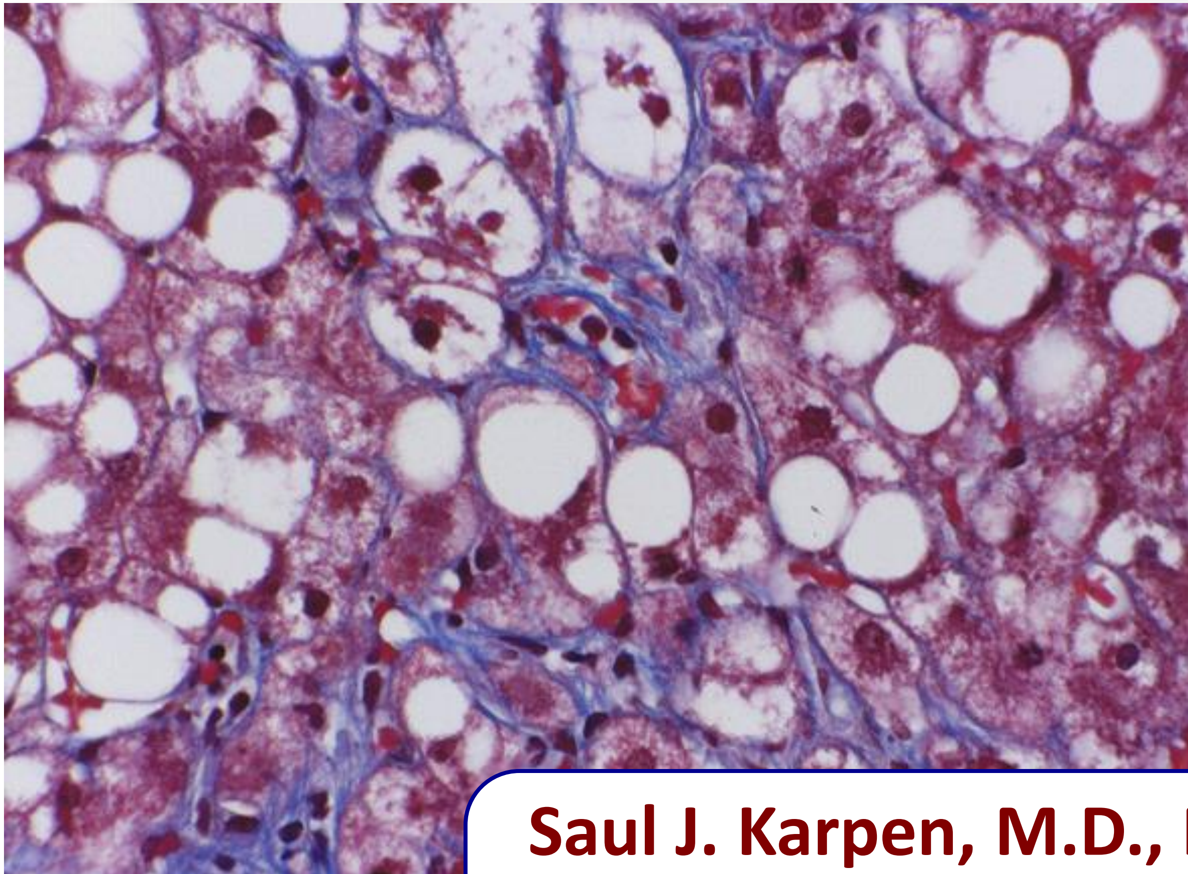
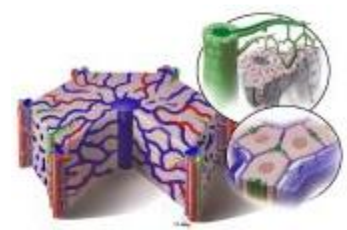
Separating the metabolic benefits from lipoprotein abnormalities associated with FXR agonists--gut vs. liver effects





Saul J. Karpen, M.D., Ph.D.

Raymond F. Schinazi Distinguished Biomedical Chair
Professor of Pediatrics

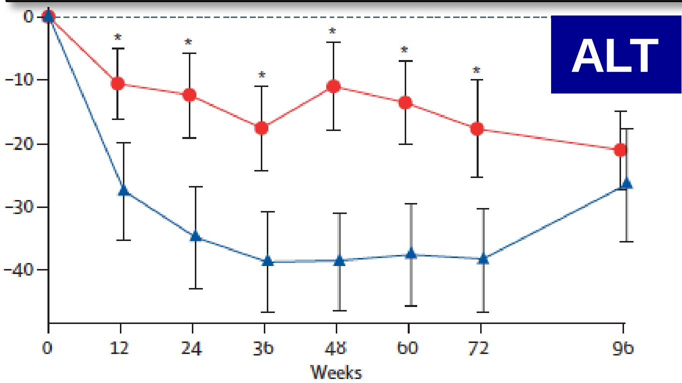
Paris July 1, 2016
No Financial Disclosures

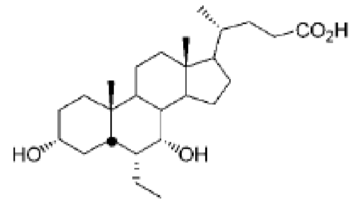
Children's℠ Healthcare of Atlanta
Dedicated to All Better

EMORY

Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial

*Brent A Neuschwander-Tetri, Rohit Loomba, Arun J Sanyal, Joel E Lavine, Mark L Van Natta, Manal F Abdelmalek, Naga Chalasani, Srinivasan Dasarathy, Anna Mae Diehl, Bilal Hameed, Kris V Kowdley, Arthur McCullough, Norah Terrault, Jeanne M Clark, James Tonascia, Elizabeth M Brunt, David E Kleiner, Edward Doo, for the NASH Clinical Research Network**

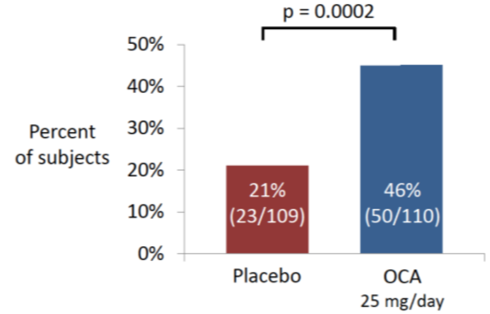


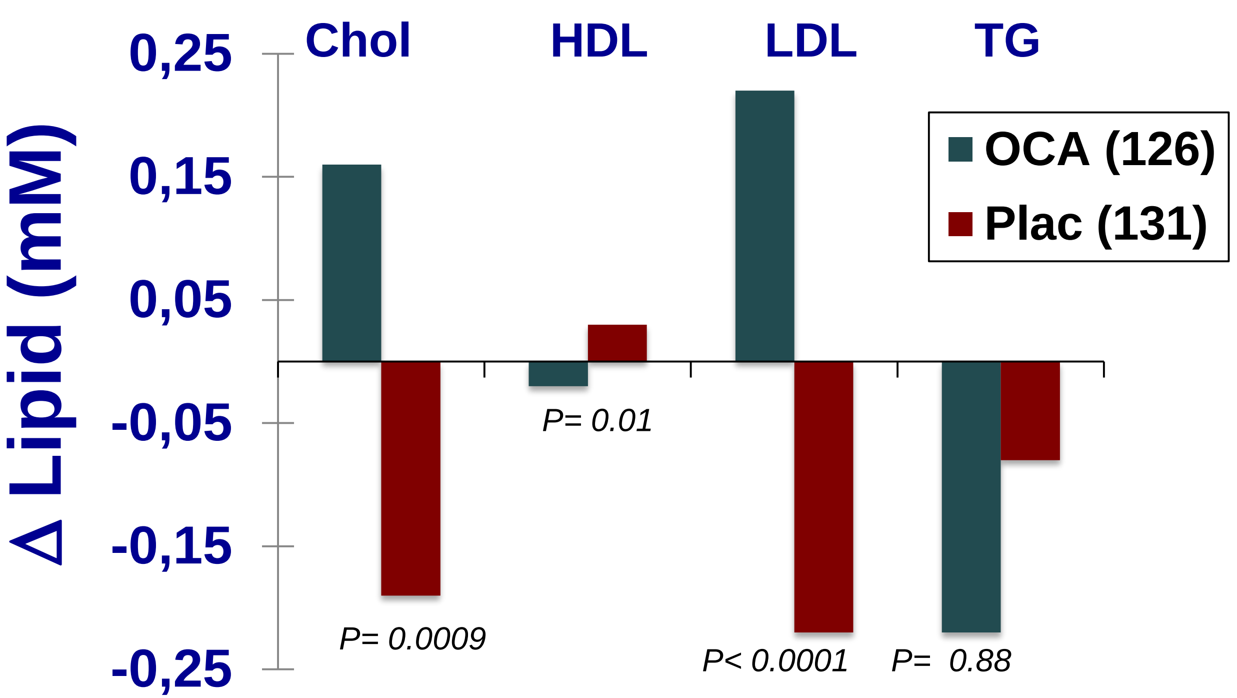


OCA/plac. x 72 w.

FLINT primary endpoint

- Improvement in NAFLD activity score* (NAS) ≥ 2 pts
  - * NAS = steatosis grade (0-3) + inflammation grade (0-3) + ballooning grade (0-2)
- No worsening of fibrosis
- Results:





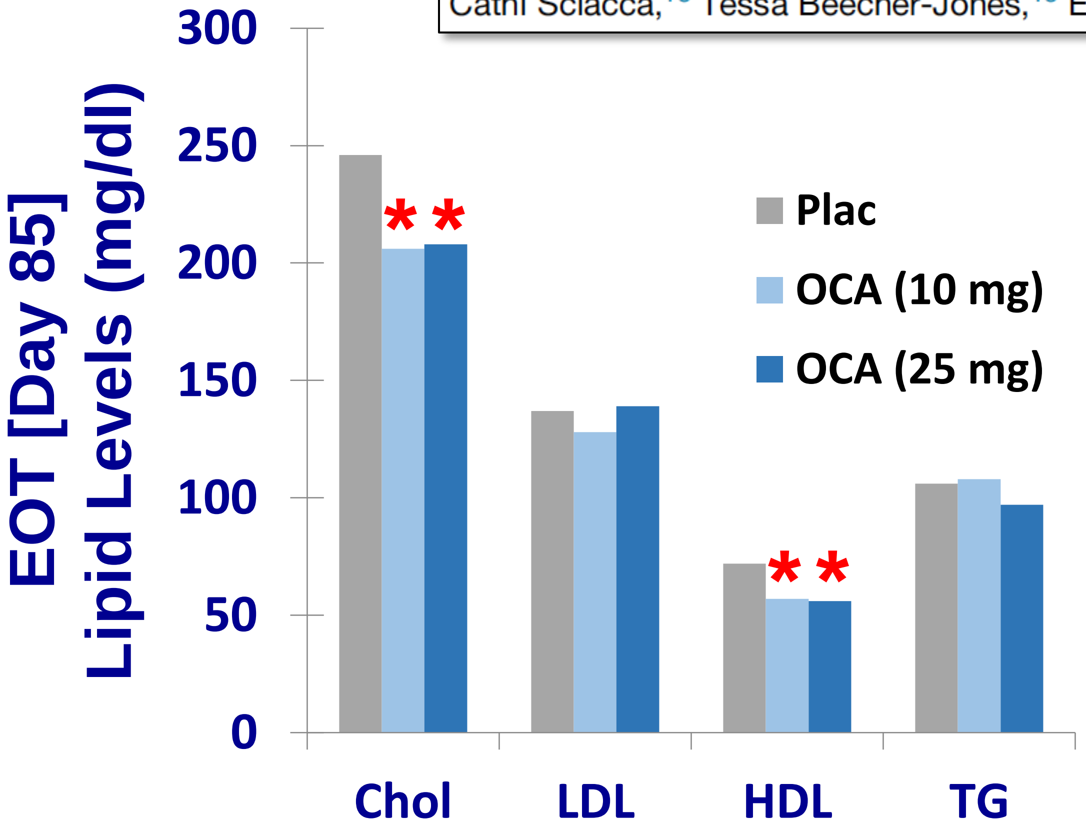
FXR AGONIST →

- ↑Chol
- ↓HDL
- ↑LDL
- ~ TG

Efficacy of Obeticholic Acid in Patients With Primary Biliary Cirrhosis and Inadequate Response to Ursodeoxycholic Acid

