UNIVERSITÉ DE LILLE
École Doctorale Biologie-Santé de Lille
Institut Pasteur de Lille

THÈSE

Pour l'obtention du grade de

DOCTEUR DE L'UNIVERSITÉ DE LILLE

Spécialité : Biomolécules, pharmacologie, thérapeutique

Présentée et soutenue publiquement par

Oscar Manuel CHÁVEZ TALAVERA

Le 5 Septembre 2019

RÔLE DES ACIDES BILIAIRES DANS LA PHYSIOPATHOLOGIE DE L'OBÉSITÉ, LA RÉSISTANCE À L'INSULINE, LE DIABÈTE DE TYPE 2, LA STÉATOSE HÉPATIQUE NON ALCOOLIQUE ET DANS LE CONTEXTE DE LA CHIRURGIE BARIATRIQUE.

Directrice de thèse : Pr. Anne MUHR-TAILLEUX

Jury :

Pr. Isabelle LECLERCQ : Rapporteure

Pr. Folkert KUIPERS : Rapporteur

Pr. Anne MUHR-TAILLEUX : Examinatrice

Pr. Bart STAELS : Examinateur

Dr. Julie CHARTON : Invitée

This thesis was prepared at the UMR1011 Inserm,
Institut Pasteur de Lille, 1 rue du professeur Calmette. 59000 Lille, France.

ACKNOWLEDGEMENTS

Merci Anne Tailleux, d'avoir été ma directrice de thèse. Comme je te l'ai dit une fois, avant de faire cette thèse je ne savais même pas ce que je ne savais pas, et tu m'as tout appris. Merci pour l'énorme patience que tu as eu avec moi, de m'avoir apporté tant de choses dans le domaine de la recherche, mais aussi au niveau humain. Tu es un modèle de chercheur et de personne à suivre, et j'ai été ravi de vivre cette heureuse thèse sous ta direction. Je vais continuer à appliquer toutes les choses que tu m'as apprises tout au long de ma carrière scientifique.

Bart Staels, thank you very much for letting me work in your team during these 4 years, for being always so generous with your knowledge and experience. You were an excellent boss and I hope we can keep on working together in the future.

Thanks to Pr. Isabelle Leclercq and Pr. Folkert Kuipers, for accepting to be my reporters and for coming to Lille to evaluate my thesis. I admire you very much and I feel much honored to have you in my jury.

Un grand merci à tous les pasteuriens qui m'ont tant aidé pendant ma thèse, et avec qui j'ai partagé des bon moments. Je tiens à remercier spécialement BrunoD et ses acides nucléiques, NathalieH et ses souris et cellules, CorinneC et ses lipoprotéines, JonathanV et ses blocs de paraffine, HélèneD & BenoitP pour tout votre soutien et questions qui m'ont aidé à préparer le grand jury.

Un grand merci aussi à tous les JK-iens, qui m'ont appris tant des choses pendant ma thèse et avec qui j'ai partagé des discussions très agréables à table.

Merci au team du CUMA, AmandineD, MostafaK et Jean-FrançoisG pour tant d'aide avec les dosages d'acides biliaires, qui ont été très importants pour ma thèse.

Thanks to CamilleM, GrégoryB, François Pattou and all his team for the fruitful collaborations that we shared.

Merci à l'EDBSL, au Dr. Sablonnière, Mr Delcroix, Mme Herminet, Mme Béguin et Mme Fofana, pour votre précieuse aide pendant cette thèse.

Un énorme merci à Emmanuelle Vallez, qui m'a appris tellement de choses à la paillasse et avec les protocoles in vivo *(qu'est-ce qu'on a fait d'OGTTs !), tu as été toujours tellement souriante et sympa, ça a été un super plaisir travailler avec toi !*

Merci à Sophie Lestavel, qui a toujours suivi ma thèse de très près, et qui m'a beaucoup aidé à préparer le concours du grand jury.

Merci à Julie Charton, pour tant d'heures d'analyse ensemble, et des discussions très agréables.

Merci à Sandrine Caron, Kad Bantubungi, Réjane Lestrelin, Sophie Lestavel et Barbara Gross de m'avoir aidé et tout appris pour donner les travaux pratiques à la Faculté de Pharmacie, je crois que l'enseignement pendant la thèse est une expérience très enrichissante et avec vous ça a été aussi très sympathique.

Merci à mi amiga Isabelle Duplan, qui m'a formé à l'expérimentation in vitro*, et avec qui j'ai partagé tellement des discussions intéressantes sur des sujets très divers.*

Valeria Spinelli, grazie per tutto il tuo aiuto e i tuoi consigli da quando sono arrivato a Lille, e per tanti bei ricordi da quando eravamo insieme in ufficio. È stato un enorme piacere poter lavorare con te ed averti come amica. Federica de Paoli, grazie per la tua amicizia all'inizio di questa tesi, e tanti shots e serate insieme!

Merci Sandrine Quemener pour ton amitié et ta bonne oreille pendant cette thèse. Merci au Dr Alexis de Boulinguiez, mon bon camarade tout le long du doctorat, et maître d'une prudence et éducation impeccable. Thanks to Artemii Nikitin for nice talks in the office and keeping me always high on caffeine. Thank you, Justine Gillard, for all your help with the acute experiments that we did while you were here in stage, and for your friendship. I really enjoyed working with you.

Thanks to QuentinT, AudreyD, CeciliaB, MargauxN, SimonP, DaniSL, GuillaumeG, BenjaminD, YannD, SarahD, and all the other PhD students with whom I shared good moments and laughs. Thanks also to ArianaM, ManjulaV, JoelH, VanessaL, DenisM, LaurentL, JulieC, LauraB and all the other post-docs for sharing your experience and good moments.

Gracias a Victoria Sanz Fernández, mi querida amiga que me heredó su departamento en Lille y que siempre me ha apoyado con sus consejos y su gran madurez.

Thanks to CarolineB, ChiaraS and JulienT for your precious help with the in vivo *experiments, for the db/db mice, glucagon dosages (and the expensive high-game sticks for glycaemia!) and for many hours of interesting discussion.*

Thank you very much Bertrand Cariou and Matthieu Wargny for the fruitful and interesting collaboration on the IT-DIAB project. Thank you Elena Osto for the interesting collaboration on the HDL-BAs and for nice discussion and analyses together.

Thank you Nikolaus Marx and Michael Lehrke for kindly receiving me in Aachen during my stage, I'm looking forward to joining your team afterwards!

Gracias a AnnieSM, SaraF, BerthaM, PatriciaOW, SamuelPDL, YolandaLV, GerardoG, TereTL, CarlosAS, ArmandoPT, ClementinaSV, CarlosGC e IrubíPP, quienes tanto me han enseñado sobre medicina e investigación y que recuerdo con tanto cariño.

Gracias Dora Elena Balmori, por enseñarme las bases de la biología y todo tu apoyo.

Ana Flisser siempre te estaré agradecido por tanto apoyo y amistad a lo largo de mi carrera. Por darme la oportunidad de formar parte del PECEM, volver a Francia y continuar con mi doctorado. Eres una de las personas más importantes en mi formación profesional y te admiro enormemente.

Merci à mes chers amis Christophe Chanoine, Alan Hansen et Béatrice Bernard. Pour etre si proches de moi pendant ma thèse, et pour tant d'années d'amitié, rire, bons moments et bières belges "spéciales" qui sont délicieuses et qui montent très vite à la tête.

Merci à Cécile Martin et Juan-Felipe Dodelin, ainsi qu'à tous mes chers amis Lillois qui ont été si importants pour mon bien être à niveau humain, et avec qui j'ai partagé tant des soirées, concerts, festivals et bonheur. Vous êtes devenus comme ma famille pendant ces 4 ans, JeanK, PaulineLV, Bertrand & MarineG, OlivierM, SarahG, XavierB, LilieH, LudoD, TommyD, MarionP, RomainC, BenoitB, RomainB, YaraG, PaulusP, MarieD, TristanF, SofianeR, QuentinD, DorianeM, VincentD, MathildeV, NaWel, ClémentP, SamuelR, ClaireVW, SoniaD, ClaudiaS, LauryS, KevinR et JavierSA.

Un grand merci à Romain Bouteiller, pour tout ton soutien et tant de bons moments pendant ma thèse, ainsi que pour ton aide précieuse dans la correction de ma rédaction en français. Je tiens aussi à remercier à SophieB & Jean-MichelB, CélineB, AnthonyD, GuettyR, LaurenceB & Jean-ClaudeB pour m'avoir accueilli chez vous et les bons moments partagés ensemble.

Finalmente, dedico esta tesis a mi familia. A mis padres, por sus consejos y palabras de aliento, así como por su incondicional apoyo a lo largo de toda mi vida, sin el cual este doctorado no habría sido posible. A mi hermana Diana, que siempre me ha cuidado y guiado mis decisiones, y que ha tanto contribuido a mi desarrollo personal. A mis abuelos Puri y Jaime. A Poncho, Pina, Rafa, Fanny, Jaime, Celina, Jaimito y Chapis.

SUMMARY

Résumé en français

En plus de leur rôle dans la solubilisation des lipides alimentaires, les acides biliaires sont des molécules de signalisation régulant leur propre métabolisme, l'homéostasie du glucose et des lipides, la dépense énergétique, la fonction cardiovasculaire et l'inflammation, en modulant le Farnesoid X Receptor (FXR) et le Takeda G protein coupled Receptor 5 (TGR5). En effet, des modifications dans les concentrations des acides biliaires sont associées aux maladies métaboliques et ce sont des candidats pour participer à la pathophysiologie de ces maladies ou prédire leur progression.

Dans la première partie de cette thèse nous avons étudié les modifications des acides biliaires dans le contexte de l'obésité, l'insulinorésistance, le diabète de type 2 et la stéatohépatite non alcoolique. Nous avons montré que les acides biliaires sont corrélés avec l'homéostasie du glucose chez l'Homme, mais qu'ils ne sont pas des prédicteurs de la bascule du prédiabète en diabète de type 2 dans un étude de cohorte.

La deuxième partie de cette thèse est dédiée à l'étude des acides biliaires dans la chirurgie bariatrique. Nos résultats ont montré que la chirurgie bariatrique réduit la recapture hépatique des acides biliaires, provoquant leur augmentation dans la circulation systémique, et que ce n'est pas l'anse biliaire mais l'anse commune qui est responsable des modifications métaboliques après la chirurgie bariatrique chez le minipig. Ensuite, nous avons montré chez l'Homme que les acides biliaires liés aux lipoprotéines de haute densité (HDL) augmentent après la chirurgie bariatrique, et que cette augmentation est corrélée avec la restauration de leurs fonctions vaso-protectrices.

Key words in French:

Acides biliaires, FXR, TGR5, Obesité, Diabète de type 2, NAFLD, Chirurgie bariatrique.

Cette thèse a été préparée à l'UMR1011 Inserm, Institut Pasteur de Lille, Université de Lille - EGID. 1 rue du professeur Calmette. 59000 Lille, France.

Title in english: Role of bile acids on the pathophysiology of obesity, insulin-resistance, type 2 diabetes, non-alcoholic fatty liver disease and in the context of bariatric surgery.

Abstract in English

In addition to their role in the solubilization of dietary lipids, bile acids are signaling molecules regulating their own metabolism, glucose and lipid homeostasis, energy expenditure, cardiovascular function and inflammation *via* the activation of the Farnesoid X Receptor (FXR) and the Takeda G protein coupled Receptor 5 (TGR5). Indeed, changes in bile acid concentrations are associated with metabolic diseases and therefore they are candidates to participate in the pathophysiology of these diseases or predict their progression.

In the first part of this thesis, we studied bile acid changes in the context of obesity, insulin resistance, type 2 diabetes and non-alcoholic steatohepatitis. We demonstrated that bile acids are correlated with glucose homeostasis in humans, but that they are not predictors for the progression from prediabetes to type 2 diabetes in a longitudinal cohort study.

In the second part of this thesis, we studied the bile acids in the context of bariatric surgery. Our results showed that bariatric surgery reduces the hepatic recapture of certain bile acids, causing them to increase in the systemic circulation. Additionally, we showed that it is not the bile limb but the common limb the one responsible for metabolic changes after bariatric surgery in the minipig. Finally, we showed in humans that bile acids linked to high-density lipoproteins (HDL) increase after bariatric surgery, and that this increase is correlated with the restoration of their vasoprotective functions.

