

Tissue Specific Modulation of cyp2c and cyp3a mRNA Levels and Activities by Diet-Induced Obesity in Mice: The Impact of Type 2 Diabetes on Drug Metabolizing Enzymes in Liver and Extra-Hepatic Tissues

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Table S1. Selected reaction monitoring for bupropion, midazolam and ebastine.

Substrate	Metabolite	SRM (m/z)	Tube Lens	Collision Energy
			(V)	(V)
Bupropion	Hydroxybupropion	256 → 139	91	28
	Hydroxybupropion-d ₆	262 → 139	91	28
Midazolam	1'-hydroxymidazolam	342 → 168	98	36
	1'-hydroxymidazolam-d ₄	346 → 168	98	36
	4'-hydroxymidazolam	342 → 234	98	22
	4'-hydroxymidazolam-d ₅	347 → 235	98	22
Ebastine	Hydroxyebastine	486 → 167	100	31
	Hydroxyebastine-d ₅	491 → 172	100	31
	Carebastine	500 → 167	100	29
	Carebastine-d ₅	505 → 172	100	29
	Desalkyl-ebastine	268 → 167	91	20
	Desalkyl-ebastine-d ₅	273 → 172	91	20

Table S2. Selected reaction monitoring for chlorzoxazone, tolbutamide, dodecanoic acid, bufuralol and repaglinide.

Substrate	Metabolite	SRM (m/z)	Tube Lens	Collision Energy
			(V)	(V)
Chlorzoxazone	Hydroxychlorzoxazone	184 → 120	-65	20
	Hydroxychlorzoxazone- ¹⁵ Nd ₂	187 → 123	-65	20
Tolbutamide	Hydroxytolbutamide	285 → 186	-50	19
	Hydroxytolbutamide-d ₉	294 → 186	-50	19
	Carboxytolbutamide	299 → 200	-65	19
	Carboxytolbutamide-d ₉	308 → 200	-65	19
Dodecanoic acid	12-Hydroxydodecanoic acid	215 → 169	-62	21
	12-Hydroxydodecanoic acid-d ₂₀	235 → 188	-62	21
Bufuralol	Hydroxybufuralol	278 → 186	77	19
	Hydroxybufuralol-d ₉	287 → 187	77	19
Repaglinide	M1-repaglinide	385 → 106	79	33
	Hydroxyrepaglinide	469 → 246	95	25

Desalkyl-ebastine-ds 273 → 172 91 20

Table S3. Total mRNA transcripts for each cyp isoform in C57BL6 mice microsomes according to diet group.

	Liver			Kidney			Heart			Lung		
	ND	LDR	HDR	ND	LDR	HDR	ND	LDR	HDR	ND	LDR	HDR
cyp2b9	2.85E-05	2.03E-02	3.79E-02	ND	ND	ND	ND	ND	ND	ND	ND	ND
cyp 2b10	6.90E-06	NQ	7.89E-06	7.04E-05	6.32E-05	4.19E-05	1.10E-05	1.57E-05	1.22E-05	8.52E-03	2.55E-02	1.93E-02
cyp 2c29	4.01E-01	1.93E-01	2.43E-01	1.80E-04	2.39E-04	1.21E-04	3.37E-05	7.33E-05	3.70E-05	5.49E-04	1.99E-03	7.19E-04
cyp 2c37	7.08E-02	8.36E-02	9.73E-02	ND	ND	ND	ND	ND	ND	ND	ND	ND
cyp 2c39	2.36E-04	3.35E-04	6.23E-04	ND	ND	ND	ND	ND	ND	ND	ND	ND
cyp 2c40	7.52E-02	3.08E-02	5.12E-02	3.30E-04	1.22E-04	2.21E-04	ND	ND	ND	ND	ND	ND
cyp 2d9	2.48E-01	3.43E-01	3.30E-01	2.28E-01	1.90E-01	2.27E-01	4.38E-06	7.19E-06	6.82E-06	3.85E-05	5.12E-05	ND
cyp 2d22	4.21E-02	4.22E-02	6.62E-02	1.03E-02	1.05E-02	1.16E-02	1.07E-02	1.14E-02	1.92E-02	2.19E-02	3.00E-02	7.73E-02
cyp 2e1	2.01E+00	3.41E+00	2.79E+00	2.51E+00	2.51E+00	2.49E+00	5.04E-03	3.29E-03	2.91E-03	9.27E-03	2.01E-02	1.88E-02
cyp 2j5	3.51E-01	3.58E-01	3.22E-01	2.48E+00	2.42E+00	2.12E+00	1.13E-05	2.11E-05	2.28E-05	5.81E-05	2.20E-04	5.58E-04
cyp 2j6	7.38E-03	5.58E-03	6.93E-03	1.44E-02	1.53E-02	1.32E-02	2.95E-03	2.17E-03	2.09E-03	2.81E-03	6.14E-03	5.67E-03
cyp3a11	6.01E-01	3.79E-02	3.88E-02	2.97E-05	1.20E-05	1.49E-05	ND	ND	ND	ND	ND	ND
cyp 3a13	3.10E-03	3.30E-03	3.15E-03	2.89E-05	4.54E-05	5.80E-05	NQ	NQ	NQ	3.51E-04	1.12E-03	7.57E-04
cyp 3a25	5.84E-02	2.11E-02	2.75E-02	1.22E-05	1.33E-05	5.42E-06	ND	ND	NQ	1.53E-05	2.15E-05	5.20E-05
cyp 4a10	1.30E-02	1.12E-02	3.25E-02	1.88E-02	1.59E-02	2.89E-02	8.49E-06	1.90E-05	1.08E-05	8.43E-05	3.35E-04	1.12E-04