Gideon M. Hirschfield,[1] Andrew Mason,[2] Velimir Luketic,[3,4] Keith Lindor,[5,6] Stuart C. Gordon,[7] Marlyn Mayo,[8] Kris V. Kowdley,[9] Catherine Vincent,[10] Henry C. Bodhenheimer Jr,[11,12] Albert Parés,[13] Michael Trauner,[14] Hanns-Ulrich Marschall,[15] Luciano Adorini,[16] Cathi Sciacca,[16] Tessa Beecher-Jones,[16] Erin Castelloe,[16] Olaf Böhm,[17] and David Shapiro[16]

Gastro 2015 PMID:25500425

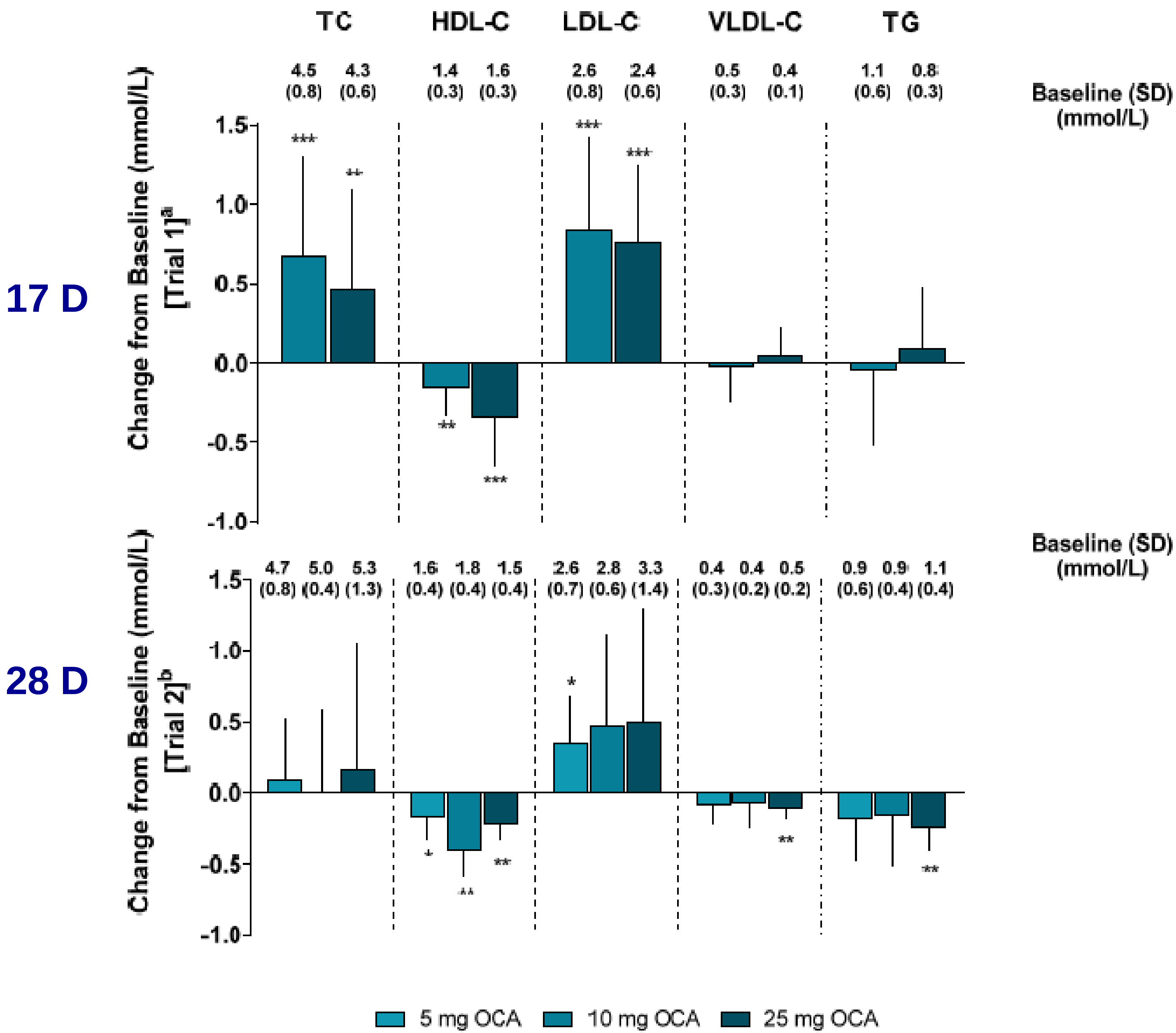


FXR AGONIST →

- ↓Chol #
- ↓HDL
- ~ LDL #
- ~ TG

: Different from NASH

OCA in 68 Healthy Volunteers: Lipid Analyses

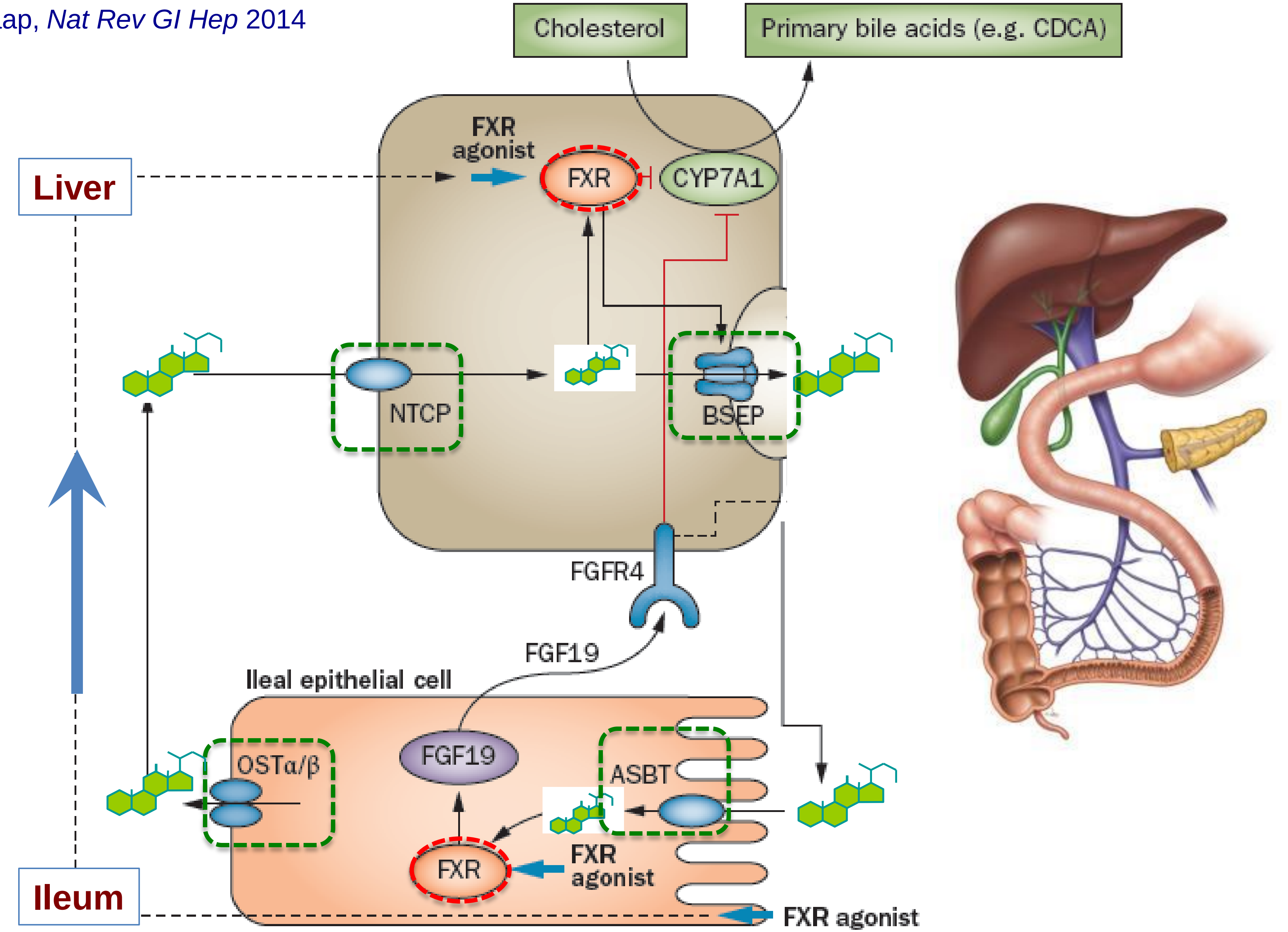


Pencek, *Diabetes, Obesity & Metabolism* 2016, PMID:27109453

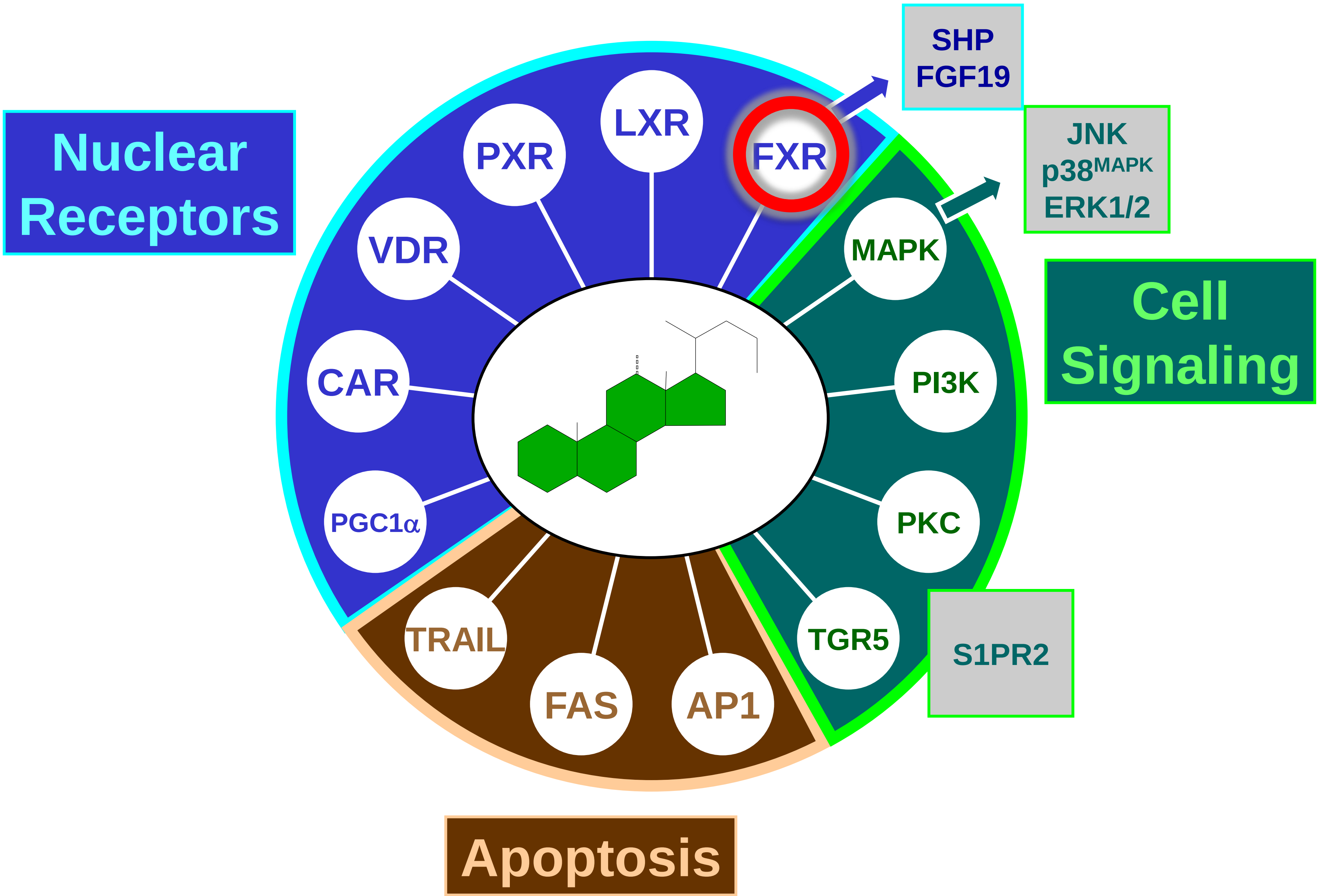
ClinicalTrials.gov Identifier: NCT01933503