Key words in English

Bile acids, FXR, TGR5, Obesity, Type 2 diabetes, NAFLD, Bariatric surgery.

This thesis was prepared at the UMR1011 Inserm, Institut Pasteur de Lille, University Lille - EGID. 1 rue du professeur Calmette. 59000 Lille, France.

Résumé substantiel en Français

La prévalence croissante de l'obésité et de ses complications représente un problème de santé publique majeur dans le monde entier, avec de graves comorbidités métaboliques, une diminution de la qualité de vie et des coûts économiques importants pour la société (Srivastava et Apovian 2018 ; Ng *et al.* 2014). La compréhension de la pathophysiologie de ces maladies permet de développer de nouvelles stratégies thérapeutiques. Cette thèse porte sur l'étude des acides biliaires dans le contexte de ces maladies, leur utilité clinique comme biomarqueurs et leurs modifications après la chirurgie bariatrique.

Les acides biliaires sont des molécules stéroïdes synthétisées dans les hépatocytes à partir du cholestérol par une série de réactions enzymatiques qui constituent deux voies. La « voie classique » est responsable de la synthèse de la plupart des acides biliaires et est initiée par le cytochrome P450 (CYP) cholestérol 7α-hydroxylase (CYP7A1), et les concentrations plasmatiques de la molécule 7α-hydroxy-4-cholesten-3-one (C4) sont corrélées avec le taux de synthèse des acides biliaires par cette voie, faisant du C4 un marqueur de la voie classique. Le reste des acides biliaires est synthétisé par la « voie alternative » qui débute avec la CYP 27α-hydroxylase (CYP27A1). Les produits hépatiques finaux de ces voies sont, chez les humains, l'acide chénodésoxycholique (CDCA), l'acide cholique (CA) et l'acide hyocholique (HCA). Chez les rongeurs, le CDCA peut être 6β-hydroxylé par le CYP2C70, pour former les espèces muricholiques (MCA) (Takahashi *et al.*, 2016). Les acides biliaires sont conjugués à la glycine ou à la taurine, pour former les acides biliaires glyco- et tauro-conjugués, respectivement.

Les acides biliaires sont ensuite sécrétés dans la bile et sont dirigés à travers les voies biliaires vers la vésicule biliaire, où ils sont stockés et concentrés pendant la période inter-digestive. Les acides biliaires sont libérés en période post-prandiale à l'intérieur du duodénum pour participer à la solubilisation et à l'absorption des lipides alimentaires. Dans l'intestin, le microbiote intestinal déconjugue les acides biliaires conjugués *via* les Hydrolases de Sel Biliaire (BSH) produisant des acides biliaires libres et de la glycine ou de la taurine. De plus, les bactéries intestinales peuvent déshydroxyler et/ou épimériser les acides biliaires primaires pour former les acides biliaires secondaires (ainsi CA devient l'acide désoxycholique (DCA), CDCA devient l'acide lithocholique (LCA) et l'acide ursodésoxycholique (UDCA), HCA devient l'acide hydéoxycholique (HDCA)).

Principalement dans l'iléon, les acides biliaires sont recapturés par le pôle apical des entérocytes et retournent dans le foie par la veine mésentérique supérieure et la veine porte hépatique. Quand le sang circule dans les sinusoïdes hépatiques, les transporteurs d'acides biliaires exprimés dans la membrane sinusoïdale des hépatocytes (sodium/bile acid

cotransporter, NTCP; organic-anion-transporting polypeptides, OATPs; organic anion transporters, OATs) les recapturent, complétant la « circulation entéro-hépatique des acides biliaires ». Ainsi, les acides biliaires sont utilisés pour la solubilisation des lipides plusieurs fois par jour, circulant efficacement entre le foie et l'intestin, et seulement 5% des acides biliaires échappent à la recapture intestinale et sont perdus dans les selles, pour être remplacés par la synthèse hépatique *de novo* à partir du cholestérol. De même, une petite fraction des acides biliaires échappe à la recapture hépatique pour atteindre le cœur par la veine cave inférieure, pour être distribuée dans la circulation systémique et atteindre ainsi les tissus périphériques (Pour une revue générale sur la synthèse et le métabolisme des acides biliaires voir : Lefebvre *et al.* 2009 ; Kuipers, Bloks, and Groen 2014 ; Chávez-Talavera *et al.* 2017).

En plus de leur rôle dans la solubilisation des lipides alimentaires, les acides biliaires sont des molécules de signalisation qui régulent leur propre métabolisme, l'homéostasie du glucose et des lipides, la dépense énergétique, la fonction cardiovasculaire et l'inflammation en modulant l'activité du récepteur nucléaire Farnesoid X (FXR) et du récepteur Takeda G-protein coupled Receptor 5 (TGR5). Les puissances différentes des espèces d'acides biliaires pour moduler FXR et le TGR5 suggèrent que des changements dans leurs proportions pourraient moduler l'homéostasie métabolique, et par conséquent les voies de signalisation des acides biliaires sont reconnues comme cibles thérapeutiques potentielles dans le traitement des troubles liés à l'obésité (Schaap, Trauner, & Jansen, 2014).

Le but de cette thèse était d'étudier les variations des acides biliaires dans la physiopathologie de différents contextes métaboliques, tels que l'obésité, l'insulinorésistance, le diabète de type 2 et l'hépatopathie grasse non alcoolique (NAFLD), et *post* chirurgie bariatrique.

Cette thèse est présentée sous la forme d'une compilation de revues et d'articles originaux, L'introduction générale est présentée dans la revue que j'ai écrite avec Anne Tailleux, Philippe Lefebvre et Bart Staels, sur la physiologie des acides biliaires, leur signalisation à l'état physiologique et dans le contexte des maladies méta-inflammatoires, ainsi que leurs applications pour le traitement des maladies métaboliques (Chávez-Talavera & Tailleux 2017 Gastroenterology). La partie décrivant les hypothèses de travail, la méthodologie et les résultats et les conclusions se décline en deux : La première partie porte sur les acides biliaires dans l'obésité, la résistance à l'insuline, le diabète de type 2 et la NAFLD. La seconde partie porte sur les acides biliaires dans la chirurgie bariatrique chez les humains et les miniporcs.

Partie I. Les acides biliaires dans le contexte de l'obésité, l'insulinorésistance, le diabète de type 2 et la NAFLD.

L'introduction de ce chapitre consiste en une revue que j'ai écrite avec le soutien de Joel Haas, Anne Tailleux et Bart Staels (Chávez-Talavera *et al.* Curr Opin Lipidol. 2019). Dans cette revue, nous avons résumé et discuté les études cliniques actuellement disponibles sur l'analyse des acides biliaires chez des patients souffrant d'obésité, d'insulinorésistance (IR), de diabète de type 2 et d'hépatopathie grasse non alcoolique (NAFLD). Les différentes études sont présentées dans un tableau, dans lequel le lecteur peut voir la grande hétérogénéité entre les paramètres métaboliques des populations étudiées choisies pour les études, ce qui pourrait expliquer les résultats très discordants rapportés. Ces maladies sont difficiles à étudier séparément, car elles sont souvent comorbides et leur gravité respective peut varier d'un patient à l'autre. Le large spectre de gravité de ces maladies métaboliques pourrait être responsable d'altérations particulières du métabolisme des acides biliaires présentes seulement à certains stades de l'histoire naturelle de chaque maladie. Par conséquent, le choix de l'état clinico-biologique des populations étudiées pourrait être crucial pour comprendre les résultats contradictoires et permettre leur interprétation appropriée. Bien que la littérature actuelle ne prouve pas clairement un rôle causal, les articles analysés dans notre revue montrent que les altérations des acides biliaires sont associées aux composantes du syndrome métabolique. Afin d'étudier un contexte métabolique particulier, les études cliniques devraient envisager de faire correspondre la sévérité de l'obésité, de l'IR ou du NAFLD entre les groupes, de sorte que les groupes comparés ne diffèrent entre eux que par le contexte métabolique étudié.

En nous basant sur les altérations des acides biliaires présentes chez les patients obèses et insulinorésistants que nous avons résumées dans notre revue Chávez-Talavera *et al.* Curr Opin Lipidol. 2019, nous nous sommes demandés si les acides biliaires avaient une association prédictive avec la conversion du prédiabète en diabète de type 2, dans une étude prospective, en collaboration avec l'équipe du Pr. Bertrand Cariou de l'UMR 1087, l'Institut du Thorax, Université de Nantes. Pour répondre à cette question scientifique, nous avons utilisé la cohorte IT-DIAB, qui est une étude prospective observationnelle de 5 ans conçue pour identifier de nouveaux biomarqueurs du risque de diabète de type 2 dans une population prédiabétique. J'ai participé à la sélection des patients et j'ai été responsable de la mesure des concentrations d'acides biliaires et de C4 dans le plasma, avec l'aide précieuse du Département de Spectrométrie de Masse (PSM-GRITA) de la Faculté de Pharmacie de l'Université de Lille, ainsi que de Matthieu Wargny de l'Université de Nantes, pour les analyses statistiques des données. J'ai rédigé le manuscrit sous la supervision

étroite d'Anne Tailleux, Bart Staels et Bertrand Cariou, et je suis donc co-premier auteur de cette publication.

En résumé, 205 patients atteints de prédiabète ont été inclus dans cette étude et ont été suivis chaque année pendant 5 ans. Les concentrations plasmatiques d'acides biliaires à jeun et de C4 ont été quantifiées dans le plasma prélevé à la première visite, et l'association entre les acides biliaires et le risque de bascule de prédiabète en diabète de type 2 a été déterminée en utilisant les modèles de risques proportionnels de Cox. Dans les analyses longitudinales, les espèces plasmatiques d'acides biliaires n'ont pas été associées indépendamment à la conversion au diabète de type 2 après ajustement aux facteurs de risque classiques du diabète de type 2. Cependant, le HCA total et le rapport entre l'HCA total et le CDCA total (reflétant le taux d'hydroxylation hépatique 6α) étaient négativement corrélés avec l'IMC et le HOMA-IR, ce qui suggère que cette espèce d'acide biliaire, souvent négligée, pourrait être liée au métabolisme du glucose (Chávez-Talavera *et al.*, article soumis. Manuscrit 3, page 45).

Pour la dernière partie de ce chapitre, j'ai pu contribuer à la mise-au-point et à la validation des mesures C4, qui n'était à cette époque pas encore validé dans notre équipe, ainsi qu'à la discussion scientifique des travaux publiés par Legry *et al.* JCEM 2018. Comme nous le discutons dans notre revue (Chávez-Talavera *et al.* Curr Opinion LIpidol 2019), de nombreuses études cliniques ont rapporté des altérations des acides biliaires dans le contexte de la NAFLD, quel que soit le statut métabolique des populations étudiées. Nous savons aussi que les altérations des acides biliaires sont associées aux comorbidités métaboliques de la NAFLD. Par conséquent, l'association entre les altérations du métabolisme des acides biliaires et les lésions histologiques nécro-inflammatoires de NASH ont été étudiés et l'analyse transcriptomique du métabolisme des acides biliaires et des acides biliaires hépatiques à jeun ont été comparés entre les patients atteints de NASH et ceux qui n'en sont pas atteints. L'originalité de cette étude est que les patients ont été soigneusement appariés par rapport à leur IMC et HOMA-IR de sorte qu'ils ne varient que sur le statut NASH. Les résultats montrent qu'il n'y a pas de différence dans l'expression génique du métabolisme hépatique des acides biliaires ni dans les concentrations plasmatiques de C4 et FGF19, un marqueur circulant d'activation inestinale de FXR. De plus, les acides biliaires plasmatiques n'étaient pas corrélés avec les lésions hépatiques de NASH mais avec les paramètres d'homéostasie du glucose, ce qui suggère que les altérations des acides biliaires sont liées au phénotype métabolique associé à NASH, plutôt qu'aux lésions histologiques proprement dites (Legry *et al.* JCEM 2018).

Partie II. Les acides biliaires dans le contexte de la chirurgie bariatrique : étude chez l'Homme et dans un modèle préclinique de miniporc (Sus scrofa).

