

Hepatic Transporters and Liver Disease

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Mechanisms of Transport in Switzerland



Conflict of Interest Disclosure

- **Commercial Financial Interest:** Co-inventor of the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®) and related technologies, which have been licensed exclusively to Qualyst Transporter Solutions, LLC, recently acquired by BioIVT
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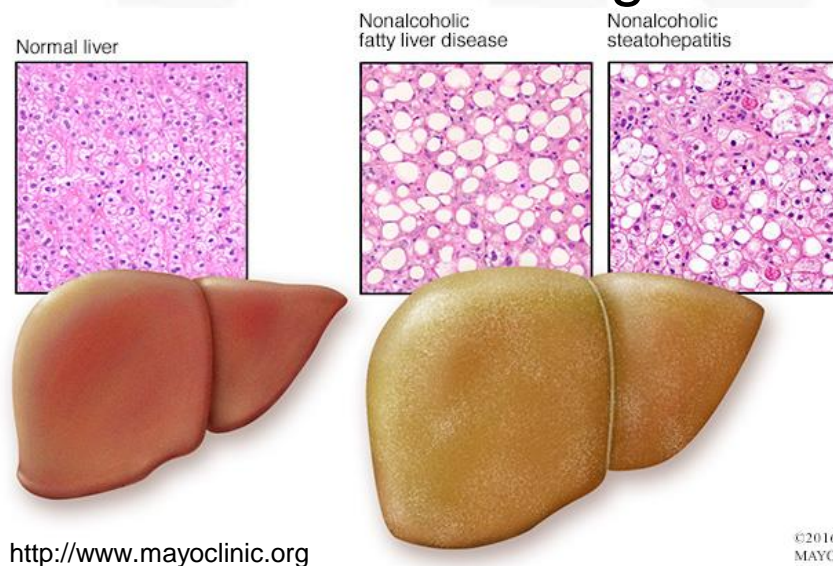
Outline

- Hepatic Transporter Expression in Patients with Nonalcoholic Steatohepatitis (NASH)
- Clinical Probes to Assess Transporter Function in NASH
 - ^{99m}Tc -Mebrofenin (MRP2 probe)
 - Morphine/Morphine Glucuronides (MRP3 probe)
- Bile Acid Concentrations in NASH
- OST α/β Induction in Liver Tissue of Patients with NASH
- Are Patients with NASH More Susceptible to Bile Acid-Mediated Drug-Induced Liver Injury (DILI)?

Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

- Worldwide prevalence of NAFLD is 25% and increasing with highest prevalence in:

– 32%	Middle East
– 31%	South America
– 27%	Asia
– 24%	USA
– 23%	Europe
– 14%	Africa



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- ~10-20% of patients with NAFLD present with the more progressive form, NASH
 - characterized by hepatocyte ballooning, steatosis, and inflammation

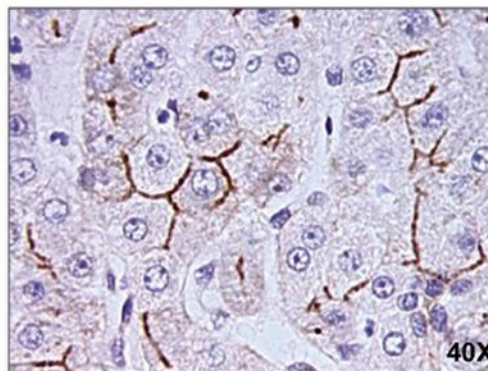
Altered Hepatic Transport Proteins in Liver Tissue from Patients with NASH

Transporter	Protein Level (Western Blot)
OATP1B1	Increased
OATP1B3	Decreased
OATP2B1	No Change
MRP3	Increased
MRP4	Increased
P-gp	Increased
BCRP	Increased
MRP2	Increased (glycosylated & unglycosylated)

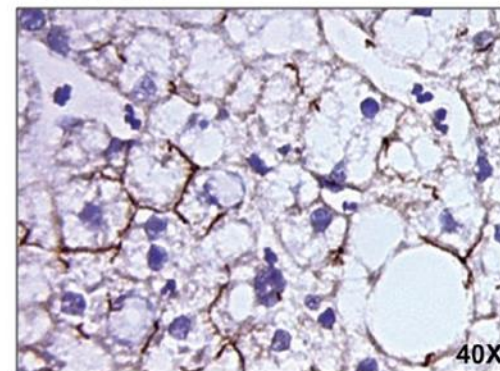
Hardwick et al., *Drug Metab Dispos*, **39**:2395, 2011;
Clarke et al., *J Hepatol*, **61**:139, 2014;
Clarke et al., *Liver Int*, **37**:1074, 2017

Immunohistochemical Staining of MRP2

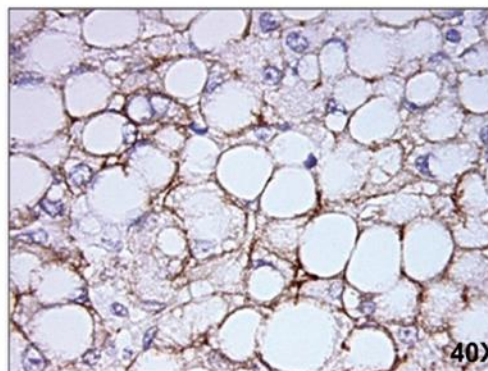
Normal



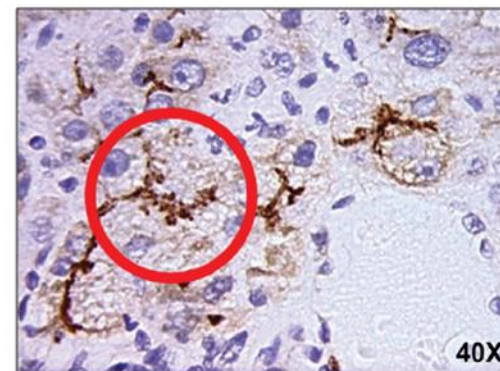
Steatosis



NASH (>5% fatty infiltration)

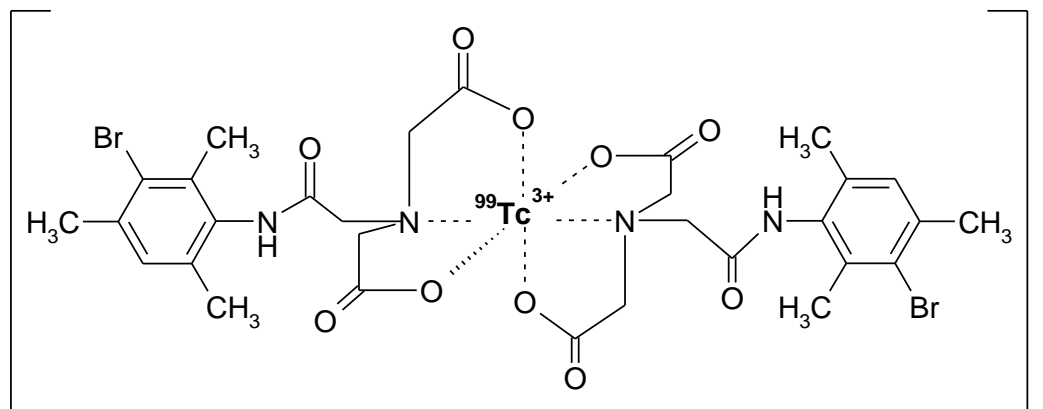


NASH (<5% fatty infiltration)