Transporters & FXR: Ileal & Hepatic Components of the EHC

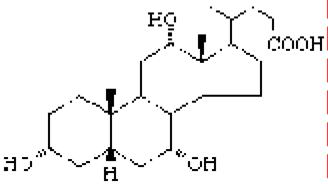
Schaap, *Nat Rev GI Hep* 2014



Multiple Molecular Roles for Bile Acids

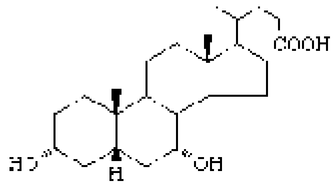


FXR Agonists



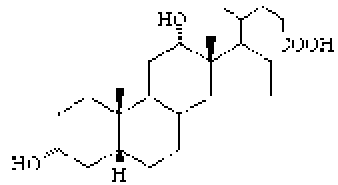
Cholic Acid

EC$_{50}$: ≈ 20 μM



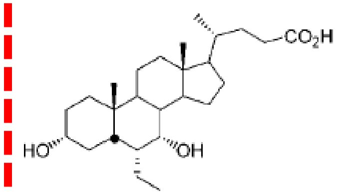
Chenodeoxycholic acid

4-10 μM



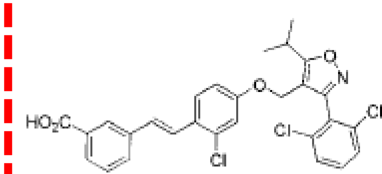
Deoxycholic acid

19-50 μM



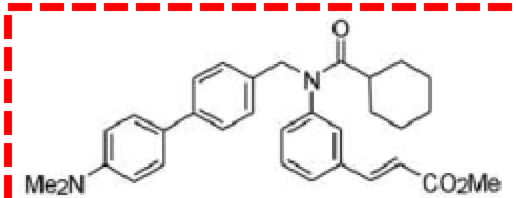
6-α-ethyl-CDCA
(Obeticholic acid)

99 nM



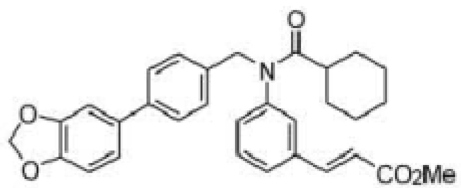
GW4064

37-80 nM



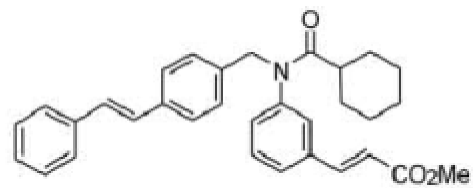
Fexaramine

25 nM



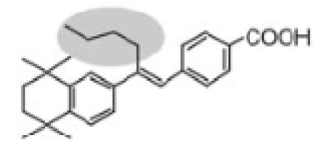
Fexarine

38 nM



Fexarene

36 nM

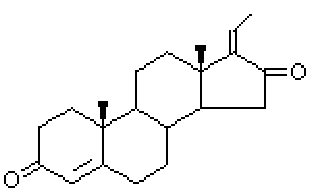


AGN31

2 μM

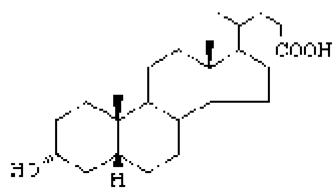
(also RXR)

FXR Antagonists



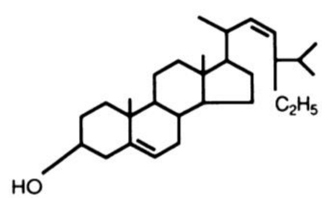
Z-Guggulsterone

IC$_{50}$: ≈ 10 μM



Lithocholic Acid

≈ 10-30 μM



Stigmasterol

≈ 10 μM

Makishima *Science* 1999
Parks *Science* 1999
Wang *Mol Cell* 1999
Urizar *Science* 2002
Yu *JBC* 2002
Pellicciari *J Med Chem* 2002
Hawkins *JCI* 2002
Dussault *JBC* 2003
Downes *Mol Cell* 2003
Carter *Ped Res* 2007

Research Article

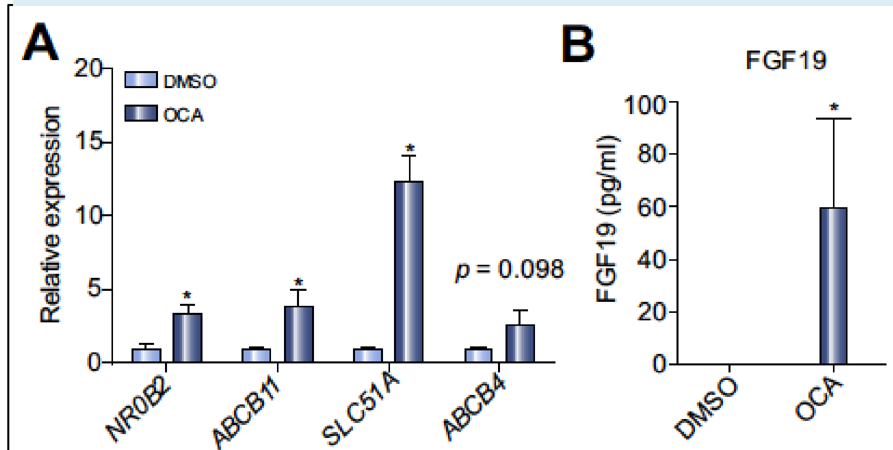
JOURNAL OF HEPATOLOGY

Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid

Noortje Ijssennagger[1], Aafke W.F. Janssen[2], Alexandra Milona[1], José M. Ramos Pittol[1], Danielle A.A. Hollman[1], Michal Mokry[3], Bark Betzel[4], Frits J. Berends[4], Ignace M. Janssen[4], Saskia W.C. van Mil[1,*,†], Sander Kersten[2,†]

2016, PMID: 26812075

OCA x 24 h: → ↑FXR targets

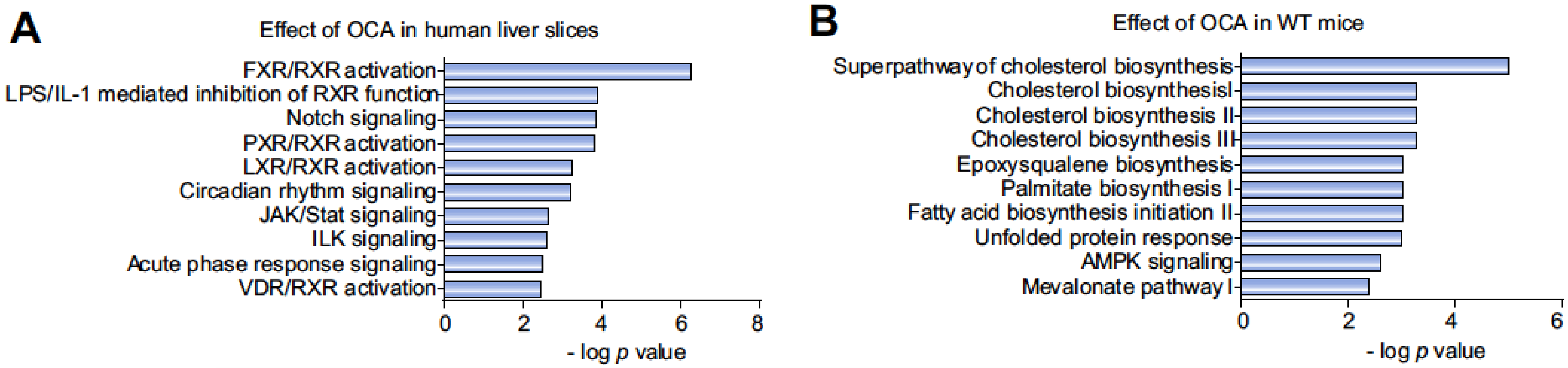


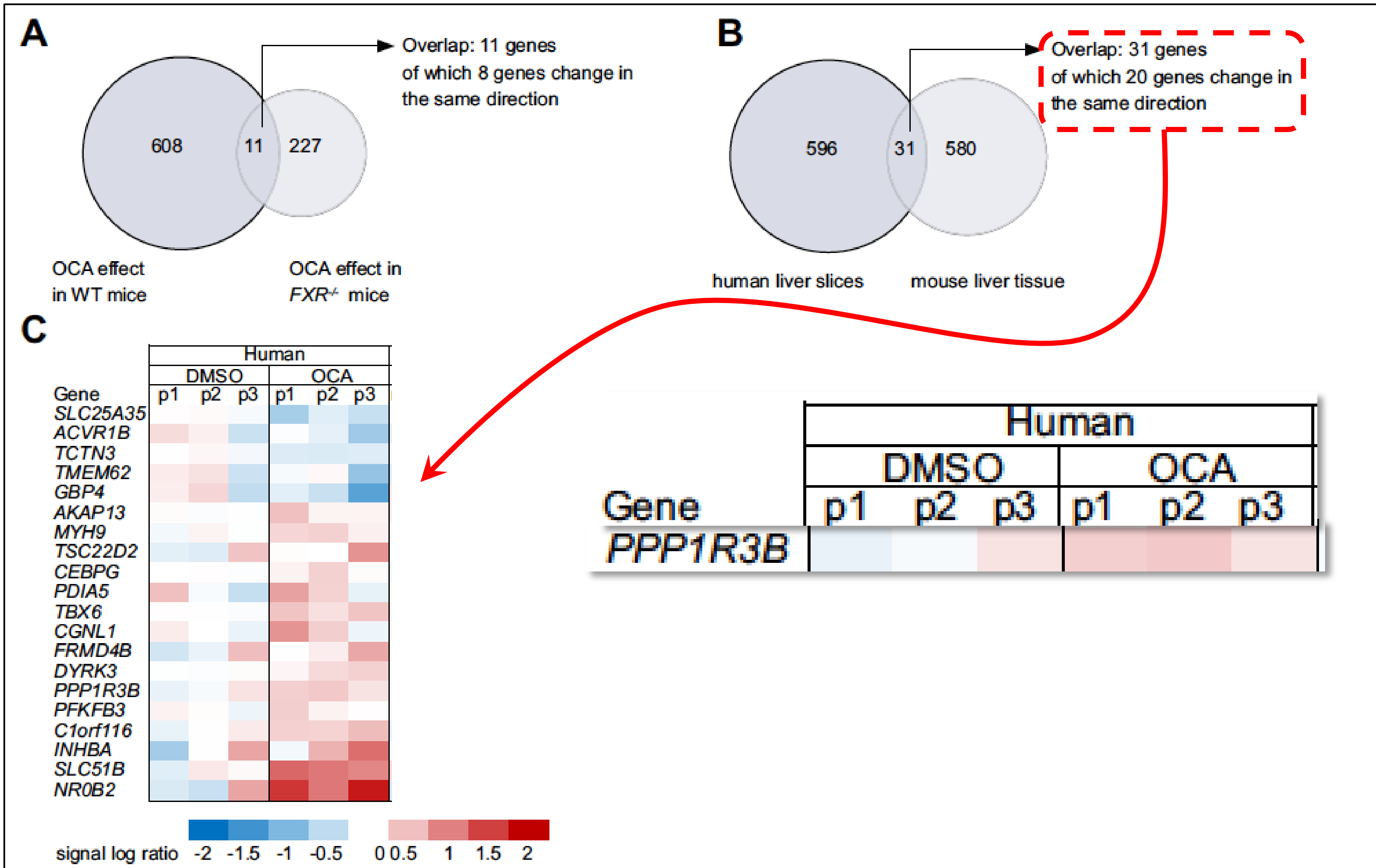
RNA: **SHP** **BSEP** **OSTα** **MDR3** **FGF19 Protein**

