XXIV International Bile Acid Meeting:
Bile Acids in Health and Disease

June 17 – 18, 2016
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Düsseldorf, Germany

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**XXIV INTERNATIONAL BILE ACID MEETING:**
**BILE ACIDS IN HEALTH AND DISEASE**

Düsseldorf (Germany)
June 17 – 18, 2016

**Scientific Organization:**
U. Beuers, Amsterdam (The Netherlands)
D. Häussinger, Düsseldorf (Germany)
V. Keitel-Anselmino, Düsseldorf (Germany)
M. Trauner, Vienna (Austria)
Session I

**Bile acid signaling in liver regeneration and tumor development**

Chair:  
D. Häussinger, Düsseldorf  
A.F. Hofman, Ja Lolla

Bile flux analysis by intravital two-photon microscopy in normal and cholestatic livers  
**J.G. Hengstler, A. Ghallab, N. Vartak, A. Vartak, R. Reif, Dortmund**  
21

Fibroblast growth factor signaling controls liver size in mice with humanized livers  
M. Grompe, W.E. Naugler, Portland  
22

The FGF15/19 signaling system FGFR4 in hepatocarcinogenesis  
M.A. Avila, Pamplona  
23

**Oral presentation of poster abstract**  
Portal vein embolization-triggered liver regeneration is accelerated by the FXR agonist obeticholic acid  
24

Session II

**Bile acids and the gut liver axis**

Chair:  
U. Beuers, Amsterdam  
D. Keppler, Heidelberg

Role of the intestinal microbiome in cholestatic liver disease  
**N.F. LaRusso, J.H. Tabibian, S.P. O’Hara, Rochester**  
27

Intestinal specific actions of nuclear bile acid receptor FXR  
A. Moschetta, Bari (no abstract)
Microbe-host interactions via microbial moderation of bile acid signatures  
C.G.M. Gahan, S.A. Joyce, Cork  

Bile acids, ceramides and a new approach for the treatment of metabolic disease  
F.J. Gonzalez, C. Jiang, C. Xie, A.D. Patterson, Bethesda, Beijing, University Park  

Gut feelings: How intestinal FXR controls fatty acid and cholesterol metabolism  

**Oral presentation of poster abstract**  
Vertical sleeve gastrectomy (VSG) in morbidly obese adolescents results in increased fibroblast growth factor 21 (FGF21) that correlates with weight loss  

**Presentation of Adolf Windaus Award**  
D. Häussinger, Düsseldorf  

**Adolf Windaus Award Lecture**  
Progress in the molecular characterization of hepatobiliary transporters  
D. Keppler, Heidelberg  

**Session III**  
**Intrahepatic signaling and bile acids as endogenous toxins**  

Chair:  
V. Keitel-Anselmino, Düsseldorf  
D.D. Moore, Houston  

Nuclear receptors FXR and PPARα regulate hepatic autophagy  
D.D. Moore, Houston (no abstract)  

Bile acids and cholangiocyte autophagy  
M. Sasaki, Kanazawa  

Soluble adenylyl cyclase regulates bile-salt-induced apoptosis in human cholangiocytes: A link to primary biliary cirrhosis  
Mechanisms of cytoprotection by TUDC
D. Häussinger, Düsseldorf
39 – 40

**Oral presentation of poster abstract**
Steroid binding to autotaxin links bile salts and lysophosphatidic acid signalling

Session III (cont.)

**Intrahepatic signaling and bile acids as endogenous toxins**

Chair:
P. Fickert, Graz
P.L.M. Jansen, Maastricht

The role of inflammation in the mechanism of bile acid-induced liver damage
S.-Y. Cai, J.L. Boyer, New Haven

TGR5 and bile acid induced liver damage
V. Keitel-Anselmino, M. Reich, L. Spomer, C. Klindt, K. Deutschmann, D. Häussinger, Düsseldorf
43 – 44

**Oral presentation of poster abstract**
Activation of intestinal bile acid receptor FXR induces membrane G protein-coupled bile acid receptor TGR5 expression and stimulates GLP-1 secretion to ameliorate metabolic disorders in diabetic mice
J.Y.L. Chiang, P. Pathak, H. Liu, S. Boehme, Rootstown

**Oral presentation of poster abstract**
Colonization of germ-free mice with a human microbiota induces FXR signaling
A. Wahlström, P. Kovatcheva-Datchary, M. Stählman, H.-U. Marschall, F. Bäckhed, Gothenburg, Copenhagen

5
Session IV

**Bile acid transporters: Role in health and disease**

**Chair:**
A. Moschetta, Bari
R.P.J. Oude Elferink, Amsterdam

Targeting Ntcp to treat metabolic and cholestatic diseases
S.F.J. van de Graaf, Amsterdam

Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: A new inborn error of metabolism with a complex phenotype
F.M. Vaz, C.C. Paulusma, H. Huidekoper, F. van Herpe,
M. de Ru, C. Lim, J. Koster, K. Ho-Mok, A.H. Bootsma, A.K. Groen,
F.G. Schaap, R.P.J. Oude Elferink, D. Cassiman, H.R. Waterham,
R.J.A. Wanders, Amsterdam, Leuven, Purmerend, Groningen,
Maastricht

Roles of ileal ASBT and OSTα-OSTβ in regulating the FXR/FGF15 pathway and bile acid-induced injury
P.A. Dawson, Atlanta

ASBT inhibitors in PBC and PSC
G. Hirschfield, Birmingham

**Oral presentation of poster abstract**
Effect of intrahepatic cholestasis of pregnancy on maternal glucose homeostasis
E. Bellafante, V. Nikolova, J. Chambers, M. Martineau,
C. Williamson, London

Session V

**Bile acid receptors and bile acid signaling as therapeutic targets**

**Chair:**
G. Hirschfield, Birmingham
M. Trauner, Vienna

Bile acids in polycystic liver diseases: Triggers of disease progression and/or potential solution for treatment?
J.M. Banales, San Sebastián

*Nor*Ursodeoxycholic acid in primary sclerosing cholangitis and non-alcoholic fatty liver disease
M. Trauner, Vienna
Role of OCA in decompensated cirrhosis
R. Mookerjee, London (no abstract)

**Oral presentation of poster abstract**
Cholic acid treatment in Zellweger spectrum disorders

**Oral presentation of poster abstract**
NorUDCA reduces liver injury and improves the metabolic state in mouse models of obesity and steatosis
D. Steinacher, T. Claudel, E. Einwallner, T. Stojakovic, M. Trauner, Vienna, Graz

List of Chairpersons, Speakers and Scientific Organizers
Poster Abstracts

1. Pregnancy alters the liver transcriptome to engage gestational metabolic and inflammatory pathways
S. Abu-Hayyeh, C. Williamson (London, GB)

2. The role of necroptosis in acute and chronic cholestasis

3. Cyp3a11 is dispensable for the formation of murine bile acids
S. Al-Dury, A. Wahlström, M. Ståhlman, F. Bäckhed, H.-U. Marschall (Gothenburg, SE; Copenhagen, DK)

4. Cafestol but not resveratrol stimulates FGF19 expression in human ileal explants

5. Bile acids are key regulators of testicular physiology and male fertility
M. Baptissart, E. Martinot, A. Vega, L. Sédès, B. Rouaisnel, K. Schoonjans, D.H. Volle (Aubiere Cedex, FR; Lausanne, CH)

6. Effect of intrahepatic cholestasis of pregnancy on maternal glucose homeostasis
E. Bellafante, V. Nikolova, J. Chambers, M. Martineau, C. Williamson (London, GB)

7. $\alpha_5\beta_1$ integrins are receptors for bile acids with a (nor-)ursodeoxycholane scaffold
M. Bonus, A. Sommerfeld, D. Häussinger, H. Gohlke (Düsseldorf, DE)

8. Autoimmune BSEP disease is curable with hematopoietic stem cell transplantation
F. Brinkert, A. Briem-Richter, V. Keitel-Anselmino, I. Müller, E. Grabhorn (Hamburg, Düsseldorf, DE)

9. Selective targeting of fxr isoform $\alpha_1$–4 by novel bile acid derivatives and lipotoxicity protection in hepg2 cells
H. Brito, S. Batista, J.A. Salvador, R.E. Castro, C.M. Rodrigues (Lisbon, Coimbra, PT)

10. Metformin protects rat hepatocytes against bile acid-induced apoptosis
M. Buist-Homan, T. Woudenberg-Vrenken, L. Conde de la Rosa, K.N. Faber, H. Moshage (Groningen, NL)
11. Soluble adenylyl cyclase regulates bile salt-induced apoptosis in human cholangiocytes

12. Activation of intestinal bile acid receptor FXR induces membrane G protein-coupled bile acid receptor TGR5 expression and stimulates GLP-1 secretion to ameliorate metabolic disorders in diabetic mice
   J.Y.L. Chiang, P. Pathak, H. Liu, S. Boehme (Rootstown, US)

13. Prevalence, clinical characteristics and outcomes of antimitochondrial type 2 seropositive patients with non-established primary biliary cholangitis

   P.H. Dixon, L. Wu, C. Williamson (London, GB; Chengdu, CN)

15. Drug-drug interactions related to inhibition of the sodium taurocholate co-transporting polypeptide (NTCP) by a novel anti-HBV peptide

16. FIC1, BSEP, and MDR3 sequencing disclosed 139 genetic variants including 63 new ones in 389 unrelated patients with suspected intrahepatic cholestasis
   C. Dröge, M. Bonus, S. Kluge, H. Gohlke, L. Schmitt, R. Kubitz, D. Häussinger, V. Keitel-Anselmino (Düsseldorf, DE)

17. Chronic central infusion of tauroliolithocholate decreases fat mass and increases brown adipose tissue triglyceride derived fatty acid uptake

18. Hormesis in cholestatic liver disease; preconditioning with low bile acid concentrations protects against bile acid-induced toxicity
   K.N. Faber, M. Buist-Homan, M. Koehorst, A.K. Groen, H. Moshage, E.M. Verhaag (Groningen, NL)

19. Absence of BSEP/ABCB11 protects from cholestatic liver injury in mice

20. Effects of ursodeoxycholic acid on FXR-mediated stimulation of FGF19 in human ileal explants
21. An experimentally validated binding mode model of TGR5 agonists
C.G.W. Gertzen, L. Spomer, S.H.J. Smits, D. Häussinger, V. Keitel-Anselmino, 
H. Gohlke (Düsseldorf, DE)

22. A novel fluorescent analogue of TUDCA reveals new mechanistic insights into 
TUDCA cytoprotection
J.F. Gilmer, J. Gavin, F. Quilty, G. Radics (Dublin, IE)

23. A novel protocol enables the differentiation of human pluripotent stem cell 
derived bipotential hepatoblasts into hepatocyte or cholangiocyte like cells 
N. Graffmann, W. Wruck, J. Adjaye (Düsseldorf, DE)

24. Bile acid biosynthesis avoiding cholesterol 
W.J. Griffiths, J. Abdel-Khalik, P.J. Crick, M. Ogundare, B.W. Bigger, 
(London, Swansea, Manchester, GB; Oakland, US; Stockholm, SE)

25. Genetic analysis of spontaneous (non-toxic) liver fibrosis in a congenic mouse 
model 
R.A. Hall, K. Hochrath, F. Lammert, F. Grünhage (Homburg, DE)

26. Enhanced ileal bile acid uptake may prevent vitamin A and/or D deficiency in 
Dutch Crohn's disease patients 
J. Heegsma, L. Wymenga, M. Hoekstra, T. Blokzijl, L. Groen, H. Groen, 
G. Dijkstra, K.N. Faber (Groningen, NL)

27. Differences in TGR5-mediated responses to bile acids and INT777 in neonatal 
and adult cardiomyocytes 
E. Ibrahim, I. Diakonov, C. Williamson, J. Gorelik (London, GB)

28. Cholic acid promotes gut epithelial proliferation in rats exposed to gamma- 
radiation 
J.-Y. Lee, N. Fuji, S. Fukiya, A. Yokota (Sapporo, Ora, Matsue, JP)

29. Vitamin D improves liver histology and hepatic gene expression in a murine 
obesity/NASH model independently of intestinal Fgf15 expression 
D. Jahn, D. Dorbath, S. Kircher, H.M. Hermanns, A. Geier (Würzburg, DE)

30. Characterisation of bile acid pathways in steroidogenic tissues 
S. Jarvis, R.M. Gadaleta, E. Want, N. Gray, S. Abu-Hayyeh, R.M.L. Winston, 
C. Williamson, C.L. Bevan (London, GB)

31. Steroid binding to autotaxin links bile salts and lysophosphatidic acid signalling 
W.-J. Keune, R. Bolier, J. Hausmann, D. Tolenaars, A. Kremer, T. Heidebrecht, 
R.P. Joosten, M. Sunkara, A. Morris, E. Matas-Rico, W.H. Moollenaar, 
A. Perrakis, R.P.J. Oude Elferink (Amsterdam, NL; Lexington, US)

33. Protective role of TGR5 in LCA induced toxic liver damage C. Klindt, K. Deutschmann, M. Reich, D. Herebian, E. Mayatepek, R. Deenen, K. Köhrer, D. Häussinger, V. Keitel-Anselmino (Düsseldorf, DE)

34. Cholic acid treatment in Zellweger spectrum disorders F.C.C. Klouwer, K. Berendse, B.G.P. Koot, E.M. Kemper, F. Schaap, H.R. Waterham, F.M. Vaz, M. Engelen, P.M.L. Jansen, R.J.A. Wanders, B.T. Poll The (Amsterdam, Maastricht, NL)


37. Bile acid-mediated hepatic differentiation of mesenchymal stem cells C. Kordes, I. Sawitza, S. Götze, D. Herebian, M. Castoldi, D. Häussinger (Düsseldorf, DE)

38. The frequent polymorphism PNPLA3 rs738409 increases hepatic steatosis but might protect against gallstone disease M. Krawczyk, R. Jiménez-Agüero, M.J. Perugorria, L. Gallego, L. Bujanda, F. Lammert, J.M. Banales (Homburg, DE; Warsaw, PL; San Sebastian, ES)


40. Bile acids regulate colonic epithelial defensin secretion: Implications for pathogenesis and therapy of inflammatory bowel disease N.K. Lajczak, V. Saint-Criq, M.S. Mroz, A. Perino, F. Murray, K. Schoonjans, S.J. Keely (Dublin, IE; Lausanne, CH)


42. Molecular regulation of adrenal function by bile acids L. Liu, A. Zaufel, J. Gumhold, E. Krones, G. Zollner, P. Fickert (Graz, AT)
43. Taurocholate induces cyclooxygenase-2 expression via the sphingosine 1-phosphate receptor 2 in a human cholangiocarcinoma cell line
R. Liu, X. Li, L. Zhang, P.B. Hylemon, H. Zhou (Richmond, US; Nanjing, CN)

44. Deletions in the cytoplasmic domain of iRhom1 and iRhom2 promote shedding of the TNF receptor by the protease ADAM17
S.K. Maney, P. Lang (Düsseldorf, DE)

45. ACOX2 deficiency: A new inborn error of bile acid biosynthesis causing persistent hypertransaminasemia

46. TGR5 activation inhibits muscular BCAA catabolism via thyroid hormone activation
T. Miyazaki, A. Honda, T. Ikegami, Y. Matsuzaki (Ibaraki, JP)

47. Raw extract from the Chinese herb Ipomoea stolonifera and its purified components are anti-inflammatory and protect against bile acid-induced apoptosis of rat hepatocytes
H. Moshage, X. Bai, Y. Chen, M. Buist-Homan, G. Shi, K.N. Faber (Groningen, NL; Shantou, CN)

48. Bile acid-dependent regulation of lysosomal biogenesis and function
T. Moustafa, T. Eichmann, K.A. Zierler, H. Wolinski, D. Kolb, J. Gumhold, P. Fickert, M. Trauner (Graz, Vienna, AT)

49. Characterization of bile acid homeostasis during liver regeneration under normal and pathological conditions
M. Mueller, S. Schultzze, N. Auer, F. Pauler, M. Trauner (Vienna, AT)

50. Gestational cholestasis is associated with white adipose tissue dysfunction
V. Nikolova, G. Papacleovoulou, E. Bellafante, C. Williamson (London, GB)

51. Bile acid malabsorption patient-reported experiences: Results of an online survey

52. Extrahepatic cholestasis induces large scale alterations in the human liver transcriptome

53. The autophagy inhibitor Rubicon is a direct FXR target in human liver and is induced in human cholestasis
K. Panzitt, H.-U. Marschall, M. Trauner, P. Fickert, M. Wagner (Graz, AT; Gothenburg, SE; Vienna, AT)
54. Antimicrobial remodelling of gut microbiota differentially affects bile acid profile, signalling and enteroprotective response in the ileum and colon of pigs
   (Barcelona, ES; Kiel, DE)

55. Impact of male cholestasis on the sperm epigenome and consequences for the health of the offspring
   V. Pataia, G. Papacleovoulou, L. Poston, C. Williamson (London, GB)

56. Recurrence of progressive familial intrahepatic cholestasis type 2 after liver transplantation with a detection of anti-BSEP antibodies
   B. Prusinskas, S. Kathemann, D. Pilic, B. Hegen, P. Küster,
   V. Keitel-Anselmino, D. Häussinger, R. Büscher, H.A. Baba, P.F. Hoyer,
   E. Lainka (Essen, Münster, Düsseldorf, DE)

57. Analysis of the bile salt export pump (ABCB11) interactome employing complementary approaches
   S. Przybylla, J. Stindt, D. Kleinschrodt, J. Schulte am Esch, D. Häussinger,
   V. Keitel-Anselmino, S.H.J. Smits, L. Schmitt (Düsseldorf, DE)

58. Inactivation of the apical sodium-dependent bile acid transporter (Asbt; Slc10a2) protects against hepatic steatosis in high fat diet-fed mice
   A. Rao, C. Ferrebee, J. Haywood, G. Wynn, W. Zhang, K.D.R. Setchell,
   S.J. Karpen, P.A. Dawson (Atlanta, Winston-Salem, Cincinnati, US)

59. ER-stress regulates bile acid uptake via downregulation of NTCP
   M. Robin, S.F.J. van de Graaf (Amsterdam, NL)

60. Pharmacokinetics, biodistribution and metabolism of obeticholic acid in rats with CCl4-induced decompensated liver cirrhosis
   A. Roda, R. Aldini, S. Spinozzi, P. Franco, M. Cont, A. D'Errico, F. Vasuri,
   A. Degiovanni, L. Adorini (Bologna, IT; San Diego, US)

61. Dual targeting of nuclear receptors ameliorates NAFLD pathogenesis in different dietary murine models
   P.M. Rodrigues, M.B. Afonso, A.L. Simão, M. Caridade, C.C. Carvalho,
   A. Trindade, A. Duarte, P.M. Borrhalho, M.V. Machado, H. Cortez-Pinto,
   C.M.P. Rodrigues, R.E. Castro (Lisbon, PT)

62. Altered bile acid homeostasis by treatment with glucocorticoids is mediated by interference with FXR/FGF19 ileum-liver crosstalk
   M.R. Romero, F.A. Al-Aqil, M.J. Monte, E. Herraez, R. Rosales, M.A. Serrano,
   F. Jimenez, L. Sanz-Ortega, R. Gonzales, C. Pizarro, J.C. Aranda, B. Ocon,
   I. Uriarte, F. Sanchez de Medina, O. Martinez-Augustin, M.A. Avila, J.J.G. Marin
   (Salamanca, Granada, Pamplona, ES)

63. Inhibiting hepatic bile acid uptake in DDC-induced cholestasis reduces serum biomarkers of liver injury
   R.L.P. Roscam Abbing, D. Stijepcevic, L. Haazen, U. Beuers,
   R.P.J. Oude Elferink, S.F.J. van de Graaf (Amsterdam, NL)
64. Vitamin A deficiency leads to mild cholestasis and a "humanized" bile acid profile in rats
A. Saeed, M.O. Hoeke, M. Hoekstra, J. Heegsma, H. Moshage, K.N. Faber
(Groningen, NL)

65. Portal vein embolization-triggered liver regeneration is accelerated by the FXR agonist obeticholic acid
F.G. Schaap, P.B. Olthof, C. van Himbeeck, F. Huisman, K.P. van Lienden,
R.F. van Golen, M. Heger, J. Verheij, I.A. Leclercq, P.L.M. Jansen,
T.M. van Gulik, S.W.M. Olde Damink
(Maastricht, Amsterdam, NL; Brussels, BE)

66. Oncostatin M contributes to non-alcoholic fatty liver disease (NAFLD)
progression in hypercholesterolemic mice
(Würzburg, DE)

67. Cholestasis reduce immune induction after viral infection
A.-K. Schupp, S. Rattay, A. Kislat, B. Homey, D. Häussinger, A. Zimmermann,
D. Graf (Düsseldorf, DE)

68. FXR agonist PX20606 reduces liver damage, fibrosis and portal hypertension in CCl4 cirrhotic rats
P. Schwabl, E. Hambruch, B.A. Payer, T.L. Schubert, B. Strobl, S. Fida,
M. Wagner, L. Garnys, M. Peck-Radosavljevic, C. Kremoser, T. Reiberger,
M. Trauner (Heidelberg, DE; Vienna, AT)

69. A mathematical model of the intestinal transit and enterohepatic circulation of bile acids
F.L.P. Sips, H.M. Eggink, P.A.J. Hilbers, M.R. Soeters, A.K. Groen,
N.A.W. van Riel (Eindhoven, Amsterdam, Groningen, NL)

70. Combined activity of NTCP and OATPs governs hepatic uptake of conjugated bile acids in vivo
D. Slijepcevic, J.M. Donkers, D. Tolenaars, D.R. de Waart, U. Beuers,
R.P.J. Oude Elferink, A.H. Schinkel, S.F.J. van de Graaf (Amsterdam, NL)

71. Postprandial transorgan bile acid kinetics: Implications for TGR5 agonism
M.R. Soeters, H.M. Eggink, F.S. van Nierop, M.G. Schooneman, A. Boelen,
A. Kalsbeek, M. Koehorst, G.A.M. Ten Have, M. Nieuwdorp, A.K. Groen,
J.A. Romijn, N.E.P. Deutz
(Amsterdam, Groningen, NL; College Station, TX, US)

72. Novel role for lymphotxin beta receptor in bile acid homeostasis after partial hepatectomy
U.R. Sorg, K. Behnke, D. Herebian, M. Reich, E. Mayatepek,
V. Keitel-Anselmino, D. Häussinger, K. Pfeffer (Düsseldorf, DE)
73. Generation of liver buds by self-condensation of human iPSC-derived MSCs, HLCs and endothelial cells
L.-S. Spitzhorn, N. Graffmann, J. Adjaye (Düsseldorf, DE)

74. TGR5 protein expression is reduced in livers of Mdr2-/- (Abcb4 -/-) mice and of patients with primary sclerosing cholangitis (PSC)
L. Spomer, M. Reich, J. Höhne, J. Hov, T. Karlsen, D. Nierhoff, D. Häussinger, V. Keitel-Anselmino (Düsseldorf, DE; Oslo, NO; Cologne, DE)

75. NorUDCA reduces liver injury and improves the metabolic state in mouse models of obesity and steatosis
D. Steinacher, T. Claudel, E. Einwallner, T. Stojakovic, M. Trauner (Vienna, Graz, AT)

76. Functional studies on monoclonal, patient-derived BSEP-reactive antibodies causing antibody-induced BSEP deficiency (AIBD)
J. Stindt, T. Tiller, C. Dröge, B. Brackertz, C. Kriegel, J. Klattig, D. Häussinger, R. Kubitz, V. Keitel-Anselmino (Düsseldorf, Munich, DE)

77. Porphyran, a functional ingredient of "Nori" improves visceral obesity and non-alcoholic fatty liver via inhibition of intestinal FXR activation

78. Bile acids regulate host weight gain and metabolism through gut microbiota modification
H. Taoka, T. Tanigaki, Y. Takashina, M. Watanabe (Fujisawa, JP)

79. BAs regulate host weight gain and metabolism through gut microbiota modification
H. Taoka, T. Tanigaki, N. Kitamura, Y. Takashina, M. Watanabe (Fujisawa, JP)

80. Toxic bile injury in mdr2 -/- mice instigates a hepatic T-lymphocyte immune response amenable to cytokine and anti-cholestatic therapy

81. Post-hepatectomy dietary challenge with cholic acid to mimic post-resectional liver failure
L. van de Laarschot, K.M.C. van Mierlo, V. Lebrun, C. van Himbeeck, P.L.M. Jansen, F.G. Schaap, S.W.M. Olde Damink, I.A. Leclercq (Maastricht, NL; Brussels, BE)

82. A FRET-based compound screen identifies novel inhibitors of the organic solute transporter alpha/beta
S.M.W. van de Wiel, S.F.J. van de Graaf (Amsterdam, NL)
83. The phospholipid flippase ATP8B1 mediates apical membrane localization of the cystic fibrosis transmembrane regulator
V.A. van der Mark, H. de Jonge, J.-C. Chang, K. Ho-Mok, S. Duijst, M.S. Carlon, R.P.J. Oude Elferink, C.C. Paulusma (Amsterdam, Rotterdam, NL; Leuven, BE)

84. Obeticholic acid enhances liver regeneration in hepatectomized mice
K.M.C. van Mierlo, V. Lebrun, C. van Himbeeck, P.L.M. Jansen, F.G. Schaap, S.W.M. Olde Damink, I.A. Leclercq (Maastricht, NL; Brussels, BE)

85. Glycodeoxycholic acid administration increases GLP-1 secretion in healthy humans
F.S. van Nierop, F.G. Schaap, F.M. Vaz, J.A. Romijn, S.W.M. Olde Damink, M.R. Soeters (Amsterdam, Maastricht, NL)

86. Colonization of germ-free mice with a human microbiota induces FXR signaling
A. Wahlström, P. Kovatcheva-Datchary, M. Ståhlman, H.-U. Marschall, F. Bäckhed (Gothenburg, SE; Copenhagen, DK)

87. Decreased intestinal glucose uptake in humans treated with the FXR agonist obeticholic acid

88. Bile acid-modulated transcript-expression in human macrophages validated by transcriptome analysis

89. Ursodeoxycholic acid and lithocholic acid exert anti-inflammatory actions in the colon: Implications for treatment of ulcerative colitis

90. Protection against oxidative stress mediated by Nrf2-Keap1 axis is impaired in primary biliary cholangitis
U. Wasik, M. Milkiewicz, P. Milkiewicz (Szczecin, PL)

91. Porphyran, a functional ingredient of "Nori" is protected from development of NASH via modulating of bile acids metabolism
M. Watanabe, K. Ishihara, N. Shimada, M. Kobayashi, Y. Takashina (Fujisawa, Yokohama, JP)

92. UDCA administration in cholestatic pregnancy can ameliorate dysregulated metabolic profile of the fetus and offspring
93. rs10488631 polymorphism of IRF5-TNPO3 confers susceptibility to primary biliary cholangitis (PBC) and is associated with abnormal liver biochemistry indexes: A single centre association study
(Szczecin, Warsaw, PL; San Diego, US)
Session I

Bile acids signaling in liver regeneration and tumor development
Bile flux analysis by intravital two-photon microscopy in normal and cholestatic livers

Jan G. Hengstler, Ahmed Ghallab, Nachiket Vartak, Amruta Vartak and Raymond Reif
IfADo (Leibniz Research Center), Ardeystrasse 67, D-44139 Dortmund, Germany

Recently, large distance two-photon imaging has been introduced that allows functional imaging of the liver of anesthetized mice with a resolution of approximately 200 nm. After tail vein injection of fluorescent bile salts their transport from the sinusoids, over the Disse space and hepatocytes into bile canaliculi can be studied. In contrast to current text book knowledge transport of bile in the canaliculi is mostly due to diffusion and much less to flow, which has major implications for pharmacokinetics of compounds, which are excreted via the biliary route. Two-photon based analysis of diffusion and convection (‘flow’) of bile salts revealed that cholestasis (e.g. after bile duct ligation) induces fundamentally different responses of the canalicular network and the interlobular bile ducts: the canaliculi showed increased diameters and protrusions into hepatocytes but no major topological alterations. In contrast, a highly reproducible sequence of topological changes was observed for the interlobular bile ducts, where cholangiocyte proliferation initially causes corrugation of the luminal duct surface, leading to an approximately five-fold increase in surface area. This is analogous to the function of villi in the intestine or sulci in the brain, where an expansion of area is achieved within a restricted volume. The increase in surface area is further enhanced by duct branching, branch elongation, and loop formation through self-joining, whereby an initially relatively sparse mesh surrounding the portal vein becomes five-fold denser through elongation, corrugation, and ramification. The number of connections between the bile duct and the lobular bile canalicular network by the canals of Hering decreases proportionally to the increase in bile duct length, suggesting that no novel connections are established. The diameter of the interlobular bile duct remains constant after BDL, a response that is qualitatively distinct from that of large bile ducts, which tend to enlarge their diameters. In conclusion, cholestasis causes adaptive remodeling that aims at optimizing the intraluminal surface area and bile salt reabsorption capacity by way of corrugation and branching of interlobular ducts (Vartak et al. Hepatology. 2016;63[3]:951–64).
Fibroblast growth factor signaling controls liver size in mice with humanized livers

Markus Grompe and Willscott E. Naugler
Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, Oregon, USA

Our laboratory has developed a system (the FRG mouse = Fah<sup>-/-</sup>/Rag2<sup>2/-</sup>/Il2r<sup>-/-</sup>) to generate mice with highly chimeric, humanized livers, containing > 90% human hepatocytes. The overall liver size of such humanized chimeras are up to 3 x larger than normal. Human hepatocytes do not recognize the fibroblast growth factor (Fgf15) produced by mouse intestine. This results in up-regulation of bile acid synthesis in the human hepatocytes and enlargement of the bile acid pool. We investigated whether abnormal bile acid signaling may be the cause of the enlarged livers in these animals.

We have previously shown that human FGF19 injections normalize bile acid synthesis in chimeric FRGN mice. To test the connection between bile acid signaling and liver size we inserted the entire genomic locus of the or human FGF19 gene (ortholog to mouse Fgf15), including regulatory sequences, into the FRGN mice to create the FRGN19 strain. Livers of FRGN19 mice and their FRGN littermates were then fully repopulated with human hepatocytes. Liver tissues were collected and bile acid pool sizes and RNA sequences were analyzed and compared with those of mice without humanized livers (controls).

Livers were larger in FRGN mice with humanized livers (average of 13% of body weight), compared with unrepopulated control FRGN mice; they also had much larger bile acid pools and aberrant bile acid signaling. In contrast livers from FRGN19 normalized to ~ 7.8% of body weight, and their bile acid pool and signaling more closely resembled that of untransplanted FRGN19 control. RNA seq transcriptomic analysis showed activation of the Hippo pathway, and immunohistochemical and revealed increased hepatocyte proliferation, but not apoptosis, in the enlarged humanized livers of FRGN mice. These abnormalities were corrected by the FGF19 transgene. Cell sorting experiments showed that although healthy human liver does not produce FGF19, non-parenchymal cells from cholestatic livers produce FGF19.

We conclude that expression of an FGF19 transgene corrects bile acid signaling defects in liver chimeric mice, resulting in normalization of bile acid synthesis, the bile acid pool, and liver size. These findings indicate that liver size is, in part, regulated by the size of the bile acid pool circulated by the liver.
The FGF15/19 signaling system FGFR4 in hepatocarcinogenesis

Matías A. Avila
Hepatology Program, CIMA-University of Navarra, Pamplona, Spain

Fibroblast growth factor 15 (FGF15), FGF19 in humans, is a bile acid-FXR induced gut-derived hormone that plays a key role in bile acid, carbohydrate and lipids metabolism. FGF15/19 is a ligand of the tyrosine kinase FGFR receptor 4 (FGFR4), which together with the klotho-beta (KLβ) trans-membrane co-receptor, are abundantly expressed in hepatocytes. FGF15 also participates in liver regeneration after partial hepatectomy by promoting the proliferation of hepatocytes and cholangiocytes. Interestingly, ectopic overexpression of FGF19 in mice induces hepatocellular carcinoma (HCC) in an FGFR4-dependent manner, and interference with FGF19-FGFR4 signaling attenuates HCC development. In humans, FGF19 is overexpressed in about 30% of HCC tumors, correlating with poor patients’ prognosis, while FGFR4 is overexpressed in 50% of cases. Therefore dysregulation of FGFR4 activation of its receptor FGFR4 promotes HCC cell growth. In an experimental model of fibrosis-associated liver cancer we have demonstrated the contribution of endogenous FGF15 to HCC development. The cellular and molecular mechanisms involved in FGF15/19-FGFR4 related hepatocarcinogenesis are not completely known, and as will be discussed may involve extensive crosstalks with other growth factors and intracellular signaling pathways. Given the recognized relevance of the FGF15/19-FGFR4 axis in hepatocarcinogenesis, and the potential implementation of therapies based on (intestinal) FXR stimulation for different liver diseases, gaining further insight into these mechanisms is highly relevant.

Here we addressed for the first time the role of endogenous Fgf15 in hepatocarcinogenesis. Fgf15+/+ and Fgf15−/− mice were subjected to a clinically relevant model of liver inflammation and fibrosis-associated carcinogenesis. Fgf15−/− mice showed less and smaller tumors, and histological neoplastic lesions were also smaller than in Fgf15+/+ animals. Importantly, ileal Fgf15 mRNA expression was enhanced in mice undergoing carcinogenesis, but at variance with human HCC it was not detected in liver or HCC tissues, while circulating FGF15 protein was clearly upregulated. Hepatocellular proliferation was also reduced in Fgf15−/− mice, which also expressed lower levels of the HCC marker alpha-fetoprotein (AFP). Interestingly, lack of FGF15 resulted in attenuated fibrogenesis. However, in vitro experiments showed that liver fibrogenic stellate cells were not direct targets for FGF15/FGF19. Conversely we demonstrate that FGF15 induces the expression of the pro-fibrogenic and pro-tumorigenic connective tissue growth factor (CTGF) in hepatocytes. These findings suggest the existence of an FGF15-triggered CTGF-mediated paracrine action on stellate cells, and an amplification mechanism for the hepatocarcinogenic effects of FGF15 via CTGF production. In summary, our observations indicate that ileal FGF15 may contribute to HCC development in a context of chronic liver injury and fibrosis.
Portal vein embolization-triggered liver regeneration is accelerated by the FXR agonist obeticholic acid

Sander Rensen\textsuperscript{1}, Frank G. Schaap\textsuperscript{1,\*}, Pim B. Olthof\textsuperscript{2,\*}, Cathy van Himbeeck\textsuperscript{1}, Floor Huisman\textsuperscript{2}, Krijn P. van Lienden\textsuperscript{3}, Rowan F. van Golen\textsuperscript{2}, Michal Heger\textsuperscript{2}, Joanne Verheij\textsuperscript{4}, Isabelle A. Leclercq\textsuperscript{5}, Peter L.M. Jansen\textsuperscript{1}, Thomas M. van Gulik\textsuperscript{2,\*}, Steven W.M. Olde Damink\textsuperscript{1,\*}

\textsuperscript{1}Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands
\textsuperscript{2}Departments of \textsuperscript{3}Surgery, \textsuperscript{4}Radiology and \textsuperscript{5}Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{5}Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, UCL, Brussels, Belgium
\textsuperscript{\*}Equally contributing first and senior authors

Introduction: The bile salt (BS) receptor FXR plays an important role in compensatory liver growth following partial liver resection. Here we explored whether FXR is involved in the regenerative response following portal vein embolization (PVE).

Methods: Adult female rabbits (n = 5–6 per group) received vehicle or the FXR agonist obeticholic acid (OCA; 10 mg/kg, daily oral gavage) for 7 days prior to and after embolization of the cranial liver lobes. Effectiveness of PVE was confirmed by portography, and caudal liver volume (CLV) was analyzed by CT-volumetric analysis at days -7, -1, +3 and +7.

Results: OCA induced a larger increase in CLV at day 3 after PVE (59.3 ± 19.2\% vs. 29.7 ± 16.1\% in controls; p = 0.001), with both groups attaining a similar volume gain after 7 days. In both groups, PVE resulted in a similar pattern of transient elevation of serum BS. Hepatic BS content was reduced (60.1 [16.0] vs. 100.1 [75.1] nmol/g in controls; p = 0.016) in the hypertrophied segments of OCA-treated animals at day +3. Reduced expression of the BS synthetic enzyme \textit{Cyp7a1} (-7.1 fold; p = 0.004) and enhanced expression of the basolateral BS efflux pump subunit \textit{Slc51b} (+6.5 fold; p = 0.004) may have contributed to this lowering. Expression of \textit{Cdc25b}, a phosphatase required for entry into mitosis, was elevated in the hypertrophic (+1.6 fold; p = 0.006) but not atrophic liver segments of OCA-treated animals. \textit{Cdc25b} expression in the non-embolized segments correlated with expression of \textit{Slc51b} (p = +0.80; p = 0.002) and \textit{Cyp7a1} (p = -0.62; p = 0.033), and tended to be associated with percentual increase in CLV at day +3 (p = +0.57; p = 0.055).

Discussion/Conclusion: OCA accelerated liver regeneration in the first 3 days after PVE in rabbits. Improved BS homeostasis and induction of proliferative genes may underlie the augmented growth rate in the initial phase after PVE. OCA treatment has potential in extending resectability and prevention of post-resectional liver failure.
Session II

Bile acids and the gut liver axis
Role of the intestinal microbiome in cholestatic liver disease

Nicholas F. LaRusso, James H. Tabibian and Steven P. O’Hara
Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

Harboring roughly $2-5 \times 10^{11}$ bacteria per gram of feces in humans, the intestinal tract embodies an incredibly complex biological ecosystem. This ‘microbiome’ includes microbes, their metabolites and degradative products, and their genomes. Receiving approximately 75% of its blood supply from the intestine via the portal circulation, the liver is thus continuously exposed to a wide repertoire of molecules, be they beneficial or noxious, originating from or influenced by the intestinal microbiome. An increasing body of literature has begun to shed light on this “gut liver axis” in the maintenance of health as well as in the pathogenesis of liver disease. In this talk, I will focus primarily on the relationship of the intestinal microbiome with primary sclerosing cholangitis (PSC), a fibroinflammatory cholangiopathy of unknown etiology and with no effective pharmacologic therapy. Several lines of evidence support a role for the microbiome in PSC. First, inflammatory bowel disease, a condition itself associated with intestinal dysbiosis, occurs in 75% of patients with PSC. Second, portal bacteremia, bacterobilia, and 16s ribosomal ribonucleic acid (RNA) in bile have all been described in PSC. Thirdly, cholangiocytes in PSC patients accumulate lipopolysaccharide (LPS) \textit{in vivo} and are hyper responsive to LPS treatment \textit{in vitro}. Fourthly, several genomic associations have been established with loci implicated in host-microbiome interactions. Fifth, treatment with select oral antibiotics offers therapeutic benefit in select patients with PSC. Finally, recent studies in germ-free MDR KO mice, an animal model of PSC, have suggested a protective influence of the microbiome on disease development.
Microbe-host interactions via microbial moderation of bile acid signatures

C.G.M. Gahan1,2,3 and S.A. Joyce1,4
1Alimentary Pharmabiotic Centre; 2School of Microbiology; 3School of Pharmacy; 4School of Biochemistry & Cell Biology, University College Cork, Cork, Ireland

Bile acids act as key signalling molecules that have the capacity to alter systemic endocrine functions in the host. Individual bile acids are capable of interacting with host cell receptors (including FXR and TGR5 receptors) to induce cellular responses in the intestine and other tissues (including the liver and adipose tissue). Gut microbes carry out unique enzymatic bile acid conversions in the host which include the liberation of unconjugated primary bile acids and the synthesis of secondary bile acids. As gut microorganisms have the capacity to significantly alter the signalling properties of bile acids we, and others, have investigated the impact of altered microbial bile acid signatures upon host physiological processes. In particular we have focused upon microbial bile salt hydrolase (BSH) activity as a gut microbial activity that has the capacity to profoundly alter both local (gastrointestinal) and systemic (hepatic) host functions. Using a functional metagenomics approach we demonstrated that BSH activity is widely distributed amongst gut bacteria and may contribute to microbial colonisation in the gut. Using both germ free and conventionally-raised mouse models we showed that gastrointestinal expression of BSH results in local bile acid deconjugation with concomitant alterations in lipid and cholesterol metabolism, signalling functions and weight gain. Key mediators of cholesterol homeostasis (Abcg5/8), gut homeostasis (RegIIIγ) and circadian rhythm (Dbp) were influenced by elevated BSH in our study. The implications of this work for the rational development of probiotics with the potential to modulate host weight gain will be discussed.
Ceramides are essential lipids in skin and nerve function, but at high concentrations can have adverse effects. Ceramide levels are positively correlated with metabolic disease in mice including obesity, insulin resistance and non-alcoholic fatty liver disease (NAFLD). Correlative studies also suggest that ceramides are associated with metabolic diseases, such as insulin resistance, in humans. Ceramide levels are regulated by secreted endocrine factors and nuclear receptors involved in control of metabolism. For example, the positive effect of FGF21 on insulin resistance is due in part to reduced cellular ceramides as a result of adiponectin stimulation of ceramide conversion to sphingosine. However, this does not explain all of the adverse effects of ceramides on metabolic diseases, and not all of the favorable metabolic effects of FGF21 could be due to lower serum and tissue ceramides. Intestinal farnesoid X receptor (FXR) was found to control the levels of serum ceramides. FXR regulates bile acid synthesis and transport in the liver, and bile acid transport in the ileum. Decreased obesity, insulin resistance and NAFLD was found in high-fat diet (HFD)-fed mice after administration of the potent intestinal FXR antagonist glycine-muricholic acid (Gly-MCA), and this was associated with decreased serum ceramides; the favorable metabolic phenotypes after Gly-MCA were reversed by injection of C16:0 ceramide. Mice lacking FXR in intestine also have lower serum ceramides and are metabolic fit; they are resistant to HFD-induced metabolic disease and this is reversed by injection of C16:0 ceramide. FXR was found to be a positive regulator of genes involved in ceramide synthesis in the small intestine. In mouse ileum, due to the presence of endogenous FXR agonists produced in the liver, these genes are activated; when Gly-MCA is administered, they are repressed which likely accounts for the decrease in serum ceramides. These studies reveal that ceramides produced in the ileum under control of FXR, influence metabolic diseases. Mechanistically, mice under intestinal FXR antagonism by Gly-MCA or lacking intestinal FXR have increased beige adipose depots that mediate weight loss through increased energy expenditure and heat production, similar to brown adipose tissue. This is due to lowering of serum ceramides through modulation of FXR signaling in the ileum. Increased adipose beiging likely accounts for the elevated energy expenditure and weight loss in obese mice treated with Gly-MCA. Inhibition of FXR signaling by bile acid derivatives could be a new therapeutic option for the treatment of metabolic disease in humans.
Gut feelings: How intestinal FXR controls fatty acid and cholesterol metabolism

Jae Myoung Suh, Sungsoon Fang, Johan W. (Hans) Jonker, Ruth T. Yu, Annette R. Atkins, Michael Downes, Ronald M. Evans
Salk Institute for Biological Studies, La Jolla, CA, USA

In regards to the feast & famine paradigm, rapid bile acid release in response to a meal selectively activates the nuclear receptor FXR in the intestine. To mimic this tissue selective effect, we built an intestinal specific FXR agonist called fexaramine. Gut specific FXR agonist Fexaramine robustly induces enteral FGF15 along with 100’s of other intestinal responsive genes (but does not activate endogenous FXR targets in liver, adrenal and kidney). Unlike systemic drugs, Fexaramine protects mice against diet-induced weight gain, reduces body-wide inflammation, enhances thermogenesis, promotes browning of white adipose tissue and represses hepatic glucose production. In addition, serum cholesterol levels are reduced, the bile acid pool is lowered and hepatic inflammation is suppressed. Together, this elevates the potential use of gut-biased ligands to control systemic problems linked to metabolic disease.
Oral presentation of poster abstract

**Vertical sleeve gastrectomy (VSG) in morbidly obese adolescents results in increased fibroblast growth factor 21 (FGF21) that correlates with weight loss**

Farooq H. Khan¹, Lindsey Shaw³, Wujuan Zhang², Rosa Maria Salazar Gonzalez⁴, Sarah Mowery⁴, Melissa Oehrle², Xueheng Zhao², Todd Jenkins², Kenneth D.R. Setchell², Thomas H. Inge³, Rohit Kohli⁴  
¹Division of General Internal Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio, USA; ²Department of Pathology and Laboratory Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA; ³Department of Pediatric Surgery, Surgical Weight Loss Program for Teens, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA; ⁴Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA

**Introduction:** Vertical sleeve gastrectomy (VSG) results in elevated bile acids (BA) and fibroblast growth factor 19 (FGF19) levels. FGF21 shares essential co-factors with FGF19 and has been shown to be increased in energy-deficit states. We studied fasting and post-prandial changes in BA and FGF19/21 physiology in morbidly obese adolescents’ post-VSG.