Results are expressed as mean N-fold differences in cyp gene relative to the average expression (using $2^{-\Delta C_t \text{ sample}}$). NQ = Not Quantifiable ($35 < C_t < 38$), ND = Not Detectable ($C_t > 38$).

Table S4. The estimated intrinsic clearance values for each substrate-probe in liver microsomes from mice fed a normal diet (ND) or a HFD (LDR; low-diet responders and HDR; high-diet responders after an eight-week period).

	Intrinsic Clearance (uL/min/mg Protein)	95% Confidence Interval
Bupropion → Hydroxybupropion		
ND	0.44	[0.40, 0.49]
LDR	0.42	[0.36, 0.51]
HDR	0.37	[0.32, 0.42]
Tolbutamide → hydroxytolbutamide		
ND	0.80	[0.78, 0.83]
LDR	0.54	[0.48, 0.62]
HDR	0.57	[0.53, 0.63]
Repaglinide → M1 repaglinide		
ND	10.6	[10.0, 11.2]
LDR	1.0	[0.9, 1.1]
HDR	1.4	[1.4, 1.5]
Repaglinide → Hydroxyrepaglinide		
ND	0.20	[0.19, 0.21]
LDR	0.10	[0.09, 0.12]
HDR	0.13	[0.13, 0.14]
Bufuralol → hydroxybufuralol		
ND	39	[37, 41]
LDR	56	[50, 63]

HDR	53	[48, 58]
Chlorzoxazone → Hydroxychlorzoxazone		
ND	43	[39, 48]
LDR	48	[44, 53]
HDR	51	[45, 58]
Ebastine → Hydroxyebastine		
ND	102	[77, 148]
LDR	65	[57, 76]
HDR	285	[245, 341]
Midazolam → 1'-hydroxymidazolam		
ND	107	[102, 11]
LDR	23	[22, 25]
HDR	41	[37, 46]
Dodecanoic acid → Hydroxydodecanoic acid		
ND	30	[26, 35]
LDR	42	[38, 48]
HDR	64	[56, 74]

Table S5. The HFD-induced change on cyp450 activities are summarized for the major drug metabolism enzymes in hepatic and extra-hepatic tissues. Results are expressed as the percentage of change compared to control mouse group (↑ or ↓ %, and—indicating no significant variation). * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ compared to ND.

Probes	HFD Groups	Liver (%)	Kidney (%)	Lung (%)	Heart (%)
Midazolam	LDR	81% ↓ †	72% ↓ †	-	N/F
(Cyp3a)	HDR	81% ↓ †	80% ↓ †	-	
Tolbutamide	LDR	32% ↓ †	49% ↑ †	-	N/F
(Cyp2c)	HDR	23% ↓ †	19% ↑ **	15% ↓ **	
Repaglinide	LDR	86% ↓ †	-	N/F	N/A
(Cyp2c)	HDR	84% ↓ †	-		
Bupropion	LDR	-	-	12% ↓ *	75% ↓ †
(Cyp2b)	HDR	23% ↓ **	36% ↓ **	13% ↓ **	83% ↓ †
Bufuralol	LDR	-	120% ↑ †	7% ↓ *	N/A
(Cyp2d)	HDR	-	111% ↑ †	28% ↓ †	
Ebastine	LDR	59% ↑ †	-	26% ↓ *	-
(Cyp2j)	HDR	30% ↑ †	31% ↓ *	59% ↓ †	-
Chlorzoxazone	LDR	20% ↑ **	11% ↑ **	32% ↑ **	29% ↓ *
(Cyp2e1)	HDR	-	17% ↓ **	-	-
Dodecanoic acid	LDR	-	-	16% ↓ *	N/F
(Cyp4a)	HDR	-	32% ↓ *	27% ↓ †	

N/A; data not available. N/F; activity not found.