La chirurgie bariatrique est le traitement le plus efficace contre l'obésité morbide, car elle induit une perte de poids et, par conséquent, améliore le métabolisme. De façon intéressante, plusieurs améliorations métaboliques consécutives à la chirurgie bariatrique surviennent avant la perte de poids corporel, et le mécanisme à l'origine de ces améliorations est inconnu. Parmi les candidats proposés pour participer à ces changements figurent les acides biliaires, puisque leurs concentrations systémiques sont augmentées et leurs proportions sont modifiées tôt après l'intervention chirurgicale dans de nombreuses études cliniques et précliniques.

L'introduction de ce chapitre est une revue résumant les changements des acides biliaires chez l'humain subissant une chirurgie bariatrique, les mécanismes sous-jacents et leur rôle potentiel de signalisation dans les améliorations métaboliques après une chirurgie bariatrique (Chávez-Talavera *et al.* Appetite 2019 soumission en cours, Manuscrit 5, Page 91).

Puisque les acides biliaires sont des candidats pour participer aux améliorations métaboliques précoces à la suite du bypass gastrique Roux-en-Y (RYGB), nous nous sommes interrogés sur le mécanisme sous-jacent aux augmentations d'acides biliaires induites par la chirurgie bariatrique. Pour répondre à cette question, nous avons fait une collaboration avec l'équipe du Pr. François Pattou (UMR 1190, EGID, Université de Lille), qui dispose d'un modèle de RYGB validé dans le modèle de miniporc de type Göttingen. Nous avons utilisé ce modèle car il présente l'avantage d'être un mammifère omnivore de taille humaine, permettant un échantillonnage continu et une analyse simultanée du sang veineux systémique et du sang préhépatique portal. Grégory Baud, doctorant sous la supervision de François Pattou au moment de l'étude, a réalisé les interventions chirurgicales, et les dosages d'acides biliaires ont été réalisés par Emmanuelle Vallez, technicienne travaillant dans notre équipe. L'interprétation des données sur les acides biliaires et la recherche des mécanismes hépatiques sous-jacents m'ont été confiés, ainsi que la rédaction du manuscrit sous la direction de ma directrice de thèse et grâce à la formation et aux contributions de Sophie Lestavel, Bart Staels et François Pattou. Comme j'étais responsable de ce projet, je suis co-premier auteur de ce manuscrit.

Puisque notre but était d'étudier le rôle du foie dans les changements des acides biliaires induits par le RYGB, nous avons analysé les acides biliaires dans le sang veineux portal et dans le sang systémique au cours d'un test de repas avant et après le RYGB, et nous avons aussi analysé l'expression des gènes du métabolisme hépatique des acides biliaires. Nous

avons démontré que, dans la période postprandiale, les acides biliaires portaux ne sont pas altérés après une chirurgie bariatrique, alors que les concentrations systémiques d'acides biliaires augmentent du fait d'une augmentation des espèces d'acides biliaires conjugués. Puisque l'organe entre le sang portal et la circulation systémique est le foie, et puisque le foie est l'un des principaux organes où se produit le métabolisme des acides biliaires, nous avons analysé dans les biopsies du foie l'expression des gènes du métabolisme des acides biliaires. Notre analyse a révélé une diminution de l'expression des gènes transporteurs responsables de la recapture hépatique des acides biliaires, ce qui suggère que le RYGB module sélectivement la recapture hépatique des acides biliaires et contribue à leurs changements plasmatiques après une chirurgie bariatrique (Chávez-Talavera & Baud *et al.* Int J Obes 2017).

Pour la section suivante de ce chapitre, nous nous sommes concentrés sur les acides biliaires intestinaux, puisqu'ils participent à la régulation de l'homéostasie du glucose *via* leurs récepteurs dans l'intestin, et que l'anse biliaire est un candidat pour être responsable des améliorations métaboliques après une chirurgie bariatrique. Nous nous sommes donc demandé si l'anse biliaire est le médiateur des changements métaboliques d'une autre technique de chirurgie bariatrique : le bypass gastrique à anastomose unique (One Anastomosis Gastric Bypass, OAGB), et si les acides biliaires intestinaux participent à ces améliorations métaboliques. Pour répondre à cette question, nous avons poursuivi la collaboration avec François Pattou, en utilisant le modèle préclinique miniporc que dans l'étude précédente. Les miniporcs ont subi une OAGB ou une chirurgie de type sham avec ou sans résection de l'anse biliaire. Le phénotype métabolique des miniporcs a été déterminé un mois après la chirurgie, y compris la composition des acides biliaires intestinaux tout au long du tractus gastro-intestinal, ainsi que les acides biliaires plasmatiques. Dr. Camille Marciniak, doctorante sous la direction de François Pattou, a réalisé les interventions chirurgicales et j'ai dosé les acides biliaires plasmatiques et les concentrations en C4 et effectué les analyses des acides biliaires. J'étais responsable de l'analyse de toutes les données sur les acides biliaires et j'ai également contribué à l'interprétation et à la rédaction du manuscrit avec Camille Marciniak. Pour cela, je suis co-premier auteur de ce manuscrit en préparation.

Ces travaux ont donné des résultats inédits dans le domaine des acides biliaires. D'abord, nous montrons pour la première fois que l'OAGB augmente les concentrations systémiques d'acides biliaires, de façon similaire au RYGB. Ensuite, nous décrivons que l'homéostasie du glucose s'améliore simultanément avec un déplacement des proportions intestinales intra-luminales de HDCA vers HCA après l'OAGB. Enfin, nous avons étudié le rôle de l'anse biliaire et de l'anse commune dans l'amélioration du métabolisme et dans les changements

des acides biliaires intra-luminaux intestinaux sur l'OAGB. Nos données montrent que la résection de l'anse biliaire (ou du segment intestinal correspondant dans le modèle sham) reproduit les changements métaboliques et d'acides biliaires après l'OAGB sans résection, ce qui suggère que la longueur de l'anse commune, mais pas celle de l'anse biliaire, intervient dans ces effets (Chávez-Talavera & Marciniak *et al.* Article en préparation, Manuscript 7, page 120).

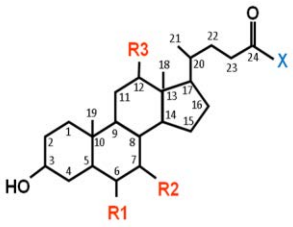
La dernière partie de ce chapitre est une étude chez l'Homme réalisée en collaboration avec l'équipe d'Elena Osto, du Laboratoire de biologie de la nutrition translationnelle de l'Université de Zurich, Zurich, Suisse.

Les acides biliaires circulent dans la circulation sanguine liés aux composants plasmatiques de la fraction dépourvue de lipoprotéines, dont l'albumine présente la plus forte affinité (Rudman et Kendall 1957 ; Ceryak, Bouscarel, et Fromm 1993). Cependant, les acides biliaires sont également transportés par les lipoprotéines (Kramer *et al.* 1979 ; Steiner *et al.* 2012). En effet, les acides biliaires transportés par les lipoprotéines, représentent 5 à 10 % des acides biliaires plasmatiques dans des conditions physiologiques (Buscher *et al.* 1987 ; Steiner *et al.* 2012 ; Hedenborg, Norman, et Ritzén 1988). De plus, les HDL sont connues pour exercer des effets vaso-protecteurs sur les cellules endothéliales, entre autres, en augmentant la production d'oxyde nitrique ou en inhibant l'apoptose. Ces propriétés des HDL sont connues pour être diminuées dans le contexte de l'obésité, et restaurées rapidement après le RYGB (Osto *et al.*, 2015). Étant donné que les acides biliaires circulants changent aussi très tôt après le RYGB, nous avons étudié les acides biliaires liés aux HDL chez l'Homme avant et après RYGB et nous avons analysé si ceux-ci sont corrélés avec l'amélioration des propriétés vaso-protectrices des HDL. Nos données montrent que le RYGB augmente les acides biliaires liés aux HDL et que l'augmentation des espèces CA et CDCA est corrélée à la restauration des propriétés anti-apoptotiques des HDL après chirurgie (Chávez-Talavera & Jomard, *et al.* Article en préparation. Manuscrit 8, page 148).

INTRODUCTION AND GENERAL OVERVIEW OF THE THESIS

The rising global prevalence of obesity and its complications represents a major public health problem worldwide, carrying severe metabolic comorbidities, decreased life quality and economic costs for society (Ng *et al.*, 2014; Srivastava & Apovian, 2018). The understanding of the pathophysiology of these diseases allows developing new strategies for their treatment. This thesis focuses on the study of bile acids in the context of these diseases, their clinical utility as biomarkers and their modifications upon bariatric surgery.

Bile acids are steroid molecules synthetized within hepatocytes from cholesterol by a series of enzymatic reactions that constitute two pathways. The “classical pathway” is responsible for the synthesis of most of bile acids and is initiated by the cytochrome P450 (CYP) cholesterol 7α-hydroxylase (CYP7A1), the plasma concentrations of the molecule 7α-hydroxy-4-cholesten-3-one (C4) are a correlated with the bile acid synthesis rate *via* this pathway. The remaining part of bile acids is synthesized by the “alternative pathway” that initiates with the CYP 27α-hydroxylase (CYP27A1). The hepatic end-products of these pathways are, in humans, the chenodeoxycholic acid (CDCA), cholic acid (CA), and the hyocholic acid (HCA). (In mice and rats, CDCA can be 6β-hydroxylated by the CYP2C70, to form the muricholic (MCA) species). Bile acids can be conjugated to glycine or taurine, to form the glyco- and tauro-conjugated bile acids, respectively.



Free BAs: X = OH
Glyco-conjugated BAs: X = NH-CH_2-CO_2H
Tauro-conjugated BAs: X = NH-CH_2-CH_2-SO_3H

$R1_{(C6)}$	$R2_{(C7)}$	$R3_{(C12)}$		Free	Glyco-BAs	Tauro-BAs
H	αOH	αOH	cholic acid	CA	GCA	TCA
H	αOH	H	chenodeoxycholic acid	CDCA	GCDCA	TCDCA
H	H	αOH	deoxycholic acid	DCA	GDCA	TDCA
H	H	H	lithocholic acid	LCA	GLCA	TLCA
βOH	αOH	H	α-muricholic acid	α-MCA	Gα-MCA	Tα-MCA
βOH	βOH	H	β-muricholic acid	β-MCA	Gβ-MCA	Tβ-MCA
αOH	βOH	H	ω-muricholic acid	ω-MCA	Gω-MCA	Tω-MCA
H	βOH	H	ursodeoxycholic acid	UDCA	GUDCA	TUDCA
αOH	αOH	H	hyocholic acid	HCA	GHCA	THCA
αOH	H	H	hyodeoxycholic acid	HDCA	GHDCA	THDCA

Figure 1. General structure of bile acids, positions of the hydroxyle groups that characterize them in the carbon 6 (R1), 7 (R2) and 12 (R3). Bile acids are called unconjugated or “Free” when there is a hydroxyle in the position X, whereas they are called “glyco-“ or “tauro-conjugated” when there is a glycine or a taurine in the position X.

Bile acids are further secreted into the bile and flow through the biliary tree into the gallbladder, where they are stocked and concentrated during the inter-digestive period. Bile acids are released postprandially inside the duodenum to participate to the alimentary lipid solubilization and absorption. Within the intestine, the gut microbiota deconjugate the conjugated bile acids *via* the Bile Salt Hydrolases (BSH), producing free bile acids and glycine or taurine. Additionally, the gut bacteria can dehydroxylate and/or epimerize the primary bile acids to form secondary bile acids (CA becomes deoxycholic acid (DCA), CDCA becomes lithocholic acid (LCA) or ursodeoxycholic acid (UDCA), HCA becomes hyodeoxycholic acid (HDCA)).