Caveats:

- ***"The hPCLS used for this study were obtained from patients with a high BMI (35–43 kg/m²)."***

- ***Dedifferentiated human cells in culture—CYP7A1 & CYP8B1 were not downregulated.***


A
Effect of OCA in human liver slices
FXR/RXR activation
LPS/IL-1 mediated inhibition of RXR function
Notch signaling
PXR/RXR activation
LXR/RXR activation
Circadian rhythm signaling
JAK/Stat signaling
ILK signaling
Acute phase response signaling
VDR/RXR activation
0 2 4 6 8
- log p value
B
Effect of OCA in WT mice
Superpathway of cholesterol biosynthesis
Cholesterol biosynthesis I
Cholesterol biosynthesis II
Cholesterol biosynthesis III
Epoxysqualene biosynthesis
Palmitate biosynthesis I
Fatty acid biosynthesis initiation II
Unfolded protein response
AMPK signaling
Mevalonate pathway I
0 2 4 6
- log p value


A
Overlap: 11 genes
of which 8 genes change in
the same direction
608
11
227
OCA effect
in WT mice
OCA effect in
FXR-/- mice
B
Overlap: 31 genes
of which 20 genes change in
the same direction
596
31
580
human liver slices
mouse liver tissue
C
Human
DMSO
OCA
Gene
p1 p2 p3 p1 p2 p3
SLC25A35
ACVR1B
TCTN3
TMEM62
GBP4
AKAP13
MYH9
TSC22D2
CEBPG
PDIA5
TBX6
CGNL1
FRMD4B
DYRK3
PPP1R3B
PFKFB3
C1orf116
INHBA
SLC51B
NR0B2
signal log ratio -2 -1.5 -1 -0.5 0 0.5 1 1.5 2
Human
DMSO
OCA
Gene
p1 p2 p3 p1 p2 p3
PPP1R3B

Essential, but seemingly contradictory effects of FXR & BA signaling in NAFLD

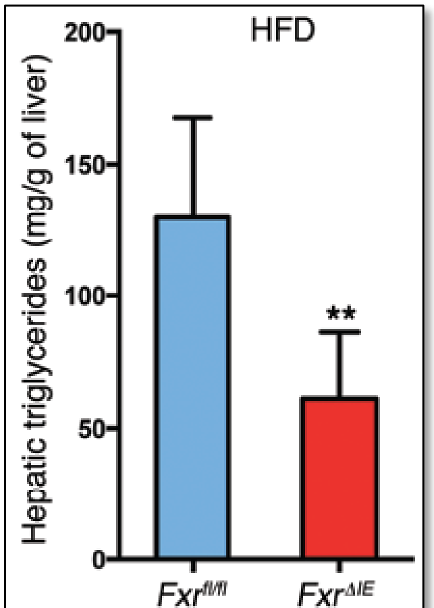
Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease

Changtao Jiang,[1,2] Cen Xie,[1] Fei Li,[1] Limin Zhang,[3,4] Robert G. Nichols,[3] Kristopher W. Krausz,[1] Jingwei Cai,[3] Yunpeng Qi,[1] Zhong-Ze Fang,[1] Shogo Takahashi,[1] Naoki Tanaka,[1] Dhimant Desai,[5] Shantu G. Amin,[5] Istvan Albert,[6] Andrew D. Patterson,[3] and Frank J. Gonzalez[1]

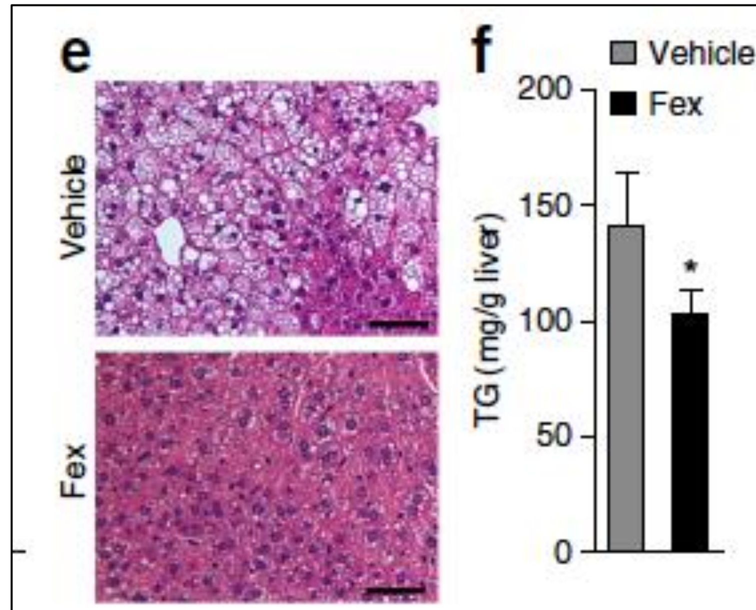
Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance

Sungsoon Fang[1], Jae Myoung Suh[1], Shannon M Reilly[2], Elizabeth Yu[1], Olivia Osborn[3], Denise Lackey[3], Eiji Yoshihara[1], Alessia Perino[4], Sandra Jacinto[1], Yelizaveta Lukasheva[1], Annette R Atkins[1], Alexander Khvat[5], Bernd Schnabl[3], Ruth T Yu[1], David A Brenner[3], Sally Coulter[6], Christopher Liddle[6], Kristina Schoonjans[4], Jerrold M Olefsky[3], Alan R Saltiel[2], Michael Downes[1] & Ronald M Evans[1,7]

Intestinal FXR ko protects against HFD-induced hepatic steatosis



Fexaramine (Intestinal FXR agonist) improves HFD-induced hepatic steatosis



Intestinal FXR <u>Antagonism</u> improves NASH in mice

Intestinal FXR <u>Agonism</u> improves NASH in mice

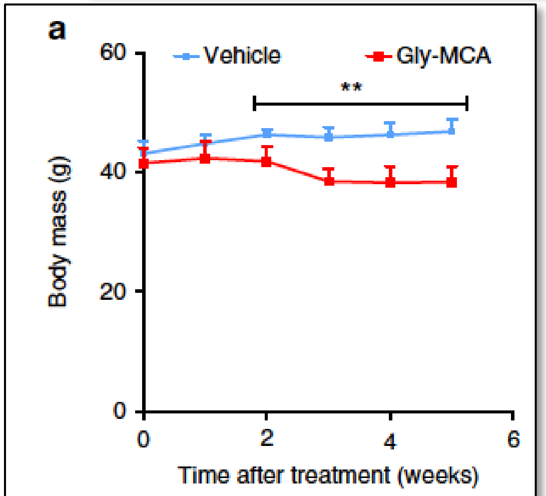
J Clin Invest. 2015;125:386–402.

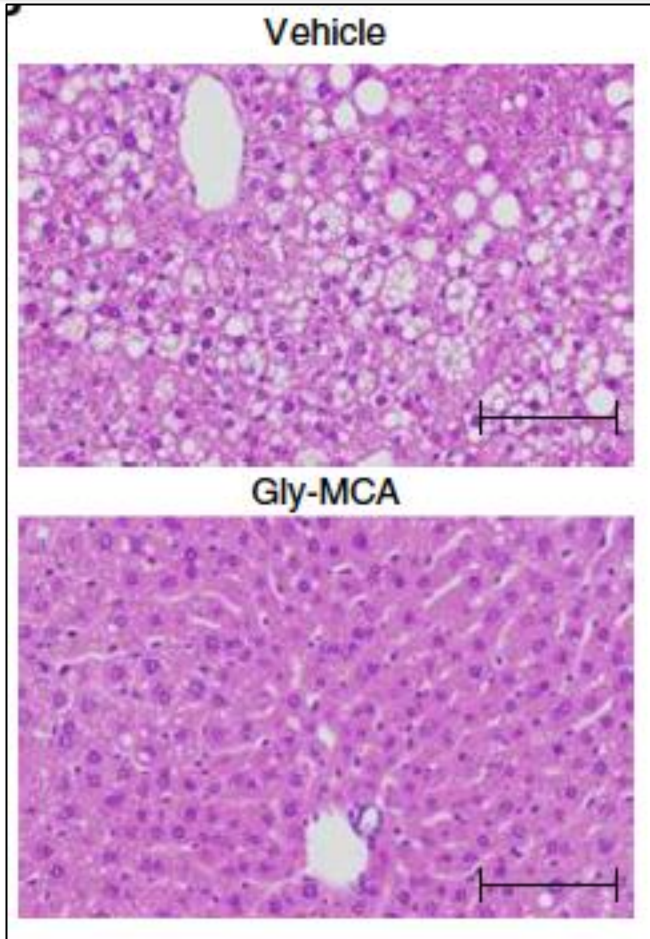
Nat Med. 2015;21:159–165.