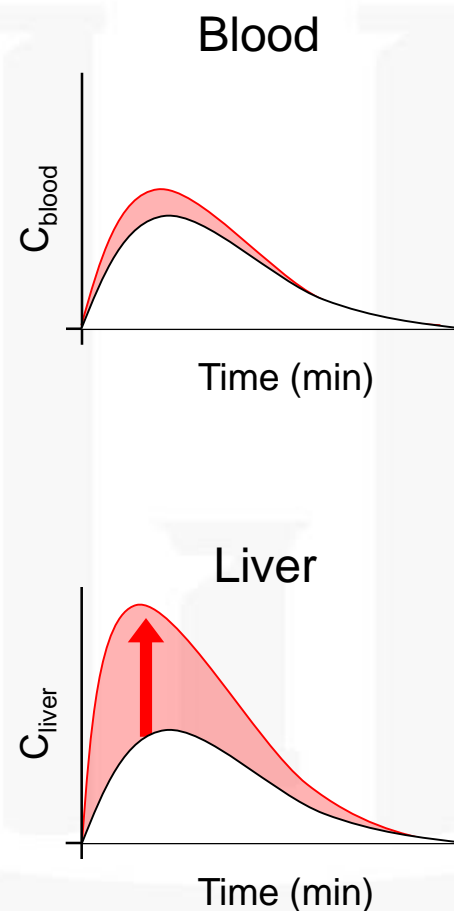
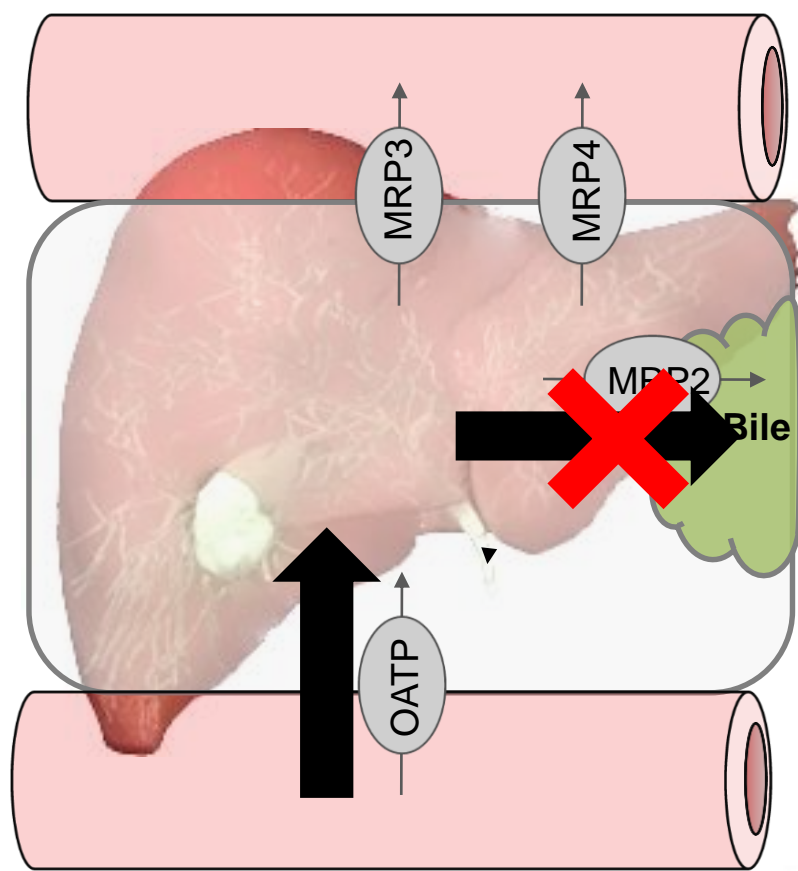
Hardwick et al., *Drug Metab Dispos*, **39**:2395, 2011

^{99m}Tc -Mebrofenin (Choletec[®]): Probe for Transporter-Mediated Hepatobiliary Excretion



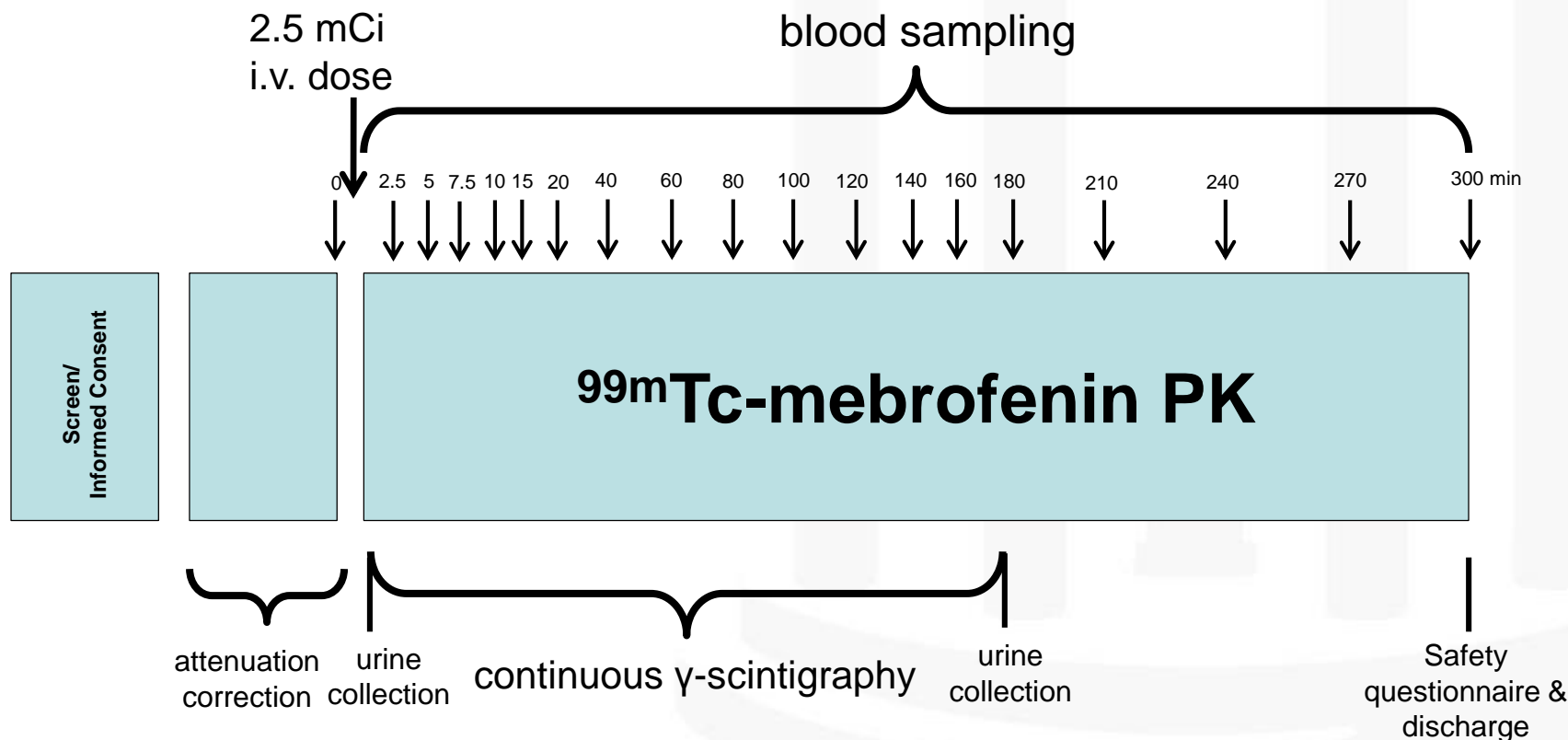
- Used clinically as a hepatobiliary imaging agent
- Liver uptake ~98%; negligible metabolism
- Urinary excretion <2% of dose
- Transporter-mediated hepatobiliary disposition
 - Hepatic uptake via OATP1B1 and OATP1B3
 - Biliary excretion via MRP2
 - Basolateral excretion via MRP3

Simulations Predict Increased Hepatic Exposure to MRP2 Substrates in NASH Patients



Clinical Study Design: ^{99m}Tc -Mebrofenin

- Healthy subjects (n=14) and biopsy-confirmed NASH patients [n=7; NAFLD activity score (NAS) ≥ 4] admitted on morning of study after overnight fast
- Attenuation correction obtained with a cobalt-57 flood source
- Subjects positioned supine under gamma camera