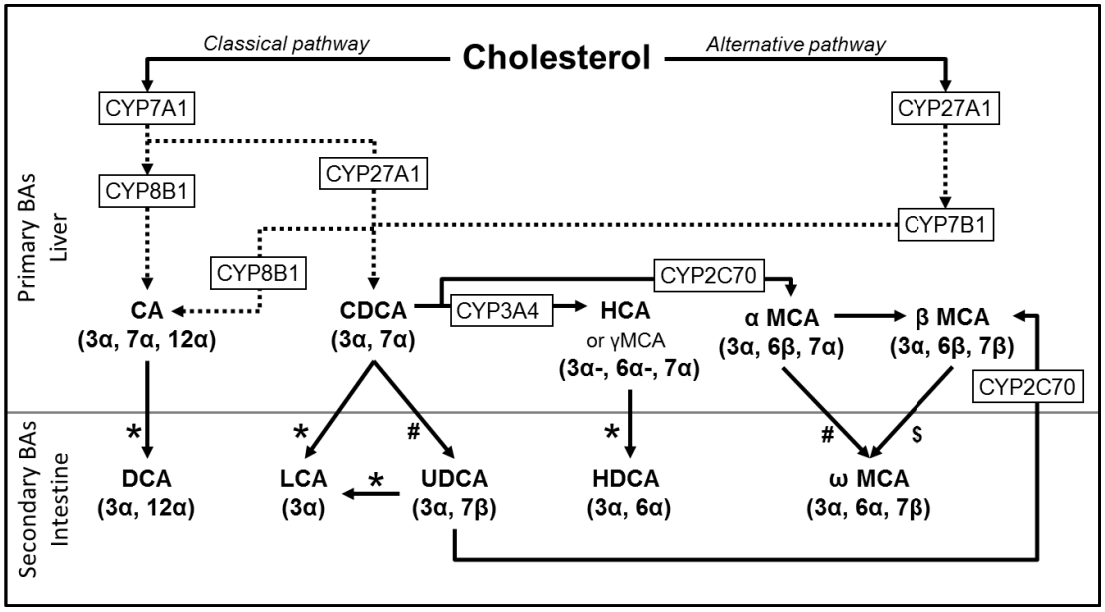


Figure 2. Main steps of hepatic and intestinal bile acid synthesis. Intestinal microbiota 7-dehydroxylation (*), 7-epimerization (#), 6-epimerization ($). From Chávez-Talavera *et al.* Appetite 2019.

Predominantly in the ileum, bile acids are recaptured at the apical pole of enterocytes and return to the liver *via* the superior mesenteric and the portal veins. As blood flows through the hepatic sinusoids, the bile acid transporters expressed in the sinusoidal membrane of the hepatocytes (sodium/bile acid cotransporter, NTCP; organic-anion-transporting polypeptides, OATPs; organic anion transporters, OATs) recapture them, completing the so called "entero-hepatic circulation" of bile acids. Thus, bile acids are used for lipid solubilization several times a day, cycling efficiently between the liver and the intestine, and only 5% of bile acids escape intestinal recapture and are lost in feces, to be replaced by hepatic *de novo* bile acid synthesis from cholesterol. Similarly, a small fraction of bile acids escape hepatic recapture to reach the heart *via* the inferior vena cava, to be distributed in the systemic circulation and reaching thus peripheral tissues, (for review on bile acid synthesis and metabolism (Chávez-

Talavera, Tailleux, Lefebvre, & Staels, 2017; Kuipers, Bloks, & Groen, 2014; Lefebvre, Cariou, Lien, Kuipers, & Staels, 2009)).

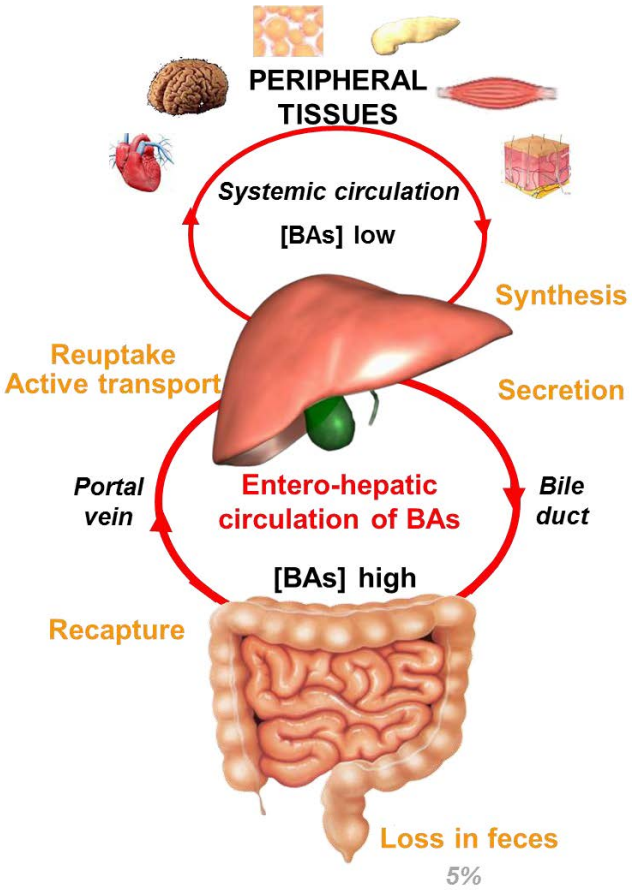


Figure 3. Entero-hepatic circulation of bile acids. Adapted from Chávez-Talavera & Spinelli, *et al.* Curr Opin Endocrinol Diabetes Obes. 2016.

In addition to their role in dietary lipid solubilization, bile acids are signaling molecules regulating their own metabolism, glucose and lipid homeostasis, energy expenditure, cardiovascular function and inflammation by activating the nuclear Farnesoid X Receptor (FXR) and the Takeda G-protein coupled Receptor 5 (TGR5). The different potencies of bile acid species to activate FXR and TGR5 suggest that changes in their proportions could modulate metabolic homeostasis, and therefore bile acid signaling pathways are recognized as potential therapeutic targets for the treatment of obesity-related disorders.

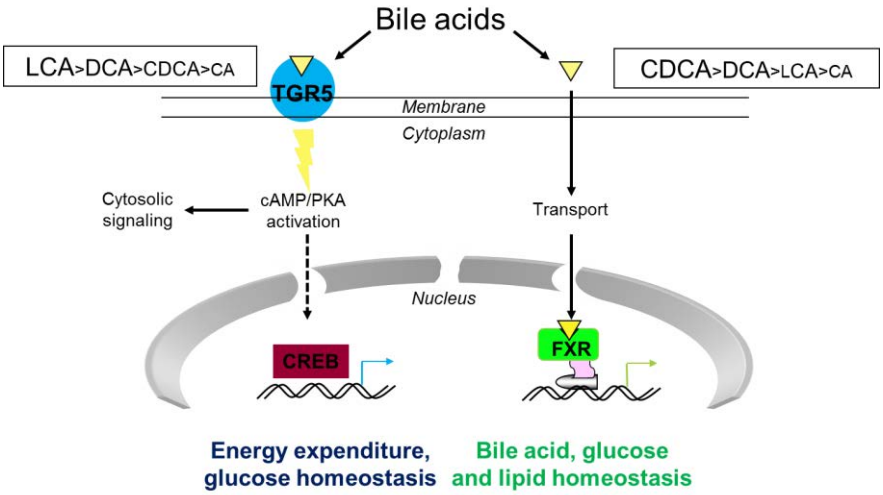


Figure 4. Bile acids modulate their receptors FXR and TGR5 with different potencies. Since TGR5 is a membrane receptor, its activation can occur by simple proximity of the bile acids with the cell, whereas FXR requires internalization and translocation of the bile acids to the nucleus.

The aim of this thesis was to study the variations of bile acids in the pathophysiology of different metabolic contexts, such as obesity, insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD), as well as in bariatric surgery. This thesis is presented as a compilation of reviews and original articles. The general introduction is presented in the review that I wrote with Anne Tailleux, Philippe Lefebvre and Bart Staels, about bile acid physiology, bile acid signaling in physiology and in the context of meta-inflammatory diseases, and the targeting of bile acid signaling pathways for the treatment of metabolic diseases **(Chávez-Talavera & Tailleux 2017 Gastroenterology**).

This thesis is organized in two parts. The **Part I** is about bile acids in the context of obesity, insulin resistance, type 2 diabetes, and NAFLD. The **Part II** is about bile acids in the context of bariatric surgery in humans and minipigs.

MANUSCRIPT NUMBER 1

Chávez-Talavera & Tailleux *et al.* Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease. Gastroenterology, 2017. May 152(7):1679-1694.e3.

Gastroenterology 2017;152:1679–1694

Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease




Oscar Chávez-Talavera Anne Tailleux Philippe Lefebvre Bart Staels

Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011 - EGID, F-59000 Lille, France

GASTROINTESTINAL IMPLICATIONS

Bile acids are signaling molecules that coordinately regulate metabolism and inflammation via the nuclear farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 (TGR5). These receptors activate transcriptional networks and signaling cascades controlling the expression and activity of genes involved in bile acid, lipid and carbohydrate metabolism, energy expenditure, and inflammation by acting predominantly in enterohepatic tissues, but also in peripheral organs. In this review, we discuss the most recent findings on the inter-organ signaling and interplay with the gut microbiota of bile acids and their receptors in meta-inflammation, with a focus on their pathophysiologic roles in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic steatohepatitis, and their potential therapeutic applications.

Keywords: Bile Acids; FXR; TGR5; Meta-Inflammation.

Enterohepatic Circulation and Metabolism of Bile Acids

Bile acids (BAs) are amphipathic steroid molecules synthesized from cholesterol in hepatocytes surrounding the hepatic central vein (perivenous hepatocytes) by the action of approximately 15 enzymes. BA synthesis occurs via 2 pathways.[1] The classical pathway, initiated by the rate-limiting enzyme cytochrome P450 cholesterol 7α-hydroxylase (CYP7A1), produces the majority of the BA pool. A fraction of the BA pool is synthesized via an alternative pathway (between 3% and 18% of total BA synthesis in healthy humans[2,3]), initiated by cytochrome P450 27α-hydroxylase (CYP27A1). The products of these pathways are the primary BA cholic acid (CA), and chenodeoxycholic acid (CDCA) in humans.[1] In rodents, α- and β-muricholic acid (MCA) are formed from CDCA and ursodesoxycholic acid (UDCA) respectively, by CYP2C70.[4] The hydrophobicity index of the BA pool, reflecting the ratio of highly hydroxylated CA(+MCA) to lower hydroxylated BA (such as CDCA), is an important parameter controlling its physiologic functions. This hydrophobicity index differs between rodents (low hydrophobicity) and humans (high hydrophobicity) due to the conversion of CDCA into MCA in rodents. CDCA and MCA synthesis is conditioned by the activity of cytochrome P450 12α-hydroxylase B1 (CYP8B1), which transforms di-hydroxylated- in tri-hydroxylated-BAs. BA synthesis, which follows a circadian rhythm controlled by the clock gene Rev-erbα[5] and the KLF15-Fgf15 axis,[6] is regulated by negative feedback mechanisms. High hepatic BA exposure inhibits BA synthesis via the farnesoid X receptor (FXR), which decreases LRH1-regulation of CYP7A1 via induction of small heterodimer protein (SHP/NR0B2) in the liver. BA also induce fibroblast growth factor-19 (FGF19, the human orthologue of murine FGF15) expression and release from the intestine, which activates the FGFR4/βKlotho receptor in the liver, thus collectively leading to inhibition of hepatic CYP7A1 and CYP8B1 expression.[7,8]

In hepatocytes, primary BAs are further conjugated to glycine—mainly in humans, or taurine—mainly in mice, at the C24 position by the enzymes BA-CoA synthase and BA-CoA–amino acid N-acetyltransferase. In addition, BA can also be sulfated by the sulfotransferase SULT2A1 (SULT2A9 in mice), or glucuronidated by UDP-glucuronosyltransferases, such as UGT2B4, UGT2B7,

Abbreviations used in this paper: apo, apolipoprotein; ASBT, apical sodium–dependent bile acid transporter; BA, bile acid; BAS, bile acid sequestrant; BAT, brown adipose tissue; BSH, bile salt hydrolase; CA, cholic acid; CCK, cholecystokinin; CDCA, chenodeoxycholic acid; CYP7A1, cytochrome P450 cholesterol 7α-hydroxylase; CYP27A1, cytochrome P450 27α-hydroxylase; CYP8B1, cytochrome P450 12α-hydroxylase B1; DCA, deoxycholic acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HFD, high-fat diet; LCA, lithocholic acid; LDL-R, low-density lipoprotein receptor; MCA, muricholic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OCA, obeticholic acid; RYGB, Roux-en-Y gastric bypass; S1PR, sphingosine-1-phosphate receptor; SHP, short heterodimer protein; T2D, type 2 diabetes; TGR5, Takeda G protein-coupled receptor 5; UDCA, ursodesoxycholic acid; VSG, vertical sleeve gastrectomy.