**Methods:** We enrolled 10 adolescents (age 17.4 ± 0.5 yr & BMI 51.5 ± 2.5 kg/m²) that underwent VSG surgery. Fasting and post-meal challenge (100 ml Ensure™) samples (till 120 minutes) were collected at 3 visits (Pre-VSG [V1], and at 1 [V2] & 3 months [V3] post-VSG) for analysis of BA, FGF19 and FGF21.

**Results:** 1 subject was excluded. As expected post-VSG, subjects lost weight over time (V2 11.8 kg ± 0.8; V3 21.9 kg ± 1.7), while post-prandial BA (V2; 60 min p = 0.001 and V3 60 min p = 0.024), and FGF19 (V2; 90 min p = 0.026; V3; 90 min p = 0.085) levels were increased. BA composition changes resulted in an improved post-prandial hydrophobicity index (V3; 30 min p = 0.030 and 60 min p = 0.033). We observed that post-VSG FGF21 levels initially increased (V2; fasting and 120 min p < 0.01), then returned towards baseline at V3 (Figure – Left Panel). There was positive correlations between the increase in postprandial BA and FGF 19 (V3; 90 min p = 0.041, R = 0.774) and fasting BA and FGF21 (V2; p = 0.003, R = 0.894). Further, we observed a correlation between rise in postprandial FGF19 and FGF21 (V2; 90 min p = 0.001, R = 0.920) but more interestingly between body weight lost (kg) and fasting FGF21 levels (V2; p = 0.012, R = 0.82; Figure – Right Panel).
Discussion/Conclusion: BA physiology is altered in obese adolescents’ post-VSG with increased serum BA, FGF19 levels, and an improved hydrophobicity index. Our study presents novel data regarding an increase in FGF21 that correlates with weight loss post-VSG. The role of FGF21 has not been studied extensively in bariatric surgery and warrants mechanistic investigation.
Progress in the molecular characterization of hepatobiliary transporters

Dietrich Keppler
German Cancer Research Center, D-69120 Heidelberg, Germany

Over the last 25 years, our understanding of the driving forces for hepatobiliary elimination and knowledge of the molecular basis of uptake and efflux transport in hepatocytes have undergone fundamental changes [1]. This refers to bile acids and many other endogenous substances as well as to drugs that are eliminated by hepatobiliary elimination. In this development, not only molecular cloning, functional characterization, and localization of transporters were decisive, but also the discovery of hereditary mutations in genes encoding sinusoidal uptake transporters and canalicular efflux pumps in humans and rodents. Uptake by passive diffusion and elimination into bile driven by the electrochemical gradient are no longer considered relevant for hepatobiliary elimination in the intact organism. Further insights into the relative roles of uptake transporters and unidirectional ATP-driven efflux pumps were obtained when we established double-transfected polarized cell lines stably expressing, as an example, the hepatocellular uptake transporter OATP1B3 and the apical (canalicular) efflux pump MRP2 [2,3].

ATP-dependent efflux transporters localized to the basolateral hepatocyte membrane, particularly MRP3 [4] and MRP4 [5,6] pump substances from hepatocytes into sinusoidal blood. Bile acids are substrates for MRP4 in the presence of reduced glutathione, which undergoes co-efflux [5,6]. These efflux pumps have been recognized in recent years to play an important compensatory role in cholestasis and to contribute to the balance between uptake and efflux of bile acids and other organic anions during the vectorial transport from sinusoidal blood into bile. This sinusoidal efflux not only enables subsequent renal elimination, but also re-uptake of substances into neighboring hepatocytes located more centrally and downstream in the sinusoid [5,6].

References:


Session III

Intrahepatic signaling and bile acids as endogenous toxins
Bile acids and cholangiocyte autophagy

Motoko Sasaki, MD, PhD
Department of Human Pathology, Kanazawa University Graduate School of Medical Sciences, Kanazawa 920-8640, Japan

Macroautophagy (a major type of autophagy) is a process of cellular self-digestion that plays a critical role in energy homeostasis and in cytoprotection to various stresses. Deregulated autophagy is caused by blockage of early and late process of autophagosome formation, which is associated with various human diseases.

Primary biliary cholangitis (PBC) is characterized by a high prevalence of serum anti-mitochondrial antibodies (AMAs) against the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) and bile duct lesions called chronic non-suppurative destructive cholangitis (CNSDC) in small bile ducts, eventually followed by extensive bile duct loss and biliary cirrhosis.

We have revealed that deregulated autophagy may be involved possibly in the autoimmune process via abnormal expression of mitochondrial antigens and also in cholangiocyte senescence in bile duct lesions in PBC. In vitro study showed that bile acids, glycochenodoxycholic acid (GCDC) and deoxycholic acid, as well as serum deprivation and oxidative stress, cause autophagy, deregulated autophagy followed by cellular senescence in cholangiocytes. Endoplasmic reticulum (ER) stress may be involved in the pathogenesis of deregulated autophagy induced by GCDC in cholangiocytes. Pretreatment with ursodeoxycholic acid (UDCA) or tauro-UDCA, which is a chemical chaperone enhancing the adaptive capacity of the ER, significantly suppressed ER stress, deregulated autophagy and cellular senescence in cholangiocytes treated with GCDC.

Bile acids may play a role in the pathogenesis of deregulated autophagy and cellular senescence thorough induction of ER stress in PBC. UDCA treatment for PBC may be effective partly via reduction of ER stress in cholangiocytes.
Soluble adenylyl cyclase regulates bile-salt-induced apoptosis in human cholangiocytes: A link to primary biliary cirrhosis

Jung-Chin Chang¹, Simei Go¹, Coen Paulusma¹, Dirk R. de Waart¹, Ulrich Beuers¹, Lonny Levin², Jochen Buck², Ronald P.J. Oude Elferink¹
¹Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands
²Department of Pharmacology, Weill Cornell University, New York, USA

The biliary tree, providing passage for millimolar bile salts, ranks among the harshest environments in the human body. In primary biliary cirrhosis the chloride/bicarbonate exchanger AE2 is downregulated and we have previously demonstrated that knockdown of the anion exchanger 2 (AE2, SLC4A2) sensitized the H69 cholangiocyte cell line not only towards bile-salt-induced apoptosis, but also to etoposide-induced apoptosis (Hohenester S. et al. [2012] Hepatology. 55:173–83). Hence, there might be a general mechanism accounting for the sensitization of AE2-deficient cholangiocytes towards pro-apoptotic agents. In fibroblasts from Ae2-/- mice we demonstrated that intracellular bicarbonate accumulation increases expression and activity of soluble adenylyl cyclase (sAC, ADCY10) (Mardones P. et al. [2008] J Biol Chem. 283:12146–53), an evolutionarily conserved enzyme that is activated by bicarbonate and fine-tuned by calcium, but not regulated by G-proteins or forskolin. On the basis of these combined results we hypothesized that bile salt-induced apoptosis in the H69 cholangiocyte cell line is regulated by soluble adenylyl cyclase.

Apoptosis, induced by incubating H69 cholangiocytes with Na-CDC (sodium chenodeoxycholate), was inhibited by sAC-inhibitors. The same was found for apoptosis induced by 1mM Na-GCDC (sodium glycochenodeoxycholate). In AE2-deficient H69 cholangiocytes, the sensitization towards both Na-CDC and Na-GCDC was prevented by sAC inhibitors, confirming that sAC is involved in regulation of bile-salt-induced apoptosis. When treated with 100μM Etoposide for 19 hours, sAC inhibitors also reduced caspase 3/7 activity. Na-CDC induced apoptosis was prevented by forskolin and the membrane-permeant cAMP analog dibutyryl-cAMP.

Conclusion: Bile-salt-induced apoptosis in H69 human cholangiocyte cell line is regulated by sAC. Our results suggest that cAMP from cytosolic sAC promotes bile-salt-induced apoptosis, whereas cAMP from transmembrane adenylyl cyclase protects against apoptosis. These results provide an important link between the observed downregulation of AE2 and increased apoptosis of cholangiocytes in primary biliary cirrhosis.
Mechanisms of cytoprotection by TUDC

Dieter Häussinger
Heinrich Heine University, Düsseldorf, Germany

Ursodeoxycholic acid is in vivo rapidly converted to its taurine conjugate taourso-deoxycholate (TUDC), which is known to exert choleretic, cytoprotective and anti-apoptotic effects in liver and provides the basis for its frequent use in the treatment of cholestatic liver disease. The choleretic effect of TUDC involves a coordinated insertion of the bile salt export pump Bsep and of the Na+-dependent taurocholate transporting peptide (Ntcp) in the canalicular and basolateral membrane of the hepatocyte1–3, respectively. Here, intracellular β1-integrins act as TUDC receptors2,4 and integrin signaling involving protein kinase A, Erks and p38MAPK triggers the insertion of intracellular Bsep and Ntcp into the respective membranes3.

Recent data demonstrate that not only the choleretic, but also the antiapoptotic effects of TUDC are mediated by an activation of α5β1-integrins5. TUDC-induced integrin activation leads to the formation of cAMP, which triggers inactivation of the CD95 and induction of a MAPK phosphatase5. It was found that TUDC inhibited the glycochendeoxycholate (GCDC)-induced activation of the CD95 death receptor at the level of association between CD95 and the epidermal growth factor receptor, a JNK-dependent step of CD95 activation. This was due to a rapid TUDC-induced β1-integrin-dependent cyclic AMP (cAMP) signal with induction of the dual specificity mitogen-activated protein (MAP) kinase phosphatase 1 (MKP-1), which prevented GCDC-induced phosphorylation of mitogen-activated protein kinase kinase 4 (MKK4) and c-Jun-NH2-terminal kinase (JNK) activation5. Furthermore, TUDC induced a protein kinase A (PKA)-mediated serine/threonine phosphorylation of the CD95, which was recently identified as an internalization signal for CD956. Accordingly, TUDC inhibited GCDC-induced CD95 activation and targeting to the plasma membrane in a β1-integrin-and PKA-dependent manner. In line with this, the β1-integrin siRNA knockdown in Ntcp-transfected HepG2 cells abolished the protective effect of TUDC against GCDC-induced apoptosis. Also nor-ursodeoxycholate (norUDCA) led to an activation of β1-integrins and Erks, but to a lesser extent than TUDC.

Hepatic stellate cells (HSC) were recently identified as liver-resident mesenchymal stem cells (MSC), which can contribute to liver regeneration7,8. TUDC was recently reported to potently stimulate hepatic differentiation of hepatic stellate cells and other mesenchymal stem cells9. Current studies suggest that TUDC-induced HSC/MSC differentiation does not involve β1-integrins, but is mediated via sphingosine-1-phosphate receptor 2 (S1PR2). In line with this, hepatic differentiation of MSC was also induced by sphingosine-1-phosphate

References:

Oral presentation of poster abstract

Steroid binding to autotaxin links bile salts and lysophosphatidic acid signalling

Willem-Jan Keune¹, Ruth Bolier²,*, Jens Hausmann¹,*, Dagmar Tolenaars², Andreas Kremer², Tatjana Heidebrecht¹, Robbie P Joosten¹, Manjula Sunkara⁴, Andrew Morris⁴, Elisa Matas-Rico³, Wouter H. Moolenaar³, Anastassis Perrakis¹,* and Ronald P.J. Oude Elferink²,*

¹Division of Biochemistry, Netherlands Cancer Institute, 1066 CX Amsterdam,; ²Tytgat Institute for Liver and Intestinal Research and Department of Hepatology & Gastroenterology, Academic Medical Center, University of Amsterdam, Amsterdam; ³Division of Cell Biology, Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands; ⁴Division of Cardiovascular Medicine, The Gill Heart Institute and Department of Veterans Affairs Medical Center Lexington, KY 40511, United States

*These authors contributed equally

Introduction: Autotaxin (ATX) generates the bioactive lipid lysophosphatidic acid (LPA) involved in multiple (patho-)physiological processes, including cholestatic pruritus. ATX protein has a tripartite active site, combining a hydrophilic groove, a hydrophobic lipid-binding pocket, and a tunnel that was proposed to function as an exit route for the product LPA.

Methods: Recombinant ATX was generated using HEK 293 Flp-In cells and crystallized. X-ray crystallography data were analysed with designated software. ATX activity upon titration with various bile salts and steroids was determined by quantification of liberated choline.

Results: Crystallography revealed electron density in the tunnel of ATX that fitted best with a 7-hydroxysterol structure. We hypothesized that the tunnel could bind bile salts. Biochemical analysis established that TUDCA and TCDCA but not 12-OH bile salts (TCA and TDCA), nor other steroids (testosterone, dexamethasone, 7-hydroxy cholesterol), act as partial non-competitive inhibitors of ATX. Co-crystallization experiments confirmed that mechanism, showing simultaneously binding TUDCA in the tunnel and LPA in the pocket. TUDCA caused half-maximal inhibition at 9 µM and maximal inhibition of 60%. Inhibition of ATX was identical with unconjugated, taurine- and glycine-conjugated UDCA and CDCA. TUDCA also inhibited endogenous ATX activity in human serum with an apparent IC₅₀ of ~ 30 µM and maximal inhibition of 60%.

Discussion/Conclusion: Bile salts without a 12-OH group inhibit ATX activity, likely by blocking the tunnel as an LPA exit route. This may explain the beneficial effect of treatment with ursodeoxycholate in patients with intrahepatic cholestasis of pregnancy. In addition, it may explain the phenomenon that in PBC patients pruritus decreases with progression of cholestasis. Conversely, at low bile salt concentrations, inhibition of the LPA exit route through the tunnel might allow the ATX-LPA to travel over a larger distance so as to reach itch nerve endings and cause itch signalling.
The role of inflammation in the mechanism of bile acid-induced liver damage

Shi-Ying Cai and James L. Boyer
Yale Liver Center, Yale University School of Medicine, New Haven, CT 06510, USA

Background: Recent studies suggest that bile acid-induced liver injury may be initiated by a cytokine mediated inflammatory response rather than by their direct cytotoxic effects. The principle argument against a direct toxic effect is that toxic bile acids never reach sufficient levels in serum in cholestasis in-vivo as required to injure hepatocytes when exposed in-vitro (Woodbright and Jaeschke. World J Gastroenterol 18:4985’12).

Key Points: Instead, when pathophysiological relevant concentrations of bile acids are exposed to mouse hepatocytes and non-parenchymal cells, in-vitro, expression of cytokines such as Ccl2 and Cxcl2 increase in hepatocytes but not non-parenchymal cells in a time and dose dependent manner. Similar findings are seen with exposure of human hepatocytes to 50 uM GCDCA.

In Mdr2/- mice hepatic cytokine expression and neutrophil infiltration occur prior to the development of signs of liver injury consistent with a role for cytokine-induced inflammatory response as a cause of subsequent liver injury. In support of this concept, Ccl2/- mice are protected from cholestatic liver injury from 1% cholic acid feeding or bile duct ligation in association with a 50% reduction in hepatic neutrophil infiltration and near absence of necrosis. Neutrophils in periportal areas of livers from cholestatic patients correlated with their serum aminotransferase elevations, further supporting a role for inflammation in cholestatic liver injury in humans. Ntcp inhibitors block bile acid-induced cytokine expression indicating that bile acids must enter the hepatocyte to elicit this effect. Once within the hepatocyte, bile acids result in damage to mitochondria and ER stress. Studies from our laboratory suggest that mitochondrial DNA may be released and activate Toll-like receptor 9 as Tlr9 agonists and TCA synergistically activate expression of Ccl2. Knockout of TLR9 or its down-stream signaling partners, MyD88/Trif significantly diminish TCA induction of Cxcl2 in mouse hepatocytes. Liver injury was also reduced in Tlr9 knockout mice after BDL.

Conclusions: These findings establish a role for the innate immune system in cholestatic liver injury and point to new targets for therapy of cholestatic liver disease.
TGR5 and bile acid induced liver damage

Verena Keitel-Anselmino, Maria Reich, Lina Spomer, Caroline Klindt, Kathleen Deutschmann and Dieter Häussinger
Department of Gastroenterology, Hepatology and Infectious Diseases, Heinrich Heine University, Düsseldorf, Germany

The G-protein coupled bile acid and neurosteroid receptor TGR5 (GPBAR-1) is expressed in various tissues in humans and rodents, including liver [1]. In the liver the receptor is localized in different non-parenchymal cells, such as sinusoidal endothelial cells (LSEC), Kupffer cells (KC), cholangiocytes as well as in hepatic stellate cells (HSC), but not in hepatocytes [1]. Activation of TGR5 in LSEC has been shown to prevent bile acid (BA)-induced apoptosis as well as to promote generation of nitric oxide, thereby modulating liver microcirculation [1]. In KC stimulation of TGR5 induces anti-inflammatory effects, while TGR5 ligands promote cell proliferation, secretion and prevent BA-dependent apoptosis in cholangiocytes [1–4]. Therefore, it was speculated the receptor has protective effects in various forms of liver damage [1]. Using TGR5 knockout mice, it could be demonstrated that absence of TGR5 hampers liver regeneration following partial hepatectomy [5]. Furthermore, in obese mice, activation of TGR5 with a synthetic ligand reduced liver triglyceride and fatty acid levels and improved steatosis on histology [6].

To study the role of TGR5 in BA induced liver damage, which is found in cholestatic liver diseases, TGR5 knockout mice were either subjected to common bile duct ligation (CBDL) for 1–7 days or fed a diet supplemented with cholic acid (CA, 0.5%) for 7 days or lithocholic acid (LCA, 1%) for 4 days. CBDL as well as BA feeding led to a more pronounced liver damage with elevated transaminases in the absence of TGR5. CBDL and LCA feeding resulted in larger areas of necrosis in livers from TGR5 knockout animals. The observed increase in liver necrosis triggered by CBDL and LCA was accompanied by a rise in the mRNA-levels of various cytokines and chemokines. Several of those were differentially regulated in TGR5 KO mice as compared to wildtype littermates. While no difference in the elevation of total serum BA levels was detected between genotypes following CBDL and CA feeding, LCA feeding led to a significantly higher rise in BA levels in both liver and serum of TGR5 knockout mice as compared to wildtype littermates. A significantly lower mRNA expression of BA exporters OSTß and MRP2 was detected in livers of TGR5 knockout mice in comparison to wildtype mice. Furthermore, reduced excretion of BA via urine and faeces in the absence of TGR5 was observed following LCA feeding.

Proliferation of hepatocytes and cholangiocytes was significantly reduced in the absence of TGR5 as determined by immunohistochemical staining for PCNA and Ki67 following CBDL and BA feeding. Moreover, isolated cholangiocytes from TGR5 knockout mice were more susceptible to BA induced cell injury as determined by MTT assay as compared to wildtype derived cells. This may represent an important mechanisms to protect cholangiocytes, which are exposed to millimolar BA concentrations, from BA mediated cytotoxicity and may therefore alleviate liver damage under cholestatic conditions. In line with this hypothesis immunofluorescence staining of TGR5 in livers from patients with primary sclerosing cholangitis (PSC) as well as from Mdr2 knockout mice revealed a significant reduction of TGR5 protein levels while the fluorescence intensity
for the cholangiocyte marker proteins cytokeratin 7 and cytokeratin 19 were unchanged. Whether this downregulation of the receptor is mediated on the mRNA or protein level as well as the underlying signaling pathways need to be determined.

BA feeding and CBDL resulted in more pronounced liver damage in TGR5 knockout mice as compared to wildtype littermates, suggesting that TGR5 exerts protective effects in models of BA overload and cholestatic liver diseases.

References:

Oral presentation of poster abstract

**Activation of intestinal bile acid receptor FXR induces membrane G protein-coupled bile acid receptor TGR5 expression and stimulates GLP-1 secretion to ameliorate metabolic disorders in diabetic mice**

John Y.L. Chiang, Preeti Pathak, Hailiang Liu and Shannon Boehme
Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH 44272, USA

**Introduction:** Bile acid receptors, farnesoid X receptor (FXR) and G-protein-coupled receptor TGR5, are co-expressed in the enteroendocrine L cells. Activation of TGR5 stimulates intestinal glucagon-like-peptide-1 (GLP-1) secretion and improves insulin sensitivity. The aim of this study is to test whether activation of intestinal FXR stimulates GLP-1 secretion and ameliorates diabetes in mice.

**Methods:** Fxr⁻/⁻, Tgr5⁻/⁻ and db/db mice were used to study the effects of an intestine FXR agonist fexaramine (FEX) on GLP-1 secretion, and glucose and insulin tolerance.

**Results:** Both FXR and TGR5 agonists stimulated intestine GLP-1 secretion, cAMP activity and intracellular Ca²⁺ uptake. Interestingly, FXR agonists induced TGR5 mRNA levels in wild type but not Fxr⁻/⁻ mice. A FXR responsive element was identified on the Tgr5 gene promoter by reporter assay and chromatin immunoprecipitation assay. Both Fxr⁻/⁻ and Tgr5⁻/⁻ mice had reduced GLP-1 secretion compared to wild type mice. Small interference RNA knockdown of both FXR and TGR5 in STC-1 cells abolished GLP-1 secretion. GLP-1 infusion in wild type mice and FEX treatment in db/db mice stimulated AKT and PKCζ phosphorylation, reduced hepatic triglycerides and gluconeogenic gene expression, and improved glucose and insulin tolerance.

**Discussion/Conclusion:** Intestinal FXR plays a key role in inducing Tgr5 expression and GLP-1 secretion to improve hepatic steatosis and insulin sensitivity in diabetic mice. This is the first report of transcriptional regulation of Tgr5 gene expression by intestinal FXR and activation of both intestinal FXR and TGR5 may coordinately regulate hepatic metabolism and protect against inflammatory intestine and liver diseases such as inflammatory bowel diseases, non-alcoholic fatty liver disease, obesity and diabetes.
Colonization of germ-free mice with a human microbiota induces FXR signaling

Annika Wahlström¹, Petia Kovatcheva-Datchary¹, Marcus Ståhlman¹, Hanns-Ulrich Marschall¹*, Fredrik Bäckhed¹,²*
¹Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine and Wallenberg Laboratory, University of Gothenburg, S-413 45 Gothenburg, Sweden; ²Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, DK-2200, Denmark; *Shared senior authorship

Introduction: The gut microbiota influences the development and progression of metabolic diseases, partly by metabolism of bile acids and modified signaling through the farnesoid X receptor (FXR). Mice that are colonized with a human microbiota can be used to study the effect of human bacteria on metabolic functions, and in this study we aim to determine how the human gut microbiota metabolizes murine bile acids and affects FXR signaling in colonized mice.

Methods: We colonized germ-free mice with fresh caecal content from a mouse donor or pre-frozen or fresh faeces from a human donor. We analyzed the gut microbiota and bile acid composition and expression of FXR target genes in ileum and liver.

Results: Caecal microbiota composition differed between mice colonized with mouse and human microbiota and the freezing process also affected microbiota composition in the humanized mice. Human and mouse microbiota reduced total bile acid levels similarly but the humanized mice produced less secondary bile acids. The human microbiota was able to induce expression of FXR target genes Fgf15 and Shp in ileum and reduce expression of Cyp7a1 in the liver. Colonization with frozen human faeces resulted in higher ratio between FXR agonistic and FXR antagonistic bile acids and higher expression of the FXR target genes compared with fresh human faeces.

Discussion/Conclusion: We show that a human microbiota can change bile acid composition and induce FXR signaling in colonized mice, but the levels of secondary bile acids produced are lower than in mice colonized with a mouse microbiota.
Session IV

Bile acid transporters: Role in health and disease
Targeting Ntcp to treat metabolic and cholestatic diseases

Stan F.J. van de Graaf
Department of Gastroenterology and Hepatology, Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

NTCP is responsible for the majority of hepatic uptake of conjugated bile acids from the portal circulation. Furthermore, NTCP forms the liver-specific receptor for Hepatitis B and Delta viruses. Myrcludex B, a lipopeptide that effectively inhibits NTCP, is currently exploited to block viral entry, forming a possible treatment option for HBV/HDV. The presentation will show evidence obtained in mice (either Ntcp knockout or Myrcludex B treated) for two additional potentially beneficial consequences of NTCP inhibition. For decades bile acids were believed to fulfill a rather passive role in metabolism as powerful detergents. This property is essential for the solubilization and absorption of dietary lipids and fat-soluble vitamins, although detrimental when bile acids accumulate inside hepatocytes. In cholestatic conditions, pharmacological NTCP inhibition has beneficial effects likely caused by reduced hepatotoxic accumulation of bile acids and stimulated urinary bile acid excretion. In addition, since the discovery of specialized bile acid-sensing proteins, such as the farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor (TGR5/GPBAR1), it has become clear that bile acids also have beneficial signaling effects which could be exploited to improve human health. We postulate that inhibition of NTCP (gene name SLC10A1) provides an excellent novel strategy to improve human health as it determines the duration of bile acid signalling by controlling how fast bile acids are removed from serum after a meal. This forms the third possible advantage of NTCP inhibition. We could indeed demonstrate that prolonged presence of bile acids in the circulation is sensed in the ileum to activate the gut-liver axis via FGF15, in line with intestine-specific FXR activation. In contrast, NTCP inhibition acutely reduces hepatic FXR activation. Consequences of NTCP inhibition on cholesterol and bile acid dynamics will be presented.
Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: A new inborn error of metabolism with a complex phenotype

Frédéric M. Vaz1,*, Coen C. Paulusma2,*, Hidde Huidekoper3, Filip van Herpe4, Minke de Ru3, Cynthia Lim5, Janet Koster1, Kam Ho-Mok2, Albert H. Bootsma1, Albert K. Groen6, Frank G. Schaap2,7, Ronald P.J. Oude Elferink2, David Cassiman8, Hans R. Waterham1 and Ronald J.A. Wanders1

1Laboratory Genetic Metabolic Disease, Academic Medical Center, Amsterdam, The Netherlands
2Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands
3Department of Pediatrics, Academic Medical Center, Amsterdam, The Netherlands
4Department of Internal Medicine, University Hospitals Leuven, Belgium
5Department of Pediatrics, Waterlandziekenhuis, Purmerend, The Netherlands
6Department of Pediatrics, University of Groningen, University Medical Center Groningen, The Netherlands
7Department of Surgery, NUTRIM School of Nutrition, Toxicology and Metabolism, Maastricht University, Maastricht, The Netherlands
8Department of Hepatology and Metabolic Center, University Hospitals Leuven, Belgium

*Both authors contributed equally to this work.

The enterohepatic circulation of bile salts is an important physiological route to recycle bile salts and ensure intestinal absorption of dietary lipids. The Na⁺-taurocholate cotransporting polypeptide SLC10A1 (NTCP) plays a key role in this process as the major transporter of conjugated bile salts from the plasma compartment into the hepatocyte. Here we present two patients with NTCP deficiency. The first was a child of 2 years old, clinically characterized by mild hypotonia, growth retardation and delayed motor milestones. Total bile salts in plasma were extremely elevated (up to 1500 µM, ref. <16.3) but there were no clinical signs of cholestatic jaundice, pruritus or liver dysfunction. Bile salt synthesis and intestinal bile salt signaling were not affected as evidenced by normal plasma 7α-hydroxy-4-cholesten-3-one (C4) and FGF19 levels. Importantly, the presence of secondary bile salts in the circulation suggested residual enterohepatic cycling of bile salts. Sequencing of the SLC10A1 gene revealed a single homozygous non-synonymous point mutation in the coding sequence of the gene resulting in an arginine to histidine substitution at position 252. Functional studies showed that this mutation resulted in a markedly reduced uptake activity of taurocholic acid. Immunofluorescence studies and surface biotinylation experiments demonstrated that the mutant protein is virtually absent from the plasma membrane. The second patient is a recently identified 30-year old female with molecularly confirmed NTCP deficiency causing persistently raised bile salts, tentatively associated with fetal loss in late pregnancy. In conclusion we describe the identification of NTCP deficiency as a new inborn error of metabolism with a relatively mild clinical phenotype but with possible negative effects on the fetus during pregnancy of an affected female. The identification of NTCP deficiency confirms that this transporter is the main import system for conjugated bile salts into the liver but also indicates that auxiliary transporters are able to sustain the enterohepatic cycle in its absence.
List of Abbreviations:

NTCP: Na\(^+\)-taurocholate cotransporting polypeptide
PFIC: progressive familial intrahepatic cholestasis
CA: cholic acid
CDCA: chenodeoxycholic acid
UDCA: ursodeoxycholic acid
DHCA: dihydroxycholestanoic acid
THCA: trihydroxycholestanoic acid
DCA: deoxycholic acid
Roles of ileal ASBT and OSTα-OSTβ in regulating the FXR/FGF15 pathway and bile acid-induced injury

Paul A. Dawson
Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322 USA

The enterohepatic circulation of bile acids is maintained by specific transporters expressed on the sinusoidal (Na+-taurocholate co-transporting polypeptide; NTCP; SLC10A1) and canalicular (Bile Salt Export Pump; BSEP; ABCB11) membranes of the hepatocyte, and the apical brush border (Apical sodium-dependent bile acid transporter; ASBT; SLC10A2) and basolateral (Organic solute transporter alpha-beta; OSTα-OSTβ; SLC51A-SLC51B) membranes of the ileal enterocyte. Whereas the regulatory and pathophysiologic effects of a block in hepatic canalicular bile acid export have been studied, little is known regarding the consequences of ileal enterocyte bile acid stasis. In ileum, bile acids activate the nuclear receptor FXR, which stimulates synthesis of the enterokine Fibroblast Growth Factor 15/19, a potent repressor of hepatic Cyp7a1 expression and bile acid synthesis. Inhibition or inactivation of the ASBT blocks intestinal bile acid absorption, suppresses ileal FXR signaling, and induces hepatic bile acid synthesis but does not appear to otherwise affect small intestinal morphology or ileal histology. By contrast, ileal enterocyte bile acid stasis secondary to inactivation of the basolateral membrane transporter OSTα in mice suppresses hepatic bile acid synthesis and markedly alters ileal morphology and histology. To determine the role of bile acids and FXR-mediated signaling in this response to ileal bile acid stasis, we examined the temporal relationship between the ileal morphological changes and initiation of the enterohepatic circulation of bile acids and the effect of inactivation of FXR or the ASBT in Ostα null mice. The findings suggest that loss of OSTα-OSTβ leads to early oxidative injury and an epithelial restitution response in ileum that is secondary to ileal enterocyte bile acid stasis.
ASBT inhibitors in PBC and PSC

Prof. Gideon Hirschfield
Professor of Autoimmune Liver Disease, University of Birmingham, UK

Cholestatic liver diseases such as PBC and PSC are characterised by a complex interaction between initiating but persisting inflammatory injury to the biliary tree, alongside profound but significant cholestasis. The cholestatic response is both protective as a means of ameliorating hepato-biliary injury, but equally represents a powerful inflammatory and pro-fibrotic stimulus itself. Additionally the characteristic and troublesome symptom of itch is driven by biliary injury and cholestasis, with evidence that total naso-biliary drainage does impact on pruritus severity in extreme instances.

Bile acids (BA) synthesised in the liver undergo a highly efficient enterohepatic circulation. In so doing there is re-uptake of nearly 95% of bile acids from the terminal ileum, in an active process co-ordinated at the ileal brush border by the membrane apical sodium-dependent bile salt transporter (ASBT). Intracellular bile acid transport is mediated by the ileal bile-acid binding protein (IBABP) cytoplasmically attached to ASBT, and BAs are secreted into the portal blood by organic solute transporter (OST) alpha/beta. Activation of FXR in the distal ileum down-regulates ASBT, induces expression of IBABP and OST alpha/beta.

There are a number of proposed mechanisms by which inhibition of ileal BA absorption may be of relevance as a future therapy in cholestatic liver diseases. ASBT inhibitors are expected to block the uptake of BAs in the terminal ileum, increase their excretion in the faeces and decrease the amount of BAs returning to the liver via enterohepatic circulation. As a result ASBT inhibition leads to a fall in total bile acid levels and FGF19 concentrations with evidence of compensatory increase in bile acid synthesis (C4) as well as reduction in total and LDL cholesterol. Other relevant changes include possible colonic mechanisms, with potential change to the gut microbiota as well as possibly changes in GLP-1 expression: heightened BA delivery to distal parts of gut, could induce GLP-1 release through direct as well as TGR5 activation.

With emergence of a variety of agents suitable for human trials, the utility of inhibition of the ASBT transporter is now being explored across PBC and PSC (as well as NASH), with a view to both reducing cholestatic/liver injury, but also addressing pruritus management.
Oral presentation of poster abstract

Effect of intrahepatic cholestasis of pregnancy on maternal glucose homeostasis

Elena Bellafante¹, Vanya Nikolova¹, Jenny Chambers¹, Marcus Martineau¹, Catherine Williamson¹ ¹Maternal and Fetal Disease Group, Women's Health Division, King's College London, UK

Introduction: Gestational Diabetes mellitus (GDM) develops in women with no previous history of glucose intolerance or insulin resistance during late pregnancy. Beside complications during gestation, GDM is associated with longer term complications for both the mother and the child such as metabolic syndrome and TD2. Women with intrahepatic Cholestasis of Pregnancy (ICP), characterised by abnormal liver function tests with elevated bile acid (BA) levels, have an increased risk of developing GDM. In this study we investigated if ICP contributes to the development of GDM by influencing the enteroinsular axis and the physiological adaptations of pancreatic islets.

Methods: GLP1 was assayed using ELISA in women with ICP and controls. C57/Blk6 mice fed with chow diet ± CA, FxrKO, Tgr5WT, and Tgr5KO were sacrificed at days 15 and 18 of pregnancy. GTT and ITT were performed at day 18 and 19, respectively.

Results: ICP women had lower postprandial GLP1 levels. The human results were supported by CA-fed mice that showed a reduction of Gcc mRNA. LCA and UDCA, natural ligands for TGR5, are increased in faeces of chow-fed mice but decreased during CA feeding, suggesting that TGR5 signalling is dampened in ICP. In line, Tgr5KO mice showed impaired GTT and insulin secretion in advanced pregnancy. Moreover, ablation of FXR, whose activity is dampened during ICP, caused glucose intolerance and insulin resistance. Physiological islet expansion and β-cell proliferation were reduced in pregnant CA-fed and FxrKO mice, and TUNEL assay showed increased apoptotic rate in islets from these mice.

Discussion/Conclusion: A cholestatic environment affects intestinal BA signalling interfering with GLP1 secretion and physiological β-cell expansion during pregnancy. These data provide a potential explanation for the increased risk of GDM and reduced GLP1 in ICP, suggesting that FXR and TGR5 deregulation increases susceptibility of women with ICP to developing GDM.
Session V

Bile acid receptors and bile acid signaling as therapeutic targets
Bile acids in polycystic liver diseases: Triggers of disease progression and/or potential solution for treatment?

Jesus M. Banales, PhD
Head, Liver Diseases Group, Biodonostia Research Institute, Donostia University Hospital, San Sebastián, Spain

Polycystic liver diseases (PLDs) are a group of hereditary/genetic cholangiopathies characterized by the development and progressive growth of hepatic cysts. Current therapies are based on surgical and pharmacological strategies, but they show short-term and modest beneficial effects. Therefore, it is necessary to determine in detail the molecular mechanisms triggering the pathogenesis of PLDs in order to find new potential targets for therapy. Ductal plate malformation during embryogenesis and abnormal cystic cholangiocyte growth and secretion are key mechanisms involved in the pathogenesis. Recently, the discovery of the presence of bile acids in the cystic fluid of PLD patients and the intrahepatic accumulation of cytotoxic bile acids in an animal model of PLD (i.e. PCK rat) point out their potential role in the pathogenesis of these diseases. On the other hand, ursodeoxycholic acid (UDCA) has emerged as a new potential therapy for PLDs showing beneficial effects both in PCK rats and in highly symptomatic patients with autosomal dominant polycystic kidney disease (ADPKD: most common type of PLD). Chronic treatment with UDCA normalizes the decreased intracellular calcium levels of cystic cholangiocytes, which results in inhibition of their hyperproliferation. Here, the role of certain bile acids in the pathogenesis of PLD and the potential therapeutic value of UDCA for the treatment of PLD will be highlighted. Moreover, future directions on the field will be discussed.
norUrsodeoxycholic acid in primary sclerosing cholangitis and non-alcoholic fatty liver disease

Michael Trauner, MD
Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria;
E-Mail: michael.trauner@meduniwien.ac.at

24-norUrsodeoxycholic acid (norUDCA) is a side-chain shortened derivate of ursodeoxycholic acid (UDCA) and lacks a methylene group in its side chain. This side-chain shortening results in relative resistance to amidation with taurine or glycine compared with UDCA. Consequently, norUDCA undergoes cholehepatic shunting (instead of undergoing a full enterohepatic circulation) resulting in ‘ductular targeting’ to bile ducts/ductules and hepatic enrichment. Importantly, cholehepatic shunting also results in a bicarbonate-rich hypercholeresis which counteracts bile acid toxicity and reinforces the biliary ‘bicarbonate umbrella’. As proof of principle, taurin-conjugated norUDCA lacks cholehepatic shunting with loss of its therapeutic efficacy. In addition, norUDCA is more hydrophilic and thereby even less toxic than its mother compound UDCA which may further help to counteract (intrinsic) biliary toxicity. As such, norUDCA (but not “conventional” UDCA) reverses sclerosing cholangitis in the experimental Mdr2/Abcb4 knockout mouse (Mdr2/Abcb4 -/-) cholangiopathy model for (primary) sclerosing cholangitis (PSC) while UDCA aggravates bile infarcts in cholestatic conditions with (complete or partial) biliary obstruction. Notably, neither norUDCA nor its mother compound UDCA have relevant affinities for dedicated bile acid receptors such as FXR or TGR5, although norUDCA has been shown to increase FXR acetylation by attenuation of SIRT-1. Moreover, norUDCA has anti-lipotoxic, anti-proliferative, anti-fibrotic as well as anti-inflammatory effects which complement stimulation of bile acid detoxification and induction of alternative bile acid export via the basolateral membrane. norUDCA also stimulates autophagy and attenuates liver injury in a mouse model of A1AT deficiency. Moreover, a recent study demonstrated beneficial effects of norUDCA (but not UDCA) on granuloma size and hepatic fibrosis in a mouse model of Schistosoma mansoni infection as world-leading cause of hepatic fibrosis and portal hypertension. The anti-inflammatory properties of norUDCA were directed to MHC class II protein expression on dendritic cells and macrophages and norUDCA reduced T-lymphocyte proliferation and serum levels of pro-fibrogenic Th2 cytokines IL-13 and IL-4. Such mechanisms could also contribute to direct anti-inflammatory and anti-fibrotic effects of norUDCA in non-cholestatic conditions.

Based on these encouraging experimental data in preclinical models and successful completion of phase I studies, norUDCA was recently tested in a double-blind, randomized, multicenter, placebo-controlled, comparative, exploratory phase II dose-finding trial in the treatment of PSC (sponsor: Dr. Falk Pharma GmbH). The primary objectives was (i) to evaluate the efficacy of three doses of norUDCA (500, 1,000 and 1,500 mg/d) vs. placebo for the treatment of PSC over 12 weeks and (ii) to identify the optimal dose of norUDCA for the treatment of PSC for further evaluation in phase III studies. Additional secondary objectives included to study the safety and tolerability of norUDCA. Patients were enrolled into the trial either as UDCA naive patients or after an UDCA-washout period of at least 8 weeks prior to baseline. This trial recruited 161 patients from 45 centers across 12 European countries. Baseline demographic data
did not differ between treatment groups. The primary efficacy endpoint was the mean relative change (%) in serum alkaline phosphatase (ALP) levels between the baseline visit and the end-of-treatment (EOT) visit. norUDCA reduced reduced serum ALP in a dose dependent fashion by 12, 17 and 26% in the 500mg/d, 1000mg/d and 1500 mg/d group, respectively. Similar does-dependent results were found for secondary endpoints such as GGT, ALT and AST, or the rate of patients with s-AP levels < 1.5 ULN at EOT. The safety profile of all tested norUDCA doses was excellent and did not differ from the placebo group. Importantly, pruritus occurred at similar and all over at low frequencies in all groups. Based on these encouraging results, a phase III trial in PSC is already in preparation.

Due to its broad mechanisms of action, norUDCA has considerable clinical potential in a wide range of cholestatic conditions/cholangiopathies beyond PSC, in particular conditions where defects of the biliary bicarbonate umbrella or MDR3/ABCB4 (directly corresponding to the Mdr2/Abcb4 -/- mouse model) as well as biliary toxicity may be involved. This may include primary biliary cirrhosis (PBC), various forms of secondary sclerosing cholangitis, prevention/treatment of non-anastomotic strictures after liver transplantation, progressive familial intrahepatic cholestasis (in particular PFIC-3 and LPAC-syndrome caused by MDR3/ABCB4 defects) and cystic fibrosis among others.

In addition to cholestatic disorders, norUDCA may also have beneficial therapeutic effects in metabolic liver diseases such as non-alcoholic fatty liver disease (NAFLD). As such, norUDCA has shown beneficial effects in various genetic and dietary mouse models of NAFLD/NASH including NEMO-/- mice (spontaneously developing NASH) and ApoE-/- mice on Western diet (developing both hepatic steatosis and atherosclerosis). Since enhanced mortality due to cardiovascular disease (CVD) is of major prognostic relevance in patients with NAFLD/NASH, therapeutic strategies aiming at both disorders are of key interest. norUDCA significantly reduced hepatic triglyceride content, hepatic inflammation and aortic plaques surface area in Western chow-fed ApoE-/- mice. These findings may open novel treatment strategies for NAFLD and CVD by targeting both conditions by norUDCA. As a result of these promising properties in preclinical models of NAFLD/NASH, a double-blind, randomized, placebo-controlled, phase II dose-finding study comparing different doses of norUDCA with placebo in the treatment of NAFLD (EudraCT Number: 2013-004605-38) has been initiated and the results are eagerly awaited.
Oral presentation of poster abstract

Cholic acid treatment in Zellweger spectrum disorders

Femke C.C. Klouwer¹,², Kevin Berendse¹,², Bart G.P. Koot³, Elles M. Kemper⁴, Frank Schaap⁵, Hans R. Waterham², Frédéric M. Vaz⁵, Marc Engelen¹, Peter M.L. Jansen⁶, Ronald J.A. Wanders², Bwee Tien Poll The¹

¹Department of Paediatric Neurology, Emma Children’s Hospital/Academic Medical Center, University of Amsterdam, The Netherlands; ²Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, The Netherlands; ³Department of Paediatric Gastroenterology, Emma Children’s Hospital/Academic Medical Center, University of Amsterdam, The Netherlands; ⁴Department of Pharmacy, Academic Medical Center, University of Amsterdam, The Netherlands; ⁵Department of Surgery, Maastricht University, The Netherlands; ⁶Department of Gastroenterology and Hepatology, Academic Medical Center, University of Amsterdam, The Netherlands

Introduction: Zellweger spectrum disorders (ZSDs) are a group of severe disabling congenital disorders resulting from a failure in peroxisome assembly, caused by autosomal recessive mutations in one of the PEX genes. At least some of the progressive and irreversible clinical abnormalities in these patients are caused by the accumulation of toxic bile acid intermediates. We hypothesize that cholic acid supplementation in these patients can stabilize progression of liver dysfunction by suppressing the endogenous bile acid synthesis and thereby decrease the accumulation of toxic and cholestatic bile acid intermediates.