Received 24 Jun 2015 | Accepted 10 Nov 2015 | Published 15 Dec 2015 DOI: 10.1038/ncomms10166 OPEN

Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction

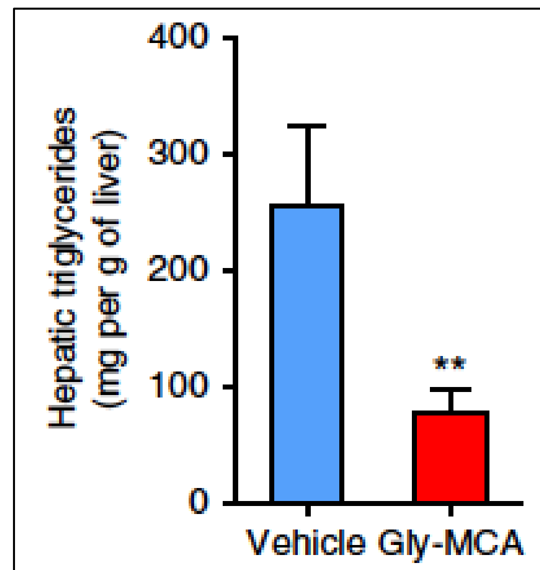
Changtao Jiang[1,2,*], Cen Xie[1,*], Ying Lv[2], Jing Li[3], Kristopher W. Krausz[1], Jingmin Shi[1], Chad N. Brocker[1], Dhimant Desai[4], Shantu G. Amin[4], William H. Bisson[5], Yulan Liu[3], Oksana Gavrilova[6], Andrew D. Patterson[7] & Frank J. Gonzalez[1]





Glyco-Muricholic Acid:

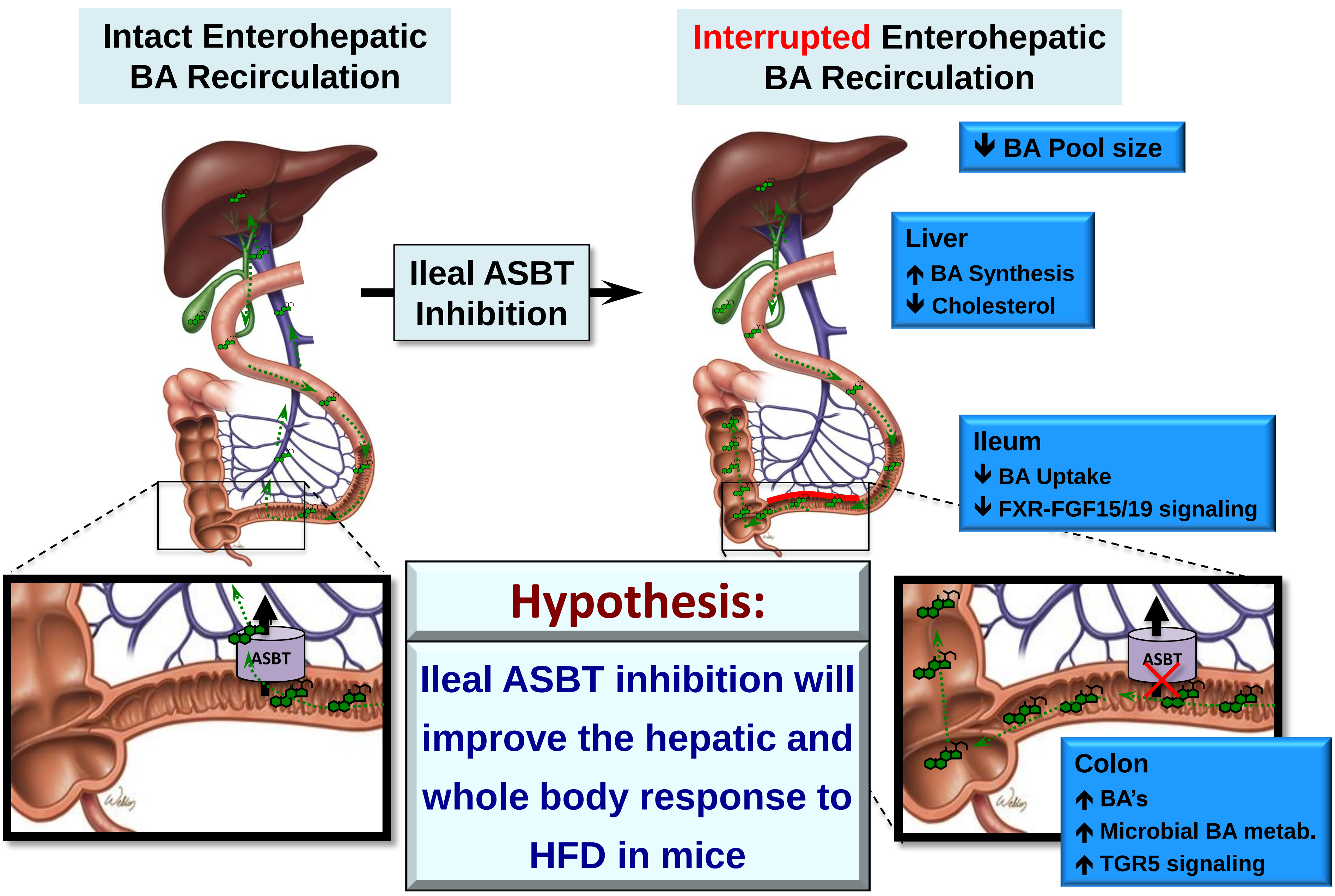
- **Intestinal FXR antag.**
- **↑ Brown Fat activity**
- **↓ Intest. Ceramides**



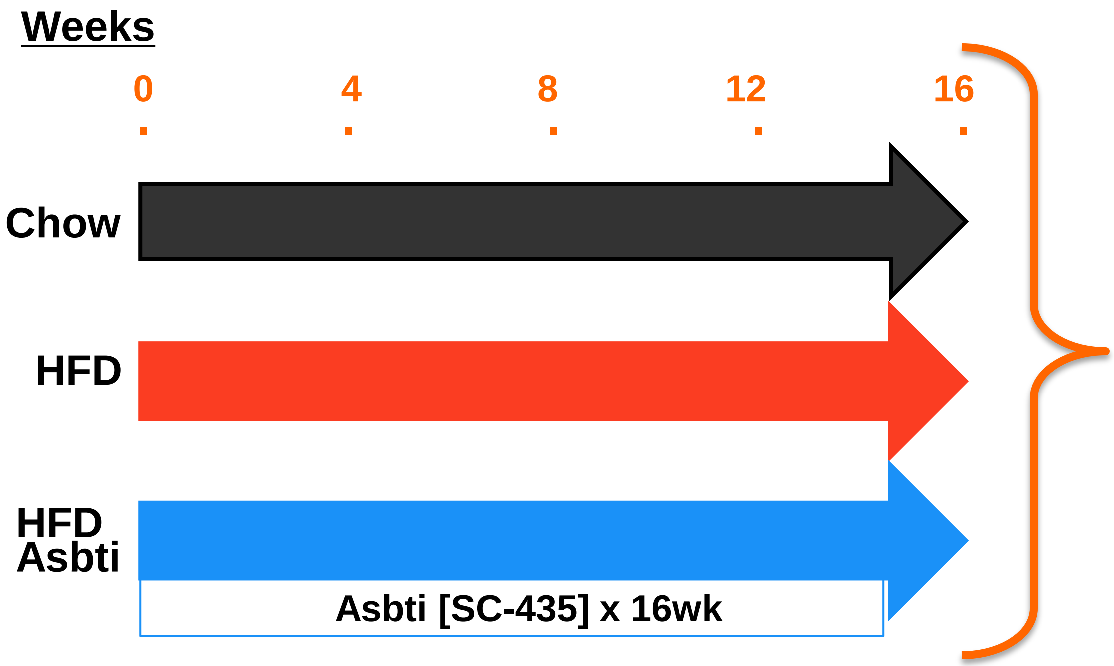
NAFLD & NASH:

FXR Agonism or FXR Antagonism

Both work → Why?


Intact Enterohepatic BA Recirculation
Interrupted Enterohepatic BA Recirculation
↓ BA Pool size
Liver
↑ BA Synthesis
↓ Cholesterol
Ileal ASBT Inhibition
Ileum
↓ BA Uptake
↓ FXR-FGF15/19 signaling
ASBT
ASBT
Colon
↑ BA's
↑ Microbial BA metab.
↑ TGR5 signaling
Hypothesis:
Ileal ASBT inhibition will improve the hepatic and whole body response to HFD in mice

Study Design & Endpoints



- Weekly Body Weights
- Weekly Caloric & fluid intake

- Week 15   GTT, ITT

- Week 16
  - Serum Liver Indices
  - Feces Bile Acids
  - Ileum RNA
  - Colon RNA
  - Liver Histology
    - Lipids & Bile Acids
    - RNA-Seq
    - RNA & Protein
    - Hydroxyproline

Mice: Male, C57BL6J, 4-6 weeks, n=7-16/group

HFD: ALIOS (45% fat; 0.2% cholesterol),
+ Added Sugars in the Drinking Water

Asbti: 0.006% SC-435, 10 mg/kg/day

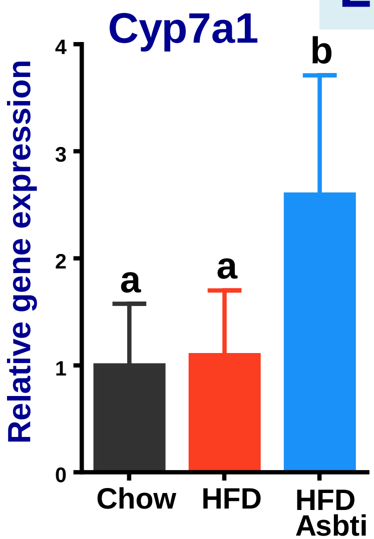
- Statistics: Mean ± SD
  - ANOVA

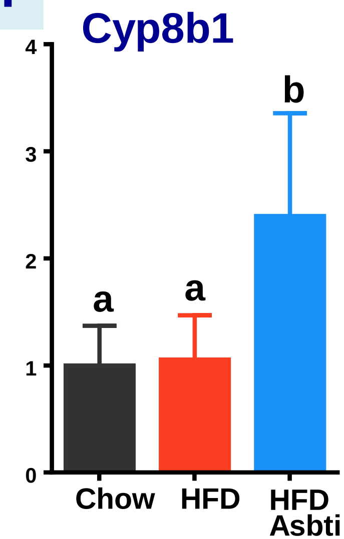
Tetri LH. *Am J Physiol GI* 2008 Nov;295(5):G987–95.

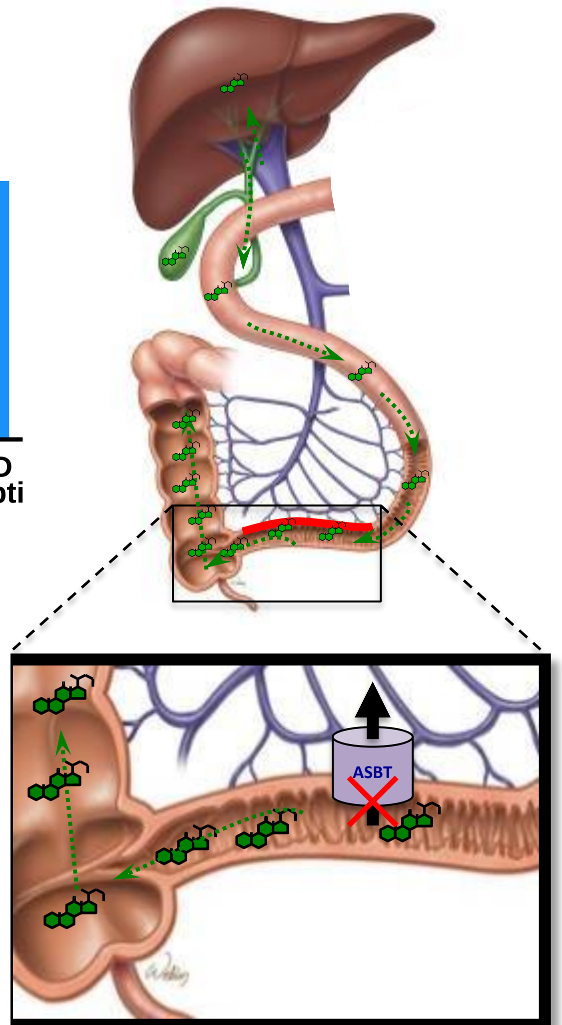
Mells *JE J Nutr Biochem*. 2015 Mar;26(3):285–92.