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2017.01.055

and UGT1A3.[1] BAs are then secreted into the bile canaliculi via the bile salt export pump and the BA transporters MRP2 and MDR1A.[1] The bile containing the secreted BA flows through the biliary tree into the gallbladder, where it is stored and concentrated during the inter-digestive period, until meal ingestion-stimulated cholecystokinin (CCK) secretion by enteroendocrine I cells induces gallbladder contraction, causing bile release into the duodenum.[1]

In the intestine, BAs activate pancreatic lipase and form micelles containing dietary fat and lipophilic vitamins (A, D, E, and K). The intestinal microbiota transforms primary BA into secondary BA: deoxycholic acid (DCA), lithocholic acid (LCA), and UDCA in humans; and DCA, LCA, ωMCA, hyodeoxycholic, and murideoxycholic acid in mice.[9] In the enterocytes from the distal ileum, 95% of intestinal BAs are actively re-absorbed through the apical sodium−dependent BA transporter (ASBT/SLC10A2) and secreted at the basolateral membrane by the heterodimeric organic solute transporters α and β. The unabsorbed 5% of intestinal BAs are either deconjugated by the gut microbiota and passively reabsorbed in the colon, or lost into feces. The absorbed BAs return to the liver through the superior mesenteric and portal veins, where they are cleared by active transporters in the sinusoidal membrane of hepatocytes (NTCP, OAT, OATP, mEH).[10] Within hepatocytes, free BAs are conjugated and secreted into bile canaliculi, along with BAs newly synthesized from cholesterol, thereby compensating for fecal loss. The small amount of BAs escaping hepatic recapture reaches the peripheral tissues via the systemic circulation. These plasma BAs circulate bound to plasma proteins—mainly albumin (approximately 80%) and lipoproteins (approximately 20%)[11]—where they may exert signaling functions on peripherally expressed BA receptors.[1]

Reciprocal Interaction Between Intestinal Microbiota and Bile Acids: Impact on Host Metabolism

In the intestinal lumen, BAs and the microbiota reciprocally control their composition. The gut microbiota transforms BAs present in the intestine at millimolar concentrations by carrying out numerous reactions, such as hydrolysis of conjugated BAs by bile salt hydrolases (BSH), 7α-dehydroxylation of CA and CDCA forming DCA and LCA, respectively, and oxidation and epimerization of hydroxyl groups at the C3, C7, and C12 positions. The gut microbiota also esterify BAs, making them more hydrophobic. Esterified BAs (ethyl-esters and long-chain fatty acid esters of LCA and polyesters of DCA) account for approximately 25% of fecal BAs.[9] Furthermore, intestinal bacteria reduce the bactericidal effect of BAs by transforming DCA and LCA into iso-DCA and iso-LCA (3β-OH epimers) via the iso-BA pathway.[12] Indeed, BAs act as antimicrobial agents by damaging bacterial membranes and altering intracellular macromolecular structures through detergent actions. Therefore, only microbial populations able to tolerate high BA concentrations can survive in the gut. Whereas free BAs are more damaging to bacterial membranes, taurine catabolic end products promote proliferation of some bacteria strains.[13]

BSH is active in *Lactobacillus, Bifidobacterium, Firmicutes, Enterococcus, Clostridium,* and *Bacteroides*, and produces free BA, taurine, and glycine. Free BAs solubilize intestinal lipids and are reabsorbed less efficiently, resulting in increased fecal BA loss and an ameliorated metabolic adaptation of the host.[14] Indeed, BSH-overexpressing *Escherichia coli* reduce host weight gain, liver triglycerides, and plasma cholesterol in conventionally raised mice by reducing intestinal cholesterol absorption and increasing hepatic uptake of plasma cholesterol for de novo BA synthesis to compensate for the fecal BA loss.[14]

Gut microbial depletion in germ-free or antibiotic-treated rodents increases the proportion of taurine-conjugated primary BA species, including TβMCA, a rodent-specific FXR antagonist (see Bile Acids: Signaling Molecules Modulating Meta-Inflammatory Diseases section), decreases the diversity of the BA pool[15] and concomitantly increases gallbladder and small intestine BA concentrations.[16] In line, treatment of mice with probiotics, such as VSL#3, which enhances deconjugation and fecal BA excretion, increases hepatic BA synthesis via down-regulation of the FXR/FGF15 axis.[17] Intriguingly, colonization of germ-free mice with human microbiota decreases the formation of secondary BA species, especially the FXR antagonist TβMCA, thereby increasing ileal FXR activity and FGF15 expression.[18] As BA receptors have different affinities for distinct BA species (see Bile Acids: Signaling Molecules Modulating Meta-Inflammatory Diseases section), the gut bacteria can modulate metabolism and host physiology by altering BA pool composition.[9]

Because type 2 diabetes (T2D), obesity and non-alcoholic fatty liver disease (NAFLD) are associated with dysbiosis[19] and changes of the BA pool size and composition,[20–22] the interaction of gut microbiota with BA metabolism and its impact on these pathophysiologic conditions is of great interest. For instance, NASH patients present dysbiosis associated with increases in BA synthesis, fecal primary BAs, and the primary to secondary BA ratio.[23]

Treatment with antibiotics alters gut bacteria composition. In antibiotic-treated mice,[24] BSH-producing *Lactobacillus* decreases, synthesis of the FXR antagonist TβMCA increases, and high-fat diet (HFD)-induced obesity, insulin resistance, and NAFLD improve.[25] However, studies in humans reported no metabolic changes after probiotic supplementation[26] or antimicrobial therapy.[27] Hence, although interplay between BA, gut microbiota, and metabolic diseases is evident, the causality and directionality of the interactions remain unclear.

Bile Acid Metabolism in Meta-Inflammatory Disorders

Cross-talk between metabolically active tissues is necessary for proper energy homeostasis. In obesity, T2D and non-alcoholic steatohepatitis (NASH), these organs often present, in combination with metabolic alterations, a chronic low-grade inflammation characterized by the recruitment of immune inflammatory cells, abnormal

cytokine and acute-phase reactant production, and inflammasome activation, referred to as "meta-inflammation."[28]

Meta-inflammatory disorders, such as obesity, T2D, and NASH, are associated with changes in BA metabolism and pool composition, as shown by a large number of observational studies (Table 1). Chronic inflammation modulates hepatic BA metabolism, as exemplified by the decrease of CYP7A1 transcription in human hepatocytes treated with interleukin 1β, which acts via the c-Jun-N-terminal kinase/c-Jun signaling pathway.[29] Total BA concentrations increase in obese patients and correlate with body mass index irrespective of T2D and NAFLD (Table 1). In T2D patients, systemic total BA concentrations are increased, in the fasting and postprandial states. However, reported changes in qualitative BA pool composition differ among studies (Table 1). Interestingly, insulin-resistant, but not T2D patients, display an increased 12α-hydroxylated to non-12α-hydroxylated BA ratio.[20]

In NASH patients, plasma BA and C4,[30] as well as hepatic BA concentrations, are increased.[23,31] In addition, hepatic BA synthesis may shift to the alternative pathway in livers of NASH patients,[21] as suggested by gene expression analysis. The higher BA exposure could lead to cytotoxicity and contribute to the pathogenesis of NAFLD.[30]

Fasting peripheral blood BA concentrations consistently increase upon bariatric surgery (Roux-en-Y gastric bypass [RYGB]) in humans (Table 1) and preclinical models.[32] Again, reported qualitative changes of the BA pool after bariatric surgery differ between studies, which may be due to differences in methodological parameters (eg, feeding state and time after surgery). The kinetics of BA pool size and composition alterations after RYGB are unclear; while some studies reported an early 2- to 3-fold increase in BAs,[33–35] others found BAs to be increased only 1 year post-RYGB[36–38] or after 20% of body weight loss.[39] Both short-term[40] and long-term[35,37,39–41] augmentations in postprandial systemic BA concentrations were reported after RYGB, with qualitative changes, such as increased conjugated BA species.[37,41] However, the changes in peripheral BA were not correlated to changes in body weight after RYGB.[42] The 12α-hydroxylated to non-12α-hydroxylated BA ratio was increased 2 years after RYGB,[35] even though insulin resistance improved. Interestingly, patients with RYGB-induced remission of T2D presented higher BA concentrations than patients without remission,[43] suggesting a role for BAs in the metabolic improvements.

There is scarce information regarding BA changes after vertical sleeve gastrectomy (VSG), biliopancreatic diversion, and laparoscopic adjustable gastric banding (Table 1). In some studies, fasting plasma BA did not change after laparoscopic adjustable gastric banding[34,39] or VSG,[44] while others reported a decrease upon 20% weight loss after laparoscopic adjustable gastric banding[39] and an increase 1 year after VSG.[38]

The mechanisms underlying the increase in peripheral BAs upon RYGB surgery are still unclear and may include increased hepatic synthesis, increased intestinal recapture potentially associated with microbiota alterations, changes in portal blood flow, and/or lower hepatic BA recapture from the portal vein.[45–48] Interestingly, germ-free mice receiving gut microbiota from RYGB-treated mice exhibit weight loss and decreased fat mass,[47] suggesting that intestinal microbiota could contribute to the metabolic improvements after RYGB via changes in the BA pool.

Alterations in FXR and TGR5 signaling could contribute to the metabolic improvements. Indeed, BA and glucagon-like peptide-1 (GLP-1) levels positively correlate after RYGB[38,49] and VSG in humans[38] and in mice.[50] FGF19 has also been reported to increase after RYGB[33,43] and VSG.[44] Direct roles for FXR and TGR5 in the metabolic improvements after VSG have been suggested, based on genetic studies in mice.[48,50,51]

Because BAs have emerged as signaling molecules regulating glucose, lipid and energy homeostasis, and inflammation, it is conceivable that changes in BA pool size and composition in metabolic diseases and upon bariatric surgery alter BA signaling pathways, impacting on metabolic parameters. However, due to methodological heterogeneity and cohort size, it is still unclear how BA pool alterations contribute to and impact on (patho)physiologic conditions in humans.

Bile Acids: Signaling Molecules Modulating Meta-Inflammatory Diseases

BA are ligands of the nuclear receptors FXR,[52] vitamin D receptor,[53] and pregnane X receptor,[54] as well as G-protein coupled receptors, such as TGR5,[55] sphingosine-1-phosphate receptor (S1PR)2,[56] and the muscarinic receptors M2/3.[57] Activation of nuclear receptors by BAs requires cellular entry, either by simple diffusion, in the case of hydrophobic free BA, or by active transport for conjugated and hydrophilic BAs. In contrast to nuclear receptors, ligands can bind directly to cell surface receptors.[1] Activation of pregnane X receptor,[54] vitamin D receptor,[53,58] and CAR[59] by BAs, mainly LCA, induce a xenobiotic detoxification response to stimulate BA excretion under cholestatic conditions.[1] We will focus on the role of BAs in the control of metabolism and inflammation via FXR, TGR5 (Figure 1), and S1PR2.