Methods: Nineteen ZSD patients were followed longitudinally prior to start of the treatment during a run in period of two years. Subsequently, all patients were treated with cholic acid during a 9-month treatment period. The bile acid spectrum and liver functions were analyzed in plasma at start, 4, 12 and 36 weeks after start of treatment. Fibroblast growth factor 19 (FGF19) and 7-alpha-hydroxy-4-cholesten-3-one (C4), as markers for feedback of the endogenous bile acid synthesis, were analyzed at start, 12 and 36 weeks after start of treatment.

Results: Both dihydroxycholestanoic acid (DHCA) and trihydroxycholestenoic acid (THCA) levels significantly decreased after 4, 12 and 36 weeks of cholic acid treatment. FGF19 and C4, respectively significantly increased and significantly decreased after 12 and 36 weeks of treatment. In patients suffering from liver cirrhosis (n = 4), cholic acid supplementation resulted in progressive cholestasis. One patient had to be excluded, due to persistent elevated bilirubin levels. No difference in liver enzymes was observed in the group of patients without liver cirrhosis.

Discussion/Conclusion: Treatment of cholic acid resulted in suppression of bile acid synthesis in the majority of the patients. However, it can be potentially harmful for patients suffering from severe liver disease, leading to progressive cholestasis. A prolonged treatment period is needed to investigate if cholic acid treatment can alter clinical outcome.
NorUDCA reduces liver injury and improves the metabolic state in mouse models of obesity and steatosis

Daniel Steinacher¹, Thierry Claudel¹, Elisa Einwallner², Tatjana Stojakovic³ and Michael Trauner¹
¹Hans Popper Laboratory of Molecular Hepatology Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria
²Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria
³Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Introduction: NorUDCA is a side-chained shortened derivative of ursodeoxycholic acid improving liver injury in mouse models of cholestatic liver injury. We aim to explore whether NorUDCA improves hepatic steatosis in mouse models of obesity and steatosis.

Methods: ob/ob mice received either a diet supplemented with 0.5% NorUDCA or chow for 6 weeks. wt/wt mice received either high-fat-diet (HFD) supplemented with 0.5% NorUDCA as prevention arm for 29 weeks or HFD alone for 29 weeks or HFD for 22 weeks following 7 weeks of HFD supplemented with 0.5% NorUDCA as treatment arm. We used metabolic cages, IPGTT and IPITT for metabolic characterizations. Food and water intake as well as bodyweight were recorded weekly. Serum biochemistry, liver histology, mRNA and protein expression were analysed.

Results: ob/ob mice treated with NorUDCA showed a significant reduction in serum AST, ALT and AP levels. mRNA expression of inflammatory markers (F4/80, Mcp1 and Tnfα) were reduced in liver. Furthermore, ER stress markers (Grp78, Chop, sXbp1 and ErDj4) were lowered. WAT/bodyweight ratio was increased in NorUDCA group, non-esterified fatty acids decreased as well as markers for improved lipid storage function Pparg2, Mpges1 and Fabp4 induced. IPGTT uncovered a significantly faster blood glucose clearance at 60 and 90 min. Despite unchanged hepatic TG content, serum TG levels were increased.

The prevention arm with NorUDCA in the DIO setting shows a clear reduction in body weight (37%), partially explained by a reduced food intake. The treatment arm shows already within 4 weeks a bodyweight reduction by 13% (despite pair feeding). The analysis of this experimental arm is still ongoing and will be available at the time of the meeting.

Discussion/Conclusion: NorUDCA treatment improves liver cell injury via reducing NASH features such as inflammation and ER stress. Moreover, we observed similar beneficial effects on WAT, resulting in an overall improved metabolic situation.
List of Chairpersons, Speakers and Scientific Organizers

Dr. Matias A. Avila  
Terapia Génica y Hepatologia  
Center for Applied Medical Research (CIMA)  
University of Navarra  
Clinica Universitaria Pamplona  
Avda. Pio XII 55  
31008 Pamplona  
Spain

Dr. Jesus M. Banales  
Department of Liver and Gastrointestinal Diseases  
Biodonostia Health Research Institute  
Donostia University Hospital  
Paseo del Doctor Beguiristain  
20014 San Sebastián  
Spain

Dr. Elena Bellafante  
Women’s Health Division  
King’s College London  
Hodgkin Building  
Guy’s Campus  
Maternal and Fetal Disease Lab.  
SE11UL London  
Great Britain

Prof. Dr. Ulrich Beuers  
Department of Gastroenterology and Hepatology, G4-216  
Tytgat Institute for Liver and Intestinal Research  
University of Amsterdam  
Meibergdreef 9  
1105 AZ Amsterdam  
The Netherlands

Dr. Ruth Bolier  
Tytgat Institute for Liver and Intestinal Research  
Amsterdam Medical Center  
Meibergdreef 69–71  
1105 BK Amsterdam  
The Netherlands

James L. Boyer, M.D.  
Professor of Medicine  
Liver Research Center, LMP 1080  
School of Medicine  
Yale University  
333 Cedar Street  
New Haven, CT 06510  
USA

John Y.L. Chiang, Ph.D.  
Distinguished University Professor  
Department of Integrative Medical Sciences  
Northeast Ohio Medical University  
4209 SR 44  
Rootstown, OH 44272  
USA

Paul A. Dawson, Ph.D.  
Professor of Pediatrics  
Department of Pediatric Gastroenterology, Hepatology and Nutrition  
Emory University School of Medicine  
Health Sciences Research Bldg.  
Suite E200  
1760 Haygood Drive  
Atlanta, GA 30322-1015  
USA

Ronald M. Evans, Ph.D.  
Professor of Biology  
The Salk Institute for Biological Studies  
Gene Expression Laboratory  
10010 North Torrey Pines Road  
La Jolla, CA 92037  
USA

Prof. Dr. Peter Fickert  
Innere Medizin I und III  
Medizinische Universität Graz  
Auenbruggerplatz 15  
8036 Graz  
Austria
Dr. Cormac G.M. Gahan  
Alimentary Pharmabiotic Centre  
School of Microbiology  
School of Pharmacy  
Food Science Bldg., Room 431  
University College Cork  
Cork  
Ireland

Frank J. Gonzalez, M.D.  
Laboratory of Metabolism  
National Cancer Institute  
National Institutes of Health  
Bldg. 37, Room 3E 24  
10 Center Drive  
Bethesda, MD 20892  
USA

Markus Grompe, M.D.  
Professor of Medicine  
Department of Molecular and Medical Genetics  
Oregon Health Sciences University  
3181 SW Sam Jackson Park Road  
Portland, OR 97201  
USA

Prof. Dr. Dieter Häussinger  
Klinik für Gastroenterologie, Hepatologie und Infektiologie  
Universitätsklinikum Düsseldorf  
Heinrich-Heine-Universität  
Moorenstr. 5  
40225 Düsseldorf  
Germany

Prof. Dr. Jan G. Hengstler  
Leibniz-Institut für Arbeitsforschung an der TU Dortmund  
Ardeystr. 67  
44139 Dortmund  
Germany

Prof. Dr. Gideon Hirschfield  
Center of Liver Research  
Institute of Biomedical Research  
University of Birmingham  
P.O. Box 363  
Birmingham B15 2TT  
Great Britain

Alan F. Hofmann, M.D., Ph.D.  
Professor of Medicine (Emeritus)  
5870 Cactus Way  
La Jolla, CA 92037  
USA

Prof. Dr. Peter L.M. Jansen  
Department of Gastroenterology and Hepatology  
NUTRIM School of Nutrition and Translational Research in Metabolism  
Maastricht University  
P.O. Box 616  
6200 MD Maastricht  
The Netherlands

Prof. Dr. Verena Keitel-Anselmino  
Klinik für Gastroenterologie, Hepatologie und Infektiologie  
Universitätsklinikum Düsseldorf  
Heinrich-Heine-Universität  
Moorenstr. 5  
40225 Düsseldorf  
Germany

Prof. Dr. Dietrich Keppler  
Deutsches Krebsforschungszentrum  
Im Neuenheimer Feld 280  
69120 Heidelberg  
Germany

Dr. Femke C.C. Klouwer  
Emma Children’s Hospital  
Department of Pediatric Neurology and Department of Pediatric Neurology/Laboratory Genetic Metabolic Diseases (F0-116)  
Academic Medical Center  
University of Amsterdam  
Meibergdreef 9  
1105 AZ Amsterdam  
The Netherlands
Rohit Kohli, M.D.
Professor of Medicine
Cincinnati Children’s Hospital
Pediatric Gastroenterology
3333 Burnet Ave.
MLC 2010
Cincinnati, OH 45229
USA

Nicholas F. LaRusso, M.D.
Professor of Medicine and Biochemistry
Gastroenterology Unit
Mayo Clinic
Box 0001
200 First Street SW
Rochester, MN 55905
USA

David D. Moore, Ph.D.
Department of Molecular and Cellular Biology
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030
USA

Dr. Raj Mookerjee
The Institute of Hepatology
69-75 Chenies Mews
London WC1E 6HX
Great Britain

Prof. Dr. Antonio Moschetta
IRCCS National Cancer Center
Università di Bari
Viale Orazio Flacco 65
70124 Bari
Italy

Prof. Dr. Ronald P.J. Oude Elferink
Tytgat Institute for Liver and Intestinal Research
Academic Medical Center S1-162
University of Amsterdam
Meibergdreef 69–71
1105 BK Amsterdam
The Netherlands

Dr. Sander Rensen
Department of Surgery
Medical Centre
Maastricht University
P.O. Box 616
The Netherlands

Dr. Motoko Sasaki
Kanazawa University
Graduate School of Medical Science
Department of Human Pathology
Takarmachi 13–1
Kanazawa 920-8640
Japan

Dr. Daniel Steinacher
Hans Popper Labor für Molekulare Hepatologie
Medizinische Klinik III
Medizinische Universität Wien
Währinger Gürtel 18–20
1090 Vienna
Austria

Prof. Dr. Michael Trauner
Klinische Abteilung für Gastroenterologie und Hepatologie
Medizinische Universität Wien
Währinger Gürtel 18–20
1090 Vienna
Austria

Dr. Stan F.J. van de Graaf
Assistant Professor
Tytgat Institute for Liver and Intestinal Research
Academic Medical Center S1–166
University of Amsterdam
Meibergdreef 69–71
1105 BK Amsterdam
The Netherlands

Dr. Fred M. Vaz
Laboratory Genetic Metabolic Diseases (Fo-224)
Academic Medical Center
University of Amsterdam
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Dr. Annika Wahlström
Sahlgrenska Academy
Institute of Medicine
Department of Molecular and Clinical Medicine
Wallenberg Laboratory
Bruna Straket 16
41345 Gothenburg
Sweden
POSTER ABSTRACTS

Poster Numbers 1 – 93

Author Index to Poster Abstracts
Pregnancy alters the liver transcriptome to engage gestational metabolic and inflammatory pathways

Shadi Abu-Hayyeh and Catherine Williamson
Women’s Health Academic Centre, King’s College, London, UK

Introduction: Pregnancy is characterised by an increase in the concentrations of bile acids and lipids, which is a necessary physiological response to support the demands of the developing fetus. However, genetic and environmental factors can perturb energy homeostasis resulting in maternal metabolic diseases.

The liver is a major site for bile and lipid metabolism. It is important to identify the hepatic metabolic pathways that are sensitive to pregnancy signals that underpin the gestational changes in bile and lipid metabolism, and are of relevance to maternal metabolic disease.

Methods: Blood was sampled longitudinally from 10–12 week old pregnant C57BL/6 mice and analysed for lipid concentrations. Two time-points preceding profound serum lipid changes were chosen to study the liver transcriptome.

Illumina microarray analysis of liver transcriptome (n = 6/group) undertaken to identify gene expression changes; Inclusion criteria: P ≤ 0.05 with ± 1.25-fold comparison cut-off. Gene ontology and upstream regulators of transcriptomic changes assessed using Ingenuity Pathway Analysis (IPA).

Results: Gestational days 4 (GD4) and GD11 precede a decrease and increase in serum lipid levels respectively, and thus are time-points of interest.

Hepatic transcriptomic analysis of GD0 (non-pregnant), GD4 and GD11 revealed clustering according to groups in a principal component analysis and resulted in 29 GD4 versus GD0 changes and 1611 GD11 versus GD0 changes, 11 shared between the two comparison groups.

IPA predicted enrichment for the GD11 versus GD0 changes in ‘immune system’ and ‘metabolic processes’. The upstream regulators of the changes modulate chemical metabolism, transcription and inflammation pathways. Interestingly, regulators of inflammation were activated whilst metabolic regulators, including modulators of FXR and LXR pathways, inhibited.

Discussion/Conclusion: These data reveal that pregnancy signals alter the expression profile of the liver, modulating networks of pathways involved in inflammation and metabolism, contributing to the biochemical profile of the maternal milieu.
The role of necroptosis in acute and chronic cholestasis

Marta B. Afonso¹, Pedro M. Rodrigues¹, André L. Simão¹, Helena Cortez-Pinto², Dimitry Ofengeim³, Joana D. Amaral¹, Rui E. Castro¹, Junying Yuan³, Cecília M.P. Rodrigues¹

¹Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; ²Gastroenterology, Hospital Santa Maria; Lisbon, Portugal; ³Department of Cell Biology, Harvard Medical School, Boston, MA, USA

Introduction: Cholestasis is a pathological condition characterized by disruption of bile flow, resulting in hepatic toxicity and inflammation. Necroptosis, a necrotic cell death pathway regulated by receptor-interacting protein 3 (RIP3), may mediate cell death and inflammation in the liver. We aimed to evaluate the role of necroptosis in patients with primary biliary cholangitis (PBC) and in mice after common bile duct ligation (BDL), a classic experimental model for acute cholestasis and secondary biliary fibrosis.

Methods: Hallmarks of necroptosis were evaluated in liver biopsies of PBC patients. C57BL/6 wild-type (WT) or RIP3-deficient (RIP3⁻/⁻) mice were subjected to BDL or sham surgeries for 3 and 14 days, with subsequent histological and biochemical analysis of hepatic damage. Necroptotic markers and the functional crosstalk between RIP3 and antioxidant response were investigated.

Results: RIP3 expression and mixed lineage kinase domain-like protein (MLKL) phosphorylation were induced in liver samples of human PBC patients, coincident with thioflavin T labelling, suggesting activation of necroptosis. BDL resulted in progressive bile duct hyperplasia, multifocal necrosis, fibrosis and inflammation. Concomitantly, necroptosis was activated as evidenced by increased RIP3 expression, sequestration of RIP3 and MLKL in the insoluble protein fraction of the liver, and augmented RIP3 kinase activity. Remarkably, RIP3 deficiency blocked BDL-induced necroinflammation at 3 and 14 days post-BDL. Serum hepatic enzymes, fibrogenic liver gene expression and oxidative stress decreased in RIP3⁻/⁻ mice at 3 days after BDL. However, at 14 days, cholestasis aggravated and fibrosis was not halted. RIP3 deficiency further associated with increased hepatic expression of heme oxygenase-1 and accumulation of iron in BDL mice.

Discussion/Conclusion: Necroptosis is triggered in PBC patients and mediates hepatic necroinflammation in BDL-induced acute cholestasis. Targeting necroptosis may represent a therapeutic strategy for acute cholestasis, although complementary approaches may be required to control progression of chronic cholestatic liver disease. (Supported by HMSP-ICT/0018/2011 and SFRH/BD/91119/2012, from FCT, Portugal.)
**Cyp3a11 is dispensable for the formation of murine bile acids**

Samer Al-Dury1, Annika Wahlström1, Marcus Ståhlman1, Fredrik Bäckhed1,2, Hanns-Ulrich Marschall1

1Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine and Wallenberg Laboratory, University of Gothenburg, S-413 45 Gothenburg, Sweden; 2Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, DK-2200, Denmark

**Introduction:** Mouse models are widely used to study the interaction between bile acids, microbiota and host metabolism. However, there are essential differences in the bile acid profiles between humans and mice. In addition to the human primary bile acids, cholic acid (CA) and Chenodeoxycholic acid (CDCA), mice also synthesize primary murine bile acids α- and β-muricholic acid (α- and βMCA), which are not found in humans. The taurine-conjugated forms of these bile acids function as FXR antagonists, which make them interesting in the context of metabolic research models. The hallmark of αMCA and βMCA is that they possess a hydroxyl group at position 6β and it is commonly assumed that the P450-enzyme CYP3A11 is responsible for the 6β-hydroxylation and hence required for the formation of these bile acids in mice; however this has not been verified. Based on these assumptions we hypothesized that mice without the Cyp3a11 gene would lack the murine 6β-hydroxylated bile acids. To test this hypothesis, we analyzed bile acid profiles in Cyp3a (8-gene) knock out mice, which lack 8 genes in the Cyp3a gene cluster including Cyp3a11, and compared them with wild type controls.

**Methods:** Bile acid composition in gallbladder, caecum and serum from male Cyp3a (8-gene) knock out mice and wild type littermate controls was analyzed with UPLC-MS/MS. Gene expression in liver was analyzed with qPCR and protein expression was measured using Western Blot.

**Results:** Bile acid analysis showed no major differences in bile acid composition between the knockout mice and their littermate controls. The percentage of total primary murine bile acids in gallbladder was slightly lower in the knockout mice; 29% compared with 25% in the wild type mice, but no differences were found in serum and caecum. QPCR analysis verified that expression of the Cyp3a11 gene was undetectable in livers from the Cyp3a (8-gene) knock out mice.

**Discussion/Conclusion:** We conclude that Cyp3a11 is dispensable for 6β-hydroxylation and formation of αMCA and βMCA in mice. Further studies are needed to explore how these murine bile acids are synthesized and to identify the enzymes involved in this process.
Cafestol but not resveratrol stimulates FGF19 expression in human ileal explants

Appleby R.N.¹, Jameie-Oskooei S.¹, Geers J.M.², Walters J.R.F.¹
¹Department of Gastroenterology, Imperial College London, UK; ²Royal College of Surgeons in Ireland, Ireland

Introduction: Farnesoid X receptor (FXR) agonists may have therapeutic benefits in NAFLD, possibly due to increased ileal FGF19 transcription. Cafestol (CAF) is a diterpene found in coffee beans that may protect against hepatic fibrosis in NAFLD. Resveratrol (RSV) is a polyphenol found in the skin of black grapes. It is an agonist of Sirtuin-1 (SIRT1), though other mechanisms of action such as up-regulation of FXR and degradation of the apical bile acid transporter (ASBT) are described. We hypothesised that CAF and RSV would increase ileal FGF19 transcription in human ileal explants.

Methods: Biopsies were obtained from the terminal ileum of 27 patients during colonoscopy. These were incubated in pairs in enriched culture media for six hours with either CAF or RSV at concentrations of 0–100 µM. To test whether CAF acted as a partial agonist, co-incubations were performed with CAF 50 µM and chenodeoxycholic acid (CDCA) 50 µM for 6 hours. Total mRNA was extracted and target gene transcription quantified by RT-PCR.

Results: CDCA 50 µM increased FGF19 expression by 106 fold compared to negative controls (p < 0.0001). Despite significant stimulation of SIRT1 at 12.5 and 25 µM (1.9 and 1.8 fold increase respectively, p < 0.05), RSV did not induce FGF19 transcription at any concentration (12.5–100 µM). Nor did it significantly effect FXR or ASBT transcription.

CAF increased ileal FGF19 transcription by 3.4 and 2 fold for 50 and 100 µM respectively (p < 0.05) compared to controls. Co-incubation of CAF 50 µM with CDCA 50 µM reduced FGF19 transcription by 95% compared to CDCA 50 µM alone (p = 0.05).

Discussion/Conclusion: Cafestol is a weak FXR agonist, but in the presence of endogenous bile acids, decreased ileal FGF19 expression. RSV has no effect on ileal FGF19. Any therapeutic effect of these compounds in NAFLD is unlikely to be due to increasing ileal FGF19.
Bile acids are key regulators of testicular physiology and male fertility

Marine Baptissart\textsuperscript{1,2,3,4,*}, Emmanuelle Martinot\textsuperscript{1,2,3,4,*}, Aurélie Vega\textsuperscript{1,2,3,4}, Lauriane Sédes\textsuperscript{1,2,3,4}, Betty Rouaisnel\textsuperscript{1,2,3,4}, Kristina Schoonjans\textsuperscript{5}, David H. Volle\textsuperscript{1,2,3,4}

\textsuperscript{1}INSERM U 1103; \textsuperscript{2}Université Clermont Auvergne, Université Blaise Pascal; \textsuperscript{3}CNRS, UMR 6293, GReD, F-63178 Aubière, France; \textsuperscript{4}Centre de Recherche en Nutrition Humaine d’Auvergne, F-63000 Clermont-Ferrand, France; \textsuperscript{5}Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

*These authors have contributed equally to this work.

Introduction: Bile acid (BA) signaling pathways control a number of physiological processes such as lipids, glucose and energy metabolisms. BAs have been defined as endocrine factors whose actions are mainly mediated by two BA responsive receptors: the nuclear receptor, FXR\textsubscript{α} (Farnesoid-X-Receptor, NR1H4), and the G-protein-coupled receptor, TGR5 (GPBAR1). Although FXR\textsubscript{α} and TGR5 have been reported to be expressed within the testes, the effects of BA signaling on testicular physiology and male fertility remain elusive.

Methods: To define the effects of BAs on testicular physiology and fertility, we exposed pre-pubertal or adult male mice to dietary BA supplementation (cholic acid). This approach was combined with the use of transgenic mouse models invalidated for the gene encoding for either TGR5 or FXR\textsubscript{α}. The involvement of BA receptors was monitored with pharmacological agonists or through siRNA experiments in vitro.

Results: From our studies, it appears that BAs have different roles in testicular physiology. We demonstrate that during pubertal age, BAs alter germ cell differentiation and survival due to lower testosterone synthesis. At the molecular level, BAs repress basal steroidogenesis via the induction expression of Shp and Dax-1, two repressors of steroidogenesis. In parallel, BA-FXR\textsubscript{α} signaling lowers the Leydig cell sensitivity to the hypothalamo-pituitary axis, the main regulator of testicular endocrine function. In adults, we showed that BA-diet alters fertility subsequent to testicular defects and lower sperm count. Elevated plasma BA levels led to germ cell sloughing and blood-testicular-barrier rupture, as well as apoptosis of post-meiotic germ cells (spermatids). The BA-TGR5 pathway plays a critical role in mediating fertility disorders, some of which are mediated within the germ cell lineage.

Discussion/Conclusion: Although more studies are needed to corroborate this correlation in humans, our mouse studies provide strong indications for deleterious effects of BAs on testicular pathophysiology and fertility.
Effect of intrahepatic cholestasis of pregnancy on maternal glucose homeostasis

Elena Bellafante¹, Vanya Nikolova¹, Jenny Chambers¹, Marcus Martineau¹, Catherine Williamson¹
¹Maternal and Fetal Disease Group, Women's Health Division, King's College London, UK

Introduction: Gestational Diabetes mellitus (GDM) develops in women with no previous history of glucose intolerance or insulin resistance during late pregnancy. Beside complications during gestation, GDM is associated with longer term complications for both the mother and the child such as metabolic syndrome and T2D. Women with intrahepatic Cholestasis of Pregnancy (ICP), characterised by abnormal liver function tests with elevated bile acid (BA) levels, have an increased risk of developing GDM.
In this study we investigated if ICP contributes to the development of GDM by influencing the enteroinsular axis and the physiological adaptations of pancreatic islets.

Methods: GLP1 was assayed using ELISA in women with ICP and controls. C57/Blk6 mice fed with chow diet ± CA, FxrKO, Tgr5WT, and Tgr5KO were sacrificed at days 15 and 18 of pregnancy. GTT and ITT were performed at day 18 and 19, respectively.

Results: ICP women had lower postprandial GLP1 levels. The human results were supported by CA-fed mice that showed a reduction of Gcc mRNA. LCA and UDCA, natural ligands for TGR5, are increased in faeces of chow-fed mice but decreased during CA feeding, suggesting that TGR5 signaling is dampened in ICP. In line, Tgr5KO mice showed impaired GTT and insulin secretion in advanced pregnancy. Moreover, ablation of FXR, whose activity is dampened during ICP, caused glucose intolerance and insulin resistance. Physiological islet expansion and β-cell proliferation were reduced in pregnant CA-fed and FxrKO mice, and TUNEL assay showed increased apoptotic rate in islets from these mice.

Discussion/Conclusion: A cholestatic environment affects intestinal BA signalling interfering with GLP1 secretion and physiological β-cell expansion during pregnancy. These data provide a potential explanation for the increased risk of GDM and reduced GLP1 in ICP, suggesting that FXR and TGR5 deregulation increases susceptibility of women with ICP to developing GDM.
α5β1 integrins are receptors for bile acids with a (nor-)ursodeoxycholane scaffold

Bonus, M.1, Sommerfeld, A.2, Häussinger, D.2, Gohlke, H.1
1Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
2Clinic for Gastroenterology, Hepatology and Infectious Diseases, Heinrich Heine University Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: Integrins are ubiquitously expressed cell adhesion receptors and the most prevalent bidirectional signaling molecules on the cell surface. A recent study combined immunofluorescence staining (IFS) experiments and molecular dynamics (MD) simulations to identify tauroursodeoxycholic acid (TUDC) as potent agonist of α5β1 integrins in hepatocytes. Activation of α5β1 leads to choleresis by FAK/c-Src/MAPK dependent signaling events. TUDC-induced integrin activation and subsequent signaling is sensitive to inhibition by the trihydroxylated taurocholic acid (TC), which tightly binds to α5β1 in MD simulations. However, effects of other bile acids on α5β1 integrin activation have not been investigated at the molecular level.

Methods: Molecular dynamics (MD) simulations were used to predict bile acid-induced conformational changes associated with integrin activation. Results from MD were compared to IFS of the active β1 integrin subunit in rat liver slices after perfusion with nor-ursodeoxycholic acid (norUDCA), tauro-nor-ursodeoxycholic acid (TnorUDCA), glycoursodeoxycholic acid (GUDC), and ursodeoxycholic acid (UDCA). NorUDCA-induced signaling was compared to signaling events induced by TUDC and hypo-osmolarity.

Results: Our results indicate that α5β1 integrins are not exclusively activated by TUDC. Nor-ursodeoxycholic acid (norUDCA), a side chain-shortened homologue of UDCA, induces conformational changes in the βA domain of α5β1 similar to the ones evoked by TUDC. Conformational changes observed with other bile acids were less pronounced. A ranking based on the extent of structural changes observed during the MD simulations correlates with results from IFS experiments on the efficacy of the bile acids. Similar to TUDC and hypo-osmolarity, norUDCA induces an integrin-dependent activation of Erk-1/2 and p38MAPK in isolated perfused rat liver.

Discussion/Conclusion: Our results indicate that norUDCA activates α5β1 integrins in a similar way but with weaker effects than TUDC. We further show that MD simulations are able to predict to what extent bile acids can induce integrin activation. Minor structural changes in the bile acids strongly influence their efficacy.
Autoimmune BSEP disease is curable with hematopoietic stem cell transplantation

Florian Brinkert1, Andrea Briem-Richter1, Verena Keitel-Anselmino2, Ingo Müller3 and Enke Grabhorn1
1. University Children’s Hospital, Pediatric Gastroenterology and Hepatology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
2. Department of Gastroenterology, Hepatology and Infectious Diseases, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany
3. Clinic for Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: PFIC-2 is caused by impaired bile salt excretion due to a defect of ABCB11, encoding the bile salt export pump (BSEP) protein and leads to cholestasis, jaundice and pruritus typically without elevation of serum gamma-glutamyltransferase (GGT). The rapidly progressing disease often leads to liver transplantation in infancy. Since the first report of recurrence of the clinical phenotype of PFIC-2 after liver transplantation, there is some evidence that an autoimmune mechanism can lead to the development of antibodies against BSEP in “BSEP-naïve” patients. The anti-BSEP antibodies can be detected in the liver tissue of the patients using immunofluorescence or immunohistochemistry by tagging anti-human immunoglobulin antibodies.

Case report: We here report a patient who suffered from genetic confirmed PFIC-2 and was transplanted from his mother at the age of 10 months. After the first transplant he developed low-GGT cholestasis mimicking BSEP disease. Despite various intensified pharmacological immunosuppression protocols in addition to IVIG, plasmapheresis/immunoabsorption and rituximab therapy, he developed liver cirrhosis and was re-transplanted. After the second transplantation preformed anti-BSEP antibodies again lead to chronic cholestasis, pruritus with low GGT. He rapidly developed liver fibrosis and showed typical signs of recurrence of PFIC-2 histologically. After an interdisciplinary discussion and approval by the ethical review board we performed a myeloablative allogeneic haematopoietic stem cell transplantation (HSCT) using a reduced-toxicity conditioning concept as salvage therapy. 3 months after HSCT without any serious complications the cholestasis disappeared, anti-BSEP antibodies decreased first in serum and later liver tissue was cleared as well.

Discussion/Conclusion: This case report shows that autoimmune BSEP disease is curable by allogeneic HSCT: 1) anti-BSEP antibodies disappeared in serum of the patient and 2) antibodies were no longer detectable in liver tissue.
From our point of view, this is the “proof of principle” of the autoimmune BSEP disease. Consequently, alternative concepts of immunoablation and HSCT, including haplo-identical HSCT when a parent was the organ donor may be considered.
Selective targeting of fxr isoforms α1–4 by novel bile acid derivatives and lipotoxicity protection in hepg2 cells

Hugo Brito¹, Salete Batista², Jorge A. Salvador², Rui E. Castro¹, Cecília M. Rodrigues¹
¹Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
²Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

Introduction: Farnesoid X receptor (FXR), a bile acid (BA)-activated nuclear receptor, plays a critical role in maintaining lipid, glucose and BA homeostasis. FXR expression is significantly decreased in livers of non-alcoholic fatty liver disease (NAFLD) patients and genetic ablation leads to hepatic steatosis and hyperlipidaemia. The FXR gene expresses four biologically active variants (FXRα1–4), regulating hepatic and lipid metabolism in an isoform-dependent manner. Activation of FXR variants α1 and α2 significantly reduces hepatic lipid accumulation. Our aim was to screen potential BA-derived FXR agonists for their ability to selectively activate different FXR isoforms and protect HepG2 cells against palmitate toxicity.

Methods: Twenty novel BA derivatives, synthesized based on the cholic (CA), deoxycholic (DCA), chenodeoxycholic (CDCA) and ursodeoxycolic (UDCA) acid scaffolds were incubated in HepG2 cells transfected with a dual-luciferase reporter construct and overexpression vector plasmids for FXRα1–4 isoforms. In parallel, BA-derivatives were co-incubated in HepG2 cells treated with 250–500 μM palmitate-BSA, for cell viability assays.

Results: As a result of the different structural modifications, BA derivatives showed differential activation of the FXRα1–4 isoforms, when compared to their precursor BAs. From the precursor BAs, only CDCA, a natural FXR ligand, significantly activated FXRα1 and α2 isoforms, with CA and UDCA displaying a modest activation of FXRα1 isoform only. Interestingly, 2 novel CA-, 1 DCA- and 4 UDCA-derivatives were stronger activators of both FXRα1 and α2, comparing with their corresponding precursors. Further, 3 novel CA-, 2 DCA-, 3 CDCA- and 4 UDCA-derivatives specifically and significantly activated FXRα3 and α4. Finally, incubation of HepG2 cells with palmitate-BSA led to up to 35% reduction in cell viability. Co-incubation of cells with CA and UDCA BA-derivatives reverted the palmitate-BSA-induced lipotoxicity.

Discussion/Conclusion: In conclusion, herein we provide a novel strategy to screen for selective agonists of FXRα1–4 isoforms and have identified new selective BA-derived FXRα1 through 4 agonists. In addition, specific derivatives appear to afford cytoprotection against lipotoxicity. The differential functional effect of these new molecules will undoubtedly contribute for a better understanding of pharmacological targeting and therapeutic efficacy of FXR agonists in liver diseases such as NAFLD.
Metformin protects rat hepatocytes against bile acid-induced apoptosis

Manon Buist-Homan, Titia Woudenberg-Vrenken, Laura Conde de la Rosa, Klaas Nico Faber, Han Moshage
Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Background: Metformin is used in the treatment of Diabetes Mellitus type II and improves liver function in patients with non-alcoholic fatty liver disease (NAFLD). Metformin has many biological effects: it lowers blood glucose, it activates AMP-activated protein kinase (AMPK), the cellular energy sensor that is sensitive to changes in the AMP/ATP-ratio, it is an inhibitor of complex I in mitochondria and it has been shown to induce ER and DNA-damage-stress response genes like GADD45beta. All these actions may also affect cell viability. Therefore, the Aim of the present study was to evaluate the effects of metformin on hepatocyte cell death.

Methods: Apoptotic cell death was induced in primary rat hepatocytes using either the bile acid glycochenodeoxycholic acid (GCDCA), the superoxide generating compound menadione or TNFα in combination with actinomycin D (actD). AMPK, mTOR and phosphoinositide-3 kinase (PI3K)/Akt were inhibited using pharmacological inhibitors. Apoptosis and necrosis were quantified by caspase-3 activation, acridine orange staining and Sytox green staining respectively.

Results: Metformin reduces GCDCA- and superoxide-induced apoptosis at 0.5 and 1 mmol/L, even when added 2 hours after GCDCA, without increasing necrotic cell death. Metformin does not protect against TNFα/ActD-induced apoptosis. The protective effect of metformin is dependent on an intact PI3-kinase/Akt pathway, but does not require AMPK/mTOR-signaling. Metformin increased the expression of the antioxidant gene HO-1 and the anti-apoptotic gene Bcl-xl but it does not affect NF-κB activation. At doses of 5 mmol/L or higher, metformin induced necrotic death of hepatocytes.

Conclusion: Metformin protects against bile acid- and superoxide-induced apoptosis, possibly via induction of anti-oxidant and anti-apoptotic genes. Therefore, metformin could be considered in the treatment of chronic liver diseases accompanied by inflammation and increased bile acids or oxidative stress, like cholestatic disorders.
Soluble adenylyl cyclase regulates bile salt-induced apoptosis in human cholangiocytes

Jung-Chin Chang¹, Simei Go¹, Coen C. Paulusma¹, Dirk R. de Waart¹, Patricia Munoz-Garrido¹,²,³, Ulrich Beuers¹, Ronald Oude Elferink¹*
¹Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
²Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute – Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastián, Spain
³National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III), Spain

Introduction: Anion exchanger 2 (AE2), the principal bicarbonate secretor in human biliary tree, is down-regulated in primary biliary cholangitis (PBC). AE2 creates a "bicarbonate umbrella" that protects cholangiocytes from the pro-apoptotic effects of bile salts by maintaining them deprotonated. We observed that knockdown of AE2 sensitized the immortalized human cholangiocytes H69 (H69 cholangiocytes) to not only bile salt-, but also etoposide-induced apoptosis. Since the toxicity of etoposide is pH-independent, there could be a more general mechanism for sensitization of AE2-deficient cholangiocytes to apoptotic stimuli. We found that AE2 deficiency led to intracellular bicarbonate accumulation and increased expression and activity of soluble adenylyl cyclase (sAC), an evolutionarily conserved bicarbonate sensor. Thus, we hypothesized that sAC regulates bile salt-induced apoptosis (BSIA).

Methods: H69 cholangiocytes and primary mouse cholangiocytes were used as models. BSIA was characterized by caspase 3/7 activity assay, immunoblotting and morphology study.

Results: sAC-specific inhibitor KH7 not only reversed sensitization to BSIA in AE2-deficient H69 cholangiocytes but even completely prevented BSIA. sAC knockdown by tetracycline inducible shRNA also prevented BSIA. In addition, sAC inhibition also reversed bile salt-induced apoptotic membrane blebbing, nuclear condensation and DNA fragmentation. Furthermore, we showed that sAC inhibition also prevented BSIA in primary mouse cholangiocytes. Mechanistically, sAC inhibition prevented Bax phosphorylation at Thr167, mitochondrial translocation of Bax and cytochrome c release, but not c-Jun N-terminal kinase activation during BSIA. Finally, BSIA in H69 cholangiocytes was inhibited by intracellular Ca²⁺ chelation, aggravated by thapsigargin, and unaffected by removal of extracellular calcium.

Discussion/Conclusion: BSIA is regulated by sAC, depends on intracellular Ca²⁺ stores and is mediated by the intrinsic apoptotic pathway. Down-regulation of AE2 in PBC sensitizes cholangiocytes to apoptotic insults by activating sAC, which may play a crucial role in disease pathogenesis.
Activation of intestinal bile acid receptor FXR induces membrane G protein-coupled bile acid receptor TGR5 expression and stimulates GLP-1 secretion to ameliorate metabolic disorders in diabetic mice

John Y.L. Chiang, Preeti Pathak, Hailiang Liu and Shannon Boehme
Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH 44272, USA

Introduction: Bile acid receptors, farnesoid X receptor (FXR) and G-protein-coupled receptor TGR5, are co-expressed in the enteroendocrine L cells. Activation of TGR5 stimulates intestinal glucagon-like-peptide-1 (GLP-1) secretion and improves insulin sensitivity. The aim of this study is to test whether activation of intestinal FXR stimulates GLP-1 secretion and ameliorates diabetes in mice.

Methods: Fxr⁻/⁻, Tgr5⁻/⁻ and db/db mice were used to study the effects of an intestine FXR agonist fexaramine (FEX) on GLP-1 secretion, and glucose and insulin tolerance.

Results: Both FXR and TGR5 agonists stimulated intestine GLP-1 secretion, cAMP activity and intracellular Ca²⁺ uptake. Interestingly, FXR agonists induced TGR5 mRNA levels in wild type but not Fxr⁻/⁻ mice. A FXR responsive element was identified on the Tgr5 gene promoter by reporter assay and chromatin immunoprecipitation assay. Both Fxr⁻/⁻ and Tgr5⁻/⁻ mice had reduced GLP-1 secretion compared to wild type mice. Small interference RNA knockdown of both FXR and TGR5 in STC-1 cells abolished GLP-1 secretion. GLP-1 infusion in wild type mice and FEX treatment in db/db mice stimulated AKT and PKCζ phosphorylation, reduced hepatic triglycerides and gluconeogenic gene expression, and improved glucose and insulin tolerance.

Discussion/Conclusion: Intestinal FXR plays a key role in inducing Tgr5 expression and GLP-1 secretion to improve hepatic steatosis and insulin sensitivity in diabetic mice. This is the first report of transcriptional regulation of Tgr5 gene expression by intestinal FXR and activation of both intestinal FXR and TGR5 may coordinately regulate hepatic metabolism and protect against inflammatory intestine and liver diseases such as inflammatory bowel diseases, non-alcoholic fatty liver disease, obesity and diabetes.
Prevalence, clinical characteristics and outcomes of antimitochondrial type 2 seropositive patients with non-established primary biliary cholangitis

Géraldine Dahlqvist, Farid Gaouar, Fabrice Carrat, Sofia Meurisse, Olivier Chazouillères, Catherine Johanet, Christophe Corpechot, Raoul Poupon & the French Network of Immunology Laboratories

1Service d’Hépatologie, Centre de Référence des Maladies Inflammatoires des Voies Biliaires (MIVB), Hôpital Saint-Antoine, Assistance Publique – Hôpitaux de Paris (APHP), Paris, France

Objective: To assess the prevalence, clinical characteristics and outcomes of patients with antimitochondrial type 2 antibodies (AMA2) and non-established primary biliary cholangitis (PBC).

Design: A prospective nationwide study of AMA2 annual incidence was conducted through an extensive network of 63 French immunology laboratories. Clinical data from 720 out of the 1318 AMA2-positive patients identified were collected. The patients were categorized as: previously diagnosed with PBC: 216 (30%); newly diagnosed with PBC: 275 (38%); and non-diagnosed with PBC: 229 (32%). The latter group was analyzed and followed-up for up to 7 years.

Results: The estimated prevalence of AMA2-positive patients with non-established PBC was 16.1 per 100,000. These patients had the following characteristics: 78% female; median age 58 years; median AMA2 titre 1:160; extra-hepatic autoimmune disorders 46%; normal serum alkaline phosphatases (ALP) 74%; elevated ALP > 1.5 times the upper limit of normal 16%; Cirrhosis 5%. Compared to those diagnosed with PBC at the same time, the patients were slightly younger, had lower sex-ratio imbalance and AMA2 titres. After exclusion of patients with abnormal ALP or cirrhosis at baseline, the estimate of the 5-year incidence rate of PBC was 19% (95% confidence interval: 5–34%). Whereas no patients died from PBC, the 5-year survival rate was 75% (63–87%) compared to 90% (83–98%) in a control population matched for age and gender (p < 0.05).

Conclusion: Nearly half of the newly detected AMA2 in clinical settings does not lead to a diagnosis of PBC. PBC is unrecognized in 16% of these cases. Only 1 in 5 patients will develop the disease after 5 years. The mortality of this population (AMA positive without PBC) is increased regardless of the PBC risk.
**Intrahepatic cholestasis of pregnancy: Serum microRNA analysis**

P.H. Dixon¹, L. Wu¹,², C. Williamson¹

1. Division of Women’s Health, King’s College London, Guy’s Campus London SE1 1UL., London, UK
2. Department of Obstetrics and Gynaecology, West China Second University Hospital Sichuan University, Chengdu, Sichuan 610041, China

**Introduction:** MicroRNAs regulate post-transcriptional gene expression by binding specific target messenger RNAs causing translational inhibition/degradation. They may be useful biomarkers, or have a role in pathophysiology. We performed serum micro RNA profiling in intrahepatic cholestasis of pregnancy (ICP), the commonest liver disease in pregnancy. Four microRNAs were selected with targets of relevance to cholestatic disease, namely miR 122 (CYP7A1), 421 (FXR), 34 (SIRT1) and 33 (ABCB11, ATP8B1).

**Methods:** Serial serum samples (2–14 per individual) were collected from 3 groups: ICP (23 treated, 8 untreated), previous ICP (5) and controls (23) with 9 cases sampled prior to disease onset. RNA (200 μl serum) was purified (miRNeasy Serum/Plasma Kit (Qiagen)) and the miScript II RT Kit was used for cDNA synthesis. MiRNAs levels were determined by RT-qPCR with the miScript SYBR® Green PCR Kit.

**Results:** Initial analysis examined the effect of gestation; only a slight decline was observed with miR 33. Strong association between miR 122 (OR 2.06, 95% CI 1.34 to 3.17, p = 0.002) and miR 421 (OR 0.45, 95% CI 0.22 to 0.92, p = 0.03) and bile acid levels was detected. Mir122 and mir34 were associated with ALT levels (OR 2.40, 95% CI 1.50 to 3.86, p = 0.001, OR 2.20, 95% CI 1.27 to 3.83, p = 0.007 respectively). Cross group comparisons identified significant differences with all miRs except miR 33. Longitudinal changes in ICP showed miR 122 increasing with disease onset and falling following treatment. miR 34 in contrast rises with diagnosis but is unchanged by treatment.

