymes. The compounds were at a concentration of 250 µM.

Compound Name	Monoisotopic Mass	Glucuronidation		
		UGT1A1	UGT2B7	UBT2B15
Phenol	94.0419	-	**Yes**	**Low**
p-Cresol	108.0575	**Low**	**Yes**	**Yes**
4-Methoxyphenol	124.0524	-	**Yes**	**Low**
4-Acetamidophenol	151.0633	-	-	-
Ferulic acid	194.0579	**Yes**	-	-
Cholic acid	408.2876	**Low**	-	-

Table S2. All validated glucuronidated metabolites with annotated confidence levels in the respective mode.

(Level 1: Validation with authentic synthetic or commercial standards; Level 2: Metabolite structure validation based on unambiguous matching of MS2 spectra with experimental spectra from literature or library sources; or identification of the molecular formula and MS2 fragmentation pattern comparison using computational tools; Level 3: MS2-validation of glucuronic acid moiety in the metabolite).

*Gluc: glucuronide, rt: retention time, Ctr: control group, EA: enzymatic treated group, CL: confidence level, F.P.: fingerprint fragment, NL: neutral loss, * asterisk represents a calculated molecular formula from the annotated aglycon (aglycon + $C_6H_8O_6$)*

#	Monoisotopic Mass	Chemical Formula	*m/z* Gluc	rt Gluc (min)	m/z Aglycon	ID	Fold Gluc (Ctr/EA)	CL	Ionization mode	F.P.	NL	Biospecimen Location
1	281.1111	*$C_{10}H_{19}NO_8$	282.1185	2.06	106.0864	HMDB0244975/ HMDB0253919	1.5	3	positive	-	no	Plasma
2	284.0894	$C_{13}H_{16}O_7$	283.0820	10.34	107.0499	HMDB0011686	*0.9*	1	negative	-	yes (low)	Feces
3	284.0900	$C_{13}H_{16}O_7$	283.0826	10.43	107.0505	HMDB0011686	31	1	negative	85 & 113	yes	Plasma
4	284.1259	* $C_{14}H_{20}O_6$	285.1333	13.19	109.1012	HMDB0246592	2.3	3	positive	-	yes (low)	Feces
5	290.0753	*$C_{10}H_{14}N_2O_8$	289.0679	5.08	113.0358	HMDB0060245	1.4	3	negative	-	no	Feces
6	295.1203	$C_{10}H_{21}N_3O_5S$	294.1129	11.98	118.0808	-	108	3	negative	85 & 113	no	Plasma
7	304.6762	$C_{32}H_{51}NO_{10}$	303.6688	16.13	127.6367	CID171038641	74	3	negative	85 & 113	no	Plasma
8	305.1606	$C_{12}H_{23}N_3O_6$	304.1532	15.68	128.1211	-	91	3	negative	85 & 113	no	Plasma
9	308.0899	$C_{15}H_{16}O_7$	307.0825	10.59	131.0504	HMDB0003441/ HMDB0032072	1.4	3	negative	85 (low) & 113	no	Feces
10	310.8840	-	309.8766	16.30	133.8445	-	1.9	3	negative	85 & 113 (low)	no	Plasma
11	312.6736	$C_{32}H_{51}NO_{11}$	311.6662	15.68	135.6341	HMDB0002579	49.9	3	negative	85 (low) & 113	no	Plasma
12	327.0955	$C_{14}H_{17}NO_8$	328.1029	7.06	152.0708	HMDB0010316	34.2	2	positive	-	yes	Plasma
			326.0881	6.78	150.0560		1732	2	negative	85 & 113	yes	
13	327.0959	$C_{14}H_{17}NO_8$	326.0885	6.72	150.0564	HMDB0010316	423	2	negative	85 & 113 & 175	yes	CSF
14	332.1838	$C_{16}H_{28}O_7$	331.1764	14.89	155.1443	HMDB0254445/ CID117762847	20	3	negative	85 & 113	no	Plasma
15	343.1269	$C_{15}H_{21}NO_8$	344.1343	6.30	168.1022	CID45040235	1.5	3	positive	-	no	Plasma
			342.1195	5.59	166.0874		2.7	3	negative	-	no	
16	343.1295	$C_{15}H_{21}NO_8$	344.1369	6.05	168.1048	CID45040235	1.5	3	positive	-	no	Feces
17	354.1684	$C_{18}H_{26}O_7$	353.1610	14.39	177.1289	HMDB0060933	*0.9*	2	negative	85 & 113 & 175	yes	CSF