FXR (*NR1H4*) is expressed in several organs, including the liver, intestine, kidneys, adrenal glands, white adipose tissue, and immune cells.[1] Natural FXR agonists are CDCA>DCA>CA>LCA, in order of decreasing potency, while Tα-, TβMCA, and possibly UDCA are antagonists.[16] Ligand-bound FXR forms a heterodimer with retinoic X receptors (α, β, or γ) to regulate target gene expression. FXR also indirectly represses gene transcription via induction of negative regulators, such as SHP, competition for other nuclear receptors (peroxisome proliferator-activated receptor−α[60]) or transcriptional coregulators (CRTC2[61]). In addition to ligands, post-translational modifications also modulate the transcriptional activity of FXR, such as O-GlcNAcylation,[62] methylation, acetylation,[63] and phosphorylation (via adenosine monophosphate−activated protein kinase and protein kinase C).[64–66] Interestingly, acetylation of FXR increases inflammation and deteriorates glucose metabolism by interfering with its sumoylation and transrepressive activity.[67]

Table 1. Influence of Obesity, Insulin Resistance, Type 2 Diabetes, Nonalcoholic Fatty Liver Disease, and Bariatric Surgery (Roux-en-Y Gastric Bypass, Vertical Sleeve Gastrectomy, Bilio-Pancreatic Diversion, and Adjustable Gastric Banding) on Peripheral Blood Bile Acid Concentrations

Case, n	Controls, n	Feeding state	Time after surgery	Methodology	Outcome	First author, year
Obesity/IR/T2D/ NAFLD			NA			
85 obese	15	Fasting	NA	Enzymatic assay	Total BA concentrations positively correlated with BMI	Prinz, 2015[1]
15 T2D	15 non-diabetic, BMI-matched controls	Fasting OGTT low-fat, medium-fat, high-fat meal test	NA	UPLC-MS/MS	Fasting: ↑ total BA concentrations compared with non-diabetic controls Postprandial: ↑ total BA concentrations in all the feeding states, positively correlated with increasing meal fat content compared with non-diabetic controls	Sonne, 2016[2]
35 T2D	200 non-diabetic patients classified into quartiles based on IR	Fasting	NA	LC-MS	T2D patients: ↑total BA concentrations without changes in the 12α-hydroxy to non-12α-hydroxy-BA ratio Non-diabetic IR patients: ↑ 12α-hydroxy to non-12α-hydroxy-BA ratio associated with ↓ insulin sensitivity and ↑ plasma TGs	Haeusler, 2013[3]
12 T2D	12 matched non-diabetic controls	Fasting meal test	NA	HPLC-MS/MS	Fasting: no changes in total BA in T2D compared with non-diabetic controls Postprandial: ↑total and Glyco-BA in T2D compared with non-diabetic controls	Vincent, 2013[4]
12 T2D	62 non-diabetic volunteers	Fasting	NA	HPLC-MS/MS	Fasting: ↑ tauro BA in T2D patients compared with non-diabetic controls	Wewalka, 2014[5]
20 T2D 22 obese	14 healthy	Fasting	NA	GC-MS	↑ total BA concentrations in obese and T2D patients compared with controls ↑DCA in T2D compared with controls ↑CA in obese patients compared with controls CDCA and CA, and to a lesser extent DCA, positively associated to IR in obese and T2D patients	Cariou, 2011[6]
7 NASH	15 healthy	Fasting postprandial	NA	LC-MS/MS	Fasting and postprandial: ↑ total BA concentrations in NASH compared with controls due to conjugated species	Ferslew, 2015[7]
RYGB						
9	—	Fasting	2 y, 4 y	HPLC-MS/MS	2 y, 4 y: ↑ total BA	Patti, 2009[8]
19	—	Fasting	1 mo, 3 mo	HPLC-MS	1 mo, 3 mo: ↑ total BA	Nakatani, 2009[9]
35	—	Fasting	3 mo	Enzymatic assay	3 mo: ↑ total BA	Jansen, 2011[10]
12	—	Fasting	4 d, 42 d	LC-MS/MS	42 d: ↑ total BA	Pournaras, 2012[11]
30	—	Fasting	1 y	Enzymatic assay, HPLC-MS/MS	1 y: ↑ total BA; ↓ tauro-BA	Simonen, 2012[12]
36 T2D-R 21 T2D-NR	37	Fasting	1 y	LC-MS/MS	1 y: ↑ total BA in T2D-R	Gerhard, 2013[13]
8	—	Fasting	After, 20% of weight loss	LC-MS	↑ total BA	Kohli, 2013[14]

Table 1. Continued

Case, n	Controls, n	Feeding state	Time after surgery	Methodology	Outcome	First author, year
21	—	Fasting	1 mo, 6 mo, 1 y, 2 y	LC-MS/MS	1 mo: ↑ total BA due to UDCA, TUDCA, GUDCA 2 y: ↑ total BA due to CA, CDCA, DCA, GDCA, HCA 6 mo, 1 y: no changes in total BA	Albaugh, 2015[15]
15	—	Fasting	1 y	LC-MS	No changes in total BA	Sachdev, 2016[16]
7	6	Fasting	1 wk, 3 mo, 1 y	GC-MS	1 wk, 3 mo: no changes in total BA 1 y: ↑ total BA	Steinert, 2013[17]
63	—	Fasting	15 mo	HPLC-MS/MS	15 mo: ↑ total BA	Werling, 2013[18]
13	—	Fasting	1 mo, 2 y	HPLC-MS/MS	1 mo: ↓ total BA 2 y: ↑ total BA and ↑ 12α-hydroxylated/non12α-hydroxylated BA ratio	Dutia, 2015[19]
13 T2D 12 non-diabetic	—	Fasting	1 wk, 3 mo, 1 y	HPLC-MS/MS	1 wk: ↓ total BA in non-diabetic patients 3 mo, 1y: ↑ total BA in T2D and non-diabetic patients	Jørgensen, 2015[20]
15	—	Meal test	1 y	LC-MS	1 y: ↑ total BA due to conjugated BA	Sachdev, 2016[16]
7	6	Meal test	1 wk, 3 mo, 1 y	GC-MS	1 wk, 3 mo: no changes in total BA 1 y: ↑ total BA	Steinert, 2013[17]
5	8	Meal test	1 wk, 4 wk, 40 wk	HPLC-MS	4 wk, 40 wk: ↑total BA	Ahmad, 2013[21]
13 T2D 12 non-diabetic	—	Meal test	1 wk, 3 mo, 1 y	HPLC-MS/MS	1 wk: ↑ total BA in non-diabetic patients 3 mo, 1 y: ↑ total BA in T2D and non-diabetic patients	Jørgensen, 2015[20]
63	—	Ogtt	15 mo	HPLC-MS/MS	15 mo: ↑ total BAs due to glycoconjugated BA	Werling, 2013[18]
13	—	Ogtt	1 mo, 2 y	HPLC-MS/MS	1 mo: unchanged total BA 2 y: ↑ total BA and ↑ 12α-hydroxylated to non-12α-hydroxylated BA ratio	Dutia, 2015[19]
8	—	Post-prandial	After, 20% of weight loss	LC-MS	↑ total BA	Kohli, 2013[14]
16 GBMIL 14 PBMIL	8	Postprandial	1 y	Enzymatic assay	1y: ↑ total BA in GBMIL and PBMIL compared with lean controls No changes between GBMIL and PBMIL	Dirksen 2013[22]
VSG						
7	6	Fasting Meal test	1 wk, 3 mo, 1 y	GC-MS	Fasting 1 wk, 3 mo: no changes; 1 y: ↑total BA Postprandial: no changes in total BA	Steinert 2013[17]
17	—	Fasting	6 mo, 1 y, 2 y	GC-MS	6 mo, 1 y, 2 y: no changes in total BA	Haluzíková, 2013[23]
Bilio-pancreatic diversion						
15	10 BMI-matched nonsurgical	Fasting	7 wk, 53 wk	LC-MS/MS	7 wk: ↑ total BA mainly due to free BA 53 wk: ↑ total BA, but lower than 7 wk	Ferrannini, 2015[24]
LAGB						
6	—	Fasting	4 d, 42 d	LC-MS/MS	No changes in total BA	Pournaras, 2012[11]
10	—	Fasting + postprandial	After 20% of weight loss	LC-MS	Fasting: ↓ total BA Postprandial: no changes in total BA	Kohli, 2013[14]

NOTE. References are found in Supplementary References 1.
BMI, body mass index; GBMIL, patients with good body mass index loss after RYGB; GC-MS, gas chromatography–mass spectrometry; IR, insulin resistance; LAGB, laparoscopic adjustable gastric banding; LC-MS, liquid chromatography–mass spectrometry; LC-MS/MS, chromatography–tandem mass spectrometry; OGTT, oral glucose tolerance test; NA, not applicable; PBMIL, patients with poor body mass index loss after RYGB; T2D, type 2 diabetes; T2D-NR, type 2 diabetes without remission; T2D-R, type 2 diabetes with remission; TG, triglycerides; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometer.

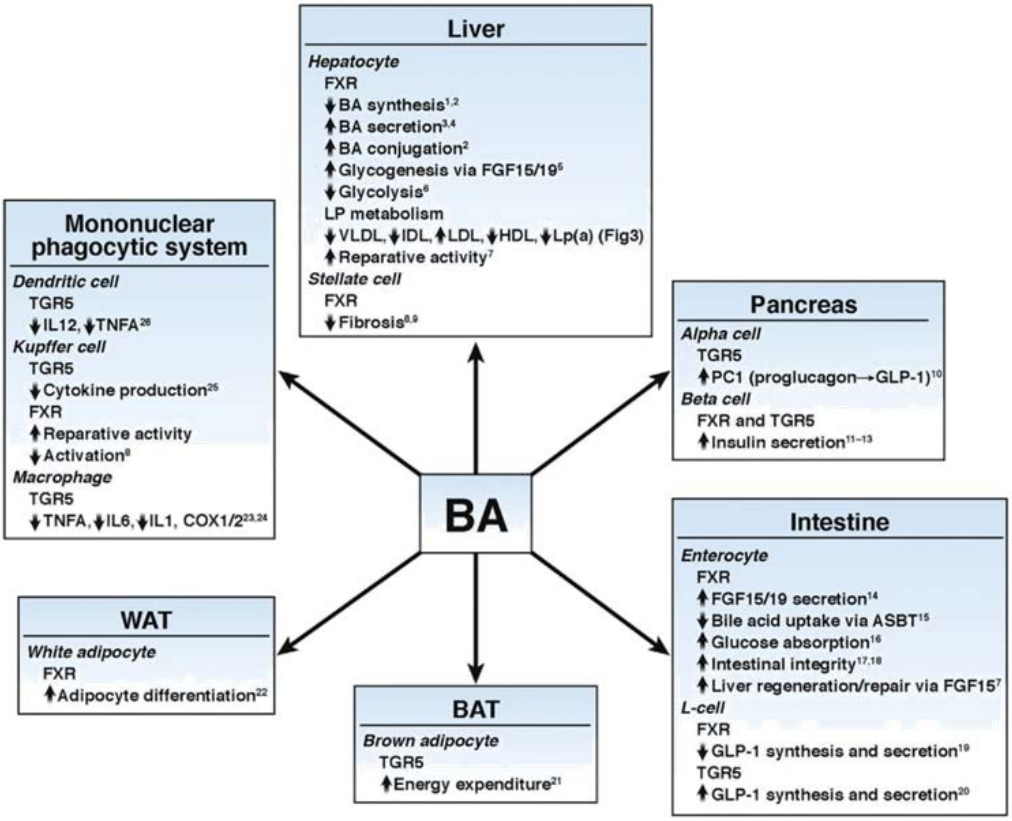


Figure 1. Role of BAs in the control of metabolic and immune homeostasis via activation of their receptors FXR and TGR5. See text and Supplementary References 2 for details. COX, cyclooxygenase; IDL, intermediary density lipoprotein; IL, interleukin; Lp, lipoprotein; Lp(a), lipoprotein (a); VLDL, very-low-density lipoprotein.