**Discussion/Conclusion:** This study has demonstrated that microRNAs with biologically relevant targets can be detected in the serum of women with ICP and that they show significant differences to controls. Further studies are warranted to determine if they represent useful biomarkers or are of relevance to disease pathophysiology.
Drug-drug interactions related to inhibition of the sodium taurocholate co-transporting polypeptide (NTCP) by a novel anti-HBV peptide

J.M. Donkers¹, M.J. Kwakkenbos³, S. Duijst¹, S. Urban⁴, R.P.J. Oude Elferink¹, S.F.J. van de Graaf¹,²
¹Tytgat Institute for Liver and Intestinal Research & ²Department of Gastroenterology & Hepatology, AMC, Amsterdam, The Netherlands; ³Aimm Therapeutics, Amsterdam, The Netherlands; ⁴German Center for Infection Research, Heidelberg University, Heidelberg, Germany

Introduction: In the liver, the sodium taurocholate co-transporting polypeptide (NTCP, SLC10A1) is the main transporter of conjugated bile acids (BA). Recently, NTCP was also identified as the entry receptor for the hepatitis B virus (HBV). Myrcludex-B, a synthetic peptide mimicking the NTCP-binding domain of HBV, also inhibits NTCP-mediated bile acid uptake. Whether this affects the pharmacokinetics of other drugs is currently unknown. This study aimed to identify compounds interfering with BA transport and/or occupation of the HBV binding site of NTCP.

Methods: Two different approaches were used to screen 1280 FDA-approved compounds in U2OS-hNTCP cells: 1) uptake assays using tritium-labelled taurocholic acid, 2) competition assays with FITC-labeled Myrcludex-B. Effects of a selection of compounds on mouse NTCP, ASBT and cell viability was analysed by taurocholate uptake- and WST-1 assays, respectively. Short term consequences of NTCP inhibition was studied in vivo by cannulation of the gallbladder and injection of radiolabeled taurocholate.

Results: BA and known NTCP inhibitors included in the screening library were amongst the top 100 hits, thereby validating both screening methods. From the largely overlapping top-hits, 12 were selected for follow-up studies. The most promising hits among these are Rosiglitazone (IC₅₀ 5.1 µM), Zafirlukast (IC₅₀ 6.5 µM), TRIAC (IC₅₀ 6.9 µM), Chicago Sky Blue 6B (IC₅₀ 7.1 µM), and Sulfasalazine (IC₅₀ 9.6 µM). All are effective in both human and mouse NTCP and largely ineffective for ASBT, a related BA transport protein. In vivo, NTCP inhibition with Myrcludex-B showed decreased clearance of serum BA.

Discussion/Conclusion: We established two complementary methods to screen for novel compounds that affect NTCP-mediated BA transport. Decreased NTCP-mediated BA transport was confirmed both in vitro and in vivo. As several of the identified compounds likely are transported via NTCP, this study identifies for which clinically relevant compounds Myrcludex B treatment could affect drug exposure or clearance.
**FIC1, BSEP, and MDR3 sequencing disclosed 139 genetic variants including 63 new ones in 389 unrelated patients with suspected intrahepatic cholestasis**

Carola Dröge¹, Michele Bonus², Stefanie Kluge¹, Holger Gohlke², Lutz Schmitt³, Ralf Kubitz¹, Dieter Häussinger¹, Verena Keitel-Anselmino¹

¹Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital, Heinrich Heine University Düsseldorf, Germany; ²Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Germany; ³Institute of Biochemistry, Heinrich Heine University Düsseldorf, Germany

**Introduction:** Familial intrahepatic cholestasis 1 (FIC1, ATP8B1), the bile salt export pump (BSEP, ABCB11), and multidrug resistance protein 3 (MDR3, ABCB4) are crucial for bile formation. Mutations in these transporters are the basis of various cholestatic liver diseases ranging from intrahepatic cholestasis of pregnancy (ICP), benign recurrent intrahepatic cholestasis (BRIC) or low phospholipid-associated cholelithiasis (LPAC) to progressive familial intrahepatic cholestasis (PFIC). Currently, gene sequencing is the method of choice to evaluate the genetic background of these cholestatic diseases.

**Methods:** To confirm diagnosis, coding exons with surrounding intron regions of ATP8B1, ABCB11, and ABCB4 of patients with cholestasis of diverging manifestation were sequenced. The potential impact of new variants was evaluated by bioinformatics tools and 3D protein modeling.

**Results:** In 131 patients with assumed FIC1 deficiency, 25 variants with 6 new ones were detected. 209 patients with supposed BSEP mutations had 72 variants including 37 novel ones. MDR3 analysis in 197 cases revealed 42 variants, 20 of them new. In a variety of patients, merely one heterozygous mutation (FIC1: 4/131, BSEP: 32/209, MDR3: 35/198), or polymorphisms or synonymous variants were detectable. These conditions are insufficient to explain severe cholestasis but may cause milder phenotypes. In patients without severe mutations, the common FIC1 variants c.3531+8G>T or p.R952Q were found in 36.6%, both BSEP polymorphisms p.V444A and p.A1028A were shown in 71.4% of the samples. Three synonymous MDR3 variants p.L59L, p.N168N, p.I237I appeared together in 22.0% of these cases.

**Discussion/Conclusion:** In this patient population, 139 variants were detected in FIC1, BSEP, and MDR3 including numerous cases with only one heterozygous or even no mutation. Other genes like TJP2 as recently described (Sambrotta et al.) in cases of low gGT cholestasis, as well as non-genomic factors probably contribute to some phenotypes. Here, we focused on variants in FIC1, BSEP, and MDR3 and their possible effects on cholestasis.
Chronic central infusion of taurolithocholate decreases fat mass and increases brown adipose tissue triglyceride derived fatty acid uptake

H.M. Eggink¹, S. Kooijman²,³, I.M. Mol²,³, R. van den Berg²,³, M. Koehorst⁴, A.K. Groen⁴,⁵, A. Boelen¹, A. Kalsbeek¹,⁶, P.C.N. Rensen²,³, J.A. Romijn⁷ and M.R. Soeters¹

¹Department Endocrinology and Metabolism, Academic Medical Centre (AMC), University of Amsterdam (UvA), Amsterdam, The Netherlands
²Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands
³Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands
⁴Departments of Pediatrics and Laboratory Medicine, University Medical Center Groningen, University of Groningen, The Netherlands
⁵Amsterdam Diabetes Center, Department of Vascular Medicine, AMC, UvA, Amsterdam, The Netherlands
⁶Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands
⁷Department of Medicine, AMC, UvA, Amsterdam, The Netherlands

Introduction: Bile acids (BAs) can function as signalling molecules in energy metabolism via the transmembrane G protein-coupled receptor, TGR5. In rodents, activation of TGR5 by BAs affects glucose and lipid metabolism. TGR5 is also present in the central nervous system, but its function is still unclear.

Methods: We investigated whether intracerebroventricular (icv) administration of taurolithocholate (tLCA), a strong TGR5 agonist, influences energy metabolism. Wild type mice were equipped with an osmotic minipump aimed for administration into the brain (icv) or into the peritoneal cavity (ip). Both groups received equal amounts of tLCA or vehicle. tLCA was dosed to reach 1 micromolar concentration in the liquor. Energy expenditure was measured using metabolic cages and body composition using MRI. After 9 days of infusion the mice underwent a lipid clearance test and were sacrificed. Blood and organs were harvested for further analysis. Group differences between the tLCA and control group were analysed using a 2-tailed Student’s t-test; data are presented as mean +/- standard error.

Results: IP infusion of tLCA had no effect on plasma BA profile, lipid or energy metabolism. However, the mice treated with central tLCA (N = 8) had significantly decreased fat mass compared to controls (N = 6) (0.43 ± 0.44 vs 1.02 ± 0.39; p = 0.02, respectively) and increased uptake of triglyceride (TG) derived fatty acids in subcapular brown adipose tissue (BAT) (28.34 ± 12.45 vs 13.23 ± 8.14; p = 0.02, respectively). This is in accordance with the trends observed in the metabolic cages (lower respiratory exchange ratio p = 0.08 and higher fat oxidation p = 0.057). No differences were found in plasma BA profile.
Discussion/Conclusion: Central administration of the strong TGR5 agonist tLCA decreases fat mass and increases BAT TG uptake, while peripheral administration of the same amounts of BA does not have an effect. Additional analysis is needed to elucidate the molecular pathways involved in these central effects of TGR5 activation.
Hormesis in cholestatic liver disease; preconditioning with low bile acid concentrations protects against bile acid-induced toxicity

Klaas Nico Faber¹,², Manon Buist-Homan¹,², Martijn Koehorst², Albert K. Groen³, Han Moshage¹,², Esther M. Verhaag¹

Departments of ¹Gastroenterology and Hepatology, ²Laboratory Medicine and ³Pediatrics, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Cholestasis is characterized by accumulation of bile acids and inflammation, causing hepatocellular damage. Still, liver damage markers are highest in acute cholestasis and drop when this condition becomes chronic, indicating that hepatocytes adapt towards the hostile environment. This may be explained by a hormetic response in hepatocytes that limits cell death during cholestasis.

Aim: To investigate the mechanisms that underlie the hormetic response that protect hepatocytes against experimental cholestatic conditions.

Methods: HepG2.rNtcp cells were preconditioned (24 h) with sub-apoptotic concentrations (0.1–50 μM) of various bile acids, the superoxide donor menadione, TNF-α or the farsenoid X receptor agonist GW4064, followed by a challenge with the apoptosis-inducing bile acid glycochenodeoxycholic acid (GCDCA; 200 μM for 4 h), menadione (50 μM, 6 h) or cytokine mixture (CM; 6 h). Levels of apoptotic and necrotic cell death, mRNA expression of the bile salt export pump (ABCB11) and bile acid sensors, as well as intracellular GCDCA levels were analyzed.

Results: Preconditioning with the pro-apoptotic bile acids GCDCA, taurocholic acid, or the protective bile acids (tauro)ursodeoxycholic acid reduced GCDCA-induced caspase-3/7 activity in HepG2.rNtcp cells. Bile acid preconditioning did not induce significant levels of necrosis in GCDCA-challenged HepG2.rNtcp cells. In contrast, preconditioning with cholic acid, menadione or TNF-α potentiated GCDCA-induced apoptosis. GCDCA preconditioning specifically reduced GCDCA-induced cell death and not CM- or menadione-induced apoptosis. The hormetic effect of GCDCA preconditioning was concentration- and time-dependent. GCDCA-, CDCA- and GW4064-preconditioning enhanced ABCB11 mRNA levels, but in contrast to the bile acids, GW4064 did not significantly reduce GCDCA-induced caspase-3/7 activity. The GCDCA challenge strongly increased intracellular levels of this bile acid, which was not lowered by GCDCA-preconditioning.

Discussion/Conclusion: Sub-toxic concentrations of bile acids in the range that occur under normal physiological conditions protect HepG2.rNtcp cells against GCDCA-induced apoptosis, which is independent of FXR-controlled changes in bile acid transport.
Absence of BSEP/ABCB11 protects from cholestatic liver injury in mice

Claudia D. Fuchs¹, Gustav Paumgartner¹, Annika Wahlström², Philipp Schwabl³, Tatjana Stojakovic⁴, Nadja Leditznig¹, Hans-Ulrich Marschall² and Michael Trauner¹
¹HansPopper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria
²Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden
³Hepatic Hemodynamic Laboratory, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria
⁴Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria

Background: Cholestasis is characterized as intrahepatic accumulation of potentially cytotoxic bile acids (BAs) which subsequently leads to liver injury reflected by disruption of hepatocellular integrity, inflammation, fibrosis, cirrhosis and increased risk for development of cancer. Bile salt export pump (BSEP/ABCB11) is the main canalicular BA transporter and therefore the rate limiting step for hepatobiliary BA secretion. In this study we aim to investigate the role of BSEP/ABCB11 in development of cholestatic liver injury.

Methods: Wildtype (WT) and ABCB11 knockout (KO) mice were subjected to common bile duct ligation (CBDL) and 3.5-Diethoxycarbonyl-1.4-dihydrocollidine (DDC) feeding as models for acute and chronic cholestasis, respectively. Liver RNA profile analysis was performed by RT-PCR. BA transporter expression was also assessed at protein levels by western blots. Serum biochemistry, hepatic hydroxyproline levels, BA content/composition as well as liver histology were assessed. Biliary pressure was also measured following BDL in the presence and absence of BSEP.

Results: In contrast to WT animals, mice lacking ABCB11 were protected from liver injury induced by 7 days of CBDL or 4 weeks of DDC feeding, as reflected by unchanged serum levels of liver transaminases (ALT, AST), alkaline phosphatase, total cholesterol and BA, whereas WT mice subjected to CBDL or DDC showed pronounced cholestatic liver injury. Notably, ABCB11 KO mice were also protected from cholestasis-induced inflammation (reflected by unchanged mRNA levels of F4/80 and MCP1) and fibrosis (reflected by liver histology and unchanged mRNA levels of Col1a1 and Col1a2 as well as αSMA protein levels) while WT animals displayed significant up-regulation of both inflammatory and fibrotic markers. Interestingly, poly-hydroxylated BAs (PHBA) were 4-fold increased in ABCB11 KO mice after CBDL when compared to cholestatic WT mice (p < 0.01). In line, mRNA expression of Cyp2b10, the downstream target of CAR – a nuclear receptor regulating BA detoxification pathways – was increased in ABCB11 KO mice after CBDL. Protein levels of BA transporter such as NTCP, OATP (sinusoidal uptake) and MRP2 (canalicular export) was reduced in WT and increased in ABCB11 KO mice under cholestatic conditions. Finally, following CBDL biliary pressure in WT mice increased up to 47 mm H₂O but remained below 11 mm H₂O in BSEP⁻/⁻ mice.
**Conclusion:** Metabolic preconditioning with subsequent changes in BA metabolism favours detoxification of potentially toxic BAs and thereby protects BSEP⁻/⁻ mice from acquired cholestatic liver and bile duct injury.
Effects of ursodeoxycholic acid on FXR-mediated stimulation of FGF19 in human ileal explants

Jenna M. Geers¹, Richard N. Appleby², Julian R.F. Walters²
¹Royal College of Surgeons in Ireland, ²Department of Gastroenterology, Imperial College London, UK

Introduction: Bile acid-mediated activation of the nuclear farnesoid X receptor (FXR) present in terminal ileum enterocytes elicits transcriptional responses on several genes, including potent stimulation of fibroblast growth factor 19 (FGF19). FGF19 mediates feed-back inhibition of bile acid synthesis in the liver. We have previously studied several different bile acids including chenodeoxycholic acid (CDCA) and obeticholic acid (OCA) and now report effects of ursodeoxycholic acid (UDCA).

Methods: Biopsies were obtained from normal terminal ileum of 20 patients during routine colonoscopy. These were incubated in enriched culture media for six hours with either UDCA 0–100 µM, CDCA 50 µM or OCA 5 µM alone, or UDCA 50–100 µM together with CDCA 50 µM or OCA 5 µM. Total mRNA was extracted and FGF19 transcription quantified by RT-PCR.

Results: In these experiments, the median fold increase of FGF19 with CDCA 50 µM and OCA 5 µM compared to controls was 67- and 75-fold respectively. UDCA alone increased FGF19 by 9-fold maximally at 100 µM, indicating weak agonist activity. Co-incubations of UDCA 100 µM and CDCA 50 µM further increased the stimulation of FGF19 by a median 2-fold change compared to CDCA 50 µM alone. Addition of UDCA to OCA 5 µM also increased FGF19 relative to OCA 5 µM alone by 2.5-fold.

Discussion/Conclusion: UDCA is a weak FXR agonist, but in contrast to the expected competitive inhibition in the presence of high affinity ligands for FXR, co-incubation with UDCA doubled transcription compared to CDCA or OCA alone. This unexpected finding may be explained by previous observations that UDCA interacts with the ileal bile acid binding protein affecting bile acid binding and FXR activation. These effects may be relevant for the therapeutic actions of OCA when this is given with UDCA to patients with PBC.
An experimentally validated binding mode model of TGR5 agonists

Gertzen, C.G.W.¹, Spomer, L.², Smits, S.H.J.³, Häussinger, D.², Keitel-Anselmino, V.², Gohlke, H.¹
¹Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
²Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Heinrich Heine University Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany
³Institute for Biochemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany

Introduction: TGR5 is the first bile acid sensing G-protein coupled bile acid receptor (GPCR)¹. High expression levels of TGR5 are found in the brain, the liver, and the gastrointestinal tract. As TGR5 influences metabolism via glucagon like peptide-1 (GLP-1) release and the regulation of thyroxin production², it is an emerging target for the treatment of metabolic diseases.³–⁵ Therefore, a wide range of TGR5 agonists have been developed so far.⁶ However, without a highly accurate binding mode model, the rational design of more potent and selective compounds is difficult.

Methods: After multi-template homology modeling, molecular docking, molecular dynamics simulations, and structure-based 3D-QSAR, several agonists were tested via cAMP reporter-gene assay and FACS analysis on TGR5 variants, which were suggested based on our binding mode model.

Results: The binding mode model resulted in a good 3D-QSAR model ($q^2 = 0.50$), thus indicating that differences in the agonist structures correlate with differences in experimentally determined pEC$_{50}$ values. The R79A mutation had a severe impact on the activity of TLC in TGR5. The Y89A mutation reduced the activity of TCDC 13-fold, while it only reduced the activity of TUDC two-fold.

Discussion/Conclusion: Our binding mode model explains why taurine-conjugated bile acids show a higher activity towards TGR5 than their unconjugated analogues. Additionally, we identified the epimer selectivity-determining residue Y89 for hydroxyl groups in position seven on the cholane scaffold. A lack of hydrogen bonding with this residue explains why TUDC is a weaker TGR5 agonist than TCDC, as the Y89A mutation shows a seven-fold higher impact on TCDC activity than on TUDC activity. This provides strong support to the validity of the binding mode model. Our binding mode model could ease the structure-based design of new TGR5 agonists.

We are grateful to the “Zentrum für Informations und Medientechnologie” (ZIM) at the Heinrich Heine University for computational support, Stefanie Lindner und Waltraud Kuß for technical assistance, and to Dr. Nadine Homeyer, Yasemin Bilgic, and Alina Völz for help with the molecular modeling. This work was supported by the Deutsche Forschungsgemeinschaft through the Collaborative Research Center SFB 974 (“Communication and Systems Relevance during Liver Damage and Regeneration”, Düsseldorf) and the Clinical Research Group KFO 217 (“Hepatobiliary Transport in Health and Disease”, Düsseldorf).
**A novel fluorescent analogue of TUDCA reveals new mechanistic insights into TUDCA cytoprotection**

John F. Gilmer, Jason Gavin, Fran Quilty, Gabor Radics
School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland

**Introduction:** Molecular chaperones are substances that assist protein folding or reduce protein unfolding and aggregation. They have potential application in a wide range of amyloid diseases and in diseases associated with improper folding and aberrant protein processing, and ER stress. The taurine conjugate of UDCA which may contribute to the therapeutic effects of UDCA (it is a major metabolite) is a very important chemical chaperone. TUDCA has shown promise in Huntington’s and ALS and in models of metabolic syndrome. Notably, the mechanism of its hepatoprotective and ER stress modulatory effects is currently unknown or at least not widely agreed. In this work, using a new synthetic taurine conjugation approach, we have produced the 3-amino dansyl analogue of TUDCA as a fluorescent reporter for TUDCA. We show that the analogue has similar cytoprotective properties and ER stress modulatory properties to TUDCA.

**Methods:** We used the hepatocarcinoma cell line HUH7 and stimulation with tunicamycin or DCA as a model of ER stress. We measured mRNA of BIP/GRP78, ATF4, CHOP and XBPu/s and PERK phosphorylation by Western Blotting at 6h. We established an in vitro model of cholestatic cytotoxic effects using DCA and GCDCA (200–300 µM) to stimulate apoptosis/necrosis in HUH7 cell culture. Cell viability with or without additional UDCA, TUDCA or fluorescent reporter was assessed using MTT and nuclear cell count on INCELL. The effect of pre and co-treatment on cell viability was assessed. Finally we used confocal microscopy to compare the distribution of the fluorescent analogue and fluorescent but unconjugated UDCA.

**Results:** The 3-alpha dansyl analog had similar effects to TUDCA on BIP, ATF4 and processing of XPB. The 3-alpha dansyl UDCA compound also inhibited protein aggregation in albumin assays. In a model of cholestasis using DCA to induce apoptosis, UDCA had no effect but pretreatment with its 3-alpha dansyl TUDCA prevented cell death similarly to TUDCA. Finally, using confocal microscopy we were able to show that the fluorescent analogue did not undergo diffusion and uptake into HUH7 cells under conditions where it was promoting survival and reducing ER stress. In contrast, fluorescent analogues of UDCA freely diffused into HUH7 cells.

**Discussion/Conclusion:** An effective new synthetic approach to taurine conjugation was used to prepare 3-alpha amino TUDCA. This is a suitable reporter for TUDCA effects on ER stress, related chaperone activity and hepatoprotection in response to toxic bile acid treatment. The fluorescent reporter analogue of TUDCA was not taken up into cells indicating that its effects and that of its parent TUDCA are mediated by interactions with cell membrane components or activation of membrane receptors.
A novel protocol enables the differentiation of human pluripotent stem cell derived bipotential hepatoblasts into hepatocyte or cholangiocyte like cells

Nina Graffmann¹, Wasco Wruck¹, James Adjaye¹
¹Institute for Stem Cell Research and Regenerative Medicine, Heinrich-Heine University, Düsseldorf, Germany

Introduction: The liver has an immense capability of self-renewal. Damaged cells are reliably replaced and even after partial hepatectomy the organ is reconstructed to its fully original size and it regains all its former functions. This is particularly challenging as liver lobules are highly vascularized structures consisting not only of hepatocytes. Cholangiocytes, epithelial cells which line the intra-hepatic bile ducts, are of major importance for the structure and function of the liver. Up to now it is not known exactly how liver regeneration takes place. Residual stem/progenitor cells could differentiate to replace the lost cells, normally quiescent hepatocytes could start proliferating or cholangiocytes could transdifferentiate in order to give rise to functional hepatocytes.

Methods: Human pluripotent stem cells (hPSCs) were differentiated into biopotential hepatoblasts that have the capability to differentiate into hepatocyte (HLCs) or cholangiocyte (CLCs) like cells. Gene expression of differentiated cells was analysed with real-time RT PCR and immunostainings. Global expression profiles were obtained by Affymetrix microarrays.

Results: In our novel protocol the cell fate decision between HLCs and CLCs is made at the stage of the biopotential hepatoblast, the last common progenitor which closely mirrors the in vivo situation. It depends highly on cell density and is influenced by NOTCH signalling. Immunostainings and global transcription analyses revealed gene expression patterns characteristic for the two different cell types. HLCs express e.g. ALBUMIN and HNF4a while CLCs are positive for EpCAM and CK19. Additionally, differentially expressed receptors and transcription factors clearly separate the two cell types and give hints for the further optimization of the differentiation protocol.

Discussion/Conclusion: In our novel protocol the cell fate decision between HLCs and CLCs is made at the stage of the biopotential hepatoblast, the last common progenitor. This closely mirrors the in vivo situation. It enables us to study the subtle differences in the differentiation of both cell types and will help us to understand human liver regeneration in more detail.
Bile acid biosynthesis avoiding cholesterol

William J. Griffiths¹, Jonas Abdel-Khalik¹, Peter J. Crick¹, Michael Ogundare¹, Brian W. Bigger², Andrew A. Morris³, Cedric H. Shackleton⁴, Peter T. Clayton⁵, Jan Sjövall⁶, Ingemar Björkhem⁶, Yuqin Wang¹

¹Swansea University, UK; ²University of Manchester, UK; ³St Mary’s Hospital Manchester, UK; ⁴Oakland Research Institute, USA; ⁵UCL Institute of Child Health, London, UK; ⁶Karolinska Institutet, Stockholm, Sweden

Bile acids are the end products of cholesterol metabolism secreted into bile and excreted in urine predominantly as glycine or taurine conjugates. They are essential for the absorption of lipids and lipid soluble compounds from the intestine and via interaction with the farnesoid X receptor (FXR) regulate their own biosynthesis. Bile acids are mostly synthesised in liver, but can also be made extrahepatically. Besides FXR, bile acids are also ligands for other nuclear receptors including the liver X receptors and the vitamin D receptor. In the current study we have investigated the bile acid content of plasma and urine from patients with a defect in cholesterol biosynthesis, i.e. Smith-Lemli-Opitz syndrome (SLOS), resulting in elevated levels of 7-dehydrocholesterol (7-DHC), an immediate precursor of cholesterol, in plasma and tissue.

Methods: Using liquid chromatography (LC)-high resolution mass spectrometry (MS) with multistage fragmentation (MSⁿ) we have analysed plasma and urine from patients with SLOS.

Results: Bile acid biosynthesis normally starts from cholesterol, however, CYP7A1 can also use 7-DHC as a substrate giving 7-oxocholesterol which can be reduced by HSD11B1 to 7β-hydroxycholesterol opening a new route to bile acid biosynthesis. The elevated levels of these sterols in plasma of SLOS patients and also those of 3β,7β-dihydroxycholestenoic and 3β,7β-dihydroxycholelenoic acids define a new and unexpected pathway for bile acid biosynthesis in SLOS patients. The importance of this pathway in SLOS is confirmed by high levels of 7β-GlcNAc conjugates in urine.

Discussion/Conclusion: Using LC-MS we have identified a novel pathway of bile acid biosynthesis in SLOS patients avoiding cholesterol and starting with 7-DHC. Retrospective analysis of LC-MS data from unaffected controls confirms that this pathway also proceeds to a minor extent in healthy individuals. The biological significance of this new pathway in healthy and SLOS affected individuals is yet to be investigated.
Genetic analysis of spontaneous (non-toxic) liver fibrosis in a congenic mouse model

Rabea A. Hall, Katrin Hochrath, Frank Lammert, Frank Grünhage
Department of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany

Background: Mutations in the ABCB4 (ATP-binding cassette, subfamily B, member 4) gene cause cholestatic liver diseases including progressive intrahepatic familial cholestasis (PFIC). Modifying genes of these diseases have yet to be identified systematically. In this study we used the Abcb4 (Mdr2) knockout (-/-) mouse model, in which the deficiency of the hepatobiliary phosphatidylcholine floppase leads to chronic cholestasis, liver injury and fibrosis. As different mouse strains show varying fibrosis susceptibility, we applied a systematic approach to elucidate the genetic control of liver fibrosis in an experimental cross of ABCB4 deficient mice.

Methods: The Abcb4-/- knockout was crossed from the fibrosis-resistant FVB-Abcb4-/- mice to the susceptible BALB/cJ strain by repeated backcrossing. To identify genetic modifiers that contribute to the fibrosis susceptibility linked to ABCB4 deficiency, we crossed these two congenic strains to generate an F2 intercross population. By quantitative trait locus (QTL) analysis differences in disease progression were mapped to polymorphic genetic regions across the whole genome. Single and two-dimensional QTL scans were applied to identify modifiers and pairwise gene interactions.

Results: Compared to FVB-Abcb4-/- mice, the BALB-Abcb4-/- mice progress to higher fibrosis stages. The heterogenic F2 population shows marked phenotypic variation. Whereas single modifiers demonstrate minor effects, gene-gene interaction scans identified a significant interaction of two QTLs on chromosomes 4 and 17. Underlying these loci we identified the genes Abcg5, Abcg8 and sterol carrier protein 2 (Scp2). These are functionally related with hepatobiliary cholesterol homeostasis and resemble creedal modifier genes.

Conclusions: The congenic Balb-Abcb4 knockout mouse allows the genomic exploration of a spontaneous, non-toxic disease model of a human gene defect. The experimental cross of the two genetic backgrounds with distinct fibrosis susceptibility enables the identification of Abcb4-dependent modifiers of cholestatic liver diseases.
Enhanced ileal bile acid uptake may prevent vitamin A and/or D deficiency in Dutch Crohn’s disease patients

Janette Heegsma1,2, Luuk Wymenga1, Mark Hoekstra1, Tjasso Blokzijl1,2, Laura Groen1, Henk Groen3, Gerard Dijkstra1, Klaas Nico Faber1

1Department of Gastroenterology and Hepatology, 2Laboratory of Medicine, Center for Liver, Digestive, and Metabolic Diseases, 3Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Crohn’s disease (CD) typically affects the terminal ileum, leading to bile acid malabsorption. Therefore, CD patients may develop hypovitaminosis A and D, fat-soluble vitamins that play a crucial role in controlling intestinal inflammation. Here, we assessed the vitamin A and D status of CD patients and investigated whether ileocecal resection and/or inflammation affects ileal expression of bile acid transporters (ASBPT, IBABP, OSTα/β), FOXP3 (regulatory T cells) and RALDH-2 (produces retinoic acid).

Methods: Serum retinol and 25-hydroxy-D3 levels were measured for 86 CD patients with and 74 CD patients without ileocecal resection. Retinol levels < 0.7 umol/L and 25(OH)D3 levels < 50 nmol/L were considered deficient. Expression of ASBPT, IBABP, OST-α, OST-β, FOXP3, and RALDH-2 in ileal biopsies was quantified by RT-qPCR.

Results: All 160 CD patients showed serum retinol levels > 0.7 umol/L. 40% of patients with, and 49% of patients without ileocecal resection showed serum 25(OH)D3 levels < 50 nmol/L, comparable to the frequency in the Dutch population. Expression of IBABP, OST-α and OST-β was significantly higher in non-inflamed ileal tissue of CD patients, compared to controls. RALDH-2 expression was significantly increased both in non-inflamed and inflamed ileum of CD patients, compared to controls. FOXP3 expression was significantly higher in inflamed ileal tissue, with no difference between uninflamed tissue from CD patients and controls.

Discussion/Conclusion: Dutch CD patients are not at risk for vitamin A deficiency, which may be due to compensatory enhancement of bile acid uptake in uninflamed ileal tissue. Moreover, the frequency of vitamin D deficiency in CD patients is similar to the general population.
Differences in TGR5-mediated responses to bile acids and INT777 in neonatal and adult cardiomyocytes

Effendi Ibrahim¹, Ivan Diakonov¹, Catherine Williamson², Julia Gorelik¹
¹Imperial College London, National Heart and Lung Institute, 4th floor, Imperial Centre for Translational and Experimental Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN, UK
²King’s College London. Maternal and Fetal Disease Group, Division of Women’s Health, Faculty of Life Sciences & Medicine, 2nd Floor, Hodgkin Building, Guy’s Campus, London SE1 1UL, UK

Introduction: The bile acid receptor TGR5 (Gpbar1) regulates bile acid metabolism, insulin resistance, bladder movement, immune responses and other functions. It is expressed in cardiac tissue, but little is known about its function in the heart. Here we studied the effect of acute exposure to bile acids (CDCA, TCDCA, UDCA, TUDCA and TCA) and 6α-ethyl-23(S)-methylcholic acid (S-EMCA/INT-777), a semisynthetic TGR5 selective agonist, in inducing cAMP release in neonatal mouse cardiomyocytes and cardiac fibroblasts as well as modulating myocyte contraction.

Methods: The level of cAMP was measured by FRET microscopy in cells isolated from transgenic mice expressing a FRET sensor pEPAC1-cAMPs. The contraction rate of myocytes isolated from wild type and TGR5KO mice was manually recorded. Acute stimulation for 15 minutes with 100 µM bile acids and INT-777 was used.

Results: Unconjugated bile acids (CDCA, UDCA) induce substantial cAMP release (FRET ratio change of more than 18%), whereas a lower response (FRET ratio change of > 7%) was seen upon stimulation with conjugated bile acids (TCDCA, TUDCA and TCA). A similar pattern was seen in cardiac fibroblasts but the response was lower than in cardiomyocytes. Interestingly, despite CDCA and UDCA inducing high cAMP release, only CDCA significantly reduces contraction of both wild type and TGR5 KO neonatal cardiomyocytes. The specific TGR5 agonist INT-777 increases cAMP release in neonatal myocytes and fibroblasts but it does not reduce, and actually slightly increases the contraction rate. However in adult myocytes it neither induces cAMP release nor slows contraction.

Discussion/Conclusion: Bile acids CDCA and UDCA and INT-777 induce high levels of cAMP release, comparable to that of adrenaline. However this does not translate into an elevated contraction rate (unlike adrenaline), which suggests different signalling compartmentation. Moreover, the reduction in cardiomyocyte contraction seen upon CDCA stimulation may not involve the TGR5 receptor.
Cholic acid promotes gut epithelial proliferation in rats exposed to gamma-radiation

Satoshi Ishizuka¹, Masahito Hagio¹², Hidehisa Shimizu¹³, Ga-Hyun Joe¹, Manami Takatsuki¹, Maiko Shiwaku¹, Ja-Young Lee¹, Nobuyuki Fujii¹, Satoru Fukiya¹, Atsushi Yokota¹

¹Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
²Faculty of Life Sciences, Toyo University, Ora 374-0193, Gunma, Japan
³Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Shimane, Japan

Introduction: Consumption of a high-fat diet increases some secondary bile acids (BAs) such as deoxycholic acid (DCA) in feces. DCA is derived from cholic acid (CA), a primary BA. We evaluated intestinal epithelial proliferation and BA metabolism in response to ingestion of CA in rats to determine the influence of a CA diet on the responses of gut epithelia to gamma-irradiation.

Methods: WKAH/HkmSlc rats were divided into two dietary groups of rats fed control diet or CA-supplemented (2 g/kg diet) diet for 10 days or 8 weeks. We measured the BA concentrations in the sera and feces in the unirradiated rats using ultra high performance liquid chromatography-electrospray ionization mass spectrometry. Some of the rats from each group were irradiated with gamma-rays (0.65 Gy/min, ⁶⁰Co) at the end of the dietary intervention, and epithelial cell proliferation in the colon was analyzed histochemically.

Results: Unirradiated CA-fed rats had high levels of DCA and CA in the sera, as well as the presence of taurocholic acid in their feces. Significant increases were observed in both epithelial proliferation and the number of epithelial cells in the colon of the CA-fed rats, and this effect was observed at 8 weeks after gamma-ray exposure. Furthermore, extracts from both cecal contents and sera of the unirradiated CA-fed rats promoted proliferation of an intestinal epithelial cell line IEC-6 cells.

Discussion/Conclusion: BAs in enterohepatic circulation promote proliferation and survival of the intestinal epithelium even after receiving DNA damage.
Vitamin D improves liver histology and hepatic gene expression in a murine obesity/NASH model independently of intestinal \textit{Fgf15} expression

D. Jahn\textsuperscript{1}, D. Dorbath\textsuperscript{1}, S. Kircher\textsuperscript{2}, H.M. Hermanns\textsuperscript{1} and A. Geier\textsuperscript{1}

\textsuperscript{1}University Hospital Würzburg, Division of Hepatology, Würzburg, Germany
\textsuperscript{2}University of Würzburg, Institute of Pathology, Würzburg, Germany

\textbf{Introduction:} The gut-derived hormone FGF19 (FGF15 in mice) regulates bile acid (BA) homeostasis and induces various metabolic effects that may help to treat obesity and NAFLD. Previous data has shown that \textit{Fgf15} transcription in mice can be induced by short-term vitamin D (VD3) administration. In the present study, we analysed whether long-term VD3 treatment ameliorates NASH in a mouse model of diet-induced obesity and whether this may be associated with changes in intestinal \textit{Fgf15} expression.

\textbf{Methods:} To induce obesity, NASH and liver fibrosis, C57BL6/J mice were fed a high-fat/high-sugar diet (HFSD) with low VD3 for 16 weeks. The effects of preventive (starting from week 1) and interventional (starting from week 12) VD3 treatment were studied on the level of liver histology and hepatic/intestinal gene expression.

\textbf{Results:} Animals receiving HFSD with low VD3 became obese and developed histologically-defined NASH and liver fibrosis after 16 weeks of feeding. This phenotype was associated with increased expression of lipogenic, inflammatory and pro-fibrotic genes in the liver and with decreased \textit{Fgf15} levels in the intestine. Interestingly, preventive but not interventional treatment with VD3 resulted in improvements of liver histology. These improvements included a significant decrease of steatosis, a trend towards lower NAFLD activity score and a slight non-significant decrease of fibrosis. In line with these changes, preventive VD3 treatment reduced the hepatic expression of certain lipogenic, inflammatory and pro-fibrotic genes. Notably, these beneficial effects occurred in the absence of increased intestinal \textit{Fgf15} expression.

\textbf{Discussion/Conclusion:} These data reveal a beneficial impact of dietary VD3 treatment on disease progression in a murine obesity/NASH model. Importantly, our observations suggest that timely initiation of VD3 supplementation (preventive vs. interventional) is a critical determinant of treatment outcome. In the applied NASH model, VD3 seems to act independently of the BA regulating hormone FGF15.
Characterisation of bile acid pathways in steroidogenic tissues


Introduction: Bile acids (BAs) are end products of cholesterol catabolism, which act as signalling molecules to regulate glucose, lipid and energy metabolism. BAs activate several receptors including the ligand sensitive transcription factor, Farnesoid X receptor (FXR) and the membrane G-protein coupled receptor, TGR5. Besides the organs physiologically in contact with BAs, like the gut and liver, BA receptors are also expressed in cholesterol-rich steroidogenic tissues, such as the testes, ovaries and adrenal glands where they regulate steroidogenesis and affect fertility. To date, there is no definitive evidence that BAs act as endogenous ligands in these tissues. Here we undertake a comparative analysis of the necessary components for functional BA pathways in steroidogenic tissues: BA receptors, ratios of BA species, transporters and enzymes.

Methods: Steroidogenic tissues (testicular, ovarian and adrenal) and liver (control) from 12-week-old C57BL/6 mice were harvested and BAs analysed in parallel with gene expression studies. Untargeted ultra-performance liquid chromatography tandem mass-spectrometry (UPLC/MS) characterised the ratios of key BA species. Relative expression of BA-activated receptors, transporters and enzymes was assessed using quantitative RT-PCR (RT-qPCR).

Results: UPLC/MS demonstrated the presence of physiologically relevant concentrations of BA species in testes, ovaries and adrenal glands. Taurocholic (TCA) and cholic acid (CA) were found in all steroidogenic tissues. Interestingly, Deoxycholic acid (DCA), which can be cytotoxic, was found in reproductive tissues. RT-qPCR confirmed expression of FXR, which can be activated by CA and DCA to a lesser degree and Tgr5, which is activated by conjugated BAs. Furthermore, BA transporters and bile acid enzymes were expressed.

Discussion/Conclusion: Here we systematically compare BAs in steroidogenic tissues and confirm expression of BA sensors and homeostasis genes, indicating that BAs may act as ligands in these tissues. Functional activation of BA pathways in steroidogenic tissue is the subject of future work.
Steroid binding to autotaxin links bile salts and lysophosphatidic acid signalling

Willem-Jan Keune1, Ruth Bolier2,*, Jens Hausmann1,*, Dagmar Tolenaars2, Andreas Kremer2, Tatjana Heidebrecht1, Robbie P. Joosten1, Manjula Sunkara4, Andrew Morris4, Elisa Matas-Rico3, Wouter H. Moolenaar2, Anastassis Perrakis1,* and Ronald P. J. Oude Elferink2,*

1Division of Biochemistry, Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands
2Tytgat Institute for Liver and Intestinal Research and Department of Hepatology & Gastroenterology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
3Division of Cell Biology, Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands
4Division of Cardiovascular Medicine, The Gill Heart Institute and Department of Veterans Affairs Medical Center Lexington, KY 40511, United States
*These authors contributed equally.

Introduction: Autotaxin (ATX) generates the bioactive lipid lysophosphatidic acid (LPA) involved in multiple (patho-) physiological processes, including cholestatic pruritus. ATX protein has a tripartite active site, combining a hydrophilic groove, a hydrophobic lipid-binding pocket, and a tunnel that was proposed to function as an exit route for the product LPA.

Methods: Recombinant ATX was generated using HEK 293 Flp-In cells and crystallized. X-ray crystallography data were analyzed with designated software. ATX activity upon titration with various bile salts and steroids was determined by quantification of liberated choline.

Results: Crystallography revealed electron density in the tunnel of ATX that fitted best with a 7-hydroxysterol structure. We hypothesized that the tunnel could bind bile salts. Biochemical analysis established that TUDCA and TCDCA but not 12-OH bile salts (TCA and TDCA), nor other steroids (testosterone, dexamethasone, 7-hydroxy cholesterol), act as partial non-competitive inhibitors of ATX Co-crystallization experiments confirmed that mechanism, showing simultaneously binding TUDCA in the tunnel and LPA in the pocket. TUDCA caused half-maximal inhibition at 9 µM and maximal inhibition of 60%. Inhibition of ATX was identical with unconjugated, taurine- and glycine-conjugated UDCA and CDCA. TUDCA also inhibited endogenous ATX activity in human serum with an apparent IC50 of ~30 µM and maximal inhibition of 60%.

Discussion/Conclusion: Bile salts without a 12-OH group inhibit ATX activity, likely by blocking the tunnel as an LPA exit route. This may explain the beneficial effect of treatment with ursodeoxycholate in patients with intrahepatic cholestasis of pregnancy. In addition, it may explain the phenomenon that in PBC patients pruritus decrease with progression of cholestasis. Conversely, at low bile salt concentrations, inhibition of the LPA exit route through the tunnel might allow the ATX-LPA to travel over a larger distance so as to reach itch nerve endings and cause itch signalling.
Vertical sleeve gastrectomy (VSG) in morbidly obese adolescents results in increased fibroblast growth factor 21 (FGF21) that correlates with weight loss

Farooq H. Khan1, Lindsey Shaw3, Wujuan Zhang2, Rosa Maria Salazar Gonzalez4, Sarah Mowery4, Melissa Oehrle2, Xueheng Zhao2, Todd Jenkins2, Kenneth D.R. Setchell2, Thomas H. Inge3, Rohit Kohli4

1Division of General Internal Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio, USA; 2Department of Pathology and Laboratory Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA; 3Department of Pediatric Surgery, Surgical Weight Loss Program for Teens, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA; 4Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA

Introduction: Vertical sleeve gastrectomy (VSG) results in elevated bile acids (BA) and fibroblast growth factor 19 (FGF19) levels. FGF21 shares essential co-factors with FGF19 and has been shown to be increased in energy-deficit states. We studied fasting and post-prandial changes in BA and FGF19/21 physiology in morbidly obese adolescents’ post-VSG.

Methods: We enrolled 10 adolescents (age 17.4 ± 0.5 yr & BMI 51.5 ± 2.5 kg/m²) that underwent VSG surgery. Fasting and post-meal challenge (100ml Ensure™) samples (till 120 minutes) were collected at 3 visits (Pre-VSG [V1], and at 1 [V2] & 3 months [V3] post-VSG) for analysis of BA, FGF19 and FGF21.