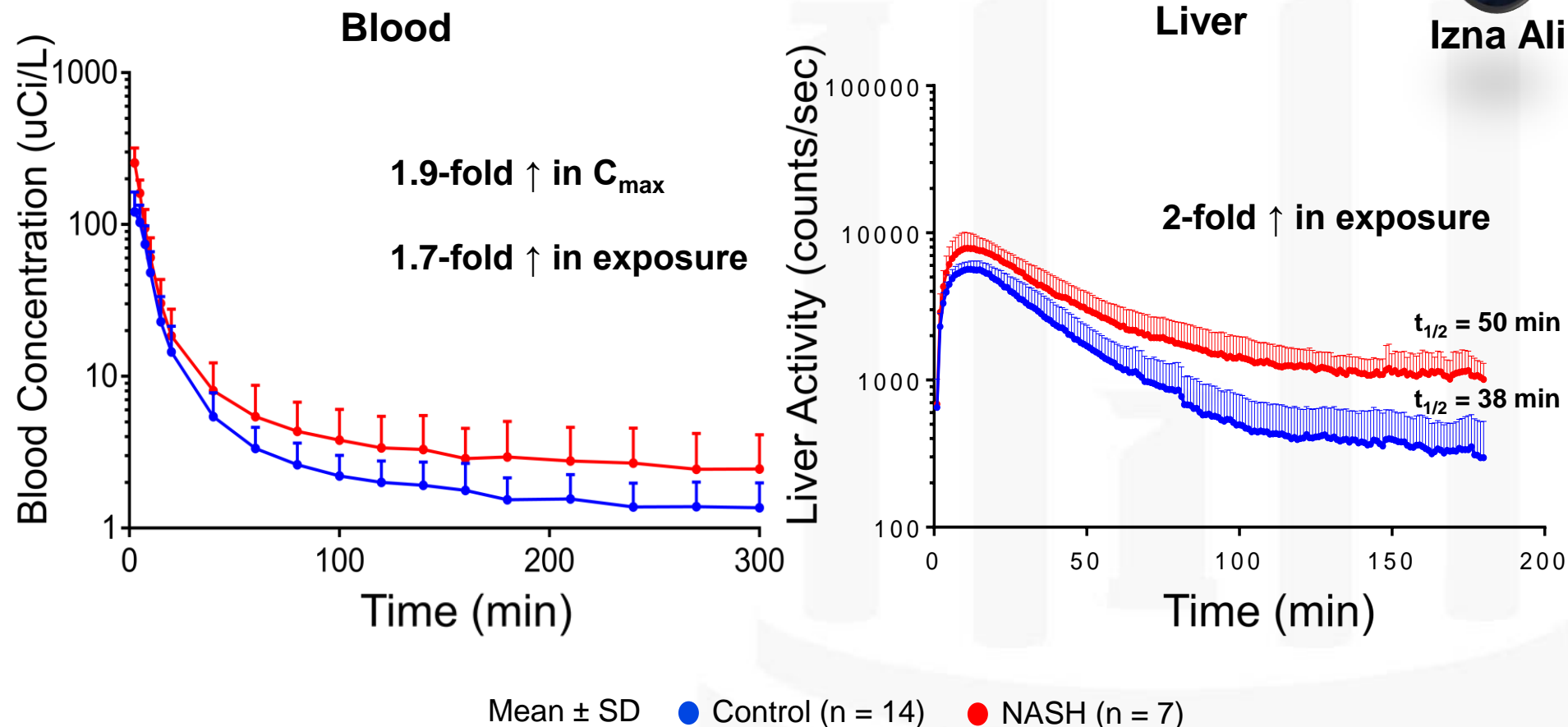


- Subjects discharged following exit exam

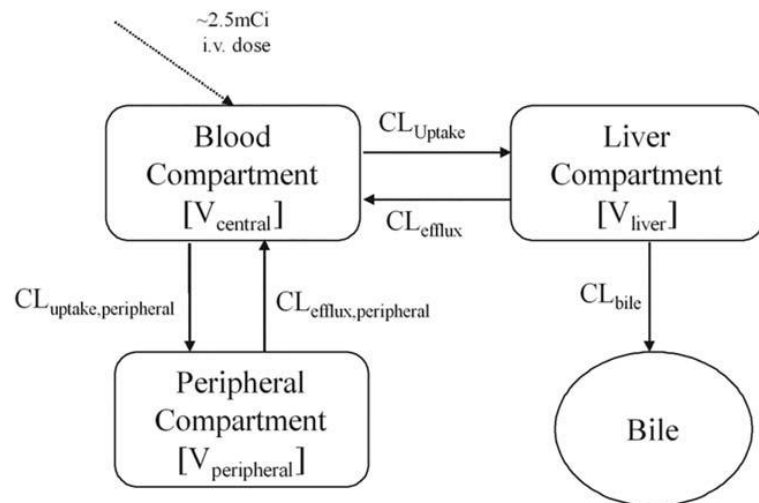
Hepatic ^{99m}Tc -Mebrofenin Exposure was Increased in Patients with NASH