TGR5 (encoded by the *GPBAR1* gene) is expressed in enteroendocrine L cells,[68,69] brown adipose tissue (BAT), white adipose tissue, skeletal muscle, gallbladder, non-parenchymal liver cells, and the brain.[1] BAs activate TGR5 with different potencies (LCA>DCA>CDCA>CA). TGR5 activation induces adenylate-cyclase to produce cyclic adenosine monophosphate, which in turn activates protein kinase A to exert immediate cytosolic effects, or activate the transcription factor cyclic adenosine monophosphate–responsive element binding protein to modulate gene expression.[1]

Conjugated BAs activate S1PR2 in hepatocytes, which activates, via the extracellular signal–regulated kinase 1/2 and Akt signaling pathways, nuclear sphingosine kinase-2. This enzyme synthesizes S1P from sphingosine, hence increasing S1P levels in the nucleus. Nuclear S1P inhibits specific histone deacetylases, thus increasing histone acetylation and inducing enzymes involved in lipid and sterol metabolism. By modulating expression of nuclear receptors (including FXR) and proteins involved in lipid and glucose metabolism (low-density lipoprotein receptor [LDL-R], sterol responsive element binding protein 1c and fatty acid synthase), S1PR2/sphingosine kinase-2/S1P signaling lowers hepatic lipid content. Indeed, S1PR2-deficient mice develop hepatic steatosis upon HFD, whereas S1PR2 overexpression prevents hepatic steatosis.[56,70]

Energy Metabolism

Distinct AT depots differentially express BA receptors, FXR and TGR5, in white[71,72] and brown[73,74] adipocytes, respectively. In addition, both receptors are also expressed in certain immune-inflammatory cells in AT, which can contribute to their anti-inflammatory and insulin-sensitizing effects. The gut microbiota promote diet-induced obesity in a FXR-dependent manner in mice.[75] In AT, FXR regulates adipocyte differentiation and functions by promoting peroxisome proliferator-activated receptor−γ activity and interfering with the Wnt/β-catenin pathway.[72,76] In BAT, TGR5 stimulates energy expenditure[73] by inducing expression of iodothyronine-deiodinase type 2, thus converting inactive thyroxine into 3,5,3′-triiodothyronine, which activates the thyroid hormone receptor to uncouple mitochondrial function and to increase thermogenesis, and peroxisome proliferator-activated receptor−γ coactivator-1α, a regulator of mitochondrial biogenesis.[73]

FXR and TGR5 may be involved in the metabolic improvement induced by VSG. The impact of VSG on body weight and glucose tolerance appears reduced in FXR-deficient mice.[48] TGR5 may mediate the effect of surgery on metabolism by enhancing production of the incretin GLP-1,[50,51] and on body weight via TGR5 activation in BAT.[50] Upon VSG in mice, TGR5 deficiency reduces the 12αhydroxylated to non-12αhydroxylated BA ratio and BA pool hydrophobicity potentially by decreasing CYP8B1.[51] BAs also modulate energy expenditure by inducing intestinal expression and secretion of FGF15/19. Administration of FGF19 to HFD-fed mice enhances the metabolic rate and insulin sensitivity and decreases body weight.[77] The increase of FGF19 associated with T2D remission after RYGB may thus also contribute to the metabolic improvements after surgery

in humans.[43] In addition, pharmacologic activation of intestinal FXR with fexaramine, a synthetic FXR agonist with intestine-restricted bioavailability, induces FGF15 in mice, reducing diet-induced weight gain, systemic inflammation, and hepatic glucose production.[78] FGF15 induces changes in BA pool composition and increases TGR5 ligand production, leading to both BAT activation, enhanced thermogenesis, and white adipose tissue browning.[78] Oral administration of CDCA to healthy humans increased BAT activity and energy expenditure likely via TGR5 activation in brown adipocytes,[74] making TGR5 a target to treat obesity.

Lipid Metabolism

FXR regulates lipid and lipoprotein metabolism by acting on hepatic lipogenesis and lipoprotein secretion, intravascular remodeling, and plasma clearance, as well as intestinal cholesterol absorption (Figure 2).

FXR reduces TG-rich lipoproteins by several mechanisms. FXR reduces lipogenesis by repressing hepatic sterol responsive element binding protein 1c expression in SHP-dependent[79] and FGF15/19-dependent manners.[80] FXR also represses microsomal triglyceride transfer protein and apolipoprotein (apo) B gene expression,[81] thus reducing very-low-density lipoprotein secretion.[79] FXR enhances lipoprotein lipase activity by increasing expression of apoCII[82]—a lipoprotein lipase activator—while reducing apoCIII[83]—a lipoprotein lipase inhibitor—stimulating intravascular lipolysis of TG-rich lipoproteins. Furthermore, FXR increases very-low-density lipoprotein receptor expression.[84] The inhibition of BA synthesis from cholesterol upon FXR activation results in increased hepatic cholesterol concentrations, therefore, LDL-R activity decreases and plasma LDL-C increases.

Levels of lipoprotein (a), an atherogenic lipoprotein, decrease due to reduced hepatic apo(a) gene expression

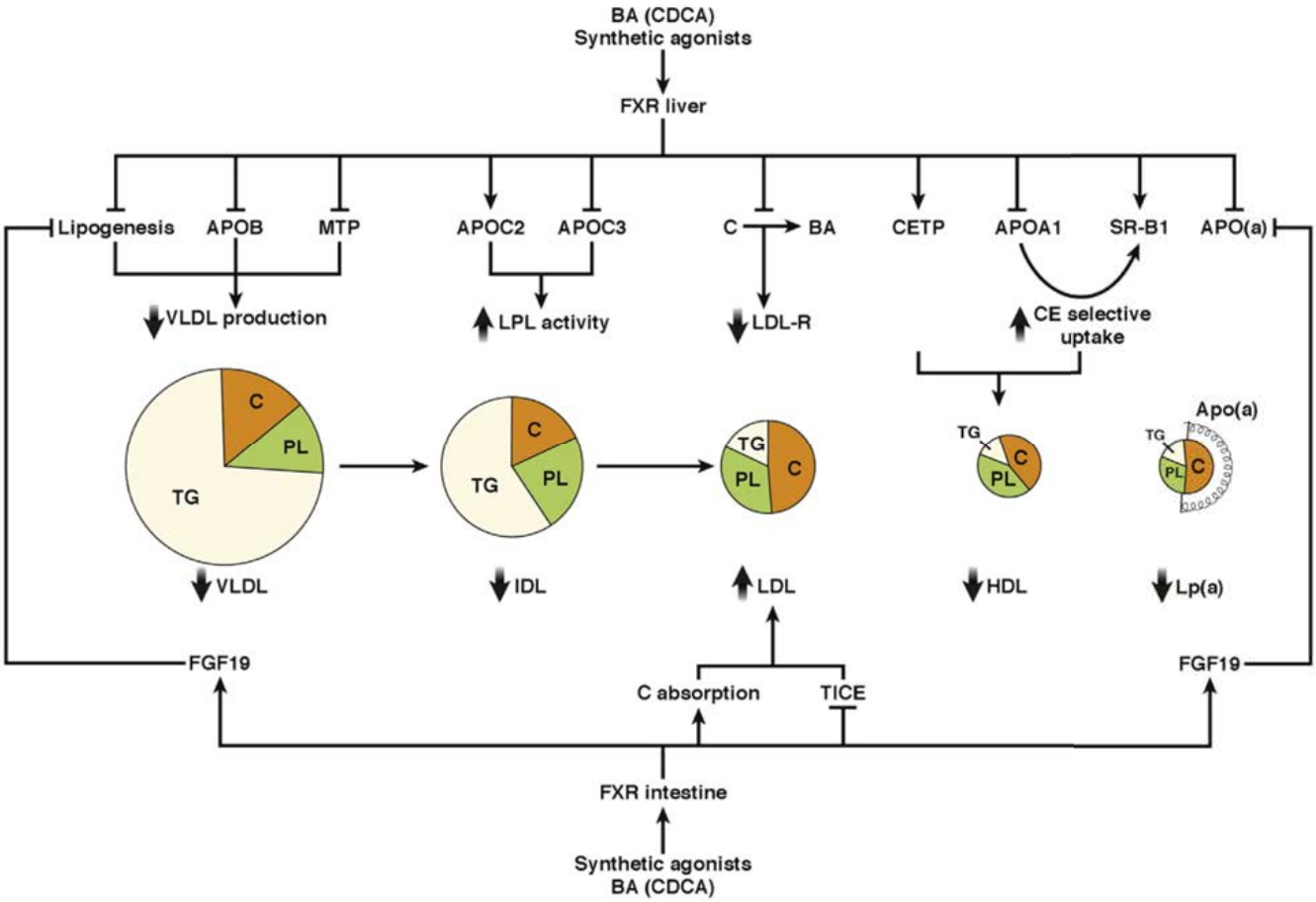


Figure 2. BAs control lipoprotein metabolism through hepatic and intestinal FXR activation. FXR and FGF15/19 decrease hepatic lipogenesis. Very-low-density lipoprotein (VLDL) secretion, apoB, and microsomal triglyceride transfer protein synthesis are inhibited by FXR. Increased ApoCII and diminished ApoCIII enhance lipoprotein lipase–mediated intravascular lipolysis, and thus promote conversion of VLDL to intermediary density lipoprotein (IDL) and LDL. FXR inhibits BA synthesis from cholesterol. As a consequence, hepatic cholesterol concentrations increase and LDL-R activity decreases. Scavenger Receptor–B1 induction enhances selective cholesteryl ester (CE) uptake, and associated with increased cholesteryl ester transfer protein (CETP) and decreased ApoA1 lowers HDL cholesterol. In rodents, FXR enhances transintestinal cholesterol excretion by changing the hydrophobicity index. This effect is thus likely to occur in the opposite direction in humans (shown in figure). Inhibition of hepatic synthesis of apo(a) by hepatic FXR and intestinal FGF19 decreases lipoprotein (a) (Lp[a]). C, cholesterol; MTP, microsomal transfer protein; SR-B1, scavenger receptor type 1; TICE, transintestinal cholesterol excretion.

through concerned actions of hepatic FXR and FGF19.[85] Interestingly, cholestatic patients present low lipoprotein (a) levels, which increase after removal of the biliary obstruction.[85] FXR also acts on reverse cholesterol transport and high-density lipoprotein (HDL) metabolism by decreasing apoA1,[86] increasing scavenger receptor−B1[87] and cholesteryl ester transfer protein[88] expression, thus increasing HDL cholesterol clearance and lowering plasma HDL cholesterol levels in vivo. FXR activation induces a combination of pro- and anti-atherogenic lipoprotein profile changes. Further attention is required to evaluate the impact of FXR activation on cardiovascular risk.

In humans, CDCA and the semi-synthetic FXR agonist obeticholic acid (OCA) (INT-747 or 6-ethyl-CDCA; Intercept Pharmaceuticals, New York, NY) increase low-density lipoprotein (LDL) cholesterol[89,90] likely via FXR-dependent CYP7A1 inhibition, thus decreasing hepatic cholesterol conversion to BA, increasing hepatic cholesterol content, and inhibiting LDL-R activity (Figure 2). In humans, CDCA treatment decreases hepatic messenger RNA levels of LDL-R and hydroxymethylglutaryl-Co-enzymeA reductase—the rate-limiting enzyme of cholesterol synthesis.[91] By contrast, in the mouse, OCA inhibits cholesterol absorption and increases reverse cholesterol transport, via hepatic, but not intestinal FXR.[92]

The hydrophobicity index and conjugation state of the BAs pool is an important determinant of intestinal dietary cholesterol and lipid absorption,[92] with a more hydrophobic BA pool being most efficient for intestinal cholesterol absorption. In line, CYP7A1$^{-/-}$ mice are protected from high-fat, high-cholesterol−induced metabolic disorders likely due to up-regulation of the alternative BA synthesis pathway and hence a more hydrophilic BA pool.[93] Importantly, studies in mice explaining the increase in reverse cholesterol transport[92] and transintestinal cholesterol excretion upon FXR activation[94] by modifications in the hydrophobicity index of the BA pool are unlikely to translate to human pathophysiology, as hydrophobic CDCA will predominate in humans vs hydrophilic MCA in rodents.

BA sequestrants (BAS) are anionic exchange resins that trap BAs in the intestinal lumen increasing fecal BA output, hence decreasing intestinal FXR activity. As a consequence, lower amounts of BA and FGF15/19 reach the liver, deactivating hepatic FXR and inducing CYP7A1-mediated conversion of cholesterol to BA, increasing LDL-R expression, and thus lowering LDL cholesterol. Concomitantly, the inhibition of lipogenesis by FXR is attenuated. In agreement, BAS decrease LDL cholesterol and increase HDL cholesterol, while also increasing plasma triglycerides and hepatic lipid accumulation. The LRC-CPPT (Lipid Research Clinics Coronary Primary Prevention Trial) showed that the BAS cholestyramine significantly reduced coronary heart disease death in hypercholesterolemic patients.[95]

Glucose Metabolism

BAs regulate glucose homeostasis by acting directly on FXR and TGR5 in the intestine, liver, and pancreas, and indirectly by promoting FXR-dependent induction of intestinal FGF15/19 (Figure 3). In humans, FGF19 is also produced by the gallbladder[96] and, in mice, FGF15 is produced in the hypothalamus, where it signals to lower glucagon production.[97]

In the intestine, FXR modulates the kinetics of glucose absorption, which is delayed in FXR-deficient mice.[98] FXR reduces postprandial glucose utilization by inhibiting hepatic glycolysis and lipogenesis,[79,99,100] whereas FGF15/19 increases glycogenesis.[101] Thus, in the postprandial state, FXR lowers splanchnic glucose utilization (Figure 3).