Results: 1 subject was excluded. As expected post-VSG, subjects lost weight over time (V2 11.8 kg ± 0.8; V3 21.9 kg ± 1.7), while post-prandial BA (V2 60 min p = 0.001 and V3 60 min p = 0.024), and FGF19 (V2 90 min p = 0.026; V3 90min p = 0.085) levels were increased. BA composition changes resulted in an improved post-prandial hydrophobicity index (V3 30 min p = 0.030 and 60 min p = 0.033). We observed that post-VSG FGF21 levels initially increased (V2 fasting and 120 min p < 0.01), then returned towards baseline at V3 (Figure – Left Panel). There was positive correlations between the increase in postprandial BA and FGF 19 (V3 90 min p = 0.041, R = 0.774) and fasting BA and FGF21 (V2 p = 0.003, R = 0.894). Further, we observed a correlation between rise in postprandial FGF19 and FGF21 (V2 90 min p = 0.001, R = 0.920) but more interestingly between body weight lost (kg) and fasting FGF21 levels (V2 p = 0.012, R = 0.82; Figure- Right Panel).
Discussion/Conclusion: BA physiology is altered in obese adolescents’ post-VSG with increased serum BA, FGF19 levels, and an improved hydrophobicity index. Our study presents novel data regarding an increase in FGF21 that correlates with weight loss post-VSG. The role of FGF21 has not been studied extensively in bariatric surgery and warrants mechanistic investigation.
Protective role of TGR5 in LCA induced toxic liver damage

Klindt C.1, Deutschmann K.1, Reich M.1, Herebian D.2, Mayatepek E.2, Deenen R.3, Körhrer K3, Häussinger D.1, Keitel-Anselmino V.1

1Heinrich-Heine-University Düsseldorf, Clinic for Gastroenterology, Hepatology and Infectious Diseases, Moorenstr. 5, Düsseldorf, Germany
2Heinrich-Heine-University Düsseldorf, Department for General Pediatrics, Neonatology and Pediatric Cardiology, Moorenstr. 5, Düsseldorf, Germany
3Genomics & Transcriptomics Laboratory (BMFZ), Heinrich-Heine University Düsseldorf, Geb. 23.12.04, Moorenstr. 5, Düsseldorf, Germany

Introduction: TGR5 (Gpbar-1) is a G-Protein-coupled cell surface-receptor for bile acids (Kawamata et al. 2003). It is expressed in several different organs and cell types, e.g. non-parenchymal liver cells. It is associated with the regulation of bile acid (BA) homeostasis and the modulation of inflammatory responses in the liver (Pols et al. 2011, Wang et al. 2011). Lithocholic acid (LCA) is a hydrophobic secondary bile acid. Feeding of a diet containing 1% LCA leads to inflammation and toxic liver damage in mice (Woolbright et al. 2014, Fickert et al. 2006). The role of TGR5 during this process is yet to be determined.

Methods: 8–12 week old TGR5 knockout (KO) and wildtype (WT) mice were sacrificed after feeding a diet containing 1% LCA (lithocholic acid) for 4 days. BAs in different body liquids were characterized. Markers for liver damage in serum, urine and stool were measured. Liver histopathology was analyzed by HE-staining and immunohistochemistry. The mRNA expression of different genes in the liver was determined by DNA microarray and Realtime-PCR.

Results: TGR5 KO mice developed a more severe liver damage as compared to WT littersmates following LCA feeding as determined by the area of necrosis of HE-stainings and a significantly higher elevation of serum AST and bilirubin levels. Proliferation of hepatocytes and cholangiocytes was significantly reduced in absence of TGR5 as determined by immunohistochemical staining for PCNA and Ki67. The observed LCA induced liver necrosis was accompanied by a considerably enlarged BA pool with an altered serum BA composition. Furthermore, DNA microarray analysis and Realtime-PCR demonstrated several genes involved in bile acid transport as well as inflammation and fibrosis being differentially regulated in the liver of TGR5 KO mice as compared to WT littersmates.

Discussion/Conclusion: In conclusion TGR5 knockout mice show reduced bile acid elimination via urine and stool leading to an enlarged bile acid pool and increased BA levels in liver resulting in more severe liver damage. This is accompanied by differential regulation of genes involved in BA metabolism and transport as well as in inflammation and proliferation.
Cholic acid treatment in Zellweger spectrum disorders

Femke C.C. Klouwer\textsuperscript{1,2}, Kevin Berendse\textsuperscript{1,2}, Bart G.P. Koot\textsuperscript{3}, Elles M. Kemper\textsuperscript{4}, Frank Schaar\textsuperscript{5}, Hans R. Waterham\textsuperscript{2}, Frédéric M. Vaz\textsuperscript{5}, Marc Engelen\textsuperscript{1}, Peter M.L. Jansen\textsuperscript{6}, Ronald J.A. Wanders\textsuperscript{2}, Bwee Tien Poll The\textsuperscript{1}

\textsuperscript{1}Department of Paediatric Neurology, Emma Children’s Hospital/Academic Medical Center, University of Amsterdam, The Netherlands; \textsuperscript{2}Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, The Netherlands; \textsuperscript{3}Department of Paediatric Gastroenterology, Emma Children’s Hospital/Academic Medical Center, University of Amsterdam, The Netherlands; \textsuperscript{4}Department of Pharmacy, Academic Medical Center, University of Amsterdam, The Netherlands; \textsuperscript{5}Department of Surgery, Maastricht University, The Netherlands; \textsuperscript{6}Department of Gastroenterology and Hepatology, Academic Medical Center, University of Amsterdam, The Netherlands

Introduction: Zellweger spectrum disorders (ZSDs) are a group of severe disabling congenital disorders resulting from a failure in peroxisome assembly, caused by autosomal recessive mutations in one of the \textit{PEX} genes. At least some of the progressive and irreversible clinical abnormalities in these patients are caused by the accumulation of toxic bile acid intermediates. We hypothesize that cholic acid supplementation in these patients can stabilize progression of liver dysfunction by suppressing the endogenous bile acid synthesis and thereby decrease the accumulation of toxic and cholestatic bile acid intermediates.

Methods: Nineteen ZSD patients were followed longitudinally prior to start of the treatment during a run in period of two years. Subsequently, all patients were treated with cholic acid during a 9-month treatment period. The bile acid spectrum and liver functions were analyzed in plasma at start, 4, 12 and 36 weeks after start of treatment. Fibroblast growth factor 19 (FGF19) and 7-alpha-hydroxy-4-cholesten-3-one (C4), as markers for feedback of the endogenous bile acid synthesis, were analyzed at start, 12 and 36 weeks after start of treatment.

Results: Both dihydroxycholestanoic acid (DHCA) and trihydroxycholestenoic acid (THCA) levels significantly decreased after 4, 12 and 36 weeks of cholic acid treatment. FGF19 and C4, respectively significantly increased and significantly decreased after 12 and 36 weeks of treatment. In patients suffering from liver cirrhosis (n = 4), cholic acid supplementation resulted in progressive cholestasis. One patient had to be excluded, due to persistent elevated bilirubin levels. No difference in liver enzymes was observed in the group of patients without liver cirrhosis.

Discussion/Conclusion: Treatment of cholic acid resulted in suppression of bile acid synthesis in the majority of the patients. However, it can be potentially harmful for patients suffering from severe liver disease, leading to progressive cholestasis. A prolonged treatment period is needed to investigate if cholic acid treatment can alter clinical outcome.
Bile salt and FGF19 signaling in the early phase after liver resection in patients with colorectal liver metastasis

Kiran V.K. Koelfat1, Kim M.C. van Mierlo, Johanne G. Bloemen1, Albert K. Groen3, Peter L.M. Jansen1, Cornelis H.C. Dejong1,3, Frank G. Schaap1, Steven W.M. Olde Damink1,4
1Department of Surgery, Maastricht University Medical Center and NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands; 2Department of Pediatrics, Laboratory of Medicine, University of Groningen, University Medical Center Groningen, The Netherlands; 3GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands; 4Institute for Liver and Digestive Health, University College London, London, United Kingdom

Introduction: Bile salts (BS) and the BS-induced enterokine FGF19/15 play an important role in liver regeneration following partial hepatectomy in rodents. Little is known about involvement of these signaling molecules in liver regeneration in humans. In this study, we explored aspects of BS/FGF19 signaling in the early phase after liver resection for colorectal liver metastasis.

Methods: Arterial, portal and hepatic venous blood were sampled in patients undergoing liver resection for colorectal liver metastasis (n = 29) shortly before (within 1 hours) and directly after (within 2 hours) liver resection. BS/FGF19 fluxes across visceral organs were calculated. Hepatic BS content and transcript levels were determined in paired liver specimens. The post-operative serum course of BS/FGF19 were assessed at postoperative day (POD) 1, 2 and 3.

Results: Partial hepatectomy induced an immediate increase in BS levels in both arterial (2.7 vs. 5.9 μmol/L; P < 0.001) and portal venous plasma (7.3 vs. 12.3 μmol/L; P < 0.001). The release of BS by the PDV (+1.2 vs. +1.6 mmol·kg⁻¹·h⁻¹; P = 0.02) and the hepatic uptake (-1.0 vs. -1.5 mmol·kg⁻¹·h⁻¹; P = 0.005) were also increased. In contrast, FGF19 levels were decreased in arterial (0.13 vs. 0.07 ng/mL; P = 0.03), portal (0.16 vs. 0.08 ng/mL; P = 0.03) and hepatic venous blood (0.14 vs. 0.08 ng/mL; P = 0.04) following liver resection. Prior to liver resection, FGF19 flux across the PDV was positive (+3.9 ng·kg⁻¹·h⁻¹) this remained stable after liver resection, indicating release of FGF19 from the PDV. Following liver resection gene expression of CYP7A1 was decreased (-2.1 fold; P < 0.001) and FXR gene expression was markedly upregulated (+6.6 fold; P < 0.001). Systemic BS levels were elevated from POD1 onwards. Subgroup analysis revealed that BS levels were higher in patients undergoing major hepatectomy (≥ 3 segments) compared with patients undergoing minor hepatectomy, on POD1 (10.2 vs. 5.0 μmol/L; P = 0.002) and POD2 (22.2 vs. 8.7 μmol/L; P = 0.03). Plasma FGF19 levels transiently peaked at POD1, with no differences in levels between the hepatectomy subgroups at any of the time points.
**Discussion/Conclusion:** Liver resection results in prompt effects on portal levels of signaling molecules participating in liver regeneration. Elevated BS flux across the remnant liver, decreased BS synthesis, upregulated FXR expression and post-operative rise of BS levels implicate early involvement of BS signaling in the regenerative process.
Hepatocyte- but not enterocyte-specific FXR deficiency accelerated non-alcoholic steatohepatitis development in mice

Bo Kong¹, Runbin Sun², Jianliang Shen¹, Yang Pan¹, Jia He¹, Quan Jin¹, Justin Schumacher¹, Le Zhan¹, Yongping Wang³, Jiye Aa², Jason R. Richardson⁴, Tracy Anthony³, Grace L. Guo¹

¹Department of Pharmacology and Toxicology, School of Pharmacy, Rutgers University, Piscataway, NJ, USA; ²Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, Jiangsu, China; ³Department of Nutritional Sciences, Rutgers University, Piscataway, NJ, USA; ⁴Northeast Ohio Medical University, Roostertown, OH, USA

Introduction: Our previous studies showed that whole-body FXR deficiency and/or altered bile acid (BA) homeostasis are involved in NASH development in mice. However, the tissue specific roles of FXR in NASH development are unclear.

Methods: Wild-type (WT; C57BL/6J), whole-body FXR knockout (FXR KO), hepatocyte-specific FXR KO (FXR⁷Hep⁻/⁻) and enterocyte-specific FXR KO (FXR⁷Int⁻/⁻) mice were fed a high-fat diet (HFD) with 60% calories from fat for 6 months. Hepatic levels of triglycerides and cholesterols were determined to assess the degree of liver steatosis. Changes in the expression of genes involved in lipid metabolism, BA homeostasis, inflammation, fibrogenesis and ER stress were determined using Q-PCR.

Results: HFD-fed WT mice developed steatosis, inflammation, and fibrosis, recapitulating human NASH characteristics. In detail, they had increased body weight, reduced insulin sensitivity, increased serum and hepatic levels of triglycerides, cholesterols and ALT activities, enhanced histology and gene expression for inflammation (IL-6, TNFα, ICAM-1), ER stress (Chop) and fibrogenesis (Collagen 1a1, TIMP-1). Furthermore, FXR KO mice showed exacerbated NASH phenotype compared to WT mice. Tissue-specific deletion of FXR affected NASH severity with the degree of NASH severity being FXR KO = FXR⁷Hep⁻/⁻ > FXR⁷Int⁻/⁻ >/= WT mice.

Discussion/Conclusion: Hepatocyte FXR plays critical roles in the protection against NASH development. The study suggests that activation of FXR in hepatocytes only may represent a better strategy for NASH treatment.
Bile acid-mediated hepatic differentiation of mesenchymal stem cells

Claus Kordes\textsuperscript{a}, Iris Sawitza\textsuperscript{a}, Silke Götze\textsuperscript{a}, Diran Herebian\textsuperscript{b}, Mirco Castoldi\textsuperscript{a}, Dieter Häussinger\textsuperscript{a}

\textsuperscript{a}Clinic of Gastroenterology, Hepatology and Infectious Diseases, \textsuperscript{b}Department of General Pediatrics, Neonatology and Pediatric Cardiology, Heinrich Heine University, Düsseldorf, Germany

Introduction: Mesenchymal stem cells (MSC) are multipotent cells, which occur in all organs as pericytes of blood vessels. Hepatic stellate cells represent a quiescent state of MSC in the normal liver. After liver injury, stellate cells become activated and can undergo developmental processes, which are not only influenced by growth factors but also by bile acids. Since bile acids such as tauroursodeoxycholic acid (TUDCA) can initiate the differentiation of MSC into hepatocyte-like cells across species boundaries, the suitability of TUDCA-treated stellate cells for the generation of artificial liver tissue was tested.

Methods: All cells were removed from rat livers by Triton X-100 and sodium dodecyl sulfate perfusion. The resulting decellularized liver scaffolds were injected with isolated rat hepatic stellate cells and constantly perfused with 2 µM TUDCA via the portal veins. The release of albumin, bile acids and vesicles into the perfusion medium was quantified and the gene expression was analyzed by Affimetrix GeneChip Arrays.

Results: Isolated stellate cells differentiated into hepatocyte-like cells in response to TUDCA treatment as indicated by the induction of albumin and bile acid release into the perfusion medium. Stellate cells further released vesicles and expressed hepatocyte-specific genes such as sodium-taurocholate-cotransporting polypeptide, organic anion-transporting polypeptide 4 and multidrug resistance protein 2. GeneChip analysis revealed a high relation of stellate cell-derived artificial liver tissue to normal rat liver, but markers of activated stellate cells such as smooth muscle actin, connective tissue growth factor and endothelin 1 were still abundant. The gene expression of stellate cell-derived artificial liver differed from freshly isolated stellate cells, which indicated cell differentiation in response to TUDCA treatment.

Conclusion: Bile acids such as TUDCA can be used for establishing artificial liver tissue from MSC and represent a novel, promising approach to generate liver tissue from patient-derived adult stem cells for therapeutic use.
The frequent polymorphism \textit{PNPLA3} rs738409 increases hepatic steatosis but might protect against gallstone disease

Marcin Krawczyk$^{1,2}$, Raúl Jiménez-Agüero$^3$, María J. Perugorria$^3$, Lander Gallego$^3$, Luis Bujanda$^3$, Frank Lammert$^1$, Jesús M. Banales$^3$

$^1$Department of Medicine II, Saarland University Medical Center, Homburg, Germany; $^2$Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland; $^3$Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute – Donostia University Hospital (HUD) – University of the Basque Country (UPV/EHU), CIBERehd, Ikerbasque, San Sebastián, Spain

\textbf{Introduction:} Gallstone disease (GD) is frequent in patients with fatty liver (NAFL). The risk of NAFL is further increased in carriers of the \textit{PNPLA3} variant p.I148M. Here, we investigate the potential association between \textit{PNPLA3} p.I148M polymorphism and development of GD.

\textbf{Methods:} We prospectively recruited a cohort of 115 individuals (39 males, BMI range 26–64 kg/m$^2$) among whom 106 were obese (i.e. BMI > 35 kg/m$^2$) scheduled for BS. Fat contents were quantified using biochemical determination of hepatic triglyceride contents (Folch) and a MRI-based equation [Jiménez-Agüero et al. BMC Med. 2014]. At the inclusion we collected data on the prevalence of gallstones and cholecystectomies (CHE). One year after BS we measured the hepatic fat content using the MRI-based equation, and calculated how many patients developed new gallstones. The \textit{PNPLA3} p.I148M polymorphism was genotyped using TaqMan assays with fluorescent detection.

\textbf{Results:} At inclusion, 36 (31.3\%) patients developed gallstones. Among them, 16 underwent CHE previously whilst in 19 individuals CHE was performed during surgery. Within 12 months after BS, new biliary stones developed in 9 (8.4\%) cases. \textit{PNPLA3} p.I148M variant was associated with increased hepatic steatosis ($P = 0.03$). Interestingly, presence of the \textit{PNPLA3} p.I148M polymorphism decreased prevalence of GD (OR = 0.44, $P = 0.02$) and decreased odds of requiring prior cholecystectomy (OR = 0.23, $P = 0.02$). On the other hand, neither this polymorphism ($P = 0.25$) nor the improvement of hepatic steatosis ($P = 0.66$) within 12 months affected the risk of developing stones.

\textbf{Discussion/Conclusion:} Our results suggest that the \textit{PNPLA3} p.I148M genotype might paradoxically decrease the risk of gallstones. Previous study suggested that \textit{PNPLA3} converts lysophosphatidic acid (LPA) into phosphatidic acid (PA) and that p.I148M is a ‘gain-of-function-mutation’ [Kumari et al. Cell Metab. 2012]. Since PA might be metabolized to phospholipids we hypothesize that increased activity of \textit{PNPLA3} could result in higher levels of biliary PS and decreased lithogeneicity of bile.
Bile acids regulate intestinal wound healing by FXR mediated inhibition of CFTR expression in human colonic epithelial cells

Natalia Katarzyna Lajczak¹, Magdalena S. Mroz¹, Vinciane Saint-Criq¹, Stephen J. Keely¹
¹Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Introduction: Epithelial restitution is an essential process for maintenance of intestinal barrier function. Increased levels of colonic bile acids have been proposed to be involved in the pathogenesis of inflammatory bowel disease (IBD) but their roles in regulating restitution are not yet known. Here, we investigated the effects of bile acids on epithelial restitution and molecular pathways involved in colonic epithelial healing.

Methods: T84 colonic epithelial cells, grown as monolayers on transparent permeable supports, were wounded by scratching with a pipette tip at T = 0. Cells were treated with either the most abundant colonic bile acid, deoxycholic acid (DCA; 150 µM), the “therapeutic” bile acid, ursodeoxycholic acid (UDCA; 100 µM), a farnesoid X receptor (FXR) agonist, GW4064 (5 µM), or a cystic fibrosis transmembrane conductance regulator (CFTR) channel blocker, CFTR(inh)-172 (10 µM). Restitution was measured as wound area after 48 h expressed as % T = 0 wound area. HEK-293 cells were transfected with vector expressing luciferase gene under control of the CFTR promoter and vectors expressing FXR. Protein expression was assessed by western blotting and cell migration by Boyden chamber assay.

Results: After 48 h post-wounding, wound closure in untreated cells was 63.3 ± 13.5% of that at T = 0, while in cells treated with DCA (150 µM) it was reduced to 24.5 ± 13.1% (n = 5; p < 0.001), whereas UDCA enhanced healing to 88 ± 4 (n = 5; p < 0.001). Furthermore, UDCA prevented inhibition of wound closure by DCA. The effects of DCA are mediated via a decrease in cell migration to 0.7 ± 0.1 fold (n = 5, p < 0.05) of that in untreated controls, rather than inhibition of cell growth. Furthermore, DCA decreased cell surface CFTR expression to 23 ± 5% of controls (n = 3, p < 0.001), while a CFTR inhibitor, CFTR(inh)-172 (10 µM), attenuated wound closure to 37 ± 2% (n = 5; p < 0.01), compared to control. Moreover, DCA decreased CFTR promoter activity, in a concentration-dependent manner that was also dependent on co-expression of FXR. Finally, GW4064 (5 µM), an agonist of FXR, mimicked DCA effects on wound healing and CFTR expression.

Discussion/Conclusion: Our data suggest that colonic bile acids differentially regulate intestinal epithelial restitution and that UDCA promotes healing and protects against the detrimental effects of DCA. Thus, manipulation of the colonic bile acid pool may prove to be a useful approach for promoting intestinal barrier function in IBD.
Bile acids regulate colonic epithelial defensin secretion: Implications for pathogenesis and therapy of inflammatory bowel disease

Natalia K. Lajczak¹, Vinciane Saint-Criq¹, Magdalena S. Mroz¹, Alessia Perino², Frank Murray¹, Kristina Schoonjans² and Stephen J. Keely¹
¹Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland
²Ecole Polytechnique Fédérale de Lausanne, Switzerland

Introduction: Increased secretion of colonic epithelial human β defensins (HβDs) has been proposed to contribute to the pathogenesis of ulcerative colitis (UC) through induction of chemokines and the consequent infiltration of mucosal immune cells. Although alterations in the colonic bile acid pool also occur in UC, the roles of bile acids in regulating HβD production are not yet known. Here, we investigated the effects of two common colonic bile acids on epithelial HβD release; deoxycholic acid (DCA), the most common of the colonic bile acids, and ursodeoxycholic acid (UDCA), which we and others have shown to exert anti-inflammatory actions in vivo models of disease.

Methods: HβD release from monolayers of T84 colonic epithelial cells or muscle-stripped sections of human colon mounted in Ussing chambers were measured by ELISA after treatment with DCA (10–150 µM) or UDCA (50–100 µM). Colonic mβD-1 and mβD-4 mRNA levels from WT and TGR5-/- mice were measured by q-PCR. Use of human and mouse tissue was approved by the Beaumont Hospital Ethics Committee.

Results: DCA significantly increased HβD-1 and HβD-2 secretion from T84 cells from 190 ± 28 pg/ml to 413 ± 34 pg/ml and 27 ± 5 pg/ml to 291 ± 2 pg/ml (n = 4; p < 0.01), respectively. Furthermore, UDCA attenuated DCA-stimulated HβD-1 and HBD-2 release to 82 ± 10 pg/ml and 95 ± 17 pg/ml, respectively (n = 4; p < 0.05). Similar effects were seen in ex vivo sections of distal human colon. Similar to DCA, (INT-777, 10 µM) a specific agonist of the bile acid receptor, TGR-5, stimulated both HβDs release from T84 cells and these responses were significantly reduced by UDCA treatment. INT777 (30 µM) failed to increase levels of mβD-1 and 4, orthologues of HβD-1 and 2, respectively, in TGR5-/- but not in WT mice. Finally, a specific inhibitor of NF-κB, BMS-34451 (25 µM) attenuated DCA (150 µM)-induced HβD-2, but not HβD-1, secretion to 27 ± 8 pg/ml (n = 6; p < 0.01).

Discussion/Conclusion: Taken together our data suggest that DCA, likely through activation of TGR5, promotes colonic epithelial HβD secretion. In contrast, UDCA inhibits HβD secretion, an effect which may underlie its anti-inflammatory actions in vivo. Thus, alterations in colonic bile acid pool may influence UC pathogenesis through alterations in HβD production.
Feedback regulation of autotaxin in mice: Why cholestatic mice don’t scratch

Jacqueline Langedijk, Dagmar Tolenaars, Ruth Bolier, Ulrich Beuers, Coen Paulusma, Ronald P.J. Oude Elferink
Tytgat Institute for Liver and Intestinal Research, AMC, Amsterdam, The Netherlands

Introduction: Serum activity of autotaxin (ATX) correlates with itch intensity in cholestatic humans [Kremer et al. 2010]. ATX converts lysophosphatidylcholine into lysophosphatidic acid (LPA). However, in cholestatic mice there is no increase in scratch behaviour and serum ATX is only marginally elevated. It was our aim to study the difference in regulation of ATX expression between humans and mice.

Method: ATX expression was measured in human and mouse fibroblasts by qPCR after incubation with (ant)agonists of LPA signaling.

Results: Addition of LPA (1 µM) to mouse fibroblasts had no effect on ATX expression because of the breakdown of LPA by lipid phosphatase (LPP). When this breakdown was inhibited by XY14 (10 µM), ATX expression was downregulated by 66%. The stable LPA analogue XY17 (1 µM) caused a downregulation of 71%. Addition of the ATX inhibitor HA155 (10 µM) increased ATX expression to 354%, showing that endogenous ATX production leads to LPA signaling.

LPA is recognized by six different G-coupled receptors. Intracellular inhibition of Gαi/o (by pertussis toxin [500 ng/mL]) and its downstream effectors PLC (U-73122; 1 µM) and PKC (Gö6983; 10 µM), led to an upregulation of 531%, 195% and 377% of control levels, respectively.

Inhibition of PKA by H89 (30 µM) strongly upregulated ATX expression (1248%). However, in the presence of H89, ATX expression could still be strongly repressed by LPA signaling, excluding a role for PKA in feedback repression of ATX. All these effects were absent in human fibroblasts.

Conclusion: In mice there is strong feedback regulation of ATX by LPA-signaling through Gαi/o, which is completely absent in humans.
Molecular regulation of adrenal function by bile acids

Lei Liu¹, Alex Zaufel¹, Judith Gumhold¹, Elisabeth Krones¹, Gernot Zollner¹, Peter Fickert¹
¹Department of Gastroenterology and Hepatology, Medical University of Graz, Austria

Introduction: Clinical studies have indicated that critical illness in liver patients is often accompanied by adrenal gland dysfunction together with elevated serum levels of bile acids. Importantly, the target receptors of bile acids such as the nuclear farnesoid X receptor (FXR), were found to be expressed at a high level in the adrenal cortex where steroid synthesis takes place. However, whether and how bile acids act directly on adrenal cortices is still widely unknown. Consequently, this research will study the effects and the possible regulatory mechanisms of bile acids on adrenal glands.

Methods: We performed 7-day or 3-week common bile duct ligation (CBDL) in wild type and FXR knock-out mice to mimic cholestasis with systemic retention of bile acids. To study the sole effects of bile acids in vivo, mice were also fed with 1% chenodeoxycholic acid (CDCA). In addition, human adrenocortical carcinoma cell line H295R was cultured and treated with various bile acids. Serum corticosterone or cortisol secretion levels, mRNA levels and protein expressions of steroidogenesis-related enzymes and cholesterol transporters, together with cholesterol concentrations in adrenals or cells, were determined.

Results: CBDL and CDCA-fed mice developed FXR-independent hypercortisolism. mRNA and protein levels of steroidogenesis-related enzymes were significantly elevated cholesterol uptake-related genes were up regulated while cholesterol efflux-related genes were repressed. In adrenals of 3-week CBDL mice, cholesteryl ester concentration decreased significantly together with enhanced HSL (hormone-sensitive lipase) protein and phosphorylation level. In vitro, conjugated CDCA significantly induced cortisol secretion of H295R cells together with induced expression of steroidogenesis-related genes and cholesterol transporters.

Discussion/Conclusion: Our results indicate that bile acids can directly affect adrenal gland function. In addition, the finding of enhanced phosphorylation of HSL suggest bile acid-dependent activation of the PKA pathway in adrenals.
Taurocholate induces cyclooxygenase-2 expression via the sphingosine 1-phosphate receptor 2 in a human cholangiocarcinoma cell line

Runping Liu, Xiaojiaoanyang Li, Luyong Zhang, Phillip B. Hylemon, and Huiping Zhou
Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University and McGuire Veterans Affairs Medical Center, Richmond, VA 23298, USA; China Pharmaceutical University, Nanjing, Jiangsu, 210009, China

Introduction: Cholangiocarcinoma (CCA) is a rare, but highly malignant primary hepatobiliary cancer with a very poor prognosis and limited treatment options. Our recent studies reported that conjugated bile acids (CBAs) promote the invasive growth of CCA via activation of sphingosine 1-phosphate receptor 2 (S1PR2). Cyclooxygenase-2 (COX-2)-derived prostaglandin E2 (PGE2) is the most abundant prostaglandin in various human malignancies including CCA. Previous studies have indicated that COX-2 was highly expressed in CCA tissues, and the survival rate of CCA patients was negatively associated with high COX-2 expression levels. It has also been reported that CBAs induce COX-2 expression, while free bile acids inhibit COX-2 expression in CCA mouse models. However, the underlying cellular mechanisms and connection between S1PR2 and COX-2 expression in CCA cells have still not been fully elucidated and is the focus of this study.

Methods: A human cholangiocarcinoma cell line HuCCT1 was used this study. A chemical antagonist of S1PR2, JTE-013, was used to inhibit S1PR2 activation and a gene specific shRNA was used to down-regulate the expression of S1PR2. S1P was used as positive control. The expression of COX-2 was determined by real-time RT-PCR and Western blot analysis. The PGE2 production was detected by ELISA. The activation of EGFR/ERK1/2/Akt-NF-κB signalling pathways were determined by Western blot analysis and immunofluorescence staining. Interaction of TCA with S1PR2 was monitored by receptor internalization assay.

Results: TCA dose- and time-dependently induced the expression of COX-2 in HuCCT1 cells. Both TCA- and S1P-induced cell proliferation and invasion were inhibited by JTE-013. TCA-induced COX-2 expression and invasiveness of HuCCT1 were also inhibited by down-regulation of S1PR2 expression with a S1PR2-shRNA or a specific chemical inhibitor of COX-2, celecoxib. In addition, TCA- and S1P-induced activation of EGFR/ERK1/2/Akt-NF-κB was markedly inhibited by JTE-013. Furthermore, both TCA and S1P significantly increased protein levels of matrix metalloproteinase 2 (MMP2) and MMP9, which was blocked by JTE-013.

Discussion/Conclusion: Elevated bile acid levels are correlated with cholangiocyte proliferation and cholangiocarcinoma by unknown mechanism. COX-2 has been linked to tumour growth. This study suggests that S1PR2 plays a critical role in TCA-induced COX-2 expression and CCA growth. Activation of S1PR2 by TCA activates ERK1/2 and Akt signalling pathways via G proteins and EGFR, which further leads to the activation of NF-κB and subsequent COX-2 expression and PGE2 synthesis. This study provides further evidence indicating that S1PR2 may represent a novel therapeutic target for CCA.
Deletions in the cytoplasmic domain of iRhom1 and iRhom2 promote shedding of the TNF receptor by the protease ADAM17

Sathish Kumar Maney, Philipp Lang
Molecular Medicine II, Heinerich Heine University, Düsseldorf, Germany

Tumor necrosis factor (TNF) is an extracellular signal that can trigger cell death through its receptor. The protease ADAM17 has a dual role in regulating TNF signaling: ADAM17 promotes TNF signaling by cleaving and releasing TNF from the cell surface, and ADAM17 dampens TNF signaling by cleaving and releasing TNF receptors from the surface. The rhomboid proteins iRhom1 and iRhom2, which lack catalytic activity, mediate the maturation and delivery of ADAM17 to the cell surface. Maney et al. found that deletions in the cytoplasmic region of iRhom1 or iRhom2, which mimic mutations in the N-terminal cytoplasmic tail of iRhom2 in some patients with susceptibility to esophageal cancer, reduced TNF signaling, despite increasing ADAM17 activity. Expression of N-terminally truncated iRhom2 in mouse fibrosarcoma cells increased the abundance of ADAM17 at the surface and the subsequent shedding of the TNF receptors, thereby suppressing TNF-induced intracellular signaling and cell death.
ACOX2 deficiency: A new inborn error of bile acid biosynthesis causing persistent hypertransaminasemia

Jose J.G. Marin¹,⁴, Maria J. Monte¹,⁴, Marta Alonso¹, Oscar Briz¹,⁴, Elisa Herraez¹,⁴, Rocio I.R. Macias¹,⁴, María J. Perez¹,²,⁴, Elisa Lozano¹,⁴, Ruba Al-Abdulla¹, Maitane Asensio¹, Francisco González San-Martín¹,²,⁴, Silvia Jiménez¹,², Beatriz Castaño¹,², Josep Argemi³,⁴, Jesus Prieto³,⁴
¹)Experimental Hepatology and Drug Targeting (HEVEFARM), IBSAL, University of Salamanca, Salamanca, Spain
²)Salamanca University Hospital, Salamanca, Spain
³)Department of Medicine, Navarra University Hospital, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain
⁴)National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Spain

Background: Inborn errors affecting bile acid synthesis are rare genetic disorders characterized by accumulation of potentially toxic intermediate metabolites.

Aim: To investigate the case of a young male suffering from persistent hypertransaminasemia of unknown origin with a suspected dysfunction in bile acid metabolism.

Methods and Results: HPLC-MS/MS analysis of his serum and urine revealed negligible levels of major bile acids. In contrast, there was evidence for the presence of tauroconjugated trihydroxycholestanoic acid (THCA), whose chemical structure was confirmed with Exact Mass determination by HPLC-TOF. Using DNA obtained from oral epithelial cells the exons of all enzymes potentially involved in the accumulation of THCA were amplified and sequenced. This revealed a homozygous mutation (c.673C>T) in exon 6 of the ACOX2 gene resulting in an amino acid change (p.Arg225Trp) in the peroxisomal enzyme ACOX2, which is involved in the shortening of the THCA side chain. ACOX2 cDNA was cloned and the mutACOX2 variant was generated by site-directed mutagenesis. Using lentiviral vectors both ACOX2 and mutACOX2 were stably expressed in human hepatoblastoma HepG2 cells. Western blot and immunofluorescence studies demonstrated that the mutation did not affect the size of the protein nor its subcellular localization at the peroxisome. However, mutACOX2 HepG2 cells showed, as compared to those expressing ACOX2, marked impairment of cholic acid (CA) production from THCA together with pronounced oxidative stress and reduced viability.

Conclusions: We describe a new inborn error of bile acid biosynthesis affecting ACOX2 causing accumulation of the toxic compound THCA. ACOX2 deficiency should be considered in the diagnostic workout of young patients with persistent unexplained hypertransaminasemia. Although cholestiramine proved to be efficient, once the molecular basis of the disease have been elucidated, administration of natural bile salts, such as UDCA + CA appears to be a more convenient therapy as it would combine hepatoprotection with down-regulation of THCA biosynthesis.
TGR5 activation inhibits muscular BCAA catabolism via thyroid hormone activation

Teruo Miyazaki¹, Akira Honda¹,², Tadashi Ikegami², Yasushi Matsuzaki²
¹Joint Research Center and ²Department of Gastroenterology, Tokyo Medical University Ibaraki Medical Center, Japan

Introduction: TGR5 activation enhances intracellular conversion of thyroid hormone (T4 → T3) through cAMP-CREB-D2 signalling pathway. Active form T3 enhances energy expenditure by stimulating glucose catabolism in skeletal muscles. However, the effects of TGR5 activation and/or thyroid hormone on the catabolism of branched-chain amino acids (BCAA), main amino acids of muscular protein, have not been elucidated. We explored the effect of TGR5 activation on BCAA catabolism in cultured human skeletal muscle cells by measuring 3-hydroxyisobutyrate (3HIB), a biomarker for BCAA catabolism.

Methods: Human cultured myotube differentiated from primary skeletal muscle myoblasts were exposed to bile acids (50 µM CA, CDCA, DCA, UDCA, or 5 µM LCA) or synthetic TGR5-ligand (0.1~10 µM 3-(2-Chlorophenyl)-N-(4-chlorophenyl)-N,5-dimethyl-4-isoxazolecarboxamide) in amino acid deficient medium with 2 mM valine and 100 nM T4. The concentrations of 3HIB in the medium were measured after 24 hours by HPLC-ESI-MS/MS.

Results: The concentration of medium 3HIB was significantly increased in the presence of valine, but was not affected by T4 alone. When 5 µM of LCA was added to the medium with T4, 3HIB concentration was significantly decreased. The addition of 50 µM of DCA also reduced 3HIB concentration, but CA, CDCA, or UDCA did not have any effects on the BCAA catabolism. The synthetic TGR-5 ligand decreased 3HIB concentration in a dose-dependent manner, but the maximum effects were observed in the presence of T4.

Discussion/Conclusion: TGR5 ligands significantly inhibited BCAA catabolism in human muscle cells, while they stimulate muscular glucose expenditure through the activation of thyroid hormone. These results suggest that the activation of TGR5 causes energy metabolic shift from BCAA to glucose catabolism, which prevents the consumption of muscular protein.
Raw extract from the Chinese herb *Ipomoea stolonifera* and its purified components are anti-inflammatory and protect against bile acid-induced apoptosis of rat hepatocytes

Han Moshage¹, Xueting Bai¹, ², Yicun Chen², Manon Buist-Homan¹, Ganggang Shi², Klaas Nico Faber¹

1. Department of Gastroenterology and Hepatology and Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
2. Department of Pharmacology, Shantou University Medical College, Shantou, China

**Background:** *Ipomoea stolonifera* (IS) is a Chinese herb that has potent anti-inflammatory properties in traditional medicine. Liver diseases are almost invariably accompanied by inflammation and loss of liver function due hepatocyte cell death. Here, we analyzed the effect of the n-butanol extract from IS (BE-IS) and five compounds purified from BE-IS (scopoletin, esculetin, umbelliferone, hesperetin and curcumin) on the inflammatory response and bile acid-induced cell death in hepatocytes and macrophages.

**Methods:** Primary rat hepatocytes were isolated from Wistar rats and treated with BE-IS and its 5 purified constituents to analyze the effects on cytokine mixture (CM)-induced inflammation and glycochenodeoxycholic acid (GCDCA: 50 µmol/L)-induced cell death. The mouse macrophage cell line RAW264.7 was treated with lipopolysaccharide to induce inflammation. iNOS and HO-1 mRNA expression were used as markers for inflammation and oxidative stress, respectively. Apoptosis was quantified by caspase-3 activity assay and determination of poly (ADP-ribose) polymerase cleavage and necrosis by lactate dehydrogenase (LDH) release.

**Results:** BE-IS and its purified compounds all inhibited CM-induced inflammation to variable extents. CM-induced iNOS mRNA expression was significantly reduced by curcumin, hesperetin and BE-IS. HO-1 mRNA expression was increased by BE-IS, curcumin and hesperetin. BE-IS dose-dependently repressed GCDCA-induced apoptosis, independent of p38, ERK or PI3k signaling. BE-IS and its constituents do not induce necrotic cell death.

**Conclusion:** Raw extracts of *Ipomoea stolonifera* and the purified compounds scopoletin, umbelliferone, hesperetin and curcumin have anti-inflammatory and cytoprotective effects on rat hepatocytes and macrophages. BE-IS is therefore a potential source for therapeutics to treat chronic cholestatic liver diseases.
Bile acid-dependent regulation of lysosomal biogenesis and function

Tarek Moustafa¹, Thomas Eichmann², Kathrin A. Zierler², Heimo Wolinski², Dagmar Kolb³, Judith Gumhold¹, Peter Fickert¹ and Michael Trauner⁴
¹Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz; ²Institute of Molecular Biosciences, University of Graz, Austria; ³Institute of Cell Biology, Histology and Embryology, and ZMF, Center for Medical Research, Medical University of Graz; ⁴Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria

Introduction: Endosomes/lysosomes play a central role in the sorting and degradation of macromolecules, thereby preventing the accumulation of dysfunctional intracellular components and recycle these macromolecules to fuel cellular need for nutrients. This process is in part coordinated by the mammalian target of rapamycin complex 1 (mTORC1) that localizes to lysosomal membranes. The 23-carbon bile acid norursodeoxycholate (norUDCA) was recently shown to affect hepatic lipid metabolism and to protect from liver fibrosis and inflammation in various mouse models of chronic liver injury. Herein we investigated the effect of norUDCA on mTORC1 signalling, autophagy, and endosome/lysosomal biogenesis.

Methods: We used lipidomic-analysis (LC/MS) to analyse lipid composition of liver and bile from mice treated with norUDCA. We studied wildtype (WT), Mdr2 knockout (Mdr2-/-), and lysosomal lipase deficient (Lal-/-) mice. Electron microscopy, polysome fractionation, immuno-histochemistry/fluorescence and immunoblotting were used.

Results: We found that norUDCA treatment in WT and Mdr2-/- animals resulted in a significant increase of hepatic lysobisphosphatidic acid (LBPA), an unconventional lipid that is mainly found in late endosomes/lysosomes. Moreover, the number of Lamp-1 positive endosomes/lysosomes was increased (~5-fold) and livers showed an accumulation of autofluorescent storage material (ceroid) in lysosomes of hepatocytes and Kupffer-cells. This storage material was similarly observed in Lal-/- mice. Treatment of animals with N-(tert-Butyl)-hydroxylamine (NtBuHA), which cleaves thioester linkage of palmitoylated proteins, resulted in lysosomal ceroid depletion. In addition, LBPA was also found in bile of norUDCA +/- NtBuHA treated animals. Lipidomic analysis identified further changes in biliary lipid composition of norUDCA treated WT and Mdr2-/- mice. Most intriguingly, norUDCA abolished mTORC1 signalling in Mdr2-/- mice, increased nuclear localization of TFEB (regulator of lysosomal biogenesis) and induced autophagy. This was paralleled by decreased polysomal formation.

Discussion/Conclusion: We provide evidence that norUDCA targets endosomal/lysosomal biogenesis and function, which influences hepatocellular and biliary lipid composition.
Characterization of bile acid homeostasis during liver regeneration under normal and pathological conditions

Michaela Mueller¹, Simon Schultze¹, Nicole Auer¹, Florian Pauler², Michael Trauner¹
¹Hans Popper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria
²CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Introduction: The liver regenerates in response to partial hepatectomy. However, if the liver remnant is too small, patients undergoing extended liver resection may suffer from hepatic failure. This is known as the small-for-size syndrome (SFSS). During partial hepatectomy (pHX) a transient increase of serum BAs promotes liver regeneration via signaling through FXR. However, little is known about BA homeostasis during impaired liver regeneration after extended hepatectomy (eHX). We performed 86% eHX in mice to characterize the exposure of the liver remnant to BAs.

Methods: 12 weeks old male C57BL/6 mice were subjected to 68% pHX and 86% eHX. We determined serum BA concentrations and expression of hepatic genes involved in BA homoeostasis after 68% pHX, 86% eHX and control mice was analyzed by RNA-deep sequencing and qRT-PCR.

Results: Mice showed decreased body weight to liver weight ratio (P > 0.01) and reduced numbers of hepatocytes undergoing mitosis (P > 0.05) at 48h upon 86% eHX compared to 68% pHX. Serum analysis revealed significantly elevated serum BAs and alkaline phosphatase levels after 86% eHX (P < 0.05). Genes regulating BA synthesis and uptake were significantly downregulated after 86% eHX (e.g. Cyp7a1, P > 0.001; Cyp8b1, P > 0.005; at 48 h after 86% eHX vs. 68% pHX). Genes involved in BA detoxification and active biliary secretion, such as Cyp2b10, Gstm2, Mdr2 and Mrp4 were upregulated after 86% eHX.

Discussion/Conclusion: Our data suggest that the remnant liver is exposed to a toxic BA overload, resulting in an adaptive response of genes involved in BA metabolism and transport. The BA overload may contribute to the inhibition of liver regeneration after 86% eHX. Currently, we validate the expression of genes in BA homeostasis by qRT-PCR and Western blotting. This study will contribute to a better understanding of BA signaling during liver regeneration and may open up new revenues for therapeutic treatment.
Gestational cholestasis is associated with white adipose tissue dysfunction

Vanya Nikolova1, Georgia Papacleovoulou1, Elena Bellafante1 and Catherine Williamson1
1Maternal and Fetal Disease Group, Women’s Health Department, King’s College London, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a liver disease characterised by maternal pruritus and raised bile acids. Women with ICP present also with increased levels of circulating cholesterol and triglycerides. White adipose tissue (WAT) is a master regulator of lipid metabolism which controls fat storage and mobilisation in response to whole-body energy balance. We hypothesised that WAT function is altered in gestational cholestasis and this contributes to the dyslipidaemic profile of the disease.

Methods: C57Bl6 mice fed normal-chow/0.5% cholic acid (CA)-supplemented diet were sacrificed on day 14 post coitum. Diet- and age-matched female mice were used as controls.