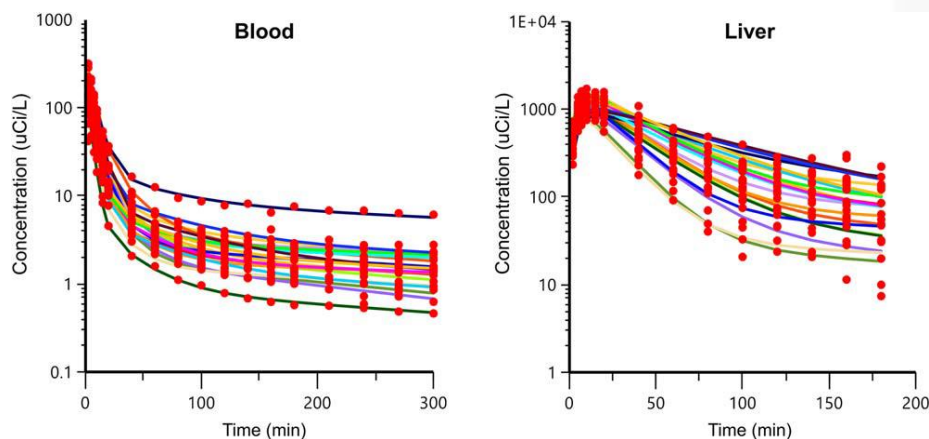
Izna Ali



Model Scheme Describing ^{99m}Tc -Mebrofenin Disposition and Parameter Estimates



Observed Data and Model Predictions for Individual Subjects

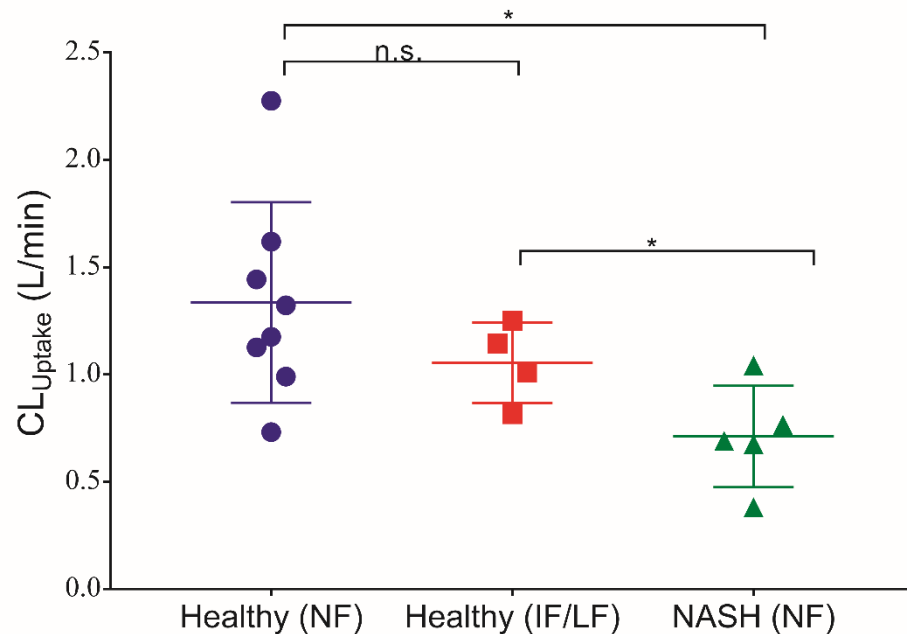


Parameters	Healthy (n=14)	NASH (n=7)
$\text{CL}_{\text{uptake}}$ (L/min)	1.14 (0.73-2.27)	0.731 ** (0.382-1.04)
$\text{CL}_{\text{efflux}}$ (L/min)	0.00800 (0.00481-0.0139)	0.00579 (0.00475-0.00903)
CL_{bile} (L/min)	0.0354 (0.0157-0.0728)	0.0171 ** (0.0110-0.0207)
V_{central} (L)	11.1 (9.55-12.5)	6.32 ** (5.69-9.69)
V_{liver} (L)	0.958 (0.527-1.39)	0.891 (0.648-1.43)

Median (range); **p<0.001

^{99m}Tc -Mebrofenin Hepatic Uptake Clearance was Lower in NASH Patients even with “Normal Function” *SLCO1B1* Genotype

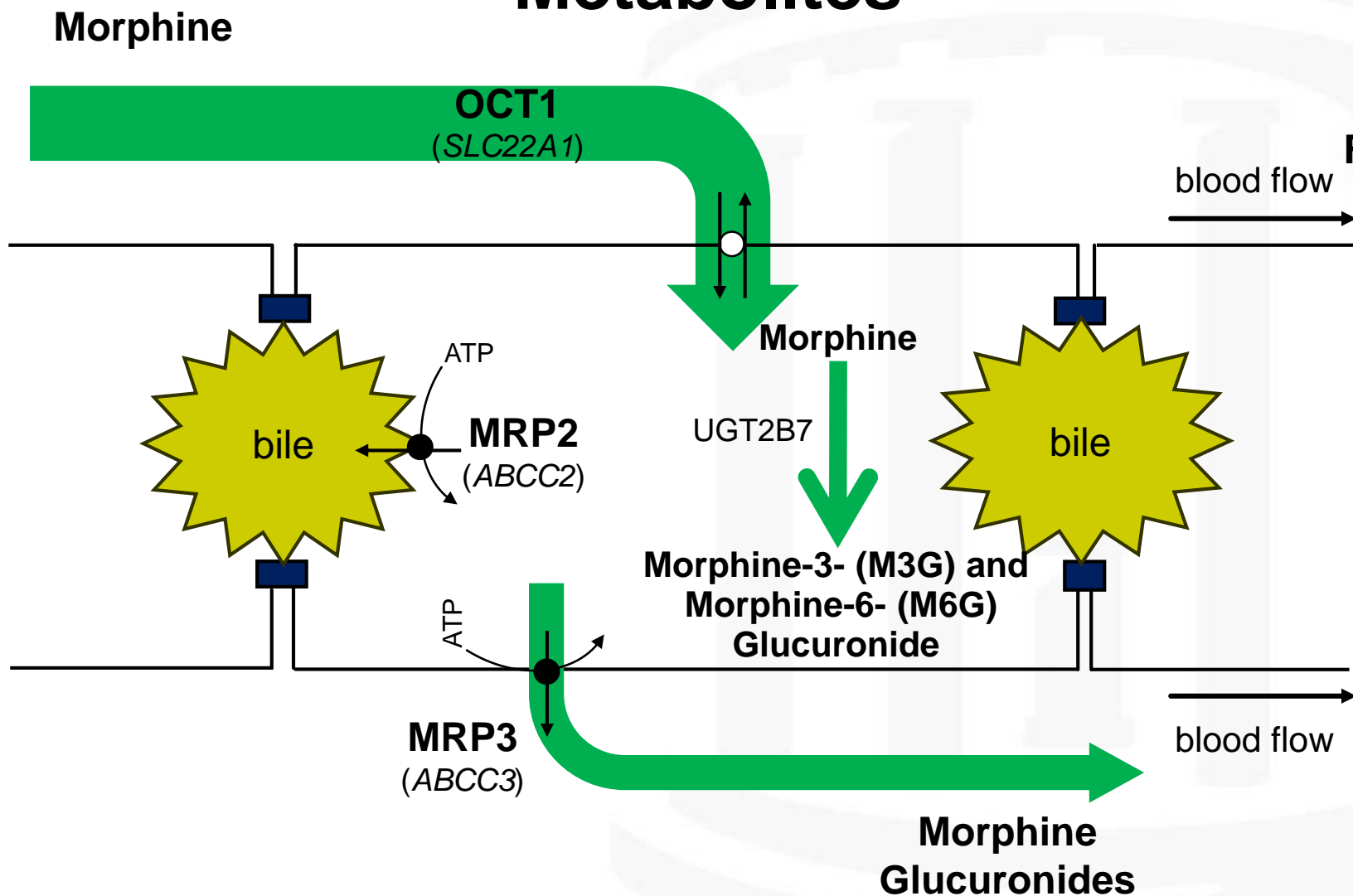
<i>SLCO1B1</i> Genotype	Healthy (# of subjects)	NASH (# of subjects)	Function
*15/*15	1		Low (LF)
*1A/*15	3		Intermediate (IF)
*1A/*14		1	Normal (NF)
*14/*14	1		
*1A/*1A	2	3	
*1B/*1B	2		
*1B/*14	1		
*1A/*1B	2	1	