SC-435 Inhibits Ileal ASBT function

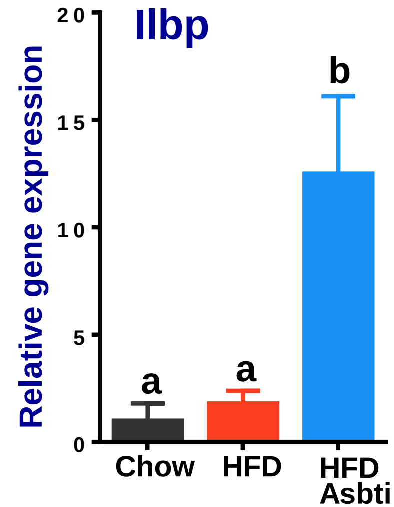
Liver



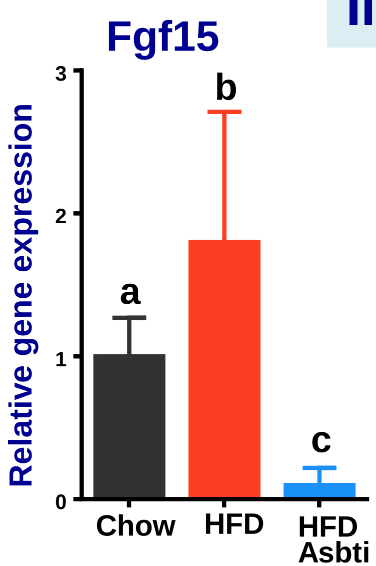


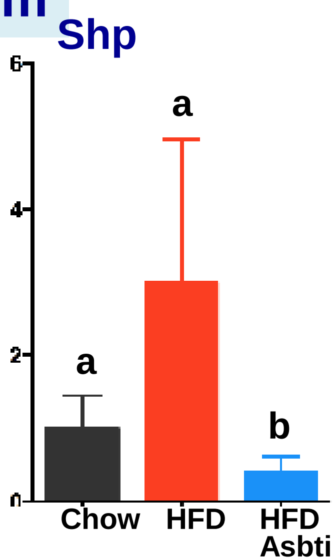


Colon

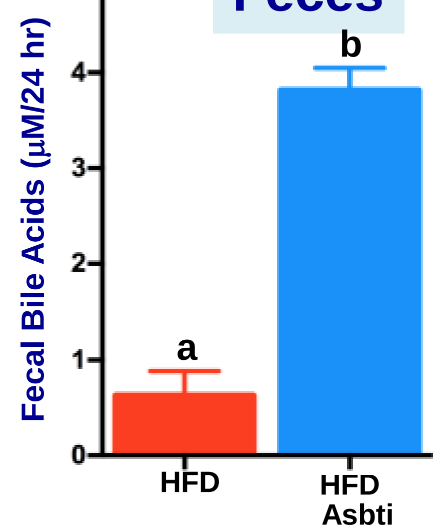


Ileum



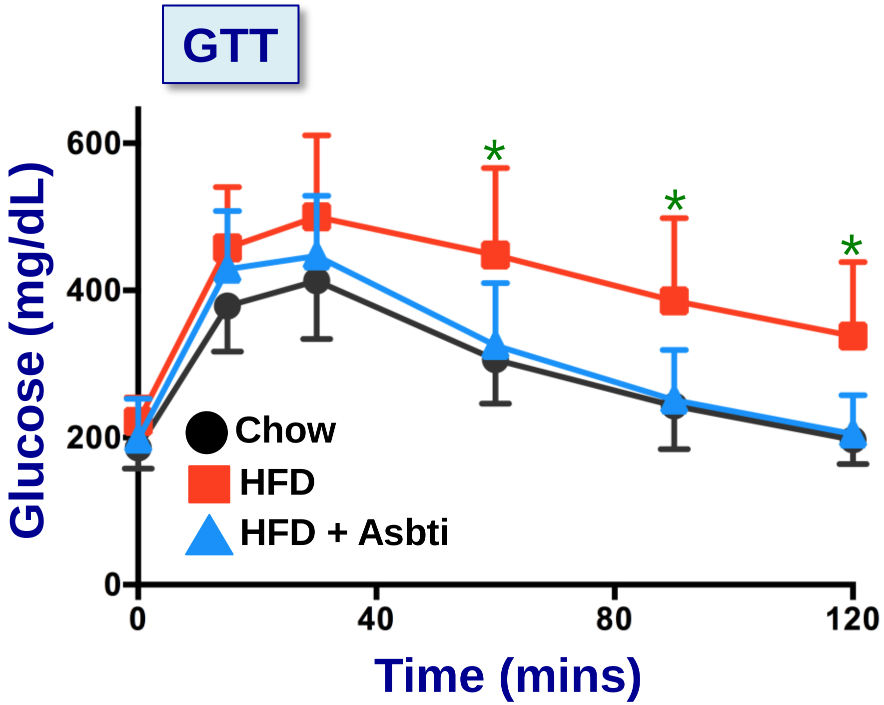


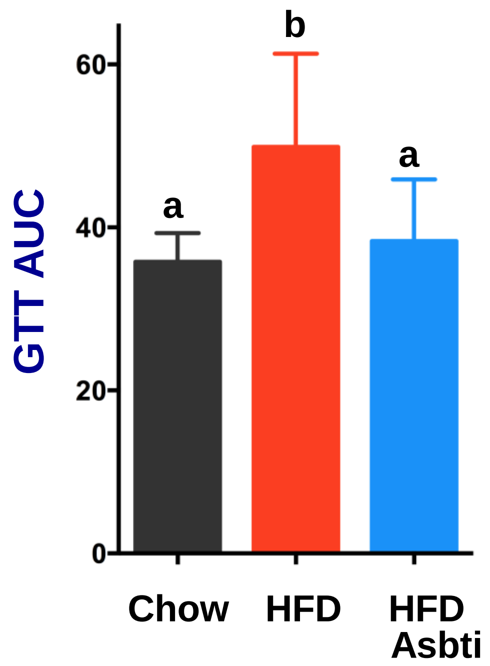
Feces



AASLD 2015

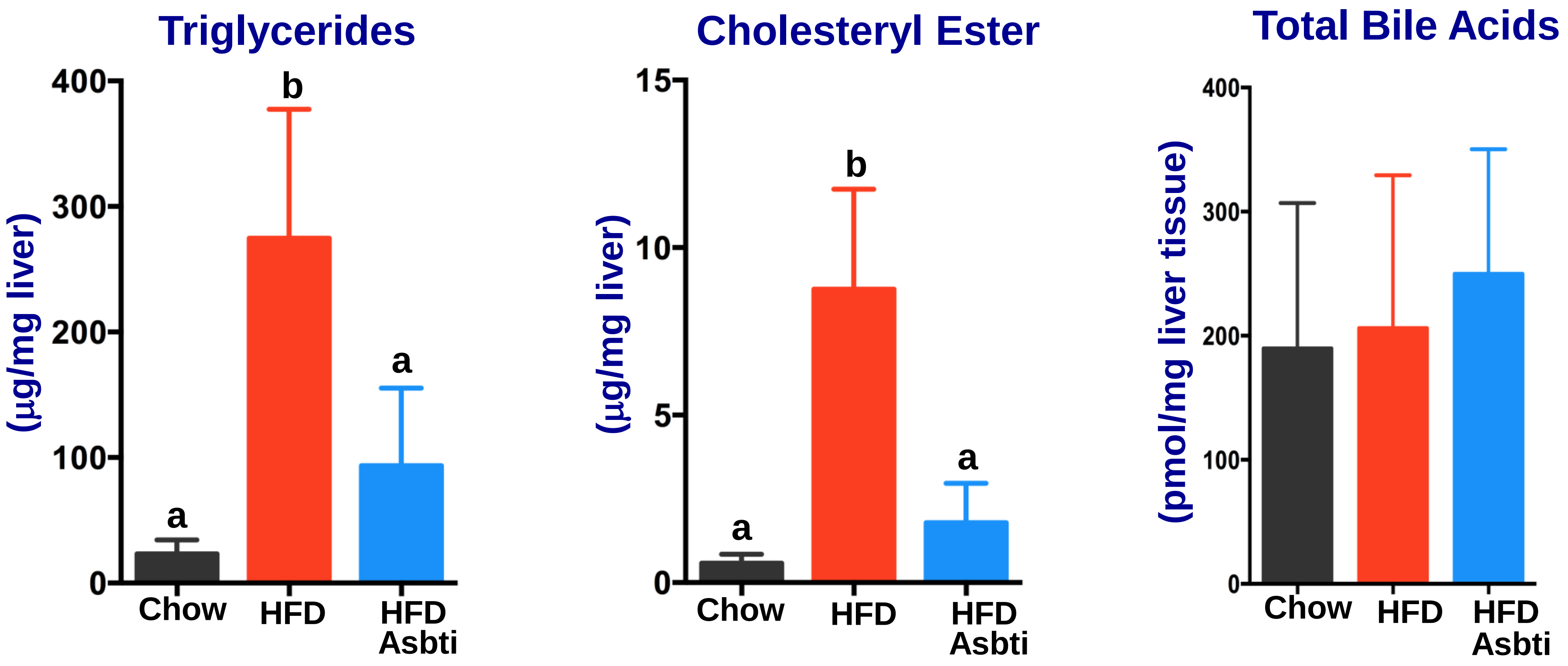
ASBTi Improves Glucose Tolerance





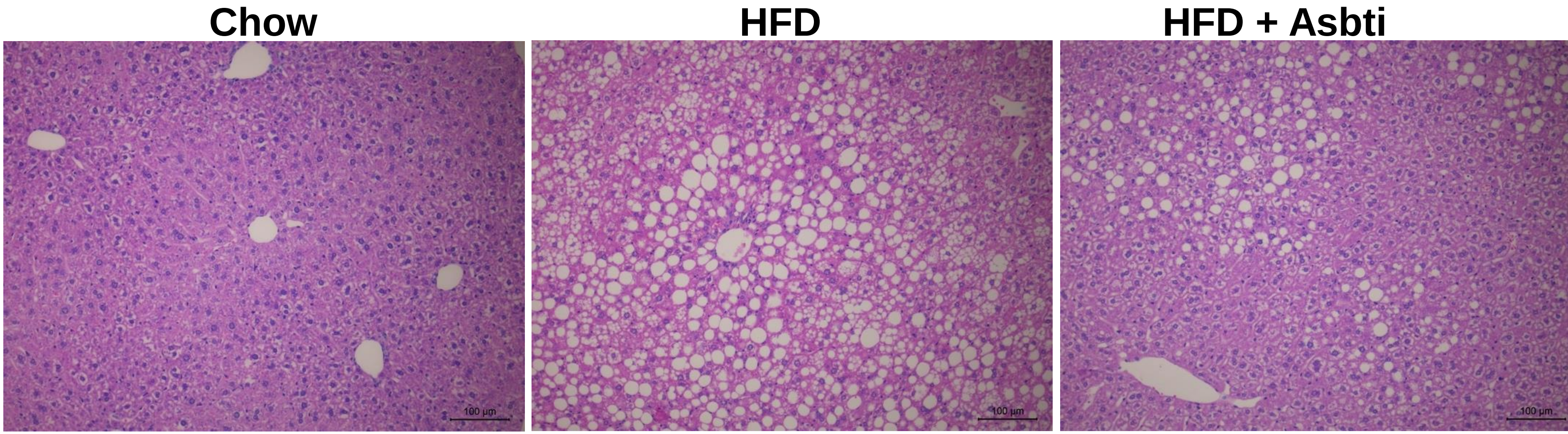
: Significantly different from HFD + Asbti