Results: Pregnant mice administered with a CA diet presented with higher levels of serum triglycerides and free fatty acids than their chow-fed counterparts. CA-fed pregnant mice had significantly reduced subcutaneous and visceral fat pad weight, triglyceride content and adipocyte size in comparison to chow-fed controls. The transcript abundance of Ppary2 and of its lipogenic targets (Ap2, Lpl, Pepck and Glut4) was reduced in WAT of pregnant CA-fed mice. Adipokine profiling revealed that in the subcutaneous fat of pregnant mice administered CA, the protein levels of the pro-inflammatory cytokines RBP4, DPP4, EMS1 and AHSG were reduced, whereas in visceral fat, RBP4 and PAI1 were decreased. In contrast, feeding of non-pregnant mice with a CA diet for a period of 14 days decreased their serum triglyceride concentrations and had no effect on their circulating free fatty acid levels, adipose tissue morphology or lipogenesis.

Discussion/Conclusion: Our data suggest that feeding of pregnant mice with a CA diet reduces adipose tissue lipogenesis and decreases WAT inflammation in a depot-specific manner thereby interfering with WAT remodelling and expansion. Failure of fat to grow and store surplus lipids that accumulate during pregnancy is likely to precipitate the development of dyslipidaemia.
Bile acid malabsorption patient-reported experiences: results of an online survey

Michelle O’Connor¹, Clare Pitchford¹, Lawrence Kelman², Ramesh Arasaradnam³, Julian R.F. Walters⁴
¹BAM Support UK; ²Bile Salt Malabsorption Facebook Group; ³University of Warwick; ⁴Imperial College London, UK

Introduction: Bile salt malabsorption/bile acid diarrhoea is increasingly being recognised as a cause of persistent, chronic diarrhoea and other related bowel symptoms but patients often receive suboptimal treatment as medical and public awareness of the condition is poor. Patient support groups, the Bile Salt Malabsorption Facebook Group and BAM Support UK, have been established recently. An online survey was conducted to provide information on how this condition affects patients and to help with dealings with medical practitioners.

Methods: Members of the Bile Salt Malabsorption Facebook Group (current membership > 1300) and BAM Support UK were invited to complete an online survey in November 2015. The first 100 responses were analysed.

Results: 91% of the respondents were female, 80% were from the UK with a wide age range. 89% had already received a diagnosis of the condition, with 62% having had a SeHCAT scan; others had been diagnosed by history or by therapeutic trial. Over 35% were diagnosed after 50. 38% had Type 2 (idiopathic, primary) and 37% type 3 (including post-cholecystectomy). 59% reported undergoing more than two tests before being diagnosed. 62% of those who knew the SeHCAT value stated this was < 5% (severe).
Symptoms had been present for > 5 years before diagnosis in over half. These symptoms included explosive, offensive, smelly or watery diarrhoea (“always” or “mostly”) in 80%, urgency in 85%, abdominal swelling/bloating in 54%, pain in 59%, at least occasional incontinence in 88% and also wind and tiredness. Treatment significantly improved diarrhoea in 60% and urgency was then experienced only occasionally in 51%. Colestyramine was the only drug used in 50% but many patients were on colesevelam or other medications. Mental health issues included embarrassment (“often” or “sometimes” in > 90%), nervousness leaving home (> 90%), depression, isolation, helplessness and low self-esteem (all > 80%), which improved with treatment. Diet avoidances, especially fat and lactose, were common (in 78%) and so was weight gain.
75% were now under continuing medical care and most were satisfied with this. Respondents had seen multiple practitioners before diagnosis but felt they were not taken seriously (35%) or dismissed (50%), had seen GPs who were unaware of the condition (28%) or were told nothing could be done (39%). Two-thirds had been diagnosed with IBS. 68% had > 10 interactions with medical professionals before diagnosis.

Discussion/Conclusion: Patient-reported issues in bile acid diarrhoea include a long history of bowel and mental health symptoms before correct diagnosis and poor awareness of the condition by medical practitioners.
Extrahepatic cholestasis induces large scale alterations in the human liver transcriptome

Steven W. Olde Damink¹, Zita Soons¹, Laura Fischer¹, Marlon J. Jetten², Danyel Jennen², Jos C. Kleinjans², Peter L. Jansen¹, Frank G. Schaap¹
¹Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands
²Department of Toxicogenomics, Maastricht University, Maastricht, The Netherlands

Introduction: Impaired formation or flow of bile results in hepatic bile salt retention and this triggers a response aimed at limiting cholestatic liver injury. The bile salt-activated transcription factor FXR mediates part of the adaptive changes in the liver, and protects against hepatic bile salt toxicity. To expand our limited knowledge of how the human liver adapts to cholestatic conditions, we profiled protein-encoding transcripts and regulatory microRNAs (miRNA) in the liver of patients with extrahepatic cholestasis.

Methods: Total RNA was isolated from liver specimens of non-cholestatic patients with pancreatic tail cancer or benign liver tumors (control, n = 9), initially jaundiced patients with periampullary malignancies receiving pre-operative biliary drainage (drained, n = 10), and patients with cholestasis due to periampullary malignancies (cholestatic, n = 9, median values: bilirubin 186 μmol/L, GGT 1055 U/L, AP 540 U/L, AST 232 U/L, ALT 388 U/L). mRNA (SureprintG3 Human GE60KV2) and miRNA (SureprintG3 Human miRNAV19) expression profiles were determined using Agilent arrays.

Results: Unsupervised clustering revealed distinct clusters of cholestatic and control patients, while drained individuals displayed inconsistent clustering within the other groups, despite normalization of serum markers of cholestasis and liver injury. For bioinformatics, data from control and cholestatic patients was used. A total of 1353 mRNAs (758 up, 595 down) and 47 miRNAs (10 up, 37 down) were differentially expressed (≥ 1.5 fold change, adjusted P value < 0.05) in cholestatic liver. Pathway analysis indicated that pathways related to metabolism (e.g. bile salt homeostasis, transport/metabolism of amino acids, glycolysis, and fatty acid degradation) and the response to wounding were differentially expressed in cholestasis. Interaction analysis identified 161 significant interactions between highly expressed miRNAs and mRNAs, and these were modulated by 13 miRNAs.

Discussion/Conclusion: In conclusion, large scale alterations in hepatic mRNA and miRNA expression are apparent in cholestasis of extrahepatic origin, with likely impact on diverse metabolic pathways.
The autophagy inhibitor Rubicon is a direct FXR target in human liver and is induced in human cholestasis

Katrin Panzitt¹, Hanns-Ulrich Marschall², Michael Trauner³, Peter Fickert¹, Martin Wagner¹
¹Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University Graz, Graz, Austria; ²Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; ³Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

Introduction: Bile acids and activation of the bile acid receptor FXR inhibit autophagy, a cellular self-digestion process necessary for cell homeostasis and regeneration. The effects and mechanisms of bile acid accumulation in human cholestatic conditions on autophagy have not been studied in detail. Moreover, it is unknown if the anticholestatic bile acid ursodeoxycholic acid (UDCA) impacts on autophagy.

Methods: Markers of autophagy (LC3, p62) have been studied by Western blot and immunohistochemistry in liver biopsies from cholestatic patients and patients treated with UDCA. Mechanistic details of bile acid (chenodeoxycholic acid (CDCA), obeticholic acid (OCA) and UDCA) effects on autophagic flux have further been studied in human HepG2 cells and primary hepatocytes using shFXR knockdown strategies as well as conventional techniques to study autophagy (e.g. chloroquine treatment, LC3-RFP-GFP transfections, electron microscopy). FXR binding to the autophagy inhibitor Rubicon has been tested independently by FXR ChIP-Seq in a cholestatic human liver sample and by FXR ChIP PCR.

Results: LC3 and p62 is increased in human cholestatic liver samples suggesting blocked autophagic flux. Mechanistically, cell line studies show that the natural and semi-synthetic FXR agonists CDCA and OCA block autophagic flux whereas UDCA increases autophagic flux. FXR ChIP-Seq in a human cholestatic liver sample reveals FXR binding to the gene of the autophagy inhibitor Rubicon, which is further confirmed by FXR ChIP-PCR. In vitro, FXR silencing reduced Rubicon, while FXR stimulation by CDCA and OCA increases Rubicon. In human cholestatic livers Rubicon is induced. In patients treated with UDCA LC3 and autophagolysosome formation are increased along with reduction of Rubicon.

Discussion/Conclusion: In human cholestatic conditions autophagy is impaired. The autophagy inhibitor Rubicon is induced in human cholestasis and is a direct functional FXR target. Conversely, anticholestatic UDCA increases autophagy along with reduction of Rubicon.
Antimicrobial remodelling of gut microbiota differentially affects bile acid profile, signalling and enteroprotective response in the ileum and colon of pigs

Jose J. Pastor\textsuperscript{a}, Aleix Gavaldá-Navarro\textsuperscript{b}, Alessandro Mere\textsuperscript{a}, Francesc Villarroya\textsuperscript{b} and Ignacio R. Ipharraguerre\textsuperscript{c}

\textsuperscript{a}Innovation Division, Lucta S.A., Parc de Recerca, Edifici Eureka, 08193 Bellaterra, Catalonia, Spain
\textsuperscript{b}Departament de Bioquímica i Biologia Molecular, Institut de Biomedicina (IBUB), Universitat de Barcelona, and CIBER Fisiopatología de la Obesidad y Nutrición, Avinguda Diagonal 645, Edifici nou Pl. -1, 08028 Barcelona, Catalonia, Spain
\textsuperscript{c}Institute of Human Nutrition and Food Science, Christian-Albrechts-University Kiel, Hermann-Rodewald-Straße 6–8, 24128 Kiel, Germany

Introduction: An expanding body of evidence links the use of antimicrobials (ANT) in animal agriculture to the worldwide spread of antibiotic-resistant bacteria. Although it is urgent to discontinue the non-therapeutic use of ANT in food-producing animals, this undertaking is hampered by the still-elusive mechanism that mediates their growth-enhancing action. Available evidence supports the hypothesis that alterations in bile acid (BA) metabolism and signalling represent a conceivable mechanism for such ANT effect.

Methods: To investigate this proposition, 24 piglets were weaned at 21 days of age and fed diets supplemented (ANT) or not (control) with amoxicillin (300 ppm), colistin sulfate (120 ppm), and zinc oxide (2500 ppm). After 35 days, animals were sacrificed and samples of target tissues were collected for later analyses.

Results: Feeding ANT enhanced weight gain (P < 0.05) and altered (P < 0.01) colonic microbial diversity and functions mainly by suppressing bacteria of the genus Lactobacillus (P < 0.0001) and Clostridium (P < 0.02). This was associated with reduced bile salt hydrolase activity (P < 0.004) but increased BA dehydroxylating capacity (P < 0.008) in colonic content of ANT-fed pigs. In ileal mucosa ANT elevated (P < 0.03) the proportion of chenodeoxycholic (CDCA) and lithocholic (LCA) acids, whereas in colonic mucosa reduced (P < 0.03) the percentage of hyocholic acid (HCA) and increased (P < 0.04) the concentration of LCA, 6-oxo-LCA, deoxycholic acid (DCA), and the ratios hyodeoxycholic acid (HDCA)/HCA, LCA/HDCA, and LCA/CDCA. These alterations were paralleled by enhanced FXR signalling in both intestinal sections; however, related changes in the expression of genes and proteins involved in gut protection against bacteria, toxins, and inflammation were more pronounced in colonic mucosa.

Discussion/Conclusion: This work demonstrates that BA regulate host enteroprotective response to ANT-mediated alterations in gut microbial composition and activity, which likely represents a mechanistic component of the growth-promoting action of ANT.
Impact of male cholestasis on the sperm epigenome and consequences for the health of the offspring

Vanessa Pataia, Georgia Papacleovoulou, Lucilla Poston and Catherine Williamson
Maternal and Fetal Disease Group, Division of Women’s Health, School of Medicine, King’s College London, London, UK

Introduction: There is accumulating evidence that the metabolic health of a male affects the epigenome of the sperm cells. Further studies have shown that changes to the sperm epigenome of fathers can have an impact on the metabolic phenotype of the sired offspring. In this study we used a mouse model to investigate whether male cholestasis can impact the sperm epigenome and alter the metabolic phenotype of the offspring.

Methods: Male mice were fed a normal-chow (NC) diet or 0.5% cholic acid (CA) supplemented diet for 10 weeks. At completion of feeding, males were mated to NC females and sperm and testes were harvested. Testes FasL mRNA expression, sperm DNA damage, global DNA methylation and hydroxymethylation and sperm microRNA content were assessed.
Females were allowed to deliver litters and the offspring were either kept on NC diet throughout life or challenged with a Western Diet (WD). Offspring were assessed for organ size, energy balance, glucose and lipid homeostasis.

Results: CA feeding for 10 weeks resulted in a significant increase in sperm DNA damage. Concomitantly, increased testicular mRNA expression of FasL was observed. After 10 weeks of CA feeding, global sperm DNA methylation and hydroxymethylation showed a trend for decreased 5-mC% and 5-hmC% DNA content. Altered sperm miR-34c_1 content was also observed. Male offspring of cholestatic fathers challenged with a WD showed increased energy expenditure and respiratory exchange rate. Increased liver size, fasting insulin levels and serum cholesterol levels were also observed in these offspring.

Discussion/Conclusion: Male cholestasis was associated with DNA damage in the sperm and increased apoptosis in the testes. Global sperm DNA methylation, hydroxymethylation and microRNA content were affected by male cholestasis. When challenged with a WD, the offspring of cholestatic males showed shifts in energy balance, organ size and fasting lipid and insulin levels.
Recurrence of progressive familial intrahepatic cholestasis type 2 after liver transplantation with a detection of anti-BSEP antibodies

Benas Prusinskas¹, Simone Kathemann¹, Denisa Pilic¹, Bianca Hegen¹, Peter Küster², Verena Keitel-Anselmino³, Dieter Häussigner³, Rainer Büscher¹, Hideo, Andreas Baba¹, Peter Friedrich Hoyer¹, Elke Lainka¹
¹University Hospital Essen, Germany; ²Clemenshospital, Münster, Germany; ³University Hospital Düsseldorf, Germany

Progressive familial intrahepatic cholestasis type 2 (PFIC-2) is an autosomal recessive disorder, which is caused by mutations in ABCB11 gene encoding bile salt export pump (BSEP). The absent function of BSEP is replaced after liver transplantation, so that liver transplantation is thought to be curative. We report about a Patient who developed posttransplant recurrence of PFIC-2 due to production of antibodies against BSEP. A today 16-year-old Patient was diagnosed with neonatal cholestasis in early infancy. Diagnosis of PFIC-2 was confirmed by homozygous status of splice site Mutation in ABCB11 gene. Liver transplantation because of end stage liver disease was performed at the age of 6. Cholestasis with normal GGT developed 8.8 years after liver retransplantation. At this time the tacrolimus level in blood was very low. A liver biopsy showed severe canalicular cholestasis and giant cell hepatitis without an evidence of chronic or acute rejection, mimicking PFIC-2. Immunofluorescence staining of normal human liver sections with patient's sera showed reactivity to BSEP. After the intensification of immunosuppressive therapy we initiated treatment with Rituximab and weekly plasmapheresis leading to stabilisation of a clinical condition and depletion of antibodies in serum. However, after nine months of intensive therapy a short break of plasmapheresis caused an immediate worsening of clinical condition, which has led to the third liver transplantation after about one year of initial recurrance. To avoid the next recurrence of PFIC-2, a stem cell transplantation has been considered.

Conclusions: A recurrence of a primary disease should be considered by the patients after liver transplantation by PFIC-2, who developed renewed cholestasis and pruritus. Beside the raising of immunosuppressive therapy, the plasmapheresis and B-cell-depletion shows a potential therapy option to influence humoral response. In a long-term, new liver and stem cell transplantation transplantation may be considered.
Analysis of the bile salt export pump (ABCB11) interactome employing complementary approaches

Susanne Przybylla¹, Jan Stindt², Diana Kleinschrodt¹, Jan Schulte am Esch³, Dieter Häussinger², Verena Keitel-Anselmino², Sander H J. Smits¹ and Lutz Schmitt¹

¹Institute of Biochemistry, Heinrich-Heine-University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany; ²Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital, Heinrich-Heine-University Düsseldorf, Moorenstraße 5, 40225 Düsseldorf, Germany; ³Department of General, Visceral and Pediatric Surgery, University Hospital, Heinrich Heine University Düsseldorf, Moorenstraße 5, 40225 Düsseldorf, Germany

Introduction: The bile salt export pump (BSEP, ABCB11) plays an essential role in the formation of bile. In hepatocytes, BSEP is localized within the apical (canalicular) membrane and a deficiency of canalicular BSEP function is associated with severe forms of cholestasis. Regulation of correct trafficking to the canalicular membrane and of activity is essential to ensure BSEP functionality and thus normal bile flow. However, little is known about the identity of interaction partners regulating function and localization of BSEP.

Methods: In our study, interaction partners of BSEP were identified in a complementary approach: BSEP interaction partners were co-immunoprecipitated from human liver samples and identified by mass spectrometry. Secondly, a membrane yeast two-hybrid assay was used to determine protein interaction partners using a human liver cDNA library. Interaction partners that were identified by membrane yeast two hybrid assays and MS were verified by in vitro interaction studies using purified proteins.

Results: The membrane yeast two-hybrid (MYTH) resulted in the identification of 37 known and unknown interaction partners of BSEP. In parallel, proteins associated with BSEP were identified by tandem mass spectrometry (MS/MS). This approach resulted in the identification of more than 500 proteins that directly or indirectly interacted with BSEP. Additionally, soluble interaction partners were verified by in vitro pull-down assays. This approach resulted in the identification of 11 so far unknown interaction partners of BSEP that were identified in all three approaches.

Discussion/Conclusion: By these complementary approaches, a set of eleven novel BSEP interaction partners was identified. With the exception of radixin, all other interaction partners were integral or membrane-associated proteins including proteins of the early secretory pathway and the bile acyl-CoA synthetase, the second last and ER-associated enzyme of bile salt synthesis.
Inactivation of the apical sodium-dependent bile acid transporter (Asbt; Slc10a2) protects against hepatic steatosis in high fat diet-fed mice

Anuradha Rao¹, Courtney Ferrebee¹, Jamie Haywood², Grace Wynn, Wujuan Zhang³, Kenneth D.R. Setchell³, Saul J. Karpen¹, Paul A. Dawson¹
¹Department of Pediatrics, Emory University School of Medicine. Atlanta, GA 30322 USA; ²Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157 USA; ³Division of Pathology and Laboratory Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229 USA

Introduction: The mechanisms underlying the development of non-alcoholic fatty liver disease (NAFLD) and progression to non-alcoholic steatohepatitis (NASH) are not fully elucidated. Bile acids (BA) and their receptors have important roles in regulating whole body metabolism, including that of hepatic lipids. We hypothesized that interruption of the enterohepatic BA circulation would modify signaling in the gut-liver axis and diminish the development of NASH in high fat diet (HFD) fed mice.

Methods: WT and Asbt−/− mice (C57Bl/6) mice were fed a HFD containing 0.2% cholesterol for 1 or 16 weeks. Body weight, food intake, liver histology, intestinal lipid absorption, liver and fecal lipids were measured. Hepatic and intestinal gene expression was measured using real-time PCR analyses.

Results: After short-term HFD-feeding, hepatic expression of Cyp7a1 and HmgCoA reductase were increased approximately 7- and 3.7-fold in Asbt−/− versus WT mice. These changes correlated with 75 and 52% reductions in hepatic BA and cholesterol content respectively, reflecting the increased fecal loss of BAs. The hepatic BA composition was dramatically altered in Asbt−/− mice, with a reduction in 6-hydroxylated BA species, increase in the BA hydrophobicity index, and proportion of FXR agonistic BAs (from 46 to 80% of the hepatic pool). Histological and biochemical assessment revealed significant triglyceride (TG) accumulation after one week of feeding that was blocked in Asbt−/− mice (hepatic TG: 56.4 versus 9.5 µg/mg liver). After long-term 16 week HFD-feeding, Asbt−/− mice were protected against hepatic accumulation of cholesterol and TG. Intestinal lipid absorption was using the sucrose-polybehenate (SPB) method and found to be similar in the WT and Asbt−/− mice (96.7 versus 95.4%).

Discussion/Conclusion: Interruption of the enterohepatic circulation of BAs protects against development of HFD-associated steatosis. Inhibition of dietary fat absorption appears to play little role in that protection.
ER-stress regulates bile acid uptake via downregulation of NTCP

Marion Robin and Stan F.J. van de Graaf
Tytgat Institute, AMC, Amsterdam, The Netherlands

Hepatocytes have a high protein synthesis capacity and thus an expanded ER. Therefore, these cells are more susceptible to experience ER-stress and subsequent activation of the unfolded protein response pathways. During cholestasis bile acid synthesis and influx is reduced to prevent or revert intrahepatic bile acid accumulation and ER-stress is induced. However, the effects of ER-stress on bile acid uptake are largely unknown. Here, we investigated the role of a moderate ER-stress on the regulation and function of the bile acid transporter NTCP.

Methods: We induced moderated ER-stress using thapsigargin treatment in osteosarcoma u2os cells and in the hepatic cell line hepg2 stably expressing NTCP. We assessed the effect of ER-stress on NTCP first via taurocholate-uptake assay, measuring NTCP activity. We then used biotinylation and western blotting analysis of total cell lysate to assess modifications in NTCP level or localisation.

Results: We show that moderate ER-stress significantly impairs the uptake of conjugated bile acid by NTCP in both u2os and hepG2 cell lines. This limited function correlates with a decreased amount of NTCP at the plasma membrane. Similarly, total NTCP levels were reduced suggesting that induction of the ER-stress response leads to NTCP degradation.

Discussion/Conclusion: Moderate ER-stress reduces NTCP protein level and leads to decreased NTCP at the membrane and limited uptake of bile acids in human HepG2 and U2OS cells. These results suggest an alternative pathway regulating NTCP and potentially affecting bile acids homeostasis during cholestasis.
Pharmacokinetics, biodistribution and metabolism of obeticholic acid in rats with CCl₄-induced decompensated liver cirrhosis

Aldo Roda¹, Rita Aldini², Silvia Spinozzi¹, Placido Franco¹, Massimiliano Cont³, Antonia D’Errico⁴, Francesco Vasuri⁴, Alessio Degiovanni⁴, Luciano Adorini⁵
¹Department of Chemistry “G. Ciamician”, University of Bologna, Italy
²Department of Pharmacy and Biotechnology, University of Bologna, Italy
³UO di Medicina Nucleare, Policlinico S.Orsola-Malpighi, Bologna, Italy
⁴“F. Addarii” Institute of Oncology and Transplant Pathology. DIMES, University of Bologna, Italy
⁵Intercept Pharmaceuticals, San Diego, USA

Introduction: In liver disease, bile acids are present in high concentration in peripheral blood. Obeticholic acid, a semisynthetic bile acid analogue and potent Farnesoid X Receptor agonist, developed for the treatment of different liver diseases could also be high in blood in diseases characterized by hepatic impairment, with enhanced exposure in different organs. Obeticholic acid pharmacokinetics, biodistribution and metabolism were therefore studied in rats with decompensated cirrhosis after oral administration to cirrhotic rats.

Methods: Decompensated liver cirrhosis was induced in rats by CCl₄ inhalation and phenobarbital administration for 13 weeks. Obeticholic acid was administered at a single oral dose of 30 mg/kg b.w. Obeticholic acid, its main metabolites and endogenous bile acids were measured at different time after administration in the main involved organs and fluids by liquid chromatography-electrospray-mass spectrometry.

Results: Plasma and hepatic concentrations of obeticholic acid were higher in decompensated cirrhosis than in controls but plasma concentration of endogenous bile acids was always higher than obeticholic acid. At 24 hours, obeticholic acid biodistribution was higher in liver (55% vs. 27%) and lower in intestine (33% vs. 57%) in cirrhotic rats compared to controls. Obeticholic acid stools excretion at 48 hours was higher in healthy than cirrhotic rats and therefore the biological half life time of obeticholic was higher in cirrhotic rat. Obeticholic acid was conjugated by the liver with taurine, glycine and 3-glucuronide in cirrhotic and mainly with taurine in control rats.

Conclusions: In decompensated cirrhosis, obeticholic acid and endogenous bile acids are similarly distributed in liver, plasma and intestine but its plasma and liver concentrations are much lower despite the high administered dose comparable to the endogenous bile acid pool. Obeticholic acid has a similar hepatic metabolism to endogenous bile acids, but is not 7α-dehydroxylated in the intestine. Obeticholic acid liver concentrations at 24 h were lower than endogenous bile acids. Despite the relatively high dose administered, obeticholic acid showed no abnormal localization in the organs or body fluids investigated in decompensated cirrhotic suggesting the safety of the compound when administered to human cirrhotic patients.
Dual targeting of nuclear receptors ameliorates NAFLD pathogenesis in different dietary murine models

Pedro M. Rodrigues¹, Marta B. Afonso¹, André L. Simão¹, Marta Caridade¹, Catarina C. Carvalho², Alexandre Trindade²,³, António Duarte²,³, Pedro M. Borralho¹, Mariana V. Machado⁴, Helena Cortez-Pinto⁴, Cecília M.P. Rodrigues¹, Rui E. Castro¹
¹Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; ²Reproduction and Development, Interdisciplinary Centre of Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal; ³Gulbenkian Institute of Science, Oeiras, Portugal; ⁴Gastrenterology, Hospital Santa Maria, Lisbon, Portugal

Introduction: microRNAs (miRNA/miRs) and bile acids constitute promising therapeutic targets for NASH. We aimed to elucidate the role of the miR-21/PPARα pathway in the liver and muscle tissues of murine NASH models and to evaluate the therapeutic potential of targeting miR-21/PPARα and FXR, using miR-21 knockout (KO) animals and obeticholic acid (OCA).

Methods: Wild-type (WT) and miR-21 KO mice were fed with chow (n = 10) or methionine and choline-deficient (MCD; n = 10) diets for 2 and 8 weeks. Alternatively, mice were fed either chow (n = 12) or fast food diet (FF; n = 12) for 25 weeks. Six animals from each group had their diet supplemented with OCA 10 mg/kg/day ( Intercept Pharmaceuticals, Inc.). Human liver biopsies were obtained from morbid obese NAFLD patients (n = 28). Liver and muscle samples were processed for histological analysis and assessment of miR-21, pro-inflammatory/pro-fibrogenic cytokines, PPARα and metabolic relevant genes, by qRT-PCR and immunoblotting. ROS levels were analysed through the use of 2′,7′-dichlorodihydrofluorescein diacetate.

Results: WT mice fed with the MCD diet presented with steatohepatitis and fibrosis. In contrast, miR-21 KO mice displayed a significant decrease in steatosis severity, liver damage, inflammation and did not develop fibrosis. WT FF-fed mice developed hepatomegaly, macrovesicular steatosis, inflammatory infiltrates and increased oxidative stress. miR-21 levels were increased in WT FF-fed mice, in both liver and muscle, concomitantly with decreased expression of PPARα, a correlation also found in NAFLD patients. Further, WT FF+OCA-fed mice exhibited decreased steatosis and miR-21 expression, compared with WT FF-fed mice. Importantly, KO FF+OCA-fed mice exhibited significantly reduced liver inflammation, oxidative stress and steatosis, in parallel with increased PPARα and key metabolic targets, such as CPT-1 and ACOX2.

Discussion/Conclusion: Altogether, miR-21 downregulation and FXR activation by OCA strongly ameliorate NASH, highlighting the therapeutic potential of novel multi-targeting therapies for diseases associated with the metabolic syndrome. (PTDC/BIM-MEC/0873/2012, SFRH/BD/88212/2012, FCT, PT).
Altered bile acid homeostasis by treatment with glucocorticoids is mediated by interference with FXR/FGF19 ileum-liver cross-talk

Romero MR$^{1,5}$, Al-Aqil FA$^1$, Monte MJ$^{1,5}$, Herraez E$^{1,5}$, Rosales R$^1$, Serrano MA$^{1,5}$, Jimenez F$^{1,5}$, Sanz-Ortega L$^1$, Gonzalez R$^{2,5}$, Pizarro C$^1$, Aranda JC$^2$, Ocon B$^3$, Uriarte I$^{4,5}$, Sanchez de Medina F$^{2,5}$, Martinez-Augustin O$^{3,5}$, Avila MA$^{4,5}$, Marin JJG$^{1,5}$

$^1$Experimental Hepatology and Drug Targeting (HEVEFARM), IBSAL, University of Salamanca. Salamanca, Spain
$^2$Dept. Pharmacology, University of Granada, Granada, Spain
$^3$Dept. Biochemistry and Molecular Biology, University of Granada, Granada, Spain
$^4$Dept. Hepatology, Center for Applied Medical Research (CIMA), IDISNA, University of Navarra, Pamplona, Spain
$^5$National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Spain

Background: Long-term treatment with glucocorticoids (GC) may have adverse effects, such as alterations in bile acid (BA) homeostasis.

Aim: To evaluate the effect of non-hepatotoxic treatment with GC on FXR/FGF19-mediated crosstalk and the expression of genes involved in BA metabolism and entero-hepatic circulation.

Methods: Three GC (dexamethasone, prednisolone and budesonide) commonly used in clinical pharmacology were used to treat (i.p., 0.25–5 mg/kg/day for 7 days) wild-type mice and KO strains Fxr$^{-/-}$, Fgf15$^{-/-}$ and GR$^{-/-}$ (lacking the intestinal GC receptor). Alexander hepatoma cells with negligible endogenous expression of FXR and CYP7A1, were transfected with FXR/RXR.

Results: In wild-type mice, GC treatment reduced, in a dose-dependent manner, ileal expression of Fgf15, whereas Asbt was up-regulated and Fxr was not modified. Using a non-hepatotoxic and non-cholestatic dose of dexamethasone (0.5 mg/kg/day for 7 days), down-regulation of intestinal Fgf15 without up-regulation of liver Cyp7a1 was found. The expression in mouse liver of other genes involved in BA homeostasis, such as Bsep, Ntcp, Mrp2 (BA transport) and Baat (BA conjugation) was not modified. In Fxr$^{-/-}$ and GR$^{-/-}$ mice, basal expression of Fgf15 was lowered. Treatment with GC resulted in further reduction of Fgf15 expression in Fxr$^{-/-}$, but not GR$^{-/-}$ mice. Liver Cyp7a1 expression was not significantly changed in any case. In contrast, in Fgf15$^{-/-}$ mice, GC treatment induced up-regulation of Cyp7a1. When Alexander cells expressing FXR/RXR were treated with GC, enhanced BSEP expression was observed. However, GW4064-induced up-regulation of FGF19 and SHP was limited by GC. This inhibition was also observed in Alexander cells transfected with empty plasmids. In contrast, GC stimulated the expression of CYP27A1, in an FXR-independent manner.

Conclusion: GC interfere with the regulation of hepatic BA synthesis by altering FXR-mediated signaling via ileal FGF19 secretion, which may lead to adverse effects accounting for hepatobiliary alterations in chronic therapy with GC.
Inhibiting hepatic bile acid uptake in DDC-induced cholestasis reduces serum biomarkers of liver injury

Reinout L.P. Roscam Abbing\textsuperscript{1*}, Davor Slijepcevic\textsuperscript{1*}, Lizette Haazen\textsuperscript{1}, Ulrich Beuers\textsuperscript{1}, Ronald P.J. Oude Elferink\textsuperscript{1}, Stan F.J. van de Graaf\textsuperscript{1}

\textsuperscript{*}Authors contributed equally to the study.

\textsuperscript{1}Tytgat Institute for Liver and Intestinal Research, Academic Medical Centre, Amsterdam, The Netherlands

Introduction: The accumulation of bile acids is a major cause of hepatocellular damage in cholestatic diseases. The Na\textsuperscript{+}-taurocholate co-transporting polypeptide (NTCP) plays a major role in the uptake of bile acids into hepatocytes, and can be specifically inhibited using Myrcludex B. We hypothesized that Myrcludex B, by blocking hepatic bile acid uptake, reduces cholestatic liver damage.

Methods: Cholestasis was induced in C57Bl6/J mice by feeding them a 3.5-Diethoxy-carbonyl-1.4-dihydrocollidine (DDC) diet for one week. Mice were injected daily with Myrcludex B or vehicle. Aminotransferase and alkaline phosphatase activity was measured in plasma. Expression levels of genes involved in bile acid homeostasis, inflammation and fibrosis were assessed by qPCR.

Results: Body weight loss was observed in both groups. This was slightly aggravated by Myrcludex B treatment. However, Myrcludex B dampened the increase in liver- and spleen size observed in DDC-fed mice. Serum aminotransferase and alkaline phosphatase levels were markedly reduced by Myrcludex B treatment. Protein levels and mRNA expression of MCP1 were reduced by Myrcludex B treatment. Fibrosis markers (Col1a1, TGF-beta, TIMP) were repressed by Myrcludex B treatment on mRNA level. These effects were less apparent on histology. Serum bile acids were elevated in vehicle treated mice and this was further increased in the Myrcludex B treated mice. The downregulation of NTCP and OATPs typically seen after induction of cholestasis was less pronounced after Myrcludex B treatment.

Discussion/Conclusion: Inhibition of NTCP-mediated bile uptake using Myrcludex B reduces serum biomarkers of cholestatic liver damage and reduces expression of proinflammatory and profibrotic genes in the liver. These results suggest that NTCP inhibition might protect the liver in acute cholestasis. However, the observed weight loss and elevated plasma bile acid levels warrant thorough analysis of long-term safety of this strategy.
**Vitamin A deficiency leads to mild cholestasis and a “humanized” bile acid profile in rats**

Ali Saeed¹, Martijn Oscar Hoeke¹, Mark Hoekstra¹, Janette Heegsma¹², Han Moshage¹, Klaas Nico Faber¹

¹Department of Gastroenterology and Hepatology, ²Laboratory of Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;

Corresponding authors: a.saeed@umcg.nl; k.n.faber@umcg.nl

**Introduction:** Vitamin A is a fat-soluble vitamin and intestinal absorption is facilitated by bile acids. The vitamin A-metabolite, 9-cis retinoic acid, regulates hepatic bile acid synthesis and transport through its interaction with the retinoic X receptor-alpha. In this study, we determined the effect of vitamin A deficiency (VAD) on bile salt synthesis, transport, bile flow and metabolism in rats.

**Methods:** Weaning male Wister rats were fed either a vitamin A sufficient (VAS) or deficient (VAD) diet for 14–17 weeks. Plasma and liver retinol and retinyl palmitate concentration was measured with reversed phase HPLC and bile acids were analysed by gas chromatography.

**Results:** Rat plasma retinol levels were normal until 12 weeks on both diets, after which they sharply dropped in rats on a VAD diet, as compared to VAS rats. Hepatic and serum retinol levels were reduced up to 99% and 85%, respectively, in rats fed a VAD diet for 17 weeks. Hepatic expression of Cyp7a1, Shp and Ntcp, Bsep was not changed, while ileal expression of Ost-α and Fgf15 was increased. VAD increased the plasma bile acid pool by 3-fold, compared to VAS controls, while the hepatic and biliary bile acid pool was not changed quantitatively. VAD strongly affected the plasma and biliary bile salt composition, with a 2.4-fold and a 10.3-fold increase in chenodeoxycholic acid (CDCA), respectively, compared to VAS controls. Also, cholic acid (CA) was increased both in plasma (17.5-fold) and bile (2.3-fold) in VAD rats, compared to VAS controls. Muricholic acids were virtually absent in plasma and bile of VAD rats.

**Discussion/Conclusion:** Vitamin A deficiency in rats leads to mild cholestasis and a more hydrophobic bile salt pool predominantly consisting of CA and CDCA, resembling the human bile salt profile. Further studies are required to define the underlying mechanisms.
Portal vein embolization-triggered liver regeneration is accelerated by the FXR agonist obeticholic acid

Frank G. Schaap¹,* , Pim B. Olthof²,* , Cathy van Himbeeck¹, Floor Huisman², Krijn P. van Lienden³, Rowan F. van Golen², Michal Heger², Joanne Verheij⁴, Isabelle A. Leclercq⁵, Peter L.M. Jansen¹, Thomas M. van Gulik²,* , Steven W.M. Olde Damink¹,*
¹Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands; Departments of ²Surgery, ³Radiology and ⁴Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ⁵Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, UCL, Brussels, Belgium
*Equally contributing first and senior authors.

Introduction: The bile salt (BS) receptor FXR plays an important role in compensatory liver growth following partial liver resection. Here we explored whether FXR is involved in the regenerative response following portal vein embolization (PVE).

Methods: Adult female rabbits (n = 5–6 per group) received vehicle or the FXR agonist obeticholic acid (OCA; 10 mg/kg, daily oral gavage) for 7 days prior to and after embolization of the cranial liver lobes. Effectiveness of PVE was confirmed by portography, and caudal liver volume (CLV) was analyzed by CT-volumetric analysis at days -7, -1, +3 and +7.

Results: OCA induced a larger increase in CLV at day 3 after PVE (59.3 ± 19.2% vs. 29.7 ± 16.1% in controls, P = 0.001), with both groups attaining a similar volume gain after 7 days. In both groups, PVE resulted in a similar pattern of transient elevation of serum BS. Hepatic BS content was reduced (60.1 [16.0] vs. 100.1 [75.1] nmol/g in controls; P = 0.016) in the hypertrophied segments of OCA-treated animals at day +3. Reduced expression of the BS synthetic enzyme Cyp7a1 (-7.1 fold; P = 0.004) and enhanced expression of the basolateral BS efflux pump subunit Slc51b (+6.5 fold; P = 0.004) may have contributed to this lowering. Expression of Cdc25b, a phosphatase required for entry into mitosis, was elevated in the hypertrophic (+1.6 fold; P = 0.006) but not atrophic liver segments of OCA-treated animals. Cdc25b expression in the non-embolized segments correlated with expression of Slc51b (p = +0.80, P = 0.002) and Cyp7a1 (p = -0.62, P = 0.033), and tended to be associated with percentual increase in CLV at day +3 (p = +0.57, P = 0.055).

Discussion/Conclusion: OCA accelerated liver regeneration in the first 3 days after PVE in rabbits. Improved BS homeostasis and induction of proliferative genes may underlie the augmented growth rate in the initial phase after PVE. OCA treatment has potential in extending resectability and prevention of post-resectional liver failure.
Oncostatin M contributes to non-alcoholic fatty liver disease (NAFLD) progression in hypercholesterolemic mice

Sonja Schubert¹, Carmen Schäfer¹, Christine Mais¹, Elke Butt², Daniel Jahn¹, Andreas Geier¹, Heike M. Hermanns¹
¹University Hospital Würzburg, Division of Hepatology, Würzburg, Germany
²Institute of Clinical Biochemistry, University of Würzburg, Würzburg, Germany

Introduction: The IL-6-type cytokine oncostatin M (OSM) is involved in the pathogenesis of inflammatory diseases, whereas its role in metabolic diseases is poorly understood. Recent publications surprisingly show protective effects of OSM on the metabolic syndrome. As hypercholesterolemia is crucial for the progression of NAFLD in humans, we investigated the influence of OSM on the pathogenesis of NAFLD in Ldlr⁻/⁻ hypercholesterolemic mice.

Methods: 11-week-old C57BL/6, Osmr⁻/⁻, Ldlr⁻/⁻ and Ldlr⁻/⁻Osmr⁻/⁻ mice were fed a Western diet for 12 weeks. Body weight was measured once a week. Quantitative PCR was used to determine mRNA expression in liver tissues, liver histology was analyzed using H&E and Sudan IV staining. Serum lipoproteins were measured by HPLC.

Results: Osmr⁻/⁻ mice showed increased body weight, cholesterol levels and hepatic steatosis compared to C57BL/6 animals. Blood glucose levels were not altered. These changes were associated with suppression of Ldlr gene expression in Osmr⁻/⁻ mice. Weight gain in Ldlr⁻/⁻ mice was similar to Osmr⁻/⁻. Serum cholesterol levels, glucose levels and hepatic lipid accumulation were elevated in Ldlr⁻/⁻ compared to C57BL/6 animals. Surprisingly, in Ldlr⁻/⁻Osmr⁻/⁻ animals body weight, cholesterol levels, glucose levels and hepatic steatosis were decreased compared to Ldlr⁻/⁻ mice. Expressions of the inflammatory genes Saa2, Ccl2 and Osm were enhanced in Ldlr⁻/⁻ mice. Cyp7a1 was strongly upregulated in Ldlr⁻/⁻Osmr⁻/⁻ whereas genes involved in triacylglyceride synthesis displayed no significant changes between Ldlr⁻/⁻ and Ldlr⁻/⁻Osmr⁻/⁻ animals.

Discussion/Conclusion: OSM influences the progression of NAFLD in a context-dependent manner. In C57BL/6 animals it has protective effects by upregulating the Ldlr expression and therefore lowering serum cholesterol levels. In Ldlr⁻/⁻ mice those beneficial effects vanish, instead OSM seems to contribute to cholesterol-induced progression of NAFLD, possibly by increasing the inflammatory state. Future studies aim to identify the mechanisms of these opposed functions of OSM and its potential as therapeutic target in the metabolic syndrome.
Cholestasis reduce immune induction after viral infection

Anna-Kathrin Schupp¹, Stephanie Rattay², Andreas Kislaf³, Bernhard Homey³, Dieter Häussinger¹, Albert Zimmermann² and Dirk Graf¹
¹Department of Gastroenterology, Hepatology and Infectious Diseases, Heinrich Heine University, University Hospital, Düsseldorf, Germany
²Institute for Virology, Heinrich-Heine-University, University Hospital, Düsseldorf, Germany
³Department of Dermatology, University Hospital, Heinrich-Heine-University, Düsseldorf, Germany

Introduction: Recently we showed that bile acids reduce mouse cytomegalovirus (MCMV) replication in primary hepatocytes. In opposite to that, bile acids impair immune-cell functions resulting in higher morbidity and mortality of cholestatic patients after bacterial and viral infections. The following study addresses the impact of cholestasis on MCMV infection and MCMV-induced immunity in mice.

Methods: The in vivo consequences of cholestatic conditions on MCMV replication was examined using the widely accepted bile duct ligation (BDL) system in mice. A panel of 19 chemokines and 6 cytokines was quantified by real time PCR in order to measure MCMV-induced immune response in liver samples. Systemic cytokine amounts were measured by a Luminex assay. Immune-cell infiltration in the liver was determined by immunohistochemistry. Virus replication was analysed by virus titration.

Results: MCMV-induced expression of pro-inflammatory cytokines and chemokines was significantly reduced in livers of BDL animals. In contrast, the anti-inflammatory cytokine IL-10 was induced by BDL. Systemic cytokine levels were also affected by BDL. Consequently a quantification of immune cell infiltration revealed decreased amounts of macrophages in livers of BDL- MCMV-infected animals. MCMV titers were not changed in BDL animals.

Discussion/Conclusion: Bile duct ligation results in a modulated expression and secretion of pro-inflammatory chemokines and cytokines in response to MCMV infection. This could be a reason for the higher susceptibility of cholestatic patients to viral infections. Since virus titers were not increased following BDL, we hypothesised that the anti-viral effect of bile acids compensates the pro-viral effect of impaired immune cell infiltration.
FXR agonist PX20606 reduces liver damage, fibrosis and portal hypertension in CCl4 cirrhotic rats

Schwabl P.¹, Hambruch E.², Payer BA.¹, Schubert T.L.¹, Strobl B.¹, Fida S.¹, Wagner M.¹, Garnys L.¹, Peck-Radosavljevic M.*, Kremoser C.*, Reiberger T.*, Trauner M.*, Hepatic Experimental Hemodynamic Laboratory of Vienna, Austria
¹Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria
²Phenex Pharmaceuticals AG, Waldhofer Str. 104, 69121 Heidelberg, Germany

Introduction: Steroidal FXR agonists attenuate liver injury and reduce portal hypertension in experimental models of cirrhosis. We aimed to assess the impact of the non-steroidal FXR agonist PX20606 (PX) on liver histology, inflammation and hemodynamics in cirrhotic rats.