Hepatic Disposition of Morphine and Metabolites

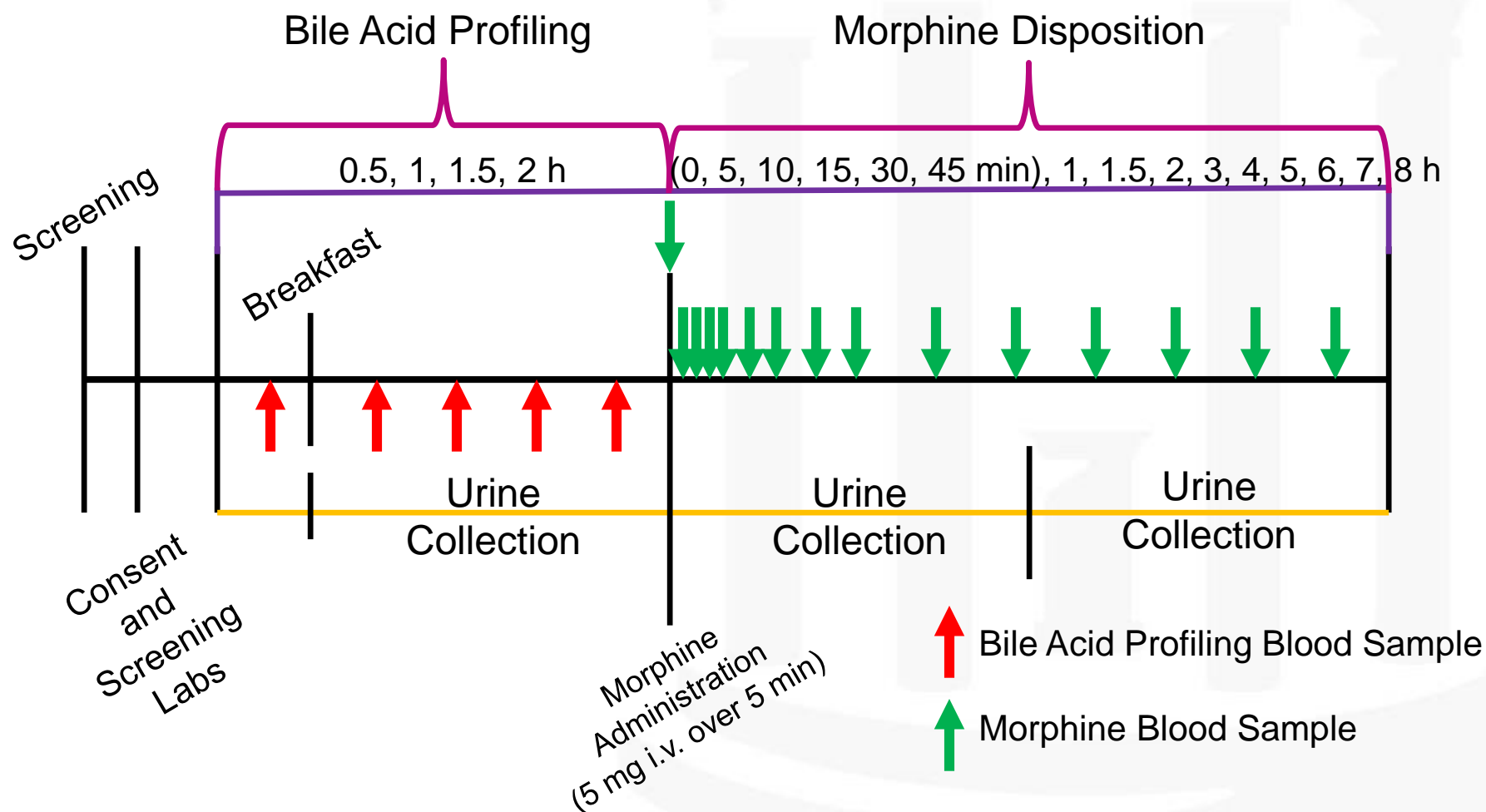


Brian Ferslew

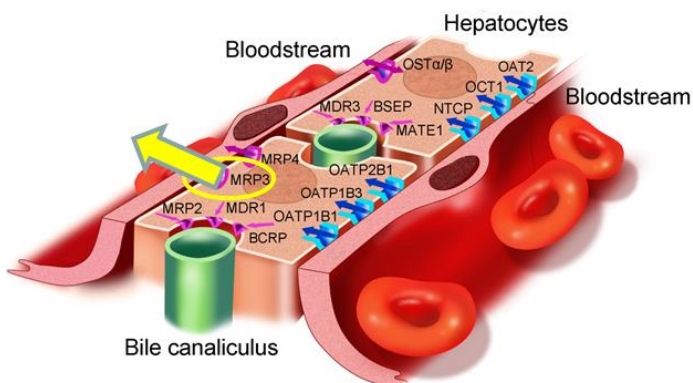
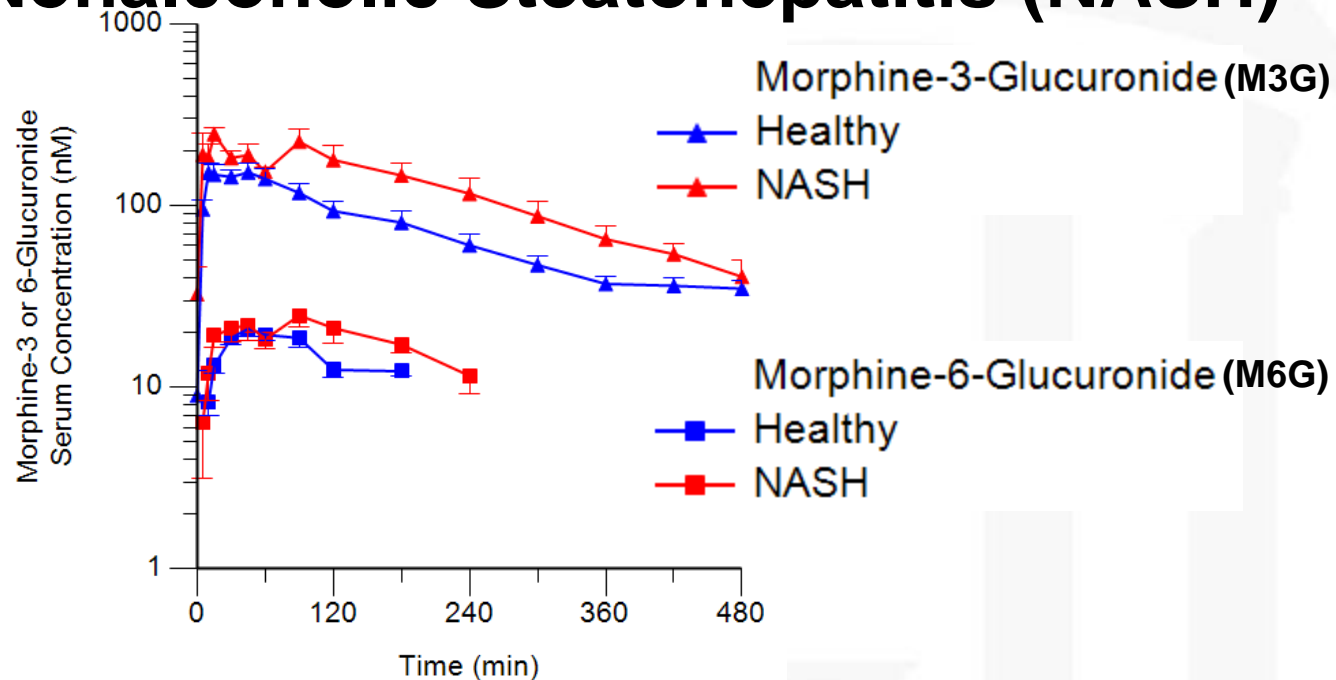
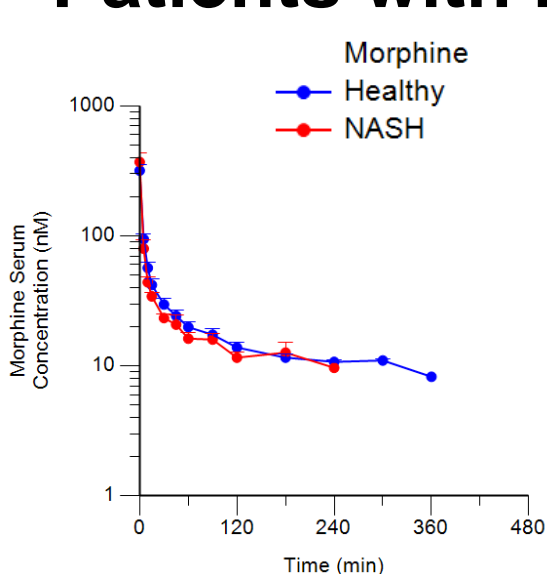


Clinical Study Design: Morphine / Glucuronides

- Healthy subjects without insulin resistance: $n=14$
- Biopsy confirmed NASH patients [NAFLD activity score (NAS) ≥ 4]: $n=7$