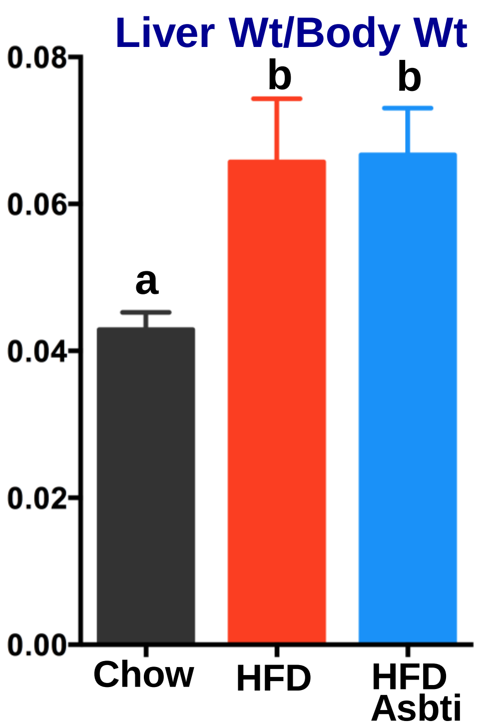
ASBTi Reduces Hepatic Lipids, But Not Total Bile Acids

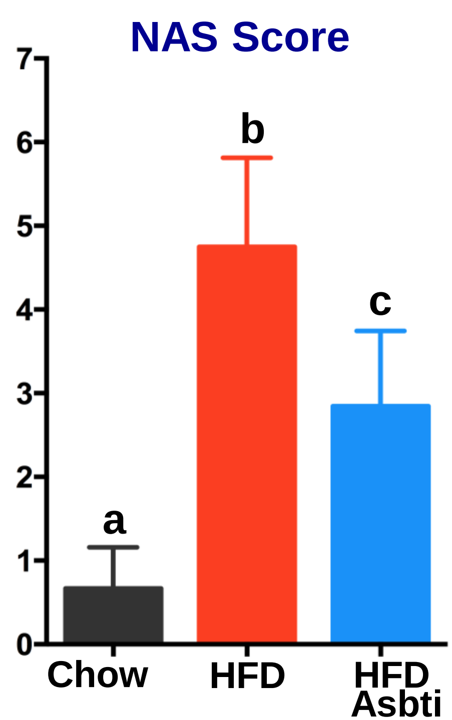


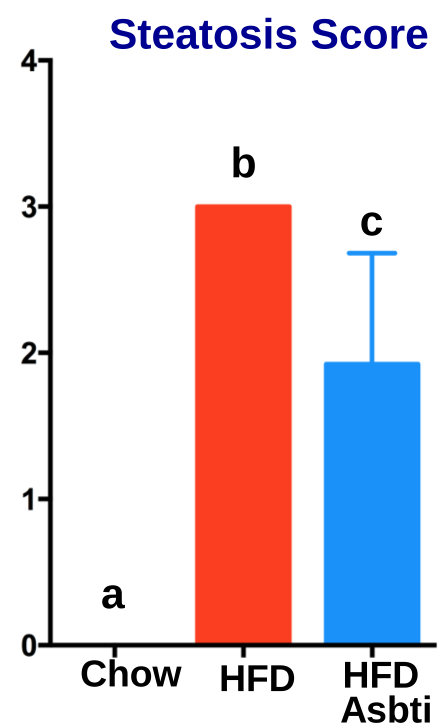
ASBTi Improves Hepatic NAS & Steatosis Scores

Chow HFD HFD + Asbti

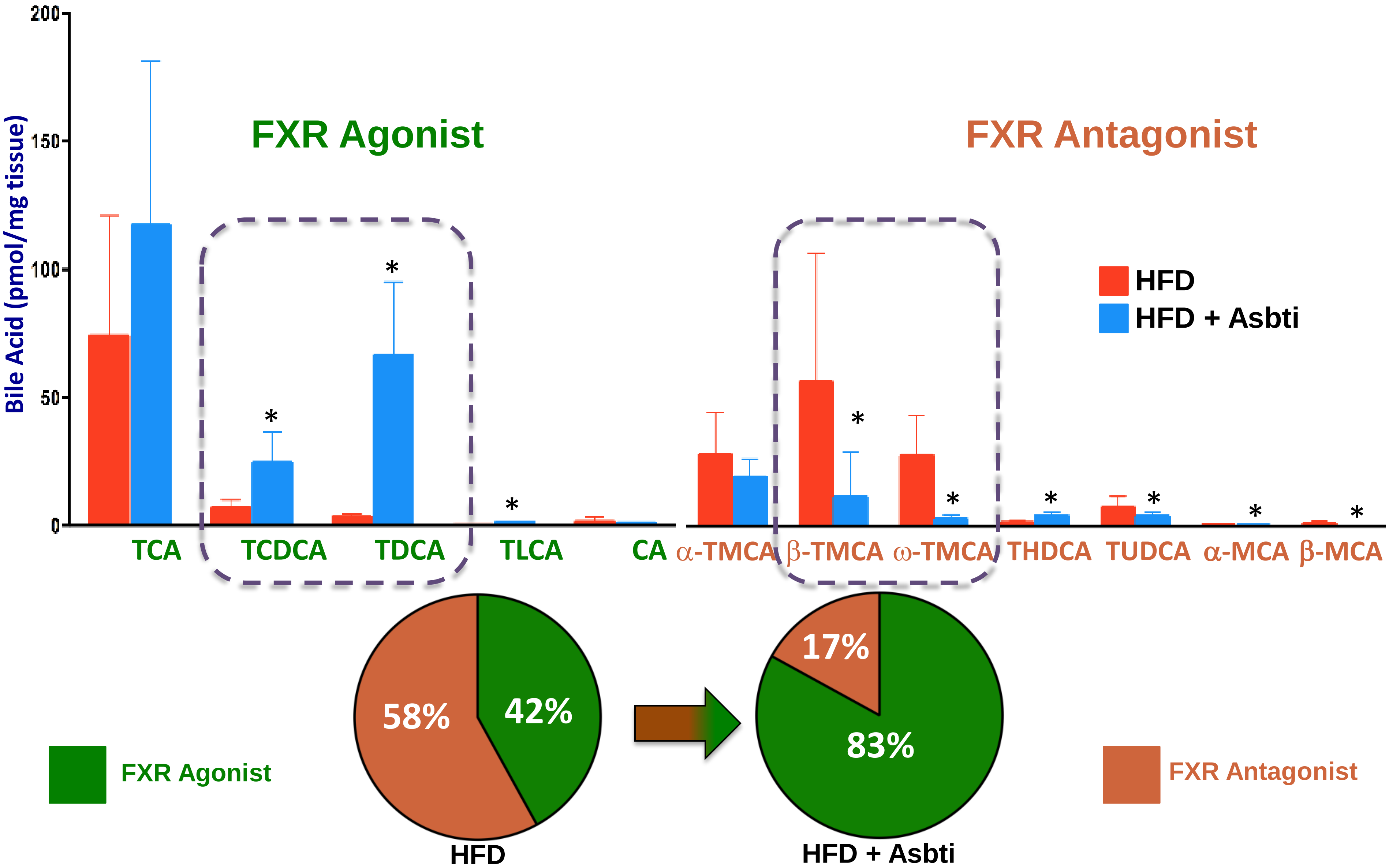




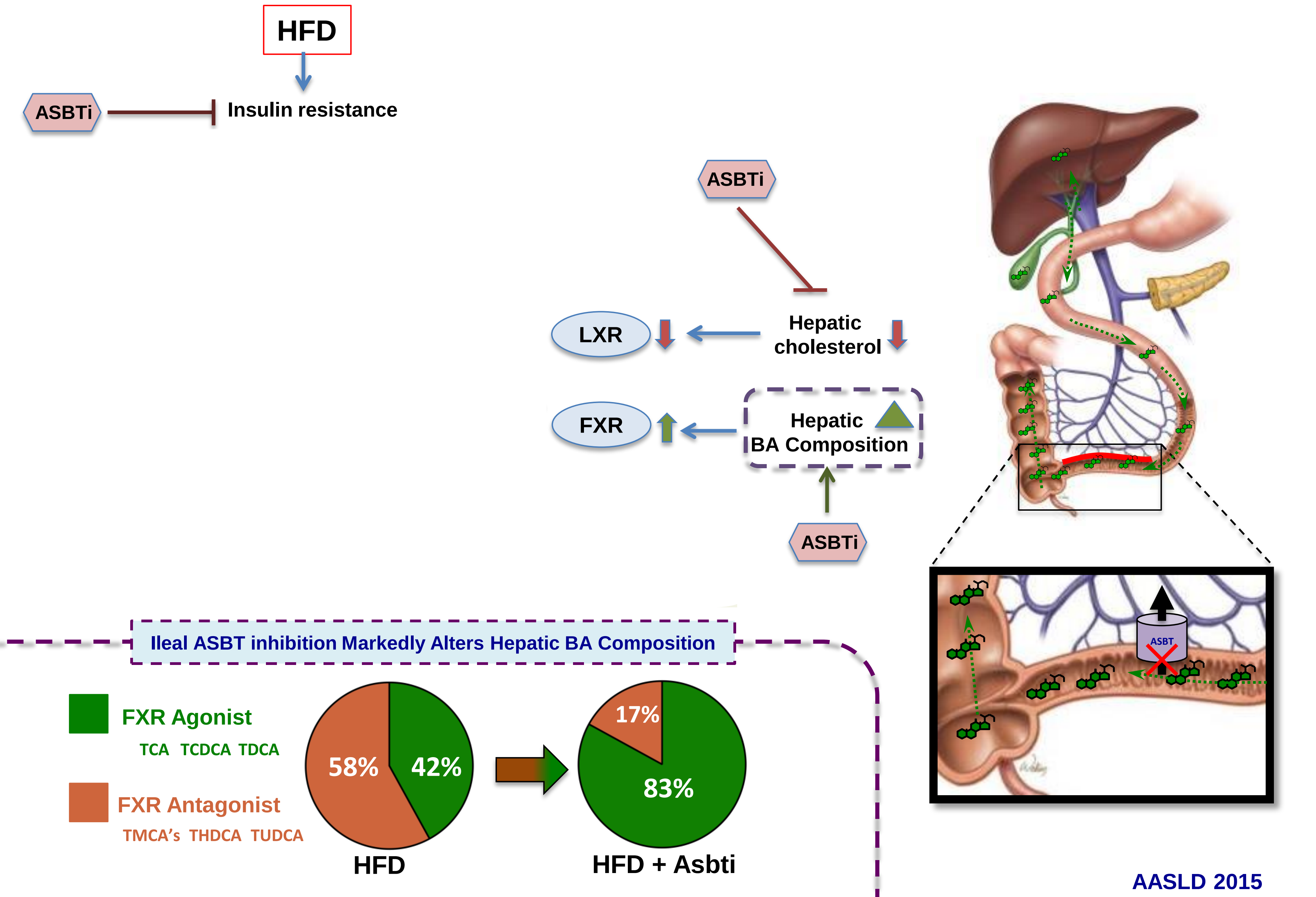




ASBTi Markedly Alters Hepatic BA Composition



Hypothesized Mechanisms of Action of ASBTi in Liver



Editorial

EASL | JOURNAL OF HEPATOLOGY

ASBT inhibitors in cholangiopathies – Good for mice, good for men?