Methods: Cirrhosis was induced in Sprague Dawley rats by carbontetrachloride (CCl4) injections (2 x/week i.p. for 12 weeks). PX (10 mg/kg/day) or placebo (DMSO) was administered per daily gavage from weeks 4 to 12. At end of treatment, mean arterial pressure (MAP), heart rate (HR), portal pressure (PP) and superior mesenteric artery blood flow (SMABF) were measured. Liver fibrosis was assessed by Sirius Red area (SRA) and content of hydroxyproline (HP). Hepatic expression of fibrogenic genes was quantified by qRT-PCR (shown as x-fold (x) of control).

Results: CCl4 rats presented with marked cirrhosis, elevated transaminases and portal hypertension compared to controls. In CCl4 rats, PX treatment significantly ameliorated liver fibrosis (SRA: 6.99 ± 3.15 vs. 3.97 ± 1.64%; p < 0.001. HP: 416 ± 85 vs. 134 ± 14 µg/g liver; p = 0.002). Accordingly AST (558 ± 23 vs. 193 ± 85 IU/ml; p = 0.008) and ALT (538 ± 233 vs. 193 ± 85 IU/ml; p = 0.008) significantly decreased under PX treatment, while plasma cholesterol or triglycerides levels remained unaffected. PX significantly decreased PP (11.9 ± 1.4 vs. 9.7 ± 1.4 mmHg; p = 0.037) and increased SMABF (8.88 ± 2.62 vs. 13.81 ± 2.81 ml/min/100 g; p = 0.021) in cirrhotic rats, while not affecting MAP (124 ± 13 vs. 116 ± 25 mmHg) or HR (330 ± 29 vs. 334 ± 52 bpm). Livers of CCl4-PX rats significantly overexpressed FXR target genes including bile salt export pump (2.5 x), small heterodimer partner (2.3 x), cystathionase (2.1 x) and dimethylargininase (1.7 x). Expression of endothelin-1 (0.45 x), PDGF-Rβ (0.51 x) and αSMA (0.61 x) were significantly reduced.

Discussion/Conclusion: PX20606 treatment reduced hepatic inflammation and fibrogenesis and thus significantly reduced portal pressure in cirrhotic rats. Hence, synthetic FXR agonists such as PX20606 represent a novel therapeutic option against liver fibrosis and portal hypertension.
A mathematical model of the intestinal transit and enterohepatic circulation of bile acids

F.L.P. Sips¹, H.M. Eggink², P.A.J. Hilbers¹, M.R. Soeters², A.K. Groen³, A.K. Groen³, N.A.W. van Riel¹,³
¹Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
²Department of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, The Netherlands
³Amsterdam Diabetes Center, Department of Vascular Medicine, Academic Medical Centre, University of Amsterdam, The Netherlands
⁴Departments of Pediatrics and Laboratory Medicine, University of Groningen, University Medical Center Groningen, The Netherlands

Introduction: Bile acids have emerged as hormone-like regulators of energy metabolism. Consequently, factors that control bile acid concentrations in various pools in the body may play an important role in the control of metabolism and thus metabolic health. The key steps in bile acid transport have been elucidated; however, as a result of the complexity of the enterohepatic circulation, a comprehensive understanding of the relative importance of these steps remains elusive. To better understand how the control of bile acid concentrations is distributed we have developed a mathematical model of bile acid circulation.

Methods: The mathematical model consists of a system of differential equations, which describe the circulation of bile acids as transportation between a series of connected compartments.

Results: The model describes the kinetics of all major bile acids in the enterohepatic circulation and their appearance in systemic circulation. Intestinal compartments are modelled in detail and transit is presumed non-homogeneous; e.g. in the terminal ileum bile acid transit is decelerated before passage into the colon. Additionally, immediately after a meal an implemented gastro-colic reflex affects a short increase of the intestinal transit speed, propelling the intestinal bile acids forward along the digestive tract. The presented model is able to reproduce main characteristics of the human postprandial bile acid response in health and following cholecystectomy.

Discussion/Conclusion: Since only a small fraction of bile acids resides in the systemic circulation while the majority of the pool is found within the enterohepatic cycle, an interesting application of the model is in model-based predictions of the sizes of enterohepatic pools of bile acids from their systemic concentrations. The model may be applicable in any circumstance in which systemic bile acid concentrations change, such as Type II diabetes, dietary intervention, or the increase of bile acid concentrations seen after Roux-and-Y gastric bypass.
Combined activity of NTCP and OATPs governs hepatic uptake of conjugated bile acids in vivo

Davor Slijepcevic¹, Joanne M. Donkers¹, Dagmar Tolenaars¹, Dirk R. de Waart¹, Ulrich Beuers¹, Ronald P.J. Oude Elferink¹, Alfred H. Schinkel*, Stan F.J. van de Graaf¹
¹Tytgat Institute for Liver and Intestinal Research & Department of Gastroenterology & Hepatology, AMC, Amsterdam, The Netherlands
2Division of Molecular Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands

Introduction: Humans with Na+-taurocholate cotransporting polypeptide (NTCP) mutations or volunteers treated with Myrcludex B, a specific NTCP inhibitor currently in anti-HBV drug trials, show marked hypercholanemia. Previously, we reported that NTCP-KO mice have an impaired hepatic uptake of conjugated bile acids (BAs). However, only a subset of NTCP-KO mice shows hypercholanemia. What underlies the normalization of serum BA levels in NTCP-KO mice is unknown. In this study, we investigated the in vivo contribution of murine OATPs to transport of conjugated BAs.

Methods: Taurocholate clearance studies were performed in wild-type and Oatp1a/1b-KO mice during gall bladder cannulation, by injection of radiolabeled taurocholate after receiving Myrcludex B (5 μg/g IV) or placebo. Myrcludex B was administrated 5 days in Oatp1a/1b-KO and Oatp1a/1b-KO mice reconstituted with human OATP1B1. Bile acid levels in serum were quantified by HPLC. Messenger RNA was quantified by RT-qPCR.

Results: All NTCP-KO mice show hypercholanemia at 4 weeks of age, and BA levels normalize in the majority of mice when reaching adulthood, suggesting partial redundancy of NTCP. The hypercholanemic adult mice display a complete absence of Oatp1a1. Oatp1a/1b-KO mice have slightly elevated serum unconjugated BA levels. In wild-type mice, injection of Myrcludex B results in delayed taurocholate clearance from the blood, but eventually all taurocholate is cleared from the blood. However, Myrcludex B completely inhibits active transport of all conjugated BA species in Oatp1a/1b-KO mice. Biliary excretion of taurocholate is < 1% of the injected bolus after 1 hour of bile collection. Cyp7a1 mRNA levels are reduced after 5-day administration of Myrcludex B, likely caused by intestinal FXR-FGF15 signaling.

Discussion/Conclusion: This in vivo study shows the contribution of Oatps as well as Ntcp to hepatic uptake of conjugated bile acids. The data suggests that in humans, NTCP contributes relatively more to the hepatic uptake of conjugated BAs than it does in mice.
Postprandial transorgan bile acid kinetics: Implications for TGR5 agonism

Soeters M.R.1, Eggink H.M.1, van Nierop F.S.1, Schooneman M.G.1, Boelen A.1, Kalsbeek A.1,2, Koehorst M.3, Ten Have G.A.M.4, Nieuwdorp M.5, Groen A.K.1,6, Romijn J.A.7 and Deutz N.E.P.4

1Department of Endocrinology and Metabolism, Academic Medical Centre (AMC), University of Amsterdam (UvA), Amsterdam, The Netherlands
2Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands
3Department of Pediatrics, University Medical Center Groningen, University of Groningen, The Netherlands
4Centre for Translational Research in Aging and Longevity, Department of Health and Kinesiology, Texas A & M University, College Station, United States
5Department of Vascular Medicine, AMC, UvA, Amsterdam, The Netherlands
6Amsterdam Diabetes Center, Department of Vascular Medicine, AMC, UvA, Amsterdam, The Netherlands
7Department of Internal Medicine, AMC, UvA, Amsterdam, The Netherlands

Introduction: The bile acid (BA) receptor Takeda G protein-coupled receptor (TGR5) is seen as an important regulator in energy metabolism. To investigate the potential of BA’s to activate TGR5 in different organs we studied fasting and postprandial concentrations of the different BA’s in a porcine transorgan flux model.

Methods: Pigs were fed a mixed meal and blood was sampled repetitively from the aorta and portal, caval, renal and hepatic veins during 4 hours. BA’s were measured by LC-MS and we calculated a TGR5 activation index using published in vitro TGR5 EC50 values. We also obtained portal and venous blood samples from 11 obese and 5 DM2 patients (fasted) in order to translate our observations to humans.

Results: Porcine fasting portal total BA concentrations were ~6 fold higher compared to other sites. Postprandial glyco-chenodeoxycholic acid (gCDCA) and glyco-hyodeoxycholic acid (gHDCA) were the most prominent portal BA (total AUC 2501 and 2403 µmol•min/L, respectively). In the peripheral veins gHDCA levels were higher as compared to gCDCA (portal and peripheral gHDCA:gCDCA-ratio: 0.95 vs 3.04; p < 0.001). Fasting BA flux in the portal vein was high and doubled after the meal. Lithocholic acid (LCA) forms with strong TGR5 agonism were present in the portal vein, but hardly detectable in other sampling sites. TGR5 activation index did not increase postprandially and was higher in the portal as compared to the peripheral veins (6.57 vs 0.64 respectively; p < 0.001). Most prominent human BA’s were ~30 fold higher in portal vein compared to other venous samples. LCA forms were only detected in the portal vein and the TGR5 activation index was again much higher in portal samples compared to peripheral samples.

Discussion/Conclusion: Portal BA concentrations are distinctly higher than in the periphery, possibly indicating a modest role for BA in TGR5 activation outside the entero-hepatic cycle.
Novel role for lymphotoxin beta receptor in bile acid homeostasis after partial heptectomy

Ursula R. Sorg¹, Kristina Behnke¹, Diran Herebian², Maria Reich³, Ertan Mayatepek², Verena Keitel-Anselmino³, Dieter Häussinger³, Klaus Pfeffer¹
¹Institute of Medical Microbiology and Hospital Hygiene, and ²Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, and ³Department of Internal Medicine, Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany

Introduction: Mice deficient for lymphotoxin beta receptor (LTbetaR-/-) show increased mortality after 70% partial heptectomy (PHx) compared to wildtype (WT) animals. Interestingly, alkaline phosphatase levels in LTbetaR-/- mice were significantly increased post PHx indicating intrahepatic cholestasis. Since FXR mediated bile acid (BA) signaling plays an important role in initiating a regenerative response after liver injury, BA homeostasis was analyzed in LTbetaR-/- animals post PHx.

Methods: PHx was performed on LTbetaR-/- and WT mice. Liver histology was evaluated and liver tissue was analyzed at various time points post PHx to determine BA levels and mRNA expression of genes associated with BA homeostasis.

Results: No significant difference between BA levels in liver tissue of non-hepatectomized LTbetaR-/- and WT mice was detected and expression levels of genes associated with BA homeostasis were comparable. However, post PHx LTbetaR-/- mice showed exacerbated liver pathology, significantly altered BA levels (in particular increased levels of more toxic BAs) and deregulated expression of genes associated with BA homeostasis (e.g. Fgf15, Ntcp) in liver tissue. In addition, RTPCR analysis detected expression of LTbetaR in cultured cholangiocytes from WT mice and, interestingly, upregulation of TGR5 and FXR expression in cultured cholangiocytes from LTbetaR-/- mice.

Discussion/Conclusion: The expression of LTbetaR in cholangiocytes from WT mice and the upregulation of TGR5 and FXR in cholangiocytes from LTbetaR-/- mice implies a role for LTbetaR mediated signaling in physiologic BA homeostasis. Also, our studies demonstrate for the first time that LTbetaR plays an important role in BA homeostasis after PHx. The overall increased toxicity of the BA pool together with the profound deregulation of the expression of genes associated with BA homeostasis could explain the exacerbated liver pathology and increased mortality of LTbetaR-/- mice after PHx. In this light, treatment of patients after liver resections with LTbetaR ligands should be explored.
Generation of liver buds by self-condensation of human iPSC-derived MSCs, HLCs and endothelial cells

Lucas-Sebastian Spitzhorn¹, Nina Graffmann¹, James Adjaye¹
¹Institute for Stem Cell Research and Regenerative Medicine, Heinrich-Heine University, Düsseldorf, Germany

Introduction: The production of bile acids is one of the key functions of the liver. In general, in vitro models of the human liver are wrought with limitations. Liver-biopsy derived primary hepatocytes, though the golden standard have several limitations (i) are scarce with a low number of healthy donors, (ii) high inter-donor variability, (iii) limited expansion in culture and (iv) rapid decline in function. Thus, the generation of hepatocyte like cells (HLCs) from induced pluripotent stem cells (iPSCs) can provide an alternative cell source [Jozefczuk J, Prigione A, Chavez L, Adjaye J. 2011]. So far these cells lack full maturity even though they express ALBUMIN and cytochrome P450 family members. Mature HLCs are needed to maximize the relevance of the experimental outcome and applicability of these cells for toxicology and drug screening. Improved maturity and functionality of human iPSC-derived HLCs has been achieved employing three-dimensional (3D) approaches incorporating MSCs and endothelial cells [Takebe et al. 2013].

Methods: Our preliminary proof of principle experiments involved mixing of iPSC-derived mesenchymal stem cells (iMSCs) with human umbilical vein endothelial cells (HUVECs) and HepG2 cells to generate 3D in vitro liver buds. We now use iMSCs, iEndothelial and HLCs derived from a single genetic background – a fetal foreskin derived iPS cell line.

Results: Within three weeks these cells aggregated and formed vascularized liver buds when cultured on artificial extracellular matrices. These buds express ALBUMIN, VIMENTIN and CD31 – an endothelial specific marker.

Discussion/Conclusion: These iPSC-derived liver buds have the added advantage of having present mesenchymal and endothelial cells from the same individual. Further studies are underway to better characterize these liver buds both molecular and biochemically (glycogen storage, bile acid production) for liver associated genes, pathways and functions.
TGR5 protein expression is reduced in livers of Mdr2-/- (Abcb4-/-) mice and of patients with primary sclerosing cholangitis (PSC)

Lina Spomer¹, Maria Reich¹, Johanna Höhne¹, Johannes Hov², Tom Karlsen², Dirk Nierhoff³, Dieter Häussinger¹, Verena Keitel-Anselmino¹
¹Clinic for Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University, Düsseldorf, Germany
²Norwegian PSC Research Center, Clinic for Specialized Medicine and Surgery, Oslo University Hospital Rikshospitalet, Oslo, Norway
³Clinic for Gastroenterology and Hepatology, University of Cologne, Cologne, Germany

Introduction: Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of unknown etiology affecting the intrahepatic and extrahepatic bile ducts and characterized by periductal fibrosis and stricture formation, which ultimately result in biliary cirrhosis and liver failure (Keitel et al. 2014). The localization of TGR5 in cholangiocytes and immune cells, the choleretic, anti-inflammatory and anti-apoptotic functions of the receptor suggest that TGR5 is important for the pathogenesis of liver diseases and in particular of biliary diseases. Additionally, an association for PSC and the frequent TGR5 exon 1 SNP rs11554825 is described, which seems associated with a lower TGR5 mRNA expression (measured in lymphoblastic cell line) (Hov et al. 2010). Aim of this study was to determine the expression level and localization of TGR5 in livers from PSC patients and Mdr2⁻ mice.

Methods: TGR5 protein localization was analyzed using immunofluorescence staining and confocal laser scanning microscopy in livers from PSC patients and controls as well as in livers from Mdr2⁻ and wildtype (WT) control mice. TGR5 protein amount in CK7 or CK19 positive cholangiocytes was determined by analysis of the mean fluorescence intensity per unit area (Axios Visio 4.8 software, LSM510 microscope). TGR5 mRNA expression in livers as well as in human macrophages (PBMC), primary mouse cholangiocytes and a human cholangiocyte cell line after stimulation with bile acids and cytokines was quantified by realtime PCR in relation to an endogenous control (HPRT1) or macrophage markers (CD14, CD163) or a cholangiocyte marker (CK19). The concentration of serum cytokine levels in Mdr2⁻ and WT mice was determined using a Luminex cytometric bead assay.

Results: The immunofluorescence analysis showed a significant reduction in TGR5 protein levels in the bile ducts of the PSC liver biopsies (n = 15) and in the bile ducts of Mdr2⁻ knockout animals (n = 6) compared to controls of human livers (n = 24; p < 0.05) and mouse livers (n = 6; p ≤ 0.01), while the protein levels of the bile duct markers CK7 and CK19 did not change. Analysis of whole liver mRNA did not reveal a significant reduction in TGR5 mRNA in PSC or Mdr2⁻ livers as compared to the respective controls. Most cholangiopathies are associated with portal inflammation in proximity to the biliary epithelium, and the initiation of inflammation plays a critical role in the pathogenesis of sclerosing cholangitis. A number of chemokines such as CCl2, CCl4, KC, MIP-2, LIX and the cytokine IL-6 were increased in serum of the Mdr2⁻ mice compared to the WT mice. While the bile salts TLC and TUDC had no effect on TGR5
mRNA expression in isolated human macrophages, the inflammatory cytokines TNFα and IL1β significantly suppressed TGR5 mRNA levels in these cells. In addition, the murine IL-8 homologues KC, LIX and MIP-2 significantly decreased TGR5 mRNA expression in cholangiocytes isolated from WT mice.

**Discussion/Conclusion:** Quantification of immunofluorescence staining demonstrated a significant downregulation of the bile acid receptor TGR5 in biliary epithelial cells of liver samples from PSC patients and Mdr2−/− mice. While incubation of human macrophages or murine cholangiocytes with different bile acids did not affect TGR5 mRNA expression, stimulation with cytokines led to a significant downregulation of the receptor mRNA levels. Whether the observed reduction of TGR5 protein levels in biliary epithelial cells in livers results from cytokine-mediated downregulation of the receptor mRNA expression is unclear. Since TGR5 exerts protective effects in cholangiocytes the downregulation of the receptor in PSC livers may render cholangiocytes more susceptible to bile acid toxicity and may also explain why feeding of a TGR5 agonist (INT-767) failed to alleviate liver damage in Mdr2−/− mice (Baghdasaryan et al. 2011).
**NorUDCA reduces liver injury and improves the metabolic state in mouse models of obesity and steatosis**

Daniel Steinacher¹, Thierry Claudel¹, Elisa Einwallner², Tatjana Stojakovic³ and Michael Trauner¹

¹Hans Popper Laboratory of Molecular Hepatology Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria
²Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria
³Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

**Introduction:** NorUDCA is a side-chained shortened derivative of ursodeoxycholic acid improving liver injury in mouse models of cholestatic liver injury. We aim to explore whether NorUDCA improves hepatic steatosis in mouse models of obesity and steatosis.

**Methods:** ob/ob mice received either a diet supplemented with 0.5% NorUDCA or chow for 6 weeks. wt/wt mice received either high-fat-diet (HFD) supplemented with 0.5% NorUDCA as prevention arm for 29 weeks or HFD alone for 29 weeks or HFD for 22 weeks following 7 weeks of HFD supplemented with 0.5% NorUDCA as treatment arm. We used metabolic cages, IPGTT and IPITT for metabolic characterizations. Food and water intake as well as bodyweight were recorded weekly. Serum biochemistry, liver histology, mRNA and protein expression were analysed.

**Results:** ob/ob mice treated with NorUDCA showed a significant reduction in serum AST, ALT and AP levels. mRNA expression of inflammatory markers (F4/80, Mcp-1 and Tnfalpha) were reduced in liver. Furthermore, ER stress markers (Grp78, Chop, sXbp1 and ErDj4) were lowered. WAT/bodyweight ratio was increased in NorUDCA group, non-esterified fatty acids decreased as well as markers for improved lipid storage function Pparg2, Mpges 1 AND Fapb4 induced. IPGTT uncovered a significantly faster blood glucose clearance at 60 and 90 min. Despite unchanged hepatic TG content, serum TG levels were increased. The prevention arm with NorUDCA in the DIO setting shows a clear reduction in body weight (37%), partially explained by a reduced food intake. The treatment arm shows already within 4 weeks a bodyweight reduction by 13% (despite pair feeding). The analysis of this experimental arm is still ongoing and will be available at the time of the meeting.

**Discussion/Conclusion:** NorUDCA treatment improves liver cell injury via reducing NASH features such as inflammation and ER stress. Moreover, we observed similar beneficial effects on WAT, resulting in an overall improved metabolic situation.
Functional studies on monoclonal, patient-derived BSEP-reactive antibodies causing antibody-induced BSEP deficiency (AIBD)

Jan Stindt¹, Thomas Tiller², Carola Dröge¹, Bettina Brackertz², Cathleen Kriegel², Jürgen Klattig², Dieter Häussinger¹, Ralf Kubitz¹, Verena Keitel-Anselmino¹
¹Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital, Heinrich-Heine University, Düsseldorf, Germany
²MorphoSys AG, Martinsried, Munich, Germany

Introduction: Inhibitory, IgG-class antibodies against the bile salt export pump (BSEP) expressed on hepatocytes may cause antibody-induced BSEP deficiency (AIBD) in patients after liver transplantation for severe BSEP mutations resulting in a PFIC-2 phenotype. Upon recurrence of PFIC-2-like symptoms, AIBD can be diagnosed by testing the patient’s serum for anti-BSEP reactivity. Since BSEP-reactive antibodies are the causative agents of this rare disease, we set out to characterize them on the molecular and functional level.

Methods: Individual, BSEP-reactive memory B cells were isolated from the peripheral blood of an AIBD patient by fluorescence-activated single cell sorting. Immunoglobulin heavy and light chain variable region gene transcripts were amplified by single cell RT-PCR and cloned into eukaryotic expression vectors for in vitro production of the encoded monoclonal antibodies (mAbs) and their corresponding monovalent Fab fragments (mFabs). All mAbs and mFabs were characterized by immunofluorescence staining of liver cryosections and BSEP-expressing cell lines, Western blot, FACS analysis and in situ perfusion of rat livers. Finally, their impact on BSEP activity was assayed in vitro using vesicular transport assays.

Results: We identified a set of five novel BSEP-reactive, patient-derived monoclonal antibodies. When used to immunostain human and rat liver cryosections, the five antibody species yielded clear canalicular staining patterns. Specific detection of BSEP was demonstrated in Western blot experiments. All mAbs and mFabs bound extracellular epitopes on BSEP as demonstrated by indirect immunofluorescence staining of liver cryosections and BSEP-expressing cell lines, Western blot, FACS analysis and in situ perfusion of rat livers. Finally, their impact on BSEP activity was assayed in vitro using vesicular transport assays.

Discussion/Conclusion: In summary, we provide a functional analysis of several patient-derived, distinct BSEP-reactive IgG species. These are part of the polyclonal antibody response causing AIBD and directly inhibit BSEP function by binding to its extracellular domains. Strikingly, we could observe stimulation of BSEP transport in vitro by one antibody species. Comparison of BSEP inhibition by mAbs and their mFab derivatives indicates that apart from direct BSEP inhibition, the antibody-mediated crosslinking of multiple BSEP transporters may also contribute to impaired transport activity of BSEP in vivo.
Porphyran, a functional ingredient of “Nori”, improves visceral obesity and non-alcoholic fatty liver via inhibition of intestinal FXR activation

Yoko Takashina¹, Kenji Ishihara², Haruka Saito¹, Tatsuya Tanigaki¹, Hiroki Taoka¹, and Mitsuhiro Watanabe¹
¹Graduate School of Media and Governance, Keio University, Fujisawa, Japan; ²Research Center for Biotechnology and Food Technology, Yokohama, Japan

Introduction: Porphyran (PP) is a major component of “Nori”, the typical Japanese food made from red algae. Previous studies have been shown that the PP protected from fat accumulation and progression of insulin resistance via the change of bile acid (BA) composition by activation of BA synthesis. To investigate the detailed mechanism of these effects of PP, we performed the DNA microarray analysis and 16S rDNA analysis of intestinal flora.

Methods: C57BL/6J mice were fed on high-fat diet mixed with 2%w/w PP for 13 weeks. Liver, ileum and colon were collected for using the DNA microarray. Total RNA was extracted from each tissues and the agilent expression array was performed using cDNA synthesized from total RNA. To characterise the gut microbial populations, 16S rDNA gene sequences were analysed by using the fecal samples.

Results: In the PP group, the signal pathway of sphingolipid synthesis was significantly inhibited in liver and C16-ceramide contents were lower in compared to the HF group by the LC/MS/MS methods. SREBP1c expression level was decreased and TG accumulation was also inhibited in liver. From the BA composition analysis, PP treatment increased Tauro-β-muricholic acid (T-β-MCA) level and decreased deoxycholic acid level in the intestine. Increase of T-β-MCA, acts as an intestinal FXR antagonist may brought the inhibition of the ceramide synthesis related genes expression. The microbiota involved in a secondary bile acid synthesis was significantly decrease in the PP group by the 16S rDNA sequencing of gut microbiota.

Discussion/Conclusion: In summary, we have shown that PP changed the BA composition and inhibited the intestinal FXR activation by the increase of T-β-MCA resulting in decrease of ceramide contents in liver and inhibition of development of fatty liver and insulin resistance.
Bile acids regulate host weight gain and metabolism through gut microbiota modification

Hiroki Taoka, Tatsuya Tanigaki, Yoko Takashina and Mitsuhiro Watanabe
Graduate School of Media and Governance, Keio University, Fujisawa, Japan

Introduction: Bile acid have roles of reducing lipogenesis genes expressions and improving hepatic triglyceride levels, insulin sensitivity and energy expenditure via FXR and TGR5. Previous studies have shown that 129S6/Sv(129) mice were less to obesity and diabetes in comparison with C57BL/6J(B6) mice. These results arise from the difference of genetic background and gut microbiota between two strain mice. Additionally, recent studies have suggested that alteration of the BA composition by microbiota improved lipid and glucose metabolism. Thus, we thought that bile acids metabolism is associated with these phenotypic differences. By comparing strain of the mice, we explore the relationship among BA, gut microbiota and metabolic regulation system.

Methods: Six-week-old B6 and 129 mice were fed on high-fat diet (D12492, Research Diet) and HF diet mixed 0.5%w/w CA (choric acid). After two months, we took blood, feces and liver from these mice to investigate lipid levels, bile acid composition and mRNA expression levels using real-time PCR. Additionally, by the use of their fecal samples, we performed pyrosequencing of the V1–V2 region of 16S rRNA genes.

Results: Bile acid treatment improved dietary obesity in B6 mice, but not in 129 mice. Not only lipogenic gene such as SREBP1c and SCD-1, but also Cyp7a1/8b1 and other bile acid-responsive gene (SHP and FGF15) expression levels were different between HF + BA treated B6 and 129 mice.
We found that BA composition and total BA were different between strains. FXR antagonistic TBMC levels decreased in HF + BA treated 129 mice. Microbiome analysis shows that closely-related species of Clostridium difficile which is deoxycholic acid-producing bacteria greatly increased in 129 strain supplemented with HF + BA.

Discussion/Conclusion: Difference of interactions between genetic background and gut microbiota in two strains changed bile acid composition, brought influence FXR and TGR5 signalling activity in the gut and liver, and caused metabolic phenotype differences.
BAs regulate host weight gain and metabolism through gut microbiota modification

Hiroki Taoka, Tatsuya Tanigaki, Naho Kitamura, Yoko Takashina and Mitsuhiro Watanabe
Keio University, Fujisawa, Kanagawa, Japan

Introduction: Bile acids (BA) have roles of improving metabolic syndrome via FXR and TGR5. Previous studies showed that 129S6/Sv mice were less to obesity in comparison with C57BL/6J (B6) mice. These results arise from the difference of genetic background and gut microbiota between two strain mice. Additionally, recent studies suggest that alteration of the BA composition by microbiota improved metabolic syndrome. Thus, we thought BA metabolism is associated with these phenotypic differences. By comparing strain of the mice, we explore the relationship among BA, gut microbiota and metabolic regulation system.

Methods: Six-week-old B6 and 129X1/Sv (129) mice were fed on high-fat diet (HF) and HF diet mixed CA (cholic acid). After two months, we took blood, feces and liver from these mice to investigate lipid levels, BA composition and mRNA expression levels using qPCR. Additionally, by the use of their fecal samples, we performed pyro-sequencing of the V1–V2 region of 16S rRNA genes.

Results: BA treatment improved dietary obesity in B6 mice, but not in 129 mice. Not only lipogenic gene such as SREBP1c and SCD-1, but also Cyp7a1/8b1 and other BA-responsive gene (SHP and FGF15) expression levels were different between HF + BA treated B6 and 129 mice.

We found that BA composition and total BAs levels were different between strains. FXR antagonistic TBMC levels decreased in HF + BA treated 129 mice. Microbiome analysis shows that secondary BA-producing bacteria greatly increased in 129 mice supplemented with HF + BA.

Discussion/Conclusion: Difference of interactions between genetic background and gut microbiota in two strains changed BA composition, brought influence FXR and TGR5 signalling activity in the gut and liver, and caused metabolic phenotype differences.
Toxic bile injury in mdr2-/ mice instigates a hepatic T-lymphocyte immune response amenable to cytokine and anti-cholestatic therapy

Amy Taylor1, Alexandra Carey1, Julia Simmons1, Celine S. Lages1, Rebekah Karns1, Nathan Salomonis2,3, Wujuan Zhang4, Kumar Shanmukhappa3,4, Tiffany Shi1, John McNulty5, Kenneth D.R. Setchell3,4 and Alexander G. Miethke1,3

1Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
2Division of Biomedical Informatics, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
3Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
4Division of Pathology and Laboratory Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA
5Shire plc, Lexington, MA, USA

Introduction: We hypothesize that T lymphocytes control hepatobiliary injury in mdr2-/ mice and are amenable to cytokine and anti-cholestatic therapy.

Methods: Hepatic lymphocyte composition was surveyed in juvenile mdr2-/ mice transgenic for FoxP3-GFP and correlated with sclerosing cholangitis (SC) phenotype. Regulatory T cells (Tregs: CD3+CD4+CD25+FoxP3+) and CD8+ lymphocytes were manipulated in-vivo with a complex of IL-2/anti-IL2 (IL2c) and depleting anti-CD8 antibody, respectively, and by treatment with the intestinal ASBT inhibitor SC-435.

Results: The SC phenotype in mdr2-/ mice developed between day 14–30 with rising serum ALT and ALP levels, accompanied by waning proportion of Tregs/CD8+ lymphocytes. Compared to age/gender-matched control mdr2-/ mice, IL2c treatment from day 7–30 increased the population of hepatic Tregs by 50%, decreased the frequency of hepatic CD8+ lymphocytes by 29%, and restored the Treg/CD8+ ratio. IL2c treatment lowered serum ALP (mean IU/L 174 IL2c vs 185 PBS; p = 0.02) and diminished liver fibrosis on histopathology (% mean area fibrosis 3.3% IL2c vs 4.9% PBS; p < 0.01). To link CD8+ lymphocytes with biliary injury, mdr2-/ mice were treated with anti-CD8 antibody from day 14–30 with a 90% reduction of hepatic CD3+CD8+ lymphocyte frequency and subsequent improvement in serum ALP levels (mean IU/L134 anti-CD8 vs 147 IgG; p = 0.04). Importantly, anti-cholestatic treatment with SC-435 between day 30–45 in mdr2-/ mice led to a 98% reduction in serum total bile acids and a more than 2-fold increase in hepatic Treg frequency (%Treg/CD3+ 7.0% SC-435 vs 3.1% control; p = 0.03). Single cell RNAseq studies revealed differential expression of genes linked to Treg identity (Ikzf2/Helios), apoptosis (Casp8), and specific cytokine effects (IL6st, Il27RA) in FACS sorted FoxP3+ Tregs from SC-435 vs control mdr2-/ mice.

Discussion/Conclusion: Tregs modulate effector T lymphocyte response and SC phenotype in murine fibrosing cholangiopathy, and their homeostasis is susceptible to cytokine and anti-cholestatic therapy.
Post-hepatectomy dietary challenge with cholic acid to mimic post-resectional liver failure

Liyanne van de Laarschot, Kim M.C. van Mierlo, Valérie Lebrun, Cathy van Himbeeck, Peter L.M. Jansen, Frank G. Schaap, Steven W.M. Olde Damink, Isabelle A. Leclercq

1Department of Surgery, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, PO BOX 616, 6200 MD Maastricht, The Netherlands
2Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium

Introduction: Post-resectional liver failure (PLF) is a dreaded complication after extended liver resection. Post-operative hyperbilirubinemia suggests that impaired hepatobiliary transport with intrahepatic accumulation of cholephiles plays an etiological role. Bile salts serve dual roles as signalling molecules engaged in liver regeneration after partial hepatectomy (PH) and biological detergents. In this study we tested the hypothesis that excessive accumulation of bile salts in the regenerating liver results in PLF.

Methods: Three months old male C57BL6/J mice were subjected to 70% PH and post-operatively challenged with control diet or diet supplemented with cholic acid (CA, 0.2, 0.5, 1.0%; n = 5–6 per group). After 48 hours mice were sacrificed, and liver injury, secretory function, and regenerative indices were assessed.

Results: Mice fed 1.0% CA diet displayed a trend towards more pronounced weight loss following PH, and had a deranged post-operative glucose course. Liver injury (transaminase elevations) and impaired hepatobiliary transport function (hyperbilirubinemia) were apparent in groups fed 0.2% or 1.0% CA diets, but not in animals fed 0.5% CA diet. No differences in liver mass recovery were observed among groups. However, the percentage of hepatocytes staining positive for the proliferation marker Ki67 were reduced in mice receiving 1.0% CA diet relative to animals fed 0.5% CA diet.

Discussion/Conclusion: A post-resectional challenge with 1.0% CA diet induces signs of liver injury and defective liver regeneration. A longer duration of the dietary challenge and/or secondary hits may further improve the model. Once a model has been established, it can be used to evaluate pharmaceutical strategies to prevent or treat PLF.
A FRET-based compound screen identifies novel inhibitors of the organic solute transporter alpha/beta

Sandra M.W. van de Wiel and Stan F.J. van de Graaf
Tytgat Institute for Liver and Intestinal Research & Department of Gastroenterology & Hepatology, AMC, Amsterdam, The Netherlands

Introduction: The organic solute transporter alpha/beta (OSTαβ) mainly facilitates transport of bile acids across the basolateral membrane of ileal enterocytes. Recently, it has been shown that deficiency of OSTα results in decreased lipid accumulation, improved insulin sensitivity and is protective for liver injury during obstructive cholestasis. Therefore, inhibition of OSTαβ might be beneficial in the treatment of many metabolic diseases. However, no inhibitors have yet been identified.

Methods: We designed a cell-based high-throughput screen to identify specific inhibitors for OSTαβ, making use of our formerly developed genetically encoded FRET-Bile Acid Sensor that enables rapid visualization of bile acid influx and efflux in living cells. We screened 1280 FDA-approved drugs of the Prestwick chemical library for compounds that potently inhibit OSTαβ-mediated bile acid efflux.

Results: Using a criterion of 20% inhibition of OSTαβ, we found 25 primary hits, that were mainly represented by steroids (9 drugs), azoles (4 drugs) and benzenoids (4 drugs). Clofazimine was the only drug specific for OSTαβ and reduced transcellular transport of [3H]-taurocholate across MDCKII monolayers expressing ASBT and OSTαβ in a dose-dependent manner. Moreover, clofazimine treatment also increased intracellular [3H]-taurocholate levels, confirming inhibition of OSTαβ. Furthermore, six hour treatment of clofazimine in differentiated Caco-2 cells led to increased activation of intestinal FXR target genes FGF19, OSTα and OSTβ. Oral administration of clofazimine (250 mg/kg) for 6 hours in mice significantly increased intestinal FXR target gene expression, confirming OSTαβ inhibition in vivo.

Discussion/Conclusion: This screen identifies clofazimine as the first selective inhibitor of OSTαβ in vitro and in vivo and confirmed the feasibility of this compound screen for bile acid transport modulators using the FRET-Bile Acid Sensor.
The phospholipid flippase ATP8B1 mediates apical membrane localization of the cystic fibrosis transmembrane regulator

Vincent A. van der Mark¹, Hugo de Jonge², Jung-Chin Chang¹, Kam Ho-Mok¹, Suzanne Duijst¹, Marianne S. Carlon³, Ronald P.J. Oude Elferink¹, Coen C. Paulusma¹
¹Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands
²Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands
³Laboratory for Molecular Virology and Gene Therapy, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium

Introduction: Progressive familial intrahepatic cholestasis type 1 (PFIC1) is caused by mutations in the gene encoding the phospholipid flippase ATP8B1. Apart from severe cholestatic liver disease, many PFIC1 patients develop extrahepatic symptoms such as pulmonary infection and sweat gland dysfunction, symptoms characteristic for cystic fibrosis (CF). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel essential for epithelial fluid transport. Demeillier et al.¹ previously reported reduced CFTR levels in liver of PFIC1 patients and in an ATP8B1-depleted human biliary epithelial cell line. The pathogenesis of CFTR down-regulation in ATP8B1 deficiency is presently unknown and is subject of the present study.

Methods: We analyzed CFTR mRNA and protein expression in ATP8B1-depleted intestinal and pulmonary epithelial cell lines. We assessed CFTR function by measuring short-circuit currents across transwell-grown ATP8B1-depleted intestinal T84 cells and by using a genetically-encoded FRET-based chloride sensor. In addition, we studied CFTR surface expression upon induction of ectopic, CMV-promoter-driven, CFTR expression by butyrate administration.

Results: We show in ATP8B1-depleted intestinal and pulmonary epithelial cells that CFTR mRNA and total protein levels were reduced by ~40% and 45%, respectively, compared to control cells. This phenotype coincided with ~20% and ~60% reduced CFTR activity when measuring short-circuit currents and FRET-based chloride signals, respectively. In ATP8B1-depleted cells, CFTR surface expression was reduced by ~60% compared to control and was not increased when ectopic CFTR protein levels were induced by butyrate administration.

Discussion/Conclusion: We conclude that ATP8B1 is essential for correct apical membrane localization of CFTR in human intestinal and pulmonary epithelial cells and that reduced CFTR mRNA in ATP8B1 deficiency is a consequence of impaired ATP8B1-mediated apical membrane targeting of CFTR or other, yet unknown apical membrane proteins. Our data provide an explanation for the CF-like phenotypes observed in ATP8B1 deficiency.

Obeticholic acid enhances liver regeneration in hepatectomized mice

Kim M.C. van Mierlo¹, Valérie Lebrun², Cathy van Himbeeck¹, Peter L.M. Jansen¹, Frank G. Schaap¹, Steven W.M. Olde Damink¹, Isabelle A. Leclercq²
¹Department of Surgery, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, PO BOX 616, 6200 MD Maastricht, The Netherlands
²Laboratory of Hepato-Gastroenterology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium

Introduction: Impaired regeneration is observed in liver failure after partial hepatectomy (PH). Tight control of bile salt homeostasis is required for efficient regeneration following PH. Bile salt signalling via intracellular bile salt receptor FXR is key to this homeostatic regulation. The aim of the study was to explore whether FXR-agonism can accelerate liver regeneration after PH.

Methods: Adult male C57Bl/6 mice were pretreated for one week with FXR agonist obeticholic acid (OCA; 10 mg/kg, daily oral gavage) or vehicle (0.5% methylcellulose, n = 13 per group). Five mice in each group were sacrificed at baseline, while the remaining mice underwent 70% PH and were sacrificed 48 hrs later. Tissues were harvested for histological and immunohistochemical examination, transcript analysis and determination of bile salt content. Glycaemia was monitored during the entire course of the experiment.

Results: Effectiveness of OCA pre-treatment was inferred from elevated expression of ileal (Fgf15, Slc51b) and reduced expression of hepatic (Cyp8b1) Fxr target genes at baseline. OCA pre-treatment did not affect pre-operative body weight or glycaemia course, or baseline hepatic or systemic bile salt levels. After PH, mitotic figures (0.0 vs. 4.8; p < 0.001) and percentage Ki67-positive hepatocytes (18.2 vs. 47.4%; p = 0.005) were higher in OCA-treated animals. Expression of cell cyclins A2 (+ 4.2 fold) and B1 (+ 5.8 fold) was elevated in OCA-treated animals, with a tendency for elevated expression of the proliferative factor Foxm1b (+ 2.3 fold, p = 0.07). Bile salt content in the regenerated liver lobes was similar in both groups. Expression of bile salt synthetic genes Cyp7a1 (- 6.3 fold) and Cyp8b1 (- 37 fold) was reduced in the OCA group.

Conclusion: The aggregate data indicates that OCA enhances hepatic regeneration following PH. Time course analysis can provide further insight whether the stimulatory effect of Fxr-agonism involves effects on bile salt homeostasis and/or a more direct effect on cellular proliferation.
Glycodeoxycholic acid administration increases GLP-1 secretion in healthy humans

van Nierop F.S.¹, Schaap F.G.², Vaz F.³, Romijn J.A.⁴, Olde Damink S.W.M.² and Soeters M.R.¹
¹Department of Endocrinology and Metabolism, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
²Department of Surgery, NUTRIM School of Nutrition, Toxicology and Metabolism, Maastricht University, Maastricht, The Netherlands
³Department of Clinical Chemistry, Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
⁴Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Introduction: Postprandial activation of the G protein-coupled bile acid (BA) receptor (TGR5) has been linked to increases in energy expenditure, glucagon-like peptide 1 (GLP-1) and insulin secretion.

Methods: We first investigated plasma BA, GLP-1, insulin and glucose levels in 9 lean males after a mixed meal test. Insulin and glycodeoxycholate (gDCA), were positively correlated at 60’ after the meal (r 0.96; p < 0.001), but neither correlated with GLP-1. To establish the possible physiological role of gDCA as a TGR5 agonist, we then investigated the effect of 750 mg gDCA. Subjects consumed a standard meal with or without gDCA on separate occasions after which blood was sampled during 4 hrs and assayed for GLP-1, glucose and insulin. Energy expenditure was measured twice postprandially.

Results: gDCA increased plasma GLP-1 at 60’ postprandially (24.1 vs 13.6 µmol/L; p < 0.01). There was a trend for higher postprandial insulin area-under-the-curve in the first hour (1055 vs 741 min*pmol/L; p = 0.10), with no effect on plasma glucose levels (1182 vs 1219 min*mmol/L; ns). gDCA plasma levels were increased only after 240’, while other BA and fibroblast growth factor 19 levels, as well as meal-induced energy expenditure were not affected by gDCA administration.

Discussion/Conclusion: The first experiment showed a correlation between gDCA and insulin levels but not between either and GLP-1. However, in the second experiment gDCA stimulated secretion of GLP-1. This may be mediated by intestinal L-cell TGR5 activation after exposure to gDCA and fits the paradigm of BA as postprandial hormones in the gastrointestinal tract. TGR5-mediated increase in energy expenditure did not occur in this acute time frame. This experiment shows that BA administration may modulate GLP-1 secretion in humans. However, timing of the BA administration as well as the form (i.e. conjugation) in which it is given may be crucial to leverage any beneficial effects.
Colonization of germ-free mice with a human microbiota induces FXR signaling

Annika Wahlström¹, Petia Kovatcheva-Datchary¹, Marcus Ståhlman¹, Hanns-Ulrich Marschall¹*, Fredrik Bäckhed¹,²*
¹Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine and Wallenberg Laboratory, University of Gothenburg, S-413 45 Gothenburg, Sweden; ²Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, DK-2200, Denmark. *Shared senior authorship.