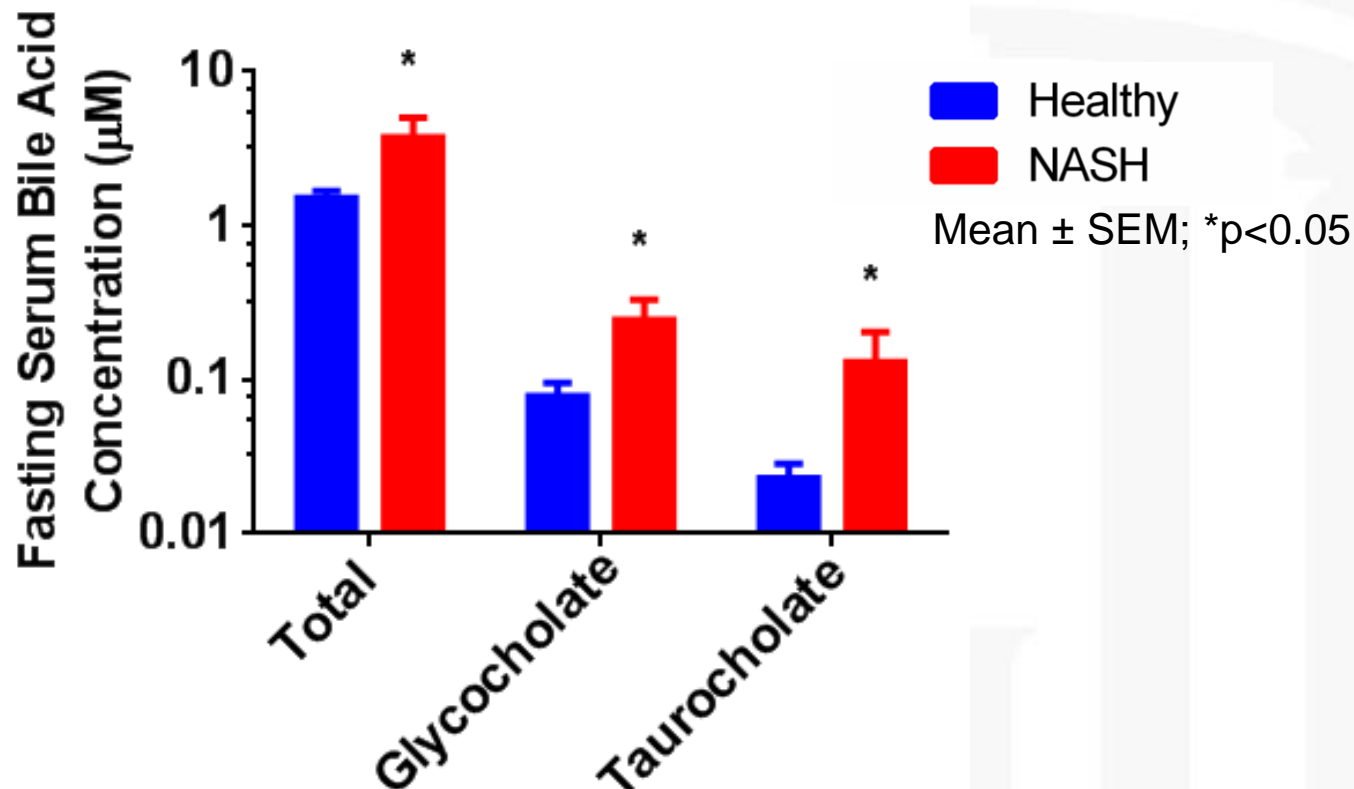
Increased M3G and M6G Serum Concentrations in Patients with Nonalcoholic Steatohepatitis (NASH)



MG Parameters	Healthy (n=14)	NASH (n=7)
C_{max} (nM)	225 (194-261)	343** (284-413)
AUC_{0-last} ($\mu M \cdot min$)	37 (32-44)	59** (42-83)
Half-life (min)	187 (153-229)	146 (104-205)

Geometric mean (95% CI); ** $p < 0.01$ t-test on log transformed data

Increased Conjugated Bile Acids in Serum of Patients with NASH



Parameter	Total Bile Acids		Glycocholate		Taurocholate	
	β	P-value	β	P-value	β	P-value
NAS+Fibrosis	0.43 (0.10)	0.004	0.03 (0.01)	0.001	0.02 (0.01)	0.006

β : regression parameter estimate (SE)

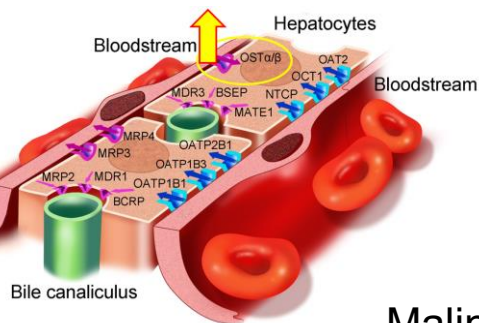
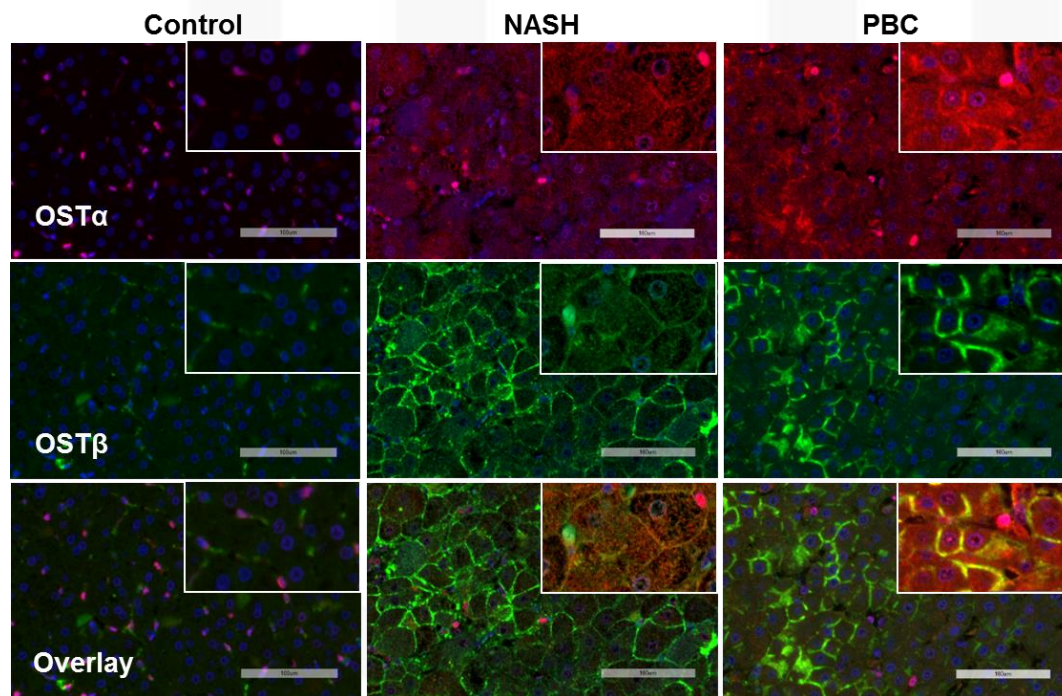
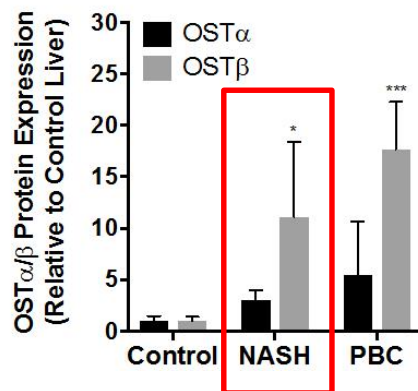
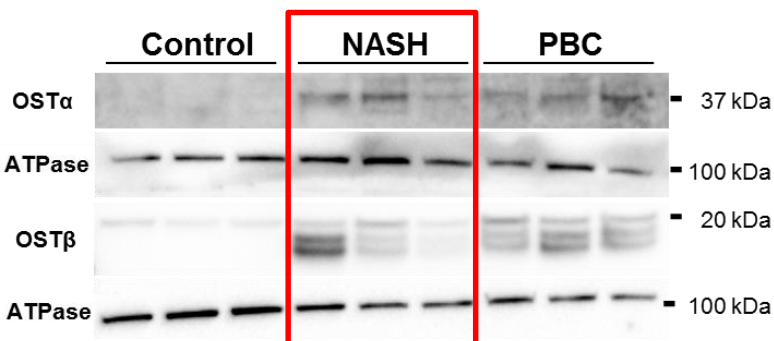
Organic Solute Transporter (OST α/β) SLC51A/B is Upregulated in Patients with Liver Disease



Melina Malinen

Is OST α/β an Overlooked “Safety Valve” for Hepatocellular Efflux of Bile Acids?