18	380.1116	*$C_{18}H_{20}O_9$	379.1042	9.23	203.0721	HMDB0033303/ HMDB0035095	1.4	3	negative	85 (low) & 113	no	Feces
19	384.0880	*$C_{17}H_{20}O_8S$	383.0806	10.60	207.0485	HMDB0034759	1.9	3	negative	-	no	Feces
20	384.0915	*$C_{14}H_{24}O_8S_2$	383.0841	11.13	207.0520	HMDB0257963/ HMDB0012210/ HMDB0060589	1.8	3	negative	-	no	Feces
21	416.2052	$C_{20}H_{32}O_9$	415.1978	14.21	239.1657	-	8.9	3	negative	85 (low) & 113	no	Plasma
22	462.2254	$C_{25}H_{34}O_8$	463.2328	16.51	287.2007	CID5460852/HMDB0010337	8.8	3	positive	-	yes	Plasma
23	463.1846	$C_{23}H_{29}NO_9$	464.1920	13.28	288.1599	HMDB0060821/ CID129627161/ CID9825634/CID46780422/CID46780423/CID45358980/CID71750840/CID117065138/CID9981841/CID101360603	31	3	positive	-	yes	Plasma
24	463.1849	$C_{23}H_{29}NO_9$	464.1923	13.07	288.1602	HMDB0060821/ CID129627161/ CID9825634/CID46780422/CID46780423/CID45358980/CID71750840/CID117065138/CID9981841/CID101360603	59	3	positive	-	yes	Plasma
25	472.2241	$C_{25}H_{32}N_2O_7$	473.2315	10.83	297.1994	CID46781815/HMDB0060717	1.2	3	positive	-	no	Feces
26	483.0951	$C_{15}H_{21}N_3O_{15}$	482.0877	8.67	306.0556	-	2171	3	negative	85 & 113	yes (low)	Plasma
27	496.2315	$C_{25}H_{36}O_{10}$	495.2241	12.76	319.1920	HMDB0240481/ CID156960878	1025	2	negative	85 (low) & 113 (low)	no	Plasma
			497.2390	13.01	321.2069		13	2	positive		yes	
28	508.2308	$C_{26}H_{36}O_{10}$	507.2234	16.15	331.1913	-	10	3	negative	85 (low) & 113 (low)	yes	Plasma
29	540.2573	$C_{27}H_{40}O_{11}$	539.2499	13.63	363.2178	HMDB0010357	18	3	negative	85 (low) & 113	no	Plasma

30	542.2730	$C_{27}H_{42}O_{11}$	541.2656	13.41	365.2335	HMDB0010320	536	3	negative	85 & 113	no	Plasma
31	568.1978	$C_{23}H_{36}O_{16}$	567.1904	14.00	391.1583	-	816	3	negative	85 & 113	no	Plasma
32	608.3218	*$C_{32}H_{48}O_{11}$	609.3292	14.30	433.2971	HMDB0014792/ HMDB0244526	1.5	3	positive	-	no	Feces

Table S3. Annotations and chemical classes of the identified glucuronidated metabolites.

** Asterisk represents a calculated molecular formula from the annotated aglycon (aglycon + $C_6H_8O_6$)*

Monoisotopic Mass	Chemical Formula	Name	Class
281.1111	*$C_{10}H_{19}NO_8$	2-Amino-2-methyl-1,3-propanediol / L-Threoninol	Amino alcohols
284.0894	$C_{13}H_{16}O_7$	*p*-Cresol glucuronide	Phenols
284.1259	*$C_{14}H_{20}O_6$	4-Vinylcyclohexene	Cycloalkenes
290.0753	*$C_{10}H_{14}N_2O_8$	L-3-Cyanoalanine	Amino acids, peptides, and analogues
295.1203	$C_{10}H_{21}N_3O_5S$	-	Unknown
304.6762	$C_{32}H_{51}NO_{10}$	Glycolithocholic acid glucuronide	Steroids and derivatives
305.1606	$C_{12}H_{23}N_3O_6$	-	Unknown
308.0899	*$C_{15}H_{16}O_7$	Cinnamaldehyde	Benzaldehydes
312.6736	$C_{32}H_{51}NO_{11}$	Glycochenodeoxycholic acid glucuronide	Steroids and derivatives
327.0955	$C_{14}H_{17}NO_8$	Acetaminophen glucuronide	Phenols
332.1838	$C_{16}H_{28}O_7$	Menthol glucuronide / Citronellyl glucuronide	Terpenoids
343.1269	$C_{15}H_{21}NO_8$	Phenylephrine glucuronide	Phenols
354.1684	$C_{18}H_{26}O_7$	Propofol glucuronide	Phenols
380.1116	*$C_{18}H_{20}O_9$	Anofinic acid / 7-Ethoxy-4-methyl-2H-1-benzopyran-2-one	Coumarins and derivatives
384.088	*$C_{17}H_{20}O_8S$	Methyl 5-(1-Propynyl)-2-thiophenepropanoate	Thiophenes and derivatives
384.0915	*$C_{14}H_{24}O_8S_2$	-	Unknown
416.2052	$C_{20}H_{32}O_9$	-	Unknown
462.2254	$C_{25}H_{34}O_8$	6-Dehydrotestosterone glucuronide	Steroids and derivatives
463.1846	$C_{23}H_{29}NO_9$	Dihydromorphine-3-glucuronide	Alkaloids and derivatives
472.2241	$C_{25}H_{32}N_2O_7$	2-Hydroxy-imipramine glucuronide	Alkaloids and derivatives
483.0951	$C_{15}H_{21}N_3O_{15}$	-	Unknown
496.2315	$C_{25}H_{36}O_{10}$	Atractyligenin 2-glucuronide	Terpenoids
508.2308	$C_{26}H_{36}O_{10}$	-	Unknown
540.2573	$C_{27}H_{40}O_{11}$	Tetrahydroaldosterone-3-glucuronide	Steroids and derivatives
542.273	$C_{27}H_{42}O_{11}$	Cortolone-3-glucuronide	Steroids and derivatives
568.1978	$C_{23}H_{36}O_{16}$	-	Unknown
608.3218	*$C_{32}H_{48}O_{11}$	Latanoprost / isopropyl 7-[3,5-dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl]hept-5-enoate	Eicosanoids