Introduction: The gut microbiota influences the development and progression of metabolic diseases, partly by metabolism of bile acids and modified signaling through the farnesoid X receptor (FXR). Mice that are colonized with a human microbiota can be used to study the effect of human bacteria on metabolic functions, and in this study we aim to determine how the human gut microbiota metabolizes murine bile acids and affects FXR signaling in colonized mice.

Methods: We colonized germ-free mice with fresh caecal content from a mouse donor or pre-frozen or fresh faeces from a human donor. We analyzed the gut microbiota and bile acid composition and expression of FXR target genes in ileum and liver.

Results: Caecal microbiota composition differed between mice colonized with mouse and human microbiota and the freezing process also affected microbiota composition in the humanized mice. Human and mouse microbiota reduced total bile acid levels similarly but the humanized mice produced less secondary bile acids. The human microbiota was able to induce expression of FXR target genes Fgf15 and Shp in ileum and reduce expression of Cyp7a1 in the liver. Colonization with frozen human faeces resulted in higher ratio between FXR agonistic and FXR antagonistic bile acids and higher expression of the FXR target genes compared with fresh human faeces.

Discussion/Conclusion: We show that a human microbiota can change bile acid composition and induce FXR signaling in colonized mice, but the levels of secondary bile acids produced are lower than in mice colonized with a mouse microbiota.
Decreased intestinal glucose uptake in humans treated with the FXR agonist obeticholic acid

Annika Wahlström¹, Samer Al-Dury¹, Anna Casselbrant², Mattias Bergentall¹, Marcus Ståhlman¹, Lars Fändriks², Fredrik Bäckhed¹, Hanns-Ulrich Marschall¹
Sahlgrenska Academy, ¹Department of Molecular and Clinical Medicine, Institute of Medicine, and ²Department of Gastrointestinal Research and Education, Institute of Clinical Sciences, University of Gothenburg, Gothenburg, Sweden

Introduction: In mice, FXR signaling has been shown to affect intestinal permeability and the gut microbiota, which itself is known to modify bile acid (BA) profiles and subsequently modulate FXR-dependent signaling. Here, we study these effects in human volunteers treated with obeticholic acid (OCA), a potent and selective FXR agonist developed for the treatment of non-viral chronic liver diseases.

Methods: As part of the randomized placebo-controlled OCAPUSH study (ClinicalTrials.gov NCT02532335), 20 healthy volunteers were administered OCA (25 mg/day) or matching placebo for three weeks. Blood and feces samples were collected at days 1 and 21 where a pushjejunoscopy was performed to sample biopsies from the small intestine. Blood and stool were investigated with UPLC-MSMS for BA profiles and markers of BA synthesis. Stool samples were also analyzed for microbiota composition by 16S-RNA sequencing and shotgun metagenomics. Intestinal biopsies were used for transcriptomics, morphology and measurements in Ussing chambers of SGLT1-mediated electrogenic responses reflecting glucose uptake.

Results: All subjects finished the trial per protocol and without adverse event. All subjects administered OCA had enriched OCA and significantly increased FGF19 and conversely, decreased C4 and endogenous BAs, as compared to subjects administered placebo. The glucose uptake in jejunum biopsies of subjects treated with OCA compared to those treated was placebo was reduced by 48.1% (p < 0.001). Our human data that FXR activation reduces jejunal glucose uptake were confirmed in murine experiments.

Discussion/Conclusion: Here we for the first time in humans (and mice) demonstrate that FXR activation substantially reduces glucose uptake from the small intestine which potentially has strong impact for the action of OCA in conditions with impaired glucose metabolism.
Bile acid-modulated transcript-expression in human macrophages validated by transcriptome analysis

Marianne Wammers¹, Anna-Kathrin Schupp¹, Johannes G. Bode¹, Karl Köhrer², Rene Deenen², Dieter Häussinger¹ and Dirk Graf¹
¹Clinic of Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Germany
²Biological and Medical Research Center (BMFZ), Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich-Heine-University Düsseldorf, Germany

Cholestasis conditions are associated with impaired innate and adaptive immunity, including monocytes, macrophages and T-cell function. Recently, we demonstrated that bile acids suppress LPS-induced cytokine expression in primary human macrophages. Moreover, bile acid treatment lead to an increased IL-10/IL-12 ratio that is characteristic for regulatory macrophages. Therefore we hypothesize that bile acid influence expression of genes, which generate the characteristic regulatory macrophages phenotype.

Based on this result, an affymetrix-based mRNA transcriptomic analysis of LPS-stimulated macrophages, treated with tauroliothocholic-acid (TLC) was implemented. While ~19245 transcripts are differentially expressed in LPS-induced macrophages, additional treatment with TLC lead to differentially expression of ~3493 transcripts compared to LPS-stimulated macrophages. First results demonstrated that TLC reduces the LPS-induced expression of CXCL chemokines on mRNA and protein level, which are involved in neutrophils and NK-cell migration. Additionally a subset of CCL chemokines is declined by bile acids, which are known to attract monocytes, DCs and neutrophils. Interestingly, TLC has not effect on LPS-induced transcripts that are associated with cell-cell contact.

Moreover the expression of several LPS-induced transcripts as Epiregulin (EREG) and Ets homologous factor (EHF3) are upregulated by adding TLC. These transcripts are involved in repair and regeneration of liver tissue but the meaning is quit unclear. MMPs are further increased by bile acid. The precise activities of macrophage-specific MMPs are necessary to orchestrate the pro- and anti-inflammatory responses by cleaving and inactivate human CXC chemokines.

These results lead to the assumption that bile acids affect the recruitment and migration of immune cells to the side of infection by reducing chemokine expression in human macrophages. Further studies will be addressed to clarify if the increased MMPs are correlated with the downregulation of chemokines after bile acid incubation.
Ursodeoxycholic acid and lithocholic acid exert anti-inflammatory actions in the colon: Implications for treatment of ulcerative colitis

Joseph B.J. Ward (PhD)¹, Orlaith B. Kelly (MD, PhD)¹, Aoife M. O'Dwyer¹, Natalia Lajczak¹, Murtaza Tambuwala (PhD)², Carolina Colliva (PhD)³, Silvia Spinozzi (PhD)³, Joan Ní Gabhann (PhD)⁴, Caroline Jefferies (PhD)⁴, Aldo Roda (PhD)³, Stephen J. Keely (PhD)¹

¹Molecular Medicine Laboratories, Royal College of Surgeons in Ireland, Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9, Ireland; ²School of Pharmacy and Pharmaceutical Sciences, Ulster University, Coleraine, Northern Ireland; ³Department of Chemistry, University of Bologna, Bologna, Italy; ⁴Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

Introduction: Ulcerative colitis (UC) is a chronic inflammatory bowel disease for which current therapies are often ineffective. The naturally-occurring secondary bile acid, ursodeoxycholic acid (UDCA), has well-established anti-inflammatory and cytoprotective actions and may therefore be effective in treating UC. Here, we aimed to investigate regulation of colonic inflammatory responses by UDCA and to determine the potential impact of bacterial metabolism on its therapeutic actions.

Methods: The anti-inflammatory efficacy of UDCA, a non-metabolisable analogue, 6-methyl-UDCA (6-MUDCA), and its primary colonic metabolite, lithocholic acid (LCA), were assessed in the murine DSS model of mucosal injury. The effects of bile acids on cytokine release (TNF-α, IL-6, IL-1β, IFN-γ) from cultured colonic epithelial cells and mouse colonic tissue in vivo were investigated. Luminal bile acids were measured by GC-MS.

Results: UDCA attenuated release of proinflammatory cytokines from colonic epithelial cells in vitro and was protective against the development of colonic inflammation in vivo. In contrast, although 6-MUDCA mimicked the effects of UDCA on epithelial cytokine release in vitro, it was ineffective in preventing inflammation in the DSS model. However, LCA was even more effective than UDCA in inhibition of epithelial cytokine release and protection against DSS-induced mucosal inflammation.

Discussion/Conclusion: UDCA and its primary metabolite, LCA, exert anti-inflammatory actions in vitro and in vivo. Furthermore, bacterial metabolism of UDCA in vivo appears to be required for it to exert its anti-inflammatory effects. Our preclinical data support the feasibility of clinical trials for UDCA in treatment of UC.
Protection against oxidative stress mediated by Nrf2-Keap1-axis is impaired in primary biliary cholangitis

Urszula Wasik¹, Małgorzata Milkiewicz¹, Piotr Milkiewicz²,³
¹Department of Medical Biology Laboratory, Pomeranian Medical University, Szczecin, Poland
²Liver and Internal Medicine Unit, Medical University of Warsaw, Warsaw, Poland
³Department of Clinical and Molecular Biochemistry, Pomeranian Medical University, Szczecin, Poland

Background and Aims: Oxidative stress contributes to pathogenic changes in primary biliary cholangitis (PBC). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is activated in response to oxidative stress and induces expression of cytoprotective genes encoding hemooxygenase 1 (HO-1) and γ-glutamate-cysteine ligase (GCLC). The Nrf2 pathway is tightly controlled both on mRNA and protein levels by micro-RNAs and Kelch-like ECH-associated protein 1 (Keap-1). Keap-1 controls the oxidation-sensitive shuttling of Nrf2 into/out of the nucleus. The stabilization of Nrf-2 occurs as a consequence of Keap-1 degradation through the autophagy pathway in a p62-dependent manner. When autophagy is inhibited, the proteins are accumulated and Keap-1 is inactivated by binding to p62. We investigated the effectiveness of Nrf2/Keap-1-axis in amelioration of oxidative stress in PBC.

Methods: Liver human specimens from non-cirrhotic (n = 14) and cirrhotic (n = 24) patients with PBC along with control tissues (n = 16) were used for the molecular analysis including Western blot/ELISA (protein) and real-time PCR (mRNA/micro-RNA).

Results: Expression of Nrf2 gene was significantly decreased in cirrhotic PBC in comparison to controls or non-cirrhotic PBC (3.5-fold, p = 0.0002, or 3.9-fold p < 0.0001, respectively). Moreover, the protein levels of the downstream target genes of Nrf2, such as HO-1 and GCLC, were decreased (2-fold, p = 0.0003 and 2.8-fold, p < 0.0001 vs. controls, respectively). Decreased Nrf2 gene expression was associated with increased level of micro-RNA-132 (3 fold, p = 0.003). Both Keap-1 and p62 protein levels were substantially increased in PBC (p = 0.001 and p < 0.0001, respectively).

Conclusions: Decreased expression of oxidative stress sensor Nrf-2 and a deterioration of autophagy was found in primary biliary cholangitis. This impairment was more advanced in patients with histological features of cirrhosis and may contribute to the liver damage in this condition. Aberrant Nrf2-Keap-1 system integrity in PBC may affect self-defending mechanisms against oxidative stress.

This study was supported by the grant no.2011/02/A/NZ5/00321 from National Science Centre in Poland.
Porphyran, a functional ingredient of “Nori”, is protected from development of NASH via modulating of bile acids metabolism

Yoko Takashina¹, Kenji Ishihara², Haruka Saito¹, Tatsuya Tanigaki¹, Hiroki Taoka¹, and Mitsuhiro Watanabe¹
¹Graduate School of Media and Governance, Keio University, Fujisawa, Japan; ²Research Center for Biotechnology and Food Technology, Yokohama, Japan

Introduction: We have shown that Porphyran (PP) inhibited progression of fatty liver and improved insulin resistance via alteration of bile acid metabolism. Moreover, Obesity induced alteration of gut microbiota was improved by the PP treatment. Non-alcoholic steatohepatitis (NASH) is characterized by increased hepatic triglyceride (TG) storage and inflammation. To explore whether PP plays a role in the etiology of NASH, we evaluated the potential of PP using the diet induced NASH model mice.

Methods: Six-week-old C57BL/6J mice were fed on NASH-induced diet containing the transfatty acids, fructose and cholesterol (D09100301, Research Diet) with/without PP for 18 weeks. Body weight and food intake were monitored weekly. We collected bloods from a tail vein monthly for monitoring for plasma TG, Cholesterol (CHO), Alanine aminotransferase (ALT) and choline. After necropsy, expression levels were analyzed using real-time PCR. The liver lipid was extracted according to the Forch method. Morphological study of major tissues, bile acid (BA) composition analysis, and gene expression analysis were conducted with samples from the model mice.

Results: C57BL/6J mice fed with NASH induced diet developed hepatic steatosis, hepatic inflammation and fibrosis for 18 weeks. Treatment with PP significantly inhibited TG accumulation in liver and decreased plasma ALT level. Plasma free choline level, is known as the biomarker for NASH decreased in PP treatment. By the Pico-Sirius red staining of collagen, PP ameliorated hepatic fibrosis. The analysis of gene expression using Q-RT-PCR has shown that PP reduced the hepatic proinflammatory genes and intestinal claudin1. Deoxycholic acid level was markedly decreased in the PP treatment in feces.

Discussion/Conclusion: These finding demonstrate that PP inhibits the hepatic steatosis and inflammation of NASH via changing BA metabolism resulting in improvement of lipid homeostasis and prevention of endotoxin influx from the tight junction in intestine.
UDCA administration in cholestatic pregnancy can ameliorate dysregulated metabolic profile of the fetus and offspring

Catherine Williamson¹, Vanessa Pataia¹, Syed A. Qadri¹, Shadi Abu-Hayyeh¹, Saraid A. Mcilvride¹, Annika Wahlström², Eugene Jansen³, Hanns-Ulrich Marschall² and Georgia Papacleovoulou¹
¹King’s College London, UK; ²University of Gothenburg, Sweden; ³RIVM, Bilthoven, The Netherlands

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a liver-specific disease of pregnancy that is characterised by increased serum bile acids (BA) and dyslipidaemia. We previously showed that maternal cholestasis causes metabolic disease in the offspring and this is associated with dyslipidaemia in the fetoplacental unit. We hypothesise that ursodeoxycholic acid (UDCA) treatment in pregnancy prevents fetoplacental dyslipidaemia and improves the offspring phenotype.

Methods: Cholestatic mice (fed 0.5% of cholic acid; CA) were fed with 0.5% UDCA during pregnancy and were either sacrificed on gestational day 18 or alternatively allowed to deliver offspring. Offspring were sacrificed at 18 weeks of age after being fed a normal chow diet or a western diet for six weeks. Metabolic profile was assessed.

Results: UDCA reversed the CA feeding-induced elevation of tauro-conjugated BA in both maternal serum and liver. Tauro-conjugated BA levels were increased in the fetal circulation of cholestatic mice compared to the maternal serum levels and this was not corrected by UDCA. UDCA had a beneficial effect on circulating and hepatic cholesterol and free fatty acid (FFA) concentrations in the mother and this was consistent with the gene expression profile of hepatic TG/FFA pathways. In the fetus, UDCA improved hepatic CA-induced cholesterol and FFA and the same effect was observed in placenta. Moreover, offspring fed a western diet had improved glucose tolerance when exposed to UDCA in utero.

Discussion/Conclusion: UDCA improves hypercholanaemia and dyslipidaemia in maternal cholestasis. UDCA improves dyslipidaemia in the fetus and glucose tolerance in the young offspring.
rs10488631 polymorphism of IRF5-TNPO3 confers susceptibility to primary biliary cholangitis (PBC) and is associated with abnormal liver biochemistry indexes: A single centre association study

Ewa Wunsch1, Aleksander Kus2, Magdalena Arlukowicz-Grabowska3, Gary L. Norman4, Rafal Ploski5, Malgorzata Milkiewicz6, Tomasz Bednarczuk2, Piotr Milkiewicz3

1Department of Clinical and Molecular Biochemistry, Pomeranian Medical University in Szczecin, Szczecin, Poland; 2Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Warsaw, Poland; 3Liver and Internal Medicine Unit, Department of General, Transplant and Liver Surgery of the Medical University of Warsaw, Warsaw, Poland; 4INOVA Diagnostics, San Diego, USA; 5Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland; 6Department of Medical Biology, Pomeranian Medical University in Szczecin, Szczecin, Poland

Background and Aims: Recent GWAS in primary biliary cholangitis (PBC) indicated its strong association with rs10488631 polymorphism of a gene encoding interferon regulatory factor 5 and transportin 3 (IRF5-TNPO3)(Nat Genet. 2010;42:655). This polymorphism has also been linked with other autoimmune conditions such as systemic lupus erythematosus or rheumatoid arthritis (Hum Mol Genet 2008;17:872). We assessed whether genetic variation of IRF5-TNPO3 is associated with susceptibility to PBC and clinical or laboratory features of the disease.

Methods: Genomic DNA was isolated from blood samples of Caucasians with PBC (n = 290) and age-matched healthy volunteers (n = 106). rs10488631 of IRF5-TNPO3 was genotyped using the MGB-TaqMan-SNP assay. Potential associations between studied SNP and clinical, biochemical, serological and quality of life measures were analyzed.

Results: A significant association between the risk allele C and PBC susceptibility (chisq = 10.0, p = 0.002) was found. CC homozygotes had higher ALT activity and cholesterol levels at the diagnosis compared to heterozygotes (231 ± 217 vs. 96 ± 119 IU/L, p = 0.02 and 428 ± 379 vs. 236 ± 86 mg/dl, p = 0.0001, respectively) and TT homozygotes (231 ± 217 vs. 102 ± 119 IU/L, p = 0.02 and 428 ± 379 vs. 248 ± 100 mg/dl, p = 0.0003). CC homozygosity tended to be associated with higher GGT levels (513 ± 759 vs. 210 ± 256 IU/L, p = 0.08) at presentation. Allelic analysis confirmed that C allele was associated with higher GGT compared to T allele (345 ± 574 vs. 210 ± 256 IU/L, p=0.02). Rheumatoid factor (RF) titers were significantly higher in CC homozygotes vs. heterozygotes (36.3 ± 75.8 vs. 6.1 ± 4.8, p < 0.0001) and TT homozygotes (36.3 ± 75.8 vs. 5.3 ± 4.0, p < 0.0001). No significant associations between IRF5-TNPO3 polymorphisms and AMA, or sp100/gp210-specific ANA titres, response to UDCA or quality of life measures were found.

Conclusions: In this homogenous group of Caucasians patients with PBC, we confirmed that rs10488631 polymorphism of IRF5-TNPO3 confers susceptibility to the diseases; though its association with phenotypic features of the disease is limited to liver biochemistry.
Financial support: Piotr Milkiewicz was supported by the grant no. 2011/02/A/NZ5/00321 from National Science Centre in Poland. Ewa Wunsch was supported by the Foundation for Polish Science and Ministry of Science and Higher Education of Republic of Poland.
Author Index to Poster Abstracts
(Name - Poster Number)

Aa, J. 36  Brackertz, B. 76
Abdel-Khalik, J. 24  Briem-Richter, A. 8
Abu-Hayyeh, S. 1, 30, 92  Brinkert, F. 8
Adjaye, J. 23, 73  Brito, H. 9
Adorini, L. 60  Briz, O. 45
Afonso, M.B. 2, 61  Buist-Homan, M. 10, 18, 47
Al-Abdulla, R. 45  Bujanda, L. 38
Al-Aqil, F.A. 62  Büsch, R. 56
Al-Dury, S. 3, 87  Butt, E. 66
Alonso, M. 45  Carey, A. 80
Amaral, J.D. 2  Caridade, M. 61
Anthony, T. 36  Carlon, M.S. 83
Appleby, R.N. 4, 20  Carrat, F. 13
Aranda, J.C. 62  Carvalho, C.C. 61
Arasaradnam, R. 51  Casselbrant, A. 87
Argemi, J. 45  Castaño, B. 45
Arlukowicz-Grabowska, M. 93  Castoldi, M. 37
Asensio, M. 45  Castro, R.E. 2, 9, 61
Auer, N. 49  Chambers, J. 6
Avila, M.A. 62  Chang, J.-C. 11, 83
Baba, H.A. 56  Chazouillères, O. 13
Bäckhed, F. 3, 86, 87  Chen, Y. 47
Bai, X. 47  Chiang, J.Y.L. 12
Banales, J.M. 38  Claudel, T. 75
Baptissart, M. 5  Clayton, P.T. 24
Batista, S. 9  Colliva, C. 89
Bednarczuk, T. 93  Conde de la Rosa, L. 10
Behnke, K. 72  Cont, M. 60
Bellafante, E. 6, 50  Corpechot, C. 13
Berendse, K. 34  Cortez-Pinto, H. 2, 61
Bergentall, M. 87  Crick, P.J. 24
Beuers, U. 11, 41, 63, 70  Dahlqvist, G. 13
Bevan, C.L. 30  Dawson, P.A. 58
de Jonge, H. 83
de Waart, D.R. 11, 70
Björkhem, I. 24  Deenen, R. 33, 88
Bloemen, J.G. 35  Degiovanni, A. 60
Blokhzijl, T. 26  Dejong, C.H.C. 35
Bode, J.G. 88  D’Errico, A. 60
Boehme, S. 12  Deutschmann, K. 33
Boelen, A. 17, 71  Deutz, N.E.P. 71
Bolier, R. 31, 41  Diakonov, I. 27
Bonus, M. 7, 16  Dijkstra, G. 26
Borralho, P.M. 61  Dixon, P.H. 14
<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donkers, J.M.</td>
<td>15, 70</td>
</tr>
<tr>
<td>Dorbath, D.</td>
<td>29</td>
</tr>
<tr>
<td>Dröge, C.</td>
<td>16, 76</td>
</tr>
<tr>
<td>Duarte, A.</td>
<td>61</td>
</tr>
<tr>
<td>Duijst, S.</td>
<td>15, 83</td>
</tr>
<tr>
<td>Eggink, H.M.</td>
<td>17, 69, 71</td>
</tr>
<tr>
<td>Eichmann, T.</td>
<td>48</td>
</tr>
<tr>
<td>Einwallner, E.</td>
<td>75</td>
</tr>
<tr>
<td>Engelen, M.</td>
<td>34</td>
</tr>
<tr>
<td>Faber, K.N.</td>
<td>10, 18, 26, 47, 64</td>
</tr>
<tr>
<td>Fändriks, L.</td>
<td>87</td>
</tr>
<tr>
<td>Ferrebee, C.</td>
<td>58</td>
</tr>
<tr>
<td>Fickert, P.</td>
<td>42, 48, 53</td>
</tr>
<tr>
<td>Fida, S.</td>
<td>68</td>
</tr>
<tr>
<td>Fischer, L.</td>
<td>52</td>
</tr>
<tr>
<td>Franco, P.</td>
<td>60</td>
</tr>
<tr>
<td>Fuchs, C.D.</td>
<td>19</td>
</tr>
<tr>
<td>Fuji, N.</td>
<td>28</td>
</tr>
<tr>
<td>Fukiya, S.</td>
<td>28</td>
</tr>
<tr>
<td>Gadaleta, R.M.</td>
<td>30</td>
</tr>
<tr>
<td>Gallego, L.</td>
<td>38</td>
</tr>
<tr>
<td>Gaouar, F.</td>
<td>13</td>
</tr>
<tr>
<td>Garnys, L.</td>
<td>68</td>
</tr>
<tr>
<td>Gavaldá-Navarro, A.</td>
<td>54</td>
</tr>
<tr>
<td>Gavin, J.</td>
<td>22</td>
</tr>
<tr>
<td>Geers, J.M.</td>
<td>4, 20</td>
</tr>
<tr>
<td>Geier, A.</td>
<td>29, 66</td>
</tr>
<tr>
<td>Gertz, C.G.W.</td>
<td>21</td>
</tr>
<tr>
<td>Gilmer, J.F.</td>
<td>22</td>
</tr>
<tr>
<td>Go, S.</td>
<td>11</td>
</tr>
<tr>
<td>Gohlke, H.</td>
<td>7, 16, 21</td>
</tr>
<tr>
<td>Gonzales, R.</td>
<td>62</td>
</tr>
<tr>
<td>Gonzales San-Martin, F.</td>
<td>45</td>
</tr>
<tr>
<td>Gorelik, J.</td>
<td>27</td>
</tr>
<tr>
<td>Götz, S.</td>
<td>37</td>
</tr>
<tr>
<td>Grabhorn, E.</td>
<td>8</td>
</tr>
<tr>
<td>Graf, D.</td>
<td>67, 88</td>
</tr>
<tr>
<td>Graffmann, N.</td>
<td>23, 73</td>
</tr>
<tr>
<td>Gray, N.</td>
<td>30</td>
</tr>
<tr>
<td>Griffiths, W.J.</td>
<td>24</td>
</tr>
<tr>
<td>Groen, A.K.</td>
<td>17, 18, 35, 69, 71</td>
</tr>
<tr>
<td>Groen, H.</td>
<td>26</td>
</tr>
<tr>
<td>Groen, L.</td>
<td>26</td>
</tr>
<tr>
<td>Grünhage, F.</td>
<td>25</td>
</tr>
<tr>
<td>Gumhold, J.</td>
<td>42, 48</td>
</tr>
<tr>
<td>Guo, G.L.</td>
<td>36</td>
</tr>
<tr>
<td>Haazen, L.</td>
<td>63</td>
</tr>
<tr>
<td>Hagio, M.</td>
<td>28</td>
</tr>
<tr>
<td>Hall, R.A.</td>
<td>25</td>
</tr>
<tr>
<td>Hambruch, E.</td>
<td>68</td>
</tr>
<tr>
<td>Haruka, S.</td>
<td>77, 91</td>
</tr>
<tr>
<td>Häussinger, D.</td>
<td>7, 16, 21</td>
</tr>
<tr>
<td>Haywood, J.</td>
<td>58</td>
</tr>
<tr>
<td>He, J.</td>
<td>36</td>
</tr>
<tr>
<td>Heegsma, J.</td>
<td>26, 64</td>
</tr>
<tr>
<td>Hegen, B.</td>
<td>56</td>
</tr>
<tr>
<td>Heger, M.</td>
<td>65</td>
</tr>
<tr>
<td>Heidebrecht, T.</td>
<td>31</td>
</tr>
<tr>
<td>Herebian, D.</td>
<td>33, 37, 72</td>
</tr>
<tr>
<td>Hermanns, H.M.</td>
<td>29, 66</td>
</tr>
<tr>
<td>Herraiz, E.</td>
<td>45, 62</td>
</tr>
<tr>
<td>Hilbers, P.A.J.</td>
<td>69</td>
</tr>
<tr>
<td>Hochrath, K.</td>
<td>25</td>
</tr>
<tr>
<td>Hoeke, M.O.</td>
<td>64</td>
</tr>
<tr>
<td>Hoekstra, M.</td>
<td>26, 64</td>
</tr>
<tr>
<td>Höhne, J.</td>
<td>74</td>
</tr>
<tr>
<td>Homey, B.</td>
<td>67</td>
</tr>
<tr>
<td>Ho-Mok, K.</td>
<td>83</td>
</tr>
<tr>
<td>Honda, A.</td>
<td>46</td>
</tr>
<tr>
<td>Hov, J.</td>
<td>74</td>
</tr>
<tr>
<td>Hoyer, P.F.</td>
<td>56</td>
</tr>
<tr>
<td>Huisman, F.</td>
<td>65</td>
</tr>
<tr>
<td>Hylemon, P.B.</td>
<td>43</td>
</tr>
<tr>
<td>Ibrahim, E.</td>
<td>27</td>
</tr>
<tr>
<td>Ikeyamg, T.</td>
<td>46</td>
</tr>
<tr>
<td>Inge, T.H.</td>
<td>32</td>
</tr>
<tr>
<td>Ipharraguerre, I.</td>
<td>54</td>
</tr>
<tr>
<td>Ishihara, K.</td>
<td>77, 91</td>
</tr>
<tr>
<td>Ishizuka, S.</td>
<td>28</td>
</tr>
<tr>
<td>Jahn, D.</td>
<td>29, 66</td>
</tr>
<tr>
<td>Jameie-Oskoei, S.</td>
<td>4</td>
</tr>
<tr>
<td>Jansen, E.</td>
<td>92</td>
</tr>
<tr>
<td>Jansen, P.L.M.</td>
<td>34, 35, 52, 65, 81, 84</td>
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<td>Jarvis, S.</td>
<td>30</td>
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<td>Jefferies, C.</td>
<td>89</td>
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<td>Jenkins, T.</td>
<td>32</td>
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<td>Jennen, D.</td>
<td>52</td>
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<td>Jetten, M.J.</td>
<td>52</td>
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<td>Jimenez, F.</td>
<td>62</td>
</tr>
<tr>
<td>Jimenez, S.</td>
<td>45</td>
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<tr>
<td>Jiménez-Agüero, R.</td>
<td>38</td>
</tr>
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<td>Name</td>
<td>Page Numbers</td>
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<td>Jin, Q.</td>
<td>36</td>
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<td>Joe, G.-H.</td>
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<td>13</td>
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<td>Joosten, R.P.</td>
<td>31</td>
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<td>Kalsbeek, A.</td>
<td>17, 71</td>
</tr>
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<td>Karlsten, T.H.</td>
<td>74</td>
</tr>
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<td>Karns, R.</td>
<td>80</td>
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<td>Karpen, S.J.</td>
<td>58</td>
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<td>Kathemann, S.</td>
<td>56</td>
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<tr>
<td>Keely, S.J.</td>
<td>39, 40, 89</td>
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<td>Keitel-Anselmino, V.</td>
<td>8, 16, 21, 33, 56</td>
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<td>Kelly, O.B.</td>
<td>89</td>
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<td>51</td>
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<td>Kemper, E.M.</td>
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<td>31</td>
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<td>Kitamura, N.</td>
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<td>76</td>
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<td>Klint, C.</td>
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<td>16</td>
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<td>Kobayashi, M.</td>
<td>91</td>
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<td>Koehorst, M.</td>
<td>17, 18, 71</td>
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<td>Koelfat, K.V.K.</td>
<td>35</td>
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<td>32</td>
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<td>Köhler, K.</td>
<td>33, 88</td>
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<td>Kolb, D.</td>
<td>48</td>
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<td>Kong, B.</td>
<td>36</td>
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<td>Kooijman, S.</td>
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<td>Kordes, C.</td>
<td>37</td>
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<tr>
<td>Kovatcheva-Datchary, P.</td>
<td>86</td>
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<td>Krawczyk, M.</td>
<td>38</td>
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<td>Kremer, A.</td>
<td>31</td>
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<td>Kremosser, C.</td>
<td>68</td>
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<td>Kriegel, C.</td>
<td>76</td>
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<td>Krones, E.</td>
<td>42</td>
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<tr>
<td>Kubitz, R.</td>
<td>16, 76</td>
</tr>
<tr>
<td>Kus, A.</td>
<td>93</td>
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<tr>
<td>Küster, P.</td>
<td>56</td>
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<td>Kwakkenbos, M.J.</td>
<td>15</td>
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<td>80</td>
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<td>Lainka, E.</td>
<td>56</td>
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<td>Lajczak, N.</td>
<td>39, 40, 89</td>
</tr>
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<td>Lammert, F.</td>
<td>25, 38</td>
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<tr>
<td>Lang, P.A.</td>
<td>44</td>
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<td>Langedijk, J.</td>
<td>41</td>
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<tr>
<td>Lebrun, V.</td>
<td>81, 84</td>
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<tr>
<td>Leclercq, I.A.</td>
<td>65, 81, 84</td>
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<td>19</td>
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<td>Lee, J.-Y.</td>
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<td>Li, X.</td>
<td>43</td>
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<td>Liu, H.</td>
<td>12</td>
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<td>42</td>
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<td>Liu, R.</td>
<td>43</td>
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<td>Lozano, E.</td>
<td>45</td>
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<td>Machado, M.V.</td>
<td>61</td>
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<td>Macias, R.I.R.</td>
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<td>66</td>
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<td>Maney, S.K.</td>
<td>44</td>
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<td>Marin, J.J.G.</td>
<td>45, 62</td>
</tr>
<tr>
<td>Marschall, H.-U.</td>
<td>3, 19, 53, 86, 87, 92</td>
</tr>
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<td>Martin, M.</td>
<td>6</td>
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<td>Martinez-Augustin, O.</td>
<td>62</td>
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<td>Martinot, E.</td>
<td>5</td>
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<tr>
<td>Matas-Rico, E.</td>
<td>31</td>
</tr>
<tr>
<td>Matsuzaki, Y.</td>
<td>46</td>
</tr>
<tr>
<td>Mayatepek, E.</td>
<td>33, 72</td>
</tr>
<tr>
<td>Mcilvride, S.A.</td>
<td>92</td>
</tr>
<tr>
<td>McNulty, J.</td>
<td>80</td>
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<tr>
<td>Mereu, A.</td>
<td>54</td>
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<td>13</td>
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<tr>
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<td>80</td>
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<tr>
<td>Milkiewicz, M.</td>
<td>90, 93</td>
</tr>
<tr>
<td>Milkiewicz, P.</td>
<td>90, 93</td>
</tr>
<tr>
<td>Miyazaki, T.</td>
<td>46</td>
</tr>
<tr>
<td>Mol, I.</td>
<td>17</td>
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<tr>
<td>Monte, M.J.</td>
<td>45, 62</td>
</tr>
<tr>
<td>Moolenaar, W.H.</td>
<td>31</td>
</tr>
<tr>
<td>Morris, A.A.</td>
<td>24, 31</td>
</tr>
<tr>
<td>Moshage, H.</td>
<td>10, 18, 47, 64</td>
</tr>
<tr>
<td>Moustafa, T.</td>
<td>48</td>
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<tr>
<td>Mowery, S.</td>
<td>32</td>
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<tr>
<td>Mroz, M.S.</td>
<td>39, 40</td>
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<td>8</td>
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<td>Müller, M.</td>
<td>49</td>
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<td>11</td>
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<td>Murray, F.</td>
<td>40</td>
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<tr>
<td>Nierhoff, D.</td>
<td>74</td>
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<td>Nieuwdorp, M.</td>
<td>71</td>
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<tr>
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<td>89</td>
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<td>Name</td>
<td>Pages</td>
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<td>Nikolova, V.</td>
<td>6, 50</td>
</tr>
<tr>
<td>Norman, G.L.</td>
<td>93</td>
</tr>
<tr>
<td>Ocon, B.</td>
<td>62</td>
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<tr>
<td>O’Connor, M.</td>
<td>51</td>
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<tr>
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<td>89</td>
</tr>
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<td>Oehrle, M.</td>
<td>32</td>
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<td>2</td>
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<td>24</td>
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<td>35, 52, 65, 81, 84, 85</td>
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<td>11, 15, 31, 41, 63, 70, 83</td>
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<td>36</td>
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<tr>
<td>Panzitt, K.</td>
<td>53</td>
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<tr>
<td>Papacleovoulou, G.</td>
<td>50, 55, 92</td>
</tr>
<tr>
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<td>54</td>
</tr>
<tr>
<td>Pataia, V.</td>
<td>55, 92</td>
</tr>
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<td>12</td>
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<tr>
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<td>49</td>
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<td>11, 41, 83</td>
</tr>
<tr>
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<td>19</td>
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<tr>
<td>Payer, B.A.</td>
<td>68</td>
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<td>Peck-Radosavljevic, M.</td>
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<td>31</td>
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<td>38</td>
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<td>Pfeffer, K.</td>
<td>72</td>
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<tr>
<td>Pilic, D.</td>
<td>56</td>
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<td>51</td>
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<td>62</td>
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<td>93</td>
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<td>34</td>
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<td>56</td>
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<td>57</td>
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<td>92</td>
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<td>Quilty, F.</td>
<td>22</td>
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<td>22</td>
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<td>58</td>
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<td>67</td>
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<td>Reiberger, T.</td>
<td>68</td>
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<td>Reich, M.</td>
<td>33, 72, 74</td>
</tr>
<tr>
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<td>17</td>
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<tr>
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<td>36</td>
</tr>
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<td>59</td>
</tr>
<tr>
<td>Roda, A.</td>
<td>60, 89</td>
</tr>
<tr>
<td>Rodrigues, C.M.P.</td>
<td>2, 9, 61</td>
</tr>
<tr>
<td>Rodrigues, P.M.</td>
<td>2, 61</td>
</tr>
<tr>
<td>Romero, M.R.</td>
<td>62</td>
</tr>
<tr>
<td>Romijn, J.A.</td>
<td>17, 71, 85</td>
</tr>
<tr>
<td>Rosales, R.</td>
<td>62</td>
</tr>
<tr>
<td>Roscam Abbing, R.L.P.</td>
<td>63</td>
</tr>
<tr>
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<td>5</td>
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<td>Saeed, A.</td>
<td>64</td>
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<tr>
<td>Saint-Criq, V.</td>
<td>39, 40</td>
</tr>
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<td>Salazar Gonzalez, R.M.</td>
<td>32</td>
</tr>
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<td>Salomonis, N.</td>
<td>80</td>
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<tr>
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<td>9</td>
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<tr>
<td>Sanchez de Medina, F.</td>
<td>62</td>
</tr>
<tr>
<td>Sanz-Ortega, L.</td>
<td>62</td>
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<tr>
<td>Sawitza, I.</td>
<td>37</td>
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<td>34, 35, 52, 65, 81, 84, 85</td>
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<td>70</td>
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<td>Schmitt, L.</td>
<td>16, 57</td>
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<tr>
<td>Schooneman, M.</td>
<td>71</td>
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<td>5, 40</td>
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<td>Schubert, S.</td>
<td>66</td>
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<td>68</td>
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<td>57</td>
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<td>49</td>
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<td>Schumacher, J.</td>
<td>36</td>
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<tr>
<td>Schupp, A.-K.</td>
<td>67, 88</td>
</tr>
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<td>Schwabl, P.</td>
<td>19, 68</td>
</tr>
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<td>5</td>
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<td>Serrano, M.A.</td>
<td>62</td>
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<td>Setchell, K.D.R.</td>
<td>32, 58, 80</td>
</tr>
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<td>91</td>
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<td>2, 61</td>
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<td>Simmons, J.</td>
<td>80</td>
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<td>69</td>
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<td>24</td>
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<td>Name</td>
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<td>Slijepcevic, D.</td>
<td>63, 70</td>
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<td>21, 57</td>
</tr>
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<td>17, 69, 71, 85</td>
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<td>7</td>
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<td>Soons, Z.</td>
<td>52</td>
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<td>Sorg, U.R.</td>
<td>72</td>
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<tr>
<td>Spinozzi, S.</td>
<td>60, 89</td>
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<tr>
<td>Spitzhorn, L.-S.</td>
<td>73</td>
</tr>
<tr>
<td>Spomer, L.</td>
<td>21, 74</td>
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<tr>
<td>Stahlman, M.</td>
<td>3, 86, 87</td>
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<td>Steinacher, D.</td>
<td>75</td>
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<td>Stindt, J.</td>
<td>57, 76</td>
</tr>
<tr>
<td>Stojakovic, T.</td>
<td>19, 75</td>
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<tr>
<td>Strobl, B.</td>
<td>68</td>
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<td>Sun, R.</td>
<td>36</td>
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<tr>
<td>Sunkara, M.</td>
<td>31</td>
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<tr>
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<td>77, 78, 79, 91</td>
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<td>28</td>
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<td>89</td>
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<td>77, 78, 79</td>
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<td>77, 78, 79</td>
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<td>80</td>
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<td>71</td>
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<td>76</td>
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<tr>
<td>Tolenaars, D.</td>
<td>31, 41, 70</td>
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<td>Trauner, M.</td>
<td>19, 48, 49, 53, 68, 75</td>
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<td>61</td>
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<td>Urban, S.</td>
<td>15</td>
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<tr>
<td>Uriarte, I.</td>
<td>62</td>
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<tr>
<td>van de Graaf, S.F.J.</td>
<td>15, 59, 63, 70, 82</td>
</tr>
<tr>
<td>van de Laarschot, L.</td>
<td>81</td>
</tr>
<tr>
<td>van de Wiel, S.M.W.</td>
<td>82</td>
</tr>
<tr>
<td>van den Berg, R.</td>
<td>17</td>
</tr>
<tr>
<td>van der Mark, V.A.</td>
<td>83</td>
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<tr>
<td>van Golen, R.F.</td>
<td>65</td>
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<tr>
<td>van Gulik, T.M.</td>
<td>65</td>
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<tr>
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<td>65, 81, 84</td>
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<tr>
<td>van Lienden, K.P.</td>
<td>65</td>
</tr>
<tr>
<td>van Mierlo, K.M.C.</td>
<td>35, 81, 84</td>
</tr>
<tr>
<td>van Nierop, F.S.</td>
<td>71, 85</td>
</tr>
<tr>
<td>van Riel, N.A.W.</td>
<td>69</td>
</tr>
<tr>
<td>Vasur, F.</td>
<td>60</td>
</tr>
<tr>
<td>Vaz, F.M.</td>
<td>34, 85</td>
</tr>
<tr>
<td>Vega, A.</td>
<td>5</td>
</tr>
<tr>
<td>Verhaag, E.M.</td>
<td>18</td>
</tr>
<tr>
<td>Verheij, J.</td>
<td>65</td>
</tr>
<tr>
<td>Villarroya, F.</td>
<td>54</td>
</tr>
<tr>
<td>Volle, D.H.</td>
<td>5</td>
</tr>
<tr>
<td>Wagner, M.</td>
<td>53</td>
</tr>
<tr>
<td>Wagner, M.</td>
<td>68</td>
</tr>
<tr>
<td>Wahlström, A.</td>
<td>3, 19, 86, 87, 92</td>
</tr>
<tr>
<td>Walters, J.R.F.</td>
<td>4, 20, 51</td>
</tr>
<tr>
<td>Wammers, M.</td>
<td>88</td>
</tr>
<tr>
<td>Wanders, R.J.A.</td>
<td>34</td>
</tr>
<tr>
<td>Wang, Y.</td>
<td>24</td>
</tr>
<tr>
<td>Wang, Y.</td>
<td>36</td>
</tr>
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<td>30</td>
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<tr>
<td>Ward, J.</td>
<td>89</td>
</tr>
<tr>
<td>Wasik, U.</td>
<td>90</td>
</tr>
<tr>
<td>Watanabe, M.</td>
<td>77, 78, 79, 91</td>
</tr>
<tr>
<td>Waterham, H.R.</td>
<td>34</td>
</tr>
<tr>
<td>Williamson, C.</td>
<td>1, 6, 14, 27, 50, 55, 92</td>
</tr>
<tr>
<td>Winston, R.M.L.</td>
<td>30</td>
</tr>
<tr>
<td>Wolinski, H.</td>
<td>48</td>
</tr>
<tr>
<td>Woudenberg-Vrenken, T.</td>
<td>10</td>
</tr>
<tr>
<td>Wruck, W.</td>
<td>23</td>
</tr>
<tr>
<td>Wu, L.</td>
<td>14</td>
</tr>
<tr>
<td>Wunsch, E.</td>
<td>93</td>
</tr>
<tr>
<td>Wymenga, L.</td>
<td>26</td>
</tr>
<tr>
<td>Wynn, G.</td>
<td>58</td>
</tr>
<tr>
<td>Yokota, A.</td>
<td>28</td>
</tr>
<tr>
<td>Yuan, J.</td>
<td>2</td>
</tr>
<tr>
<td>Zaufel, A.</td>
<td>42</td>
</tr>
<tr>
<td>Zhan, L.</td>
<td>36</td>
</tr>
<tr>
<td>Zhang, L.</td>
<td>43</td>
</tr>
<tr>
<td>Zhang, W.</td>
<td>32, 58, 80</td>
</tr>
<tr>
<td>Zhao, X.</td>
<td>32</td>
</tr>
<tr>
<td>Zhou, H.</td>
<td>43</td>
</tr>
<tr>
<td>Zierler, K.A.</td>
<td>48</td>
</tr>
<tr>
<td>Zimmermann, A.</td>
<td>67</td>
</tr>
<tr>
<td>Zollner, G.</td>
<td>42</td>
</tr>
</tbody>
</table>
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