Patients with Nonalcoholic Steatohepatitis (NASH) and Primary Biliary Cirrhosis (PBC)



Chenodeoxycholic Acid (CDCA) and Obeticholic Acid (OCA) Treatment of Sandwich-Cultured Human Hepatocytes Increased OST α/β and BSEP mRNA, Protein and Function (100 μ M treatment x 72 hr; n=3)



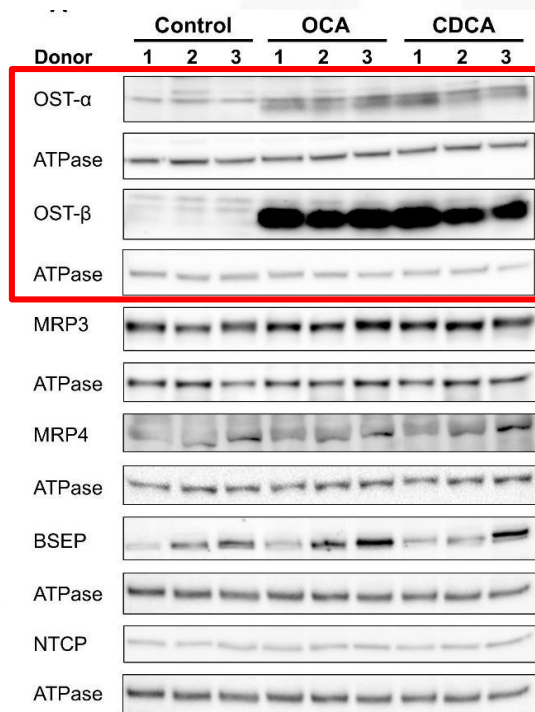
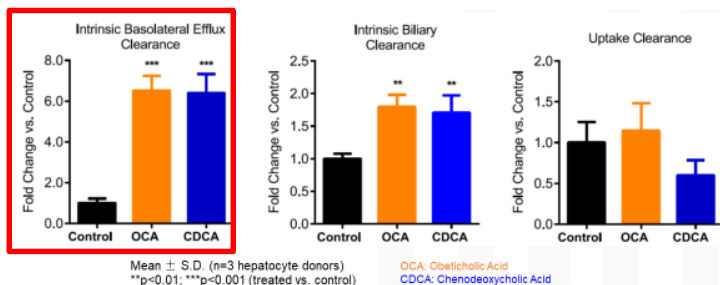
Cen Guo

CDCA Treatment

Transporter	mRNA (fold increase)	Protein (fold increase)
OST α	3-7	>3
OST β	21-187	13
BSEP	2-8	2

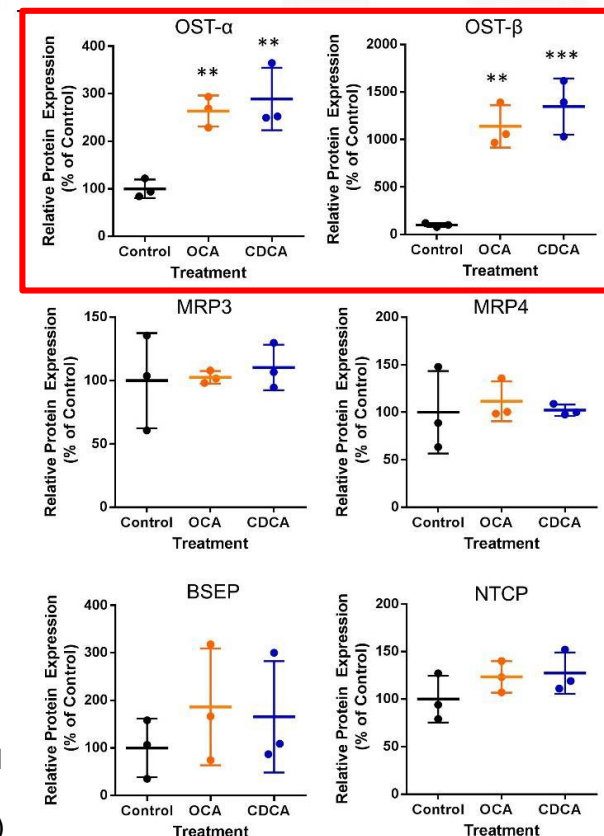
Data expressed as relative fold change

Fold Change in d_8 -Taurocholate Clearance



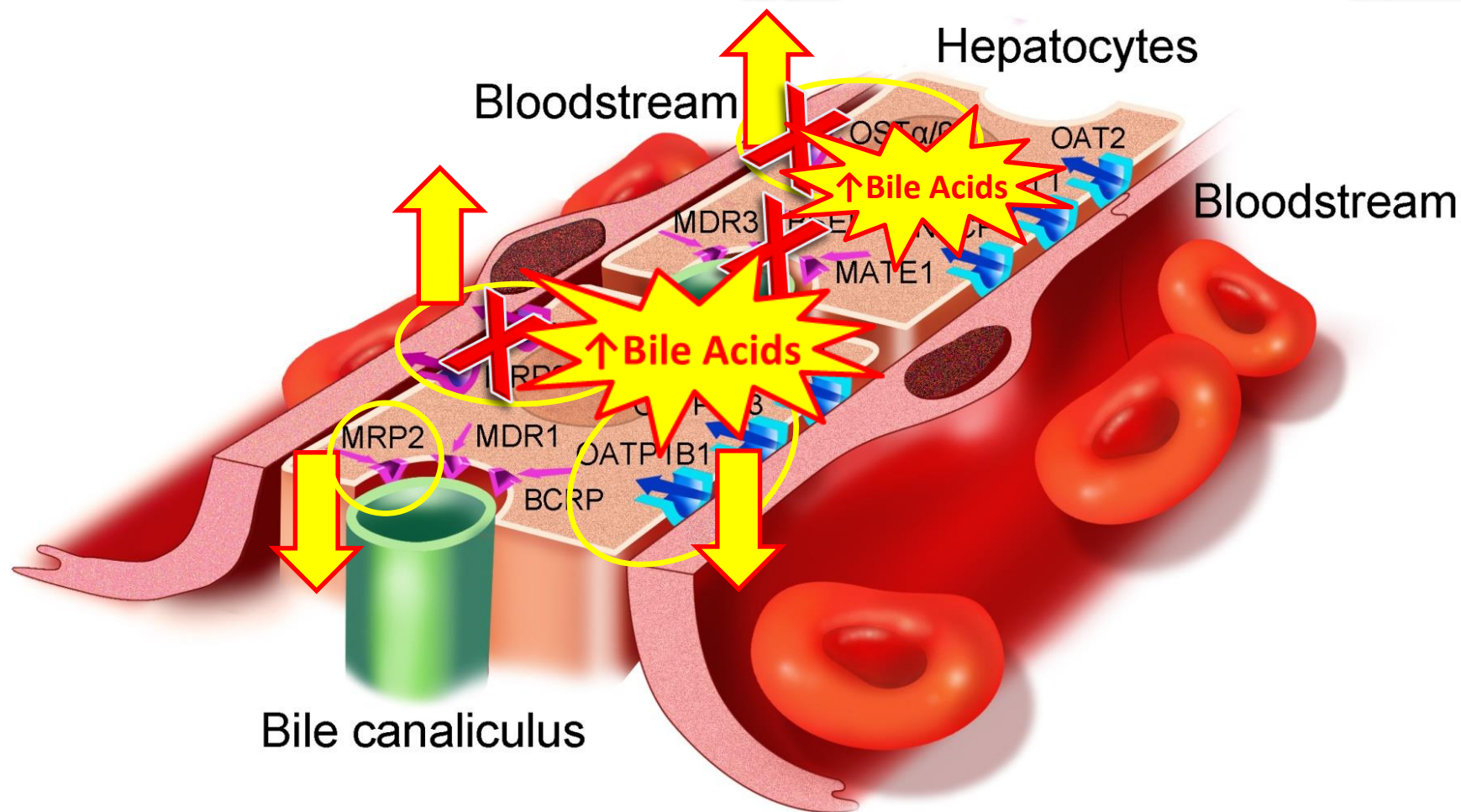
Mean \pm S.D. expressed as % of control
(n = 3 hepatocyte donors)

p<0.01; *p<0.001 (treated vs. control)