Raoul Poupon*

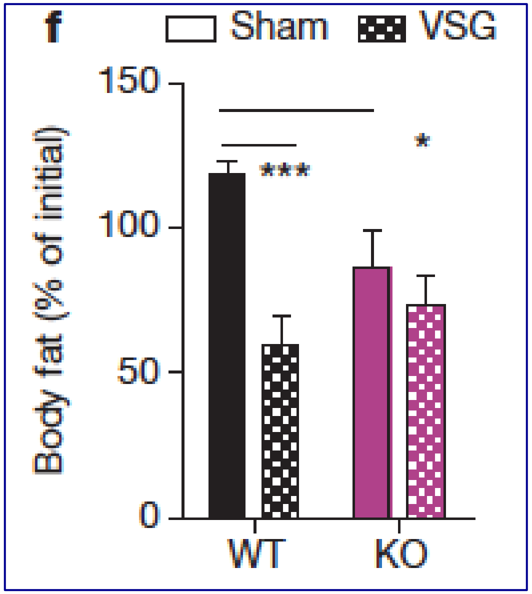
UPMC Univ Paris 06, INSERM, UMR_S 938, Service d'Hépatologie et Centre de Référence des Maladies Inflammatoires des Voies Biliaires, Hôpital Saint-Antoine, AP-HP, Paris, France

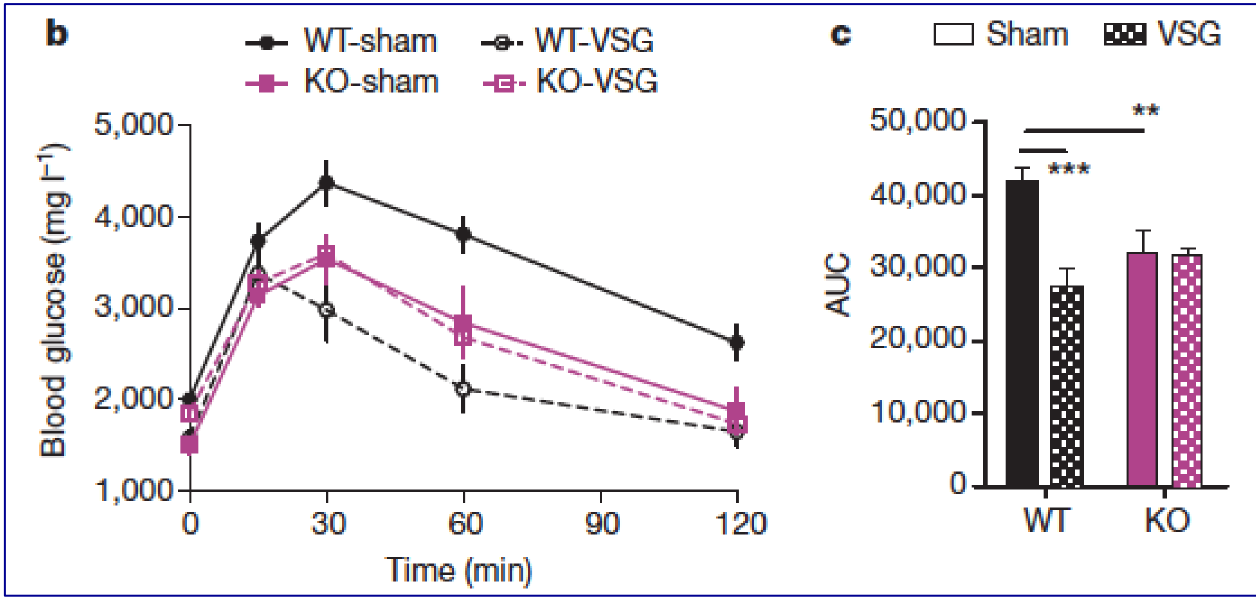
March 2016, PMID: 26708144

FXR is a molecular target for the effects of vertical sleeve gastrectomy

Nature. 2014 Mar 26.

Karen K. Ryan[1], Valentina Tremaroli[2], Christoffer Clemmensen[1,3], Petia Kovatcheva-Datchary[2], Andriy Myronovych[4], Rebekah Karns[5], Hilary E. Wilson-Pérez[1], Darleen A. Sandoval[1], Rohit Kohli[4], Fredrik Bäckhed[2,6] & Randy J. Seeley[1]

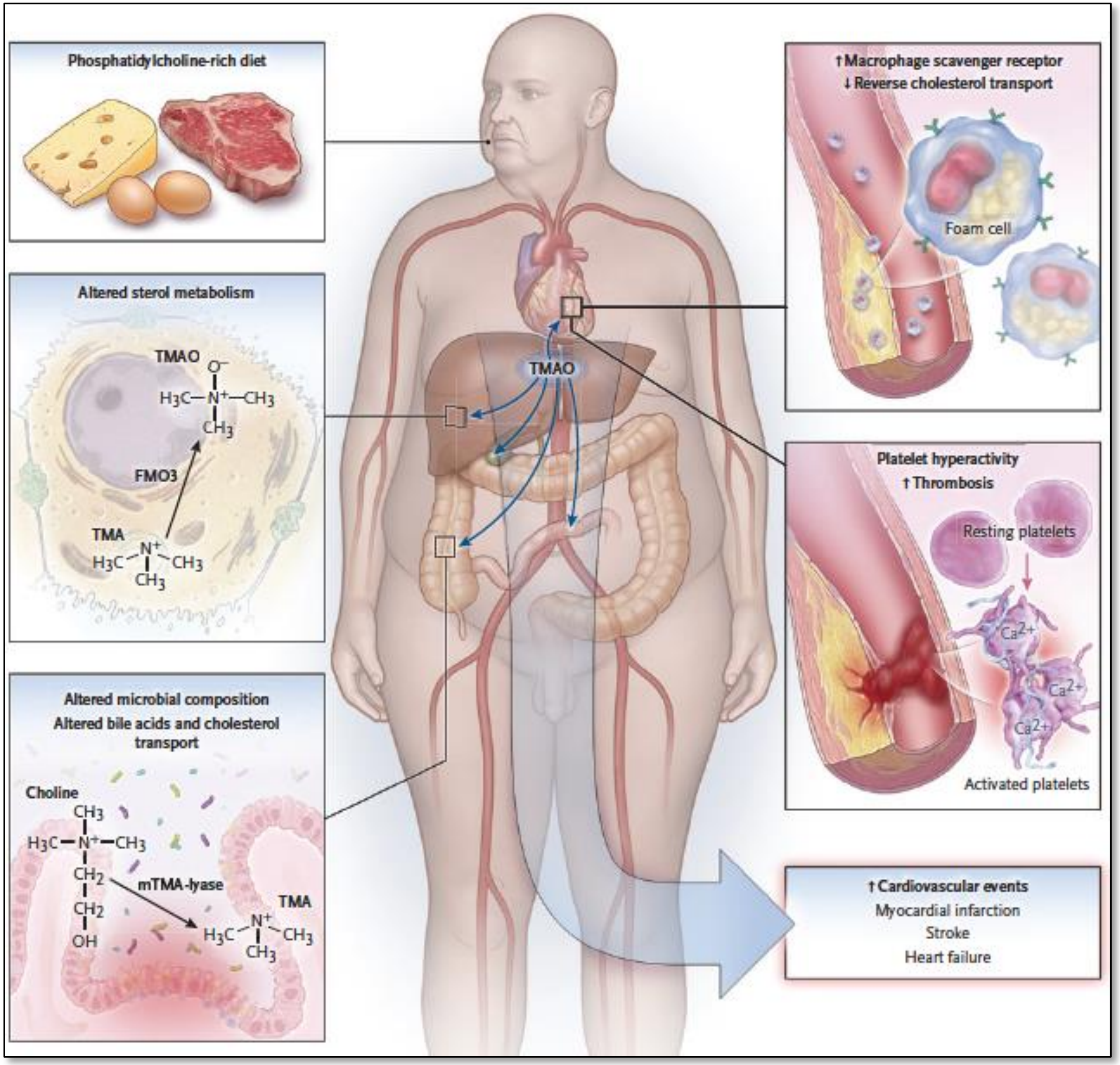




Intact BA signaling, through its receptor, FXR, mediates the response to Bariatric Surgery

HFD, 11w of VSG in mice **KO = $FXR^{-/-}$**

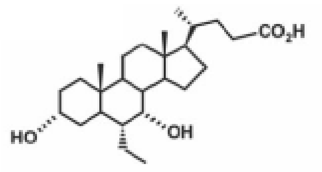
Interaction of Diet (PC), microbes, BAs, Genes (FMO3) → CV Disease



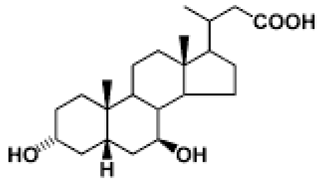
Tilg *NEJM* June 23, 2016

Bile acid based therapeutic trials (~ 200 in clinicaltrials.gov)

Glycocholic Acid: **BA Synthesis Defect**

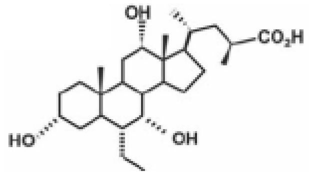


FXR agonists: Obeticholic Acid **NASH** **PBC** **PSC** **BA diarrhea** **Alcohol** **Fibrosis**

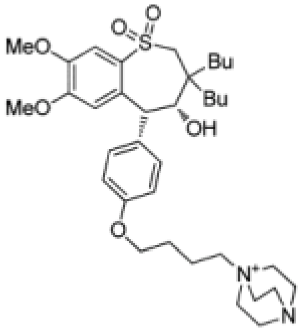


NorUDCA: **PSC**

TGR5 agonists: **Satiety** **Constipation**



ASBT inhibitors: **Pruritus in cholestasis (ALGS, PFIC's)** **IBS-C** **PSC**



BA Sequestrant: Colesevelam **Diabetes** **NASH** **Obesity**

Summary: FXR & the Lipids in NASH

- **Bile acid (BA) biology: Opportunities for discovering new linked components of the Gut-Liver-Microbe-Gene Axis**
  - Differential effects of FXR & BAs in Ileum, Colon, Liver, Fat, …
  - Individual BA’s have distinct functional properties
- **FXR Agonists in NAFLD & NASH: Lipid Issues**
  - Reduces CYP7A1 & BA synthesis
  - ↑Total Cholesterol
  - ↑ LDL
  - ↓ HDL
- **Further evidence that we will need to attack NASH from multiple therapeutic angles**
  - FXR Agonism & Antagonism both improve NASH in mice
  - ASBT inhibition improves NASH in mice
    - Reduces Hepatic TG & Cholesterol

Emory University (Saul-Paul Lab)

Saul Karpen, MD, PhD
Paul Dawson, PhD
Astrid Kosters, PhD
Anuradha Rao, PhD
Angelica Amanso, PhD
JP Berauer, MD
Gina Ramirez

Anya Mezina,MD MSCR
Courtney Ferrebee
Jamie Mells, PhD
Kim Pachura
Jianing Li, PhD
Grace Wynn
Prabhu Shankar, MD

Emory University

Hong Yin, MD (Pathology)

Dean Jones, PhD (Metabolomics)
Sophia Banton
Shuzhao Li

Hao Wu, PhD (School of Public Health)

Brad Keller, PhD (Lumena/Shire)

Cincinnati Children's

Ken Setchell, PhD

Wujuan Zhang, PhD

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LDL Pathway targets

	Human					
	DMSO			OCA		
Gene	p1	p2	p3	p1	p2	p3
PPP1R3B						

HDL Pathway targets

Genes ↓ by OCA:

- **Abca1**
- **Tgm2**
- **Fgl1**
- **Npc1l1**
- **Angptl4**
- **Hif1**
- **Ghr**