OCA: Obeticholic Acid
CDCA: Chenodeoxycholic Acid

Altered Hepatic Transporter Function in NASH Patients May Increase Hepatic and/or Systemic Exposure to Drugs and Susceptibility to Drug-Induced Liver Injury



Köck and Brouwer, *Clin Pharmacol Ther*, **92**:599, 2012
(Adapted from Ho and Kim, *Clin Pharmacol Ther*, **78**:260, 2005)

Graduate Students/ Postdoctoral Fellows/ Visiting Scholars

- Izna Ali
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- Melina Malinen
- Jason Slizgi
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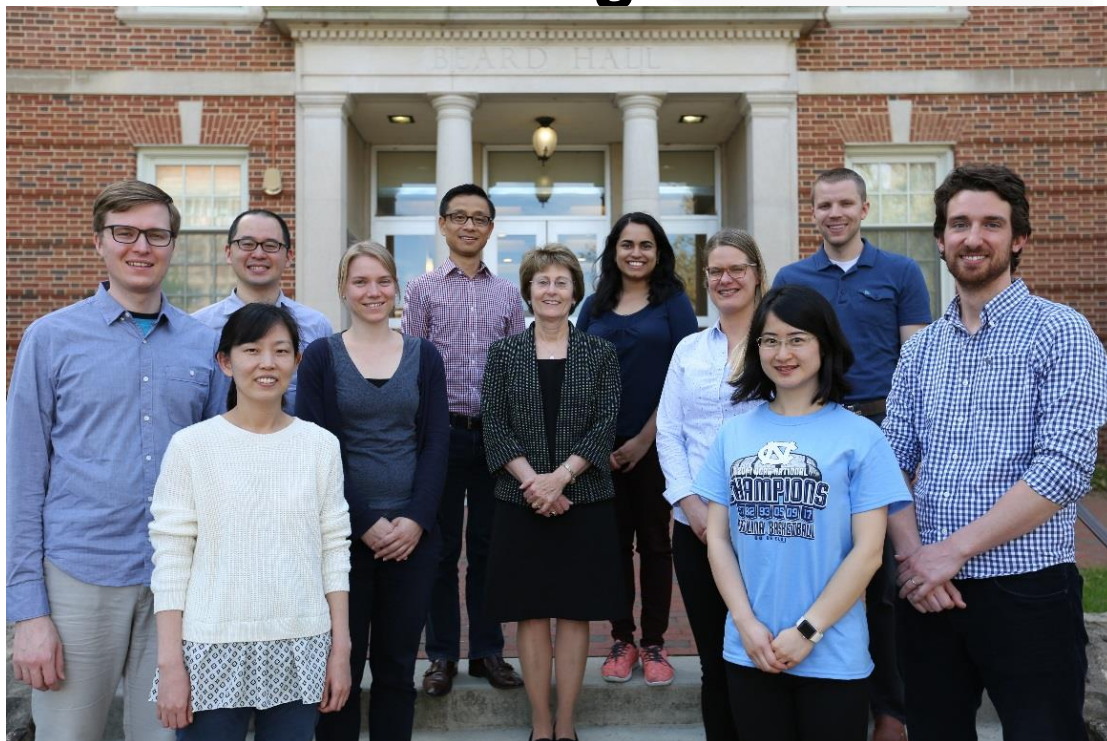
Collaborators

- | | |
|-------------------|----------------|
| ➤ Sid Barritt | Arlene Bridges |
| ➤ Marija Ivanovic | Wei Jai |
| ➤ Mikko Niemi | Mary Paine |
| ➤ Paul Stewart | Paul Watkins |

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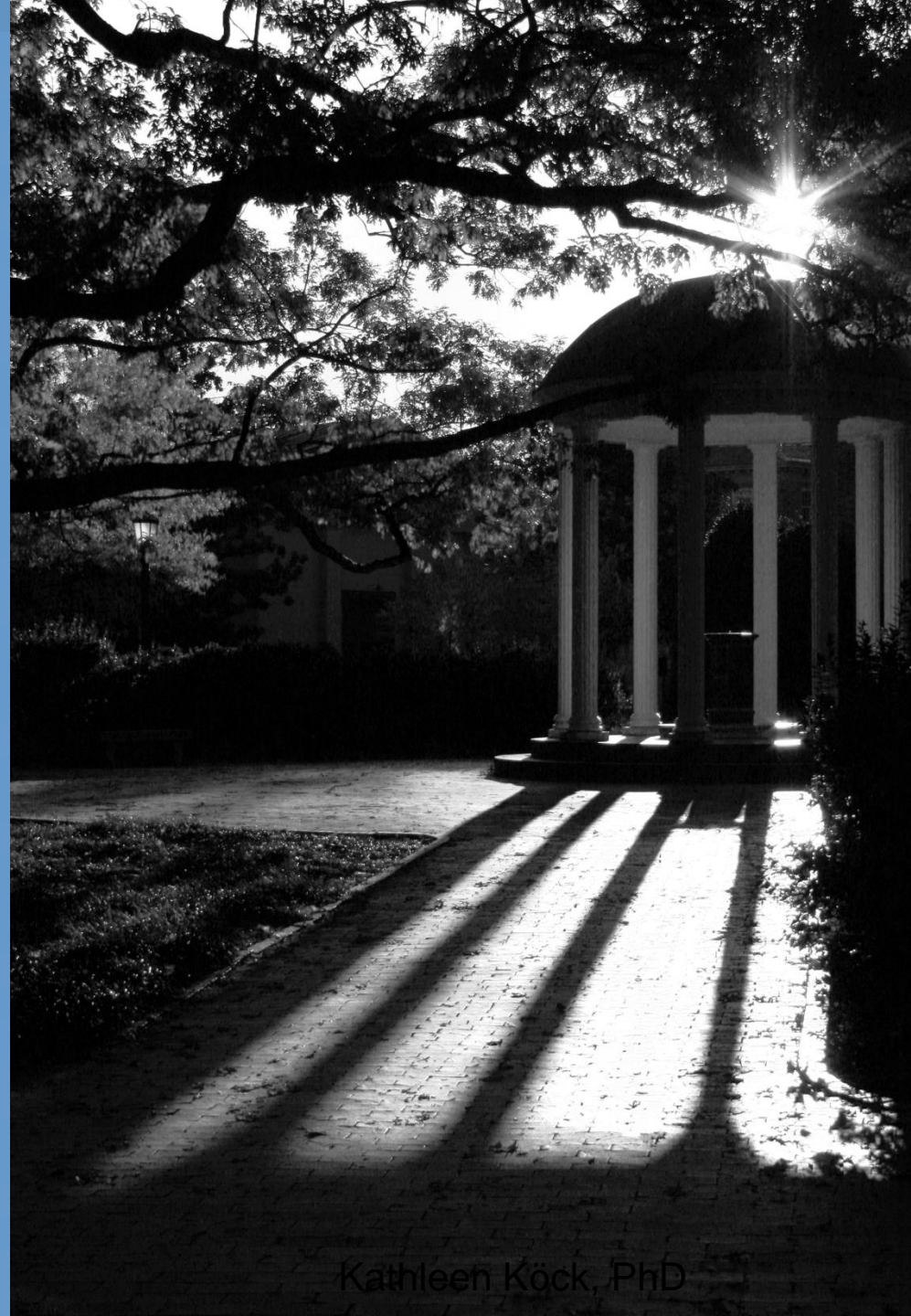


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