In enteroendocrine L cells, BAs regulate the production and secretion of GLP-1 via opposite effects on TGR5 and FXR. Activation of TGR5, the expression of which parallels L-cell density along the gastrointestinal tract with maximal expression in the colon, induces preproglucagon gene expression and GLP-1 secretion.[69,102] Because TGR5 is rather expressed at the basolateral than at the apical L-cell membrane, absorption and local release of its agonists appears to be a prerequisite for its activation.[103] By contrast, FXR activation represses preproglucagon gene expression and GLP-1 secretion in the ileum by inhibiting glycolysis and carbohydrate responsive element binding protein activity in L cells.[104] Because TGR5-mediated cytosolic signaling is rapid, whereas transcriptional regulation by FXR is slow, and because FXR expression is more proximal than TGR5 in the intestine, these receptors likely exert opposing effects on GLP-1 production, which are, however, separated in time and space.

FXR and TGR5 are both expressed in pancreatic β-cells, where they positively regulate synthesis and glucose-induced secretion of insulin.[105,106] In addition, TGR5 activation in pancreatic α-cells induces pro-convertase-1 expression, shifting glucagon production to GLP-1, hence increasing β-cell mass and function in a paracrine manner.[107] Perturbations in hepatic glucose metabolism alter BA synthesis, impacting on FXR-regulated β-cell glucose-stimulated insulin secretion,[108] identifying a liver−pancreas BA signaling connection.

BAS treatment also improves glucose homeostasis, and colesevelam is a US Food and Drug Administration−approved oral antidiabetic drug. Chronic treatment with BAS deactivates FXR in intestinal L cells, enterocytes, and hepatocytes, increasing GLP-1 synthesis and secretion, decreasing intestinal glucose absorption, enhancing hepatic glycolysis and lipogenesis, thus promoting splanchnic glucose utilization[109] (Figure 3). In accordance, pharmacologic inactivation or genetic deficiency of FXR in the intestine improves energy and glucose homeostasis[110,111] and NASH due to decreased intestinal ceramide production.[25] In addition, the BAS sevelamer decreases steatosis, lobular inflammation, and endotoxemia in Western diet−fed mice.[112] Together, most observations suggest that inactivation of intestinal FXR results in an improved metabolic profile, although treatment with the intestinal-selective FXR agonist fexaramine was also reported to improve metabolism.[78]

Inhibition or deficiency of CYP8B1 improves glucose homeostasis by increasing GLP-1 in mice. The decreased CA/MCA ratio upon CYP8B1-deficiency mice impairs micellar absorption of fats and nutrients, increasing luminal free fatty

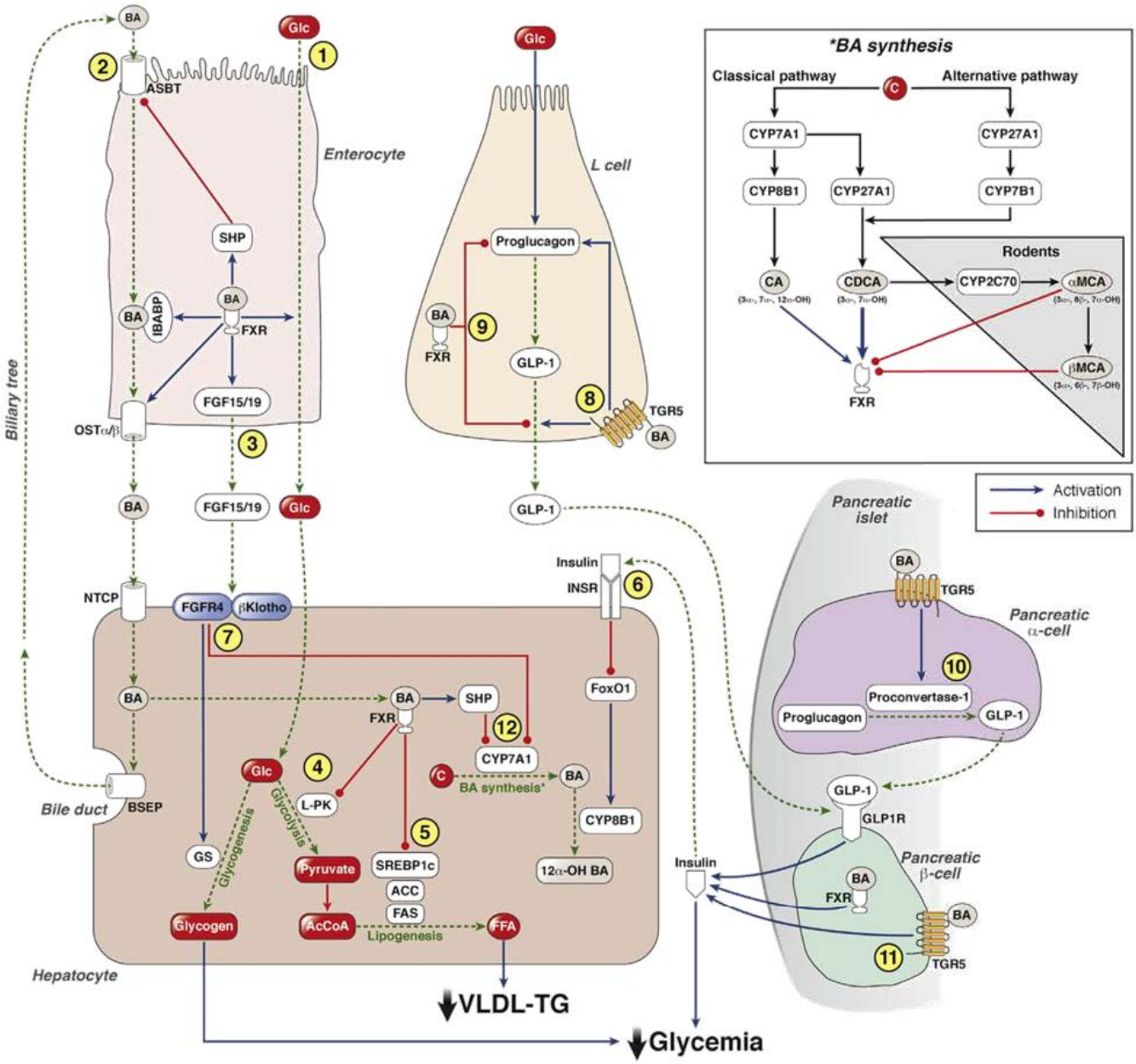


Figure 3. Modulation of glucose homeostasis by BAs. FXR activation in the enterocyte modulates the absorption of glucose (*1*) and BA (*2*), and induces FGF15/19 secretion (*3*). In the liver, FXR activation decreases glycolysis and lipogenesis via inhibition of carbohydrate responsive element binding protein (ChREBP) (*4*) and sterol responsive element binding protein 1 (SREBP1c) (*5*), respectively, hence decreasing very-low-density lipoprotein (VLDL)/triglyceride (TG) production. Insulin via the insulin receptor (INSR) (*6*) modulates the proportion of 12α-OH BA by repressing FoxO1, a CYP8B1 activator. Intestinal FGF15/19 activates hepatic FGFR4/βKlotho (*7*), which decreases glycogen synthase (GS) phosphorylation by inhibiting glycogen synthase kinase 3 (GSK3) β, increasing glycogenesis and decreasing glycemia. In entero-endocrine L cells, BA increase via TGR5 (*8*) or decrease via FXR through ChREBP (*9*) proglucagon gene expression and GLP-1 secretion. TGR5 activation in pancreatic α-cells induces pro-convertase-1, shifting proglucagon processing from glucagon to GLP-1 (*10*). FXR and TGR5 activation in pancreatic β-cells promotes insulin secretion, lowering glycemia (*11*). BA synthesis in the hepatocyte is decreased by FGF15/19-FGFR4/βKlotho activation and FXR activation via SHP-LRH-1 (*12*). AcCoA, acetyl co-enzyme A; BSEP, bile salt export protein; L-PK, liver pyruvate kinase; OST α/β, organic solute transporters α and β; VLDL (very low density lipoprotein).

acids in the ileum and GLP-1 secretion.[113] Furthermore, MCA may inhibit intestinal FXR, which enhances GLP-1 production.[104] However, translation of these findings to humans is unlikely (see Lipid Metabolism section).

Initial studies suggested a role of FXR in the regulation of hepatic gluconeogenesis by decreasing the expression of the rate-limiting enzymes phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fructose-1,6-biphosphatase-1.[114,115] However, other studies reported that FXR activation induces phosphoenolpyruvate carboxykinase[116] and that phosphoenolpyruvate carboxy-kinase and glucose-6-phosphatase are lowered in FXR-deficient

mice.[99,115] FXR-deficient mice submitted to a fasting–refeeding schedule presented an accelerated response to high carbohydrate refeeding with induction of glycolytic and lipogenic genes and a pronounced repression of gluconeogenic genes, with concomitant hypoinsulinemia and hypoglycemia.[99] These studies all employed different conditions in which gluconeogenesis was evaluated (eg, in vivo, in vitro, fasting, refeeding, and HFD). Thus, the exact role of FXR in fasting-induced gluconeogenesis is still unclear and requires further studies. Similarly conflicting data exist in humans, because OCA treatment increases insulin-sensitivity measured using hyperinsulinemic–euglycemic clamps in T2D/NASH patients,[117] whereas homeostasis model assessment-estimated insulin resistance increased in NASH patients in the FLINT (Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment) trial.[90]

Immune Function

FXR and TGR5 are expressed in several immune cell types. TGR5 exerts anti-inflammatory activities, decreasing cytokine production in monocytes, macrophages,[118] Kupffer,[119] and human dendritic cells.[120] TGR5 activation reduces HFD-induced glucose intolerance, insulin resistance, and inflammation by inhibiting NLRP3 inflammasome activation via the TGR5-cyclic adenosine monophosphate–protein kinase A axis in mice.[121] Furthermore, TGR5 activation protects against lipopolysaccharide-induced inflammation,[122] atherosclerosis,[123] and experimental autoimmune encephalitis.[118]

Overexpression of CYP7A1 decreases hepatic inflammatory cell infiltration, pro-inflammatory cytokine production, and fibrosis in methionine/choline-deficient diet-fed mice by decreasing hepatic free cholesterol, oxidative stress in an FXR-dependent, but not TGR5-dependent, manner.[124] Intestinal FXR activation with OCA decreased the pro-inflammatory genes interleukin 1β, interleukin 6, and macrophage chemoattractant protein-1 in the colon, lowering trinitrobenzensulfonic acid or dextran sodium sulfate–induced inflammation in mouse colonic mucosa.[125,126] In addition, FXR-deficiency impairs intestinal barrier function, which can enhance hepatic lipopolysaccharide exposure and inflammation.[127]

Nonalcoholic Steatohepatitis and Nonalcoholic Fatty Liver Disease

NAFLD is a progressive liver disease, which initiates with hepatic steatosis and can progress to inflammation and hepatocyte ballooning (NASH), fibrosis, cirrhosis, and, finally, hepatocarcinoma. Besides contributing to liver-related mortality, NAFLD is also strongly associated with high cardiovascular disease risk. Promoter hypermethylation is inversely correlated to the expression of the CYP27A1, organic solute transporter α, BA-CoA synthase and OATP genes in NAFLD livers, which could lead to liver and systemic toxicity.[128] FXR activation may reduce NAFLD, as it reduces steatosis by inhibiting lipogenesis, decreases chemically induced hepatic inflammation and fibrosis in rats,[129] and maintains intestinal barrier integrity, thus protecting the liver from bacteria-derived inflammatory signals.[127] Because FXR expression is low in quiescent and activated stellate cells, its effects on fibrosis may be indirect.[130]

A recent large randomized placebo-controlled trial in patients with biopsy-diagnosed NASH showed that OCA treatment improves the histologic NAFLD activity score and fibrosis of the liver.[90] However, OCA treatment also reduces HDL cholesterol and increases LDL cholesterol and homeostasis model assessment–estimated insulin resistance. In line, OCA reduces the secretion of inflammatory and fibrotic factors, but increases apoB secretion in an in vitro reconstituted human liver model.[131] These effects on lipids, also observed in healthy individuals,[132] and glucose homeostasis are potentially restricting the clinical use of such semi-synthetic BA compounds and call for the design of selective BA receptor modulators devoid of such side effects.

Therapeutic Modulation of Bile Acid Metabolism

Given the role of BA signaling in the regulation of metainflammation, altering the BA pool and BA receptor activities can be valuable therapeutic options to treat metainflammatory disorders.[133,134]

Takeda G Protein–Coupled Receptor 5

Based on their ability to promote energy expenditure in BAT, GLP-1 secretion in enteroendocrine L cells, and anti-inflammatory properties, TGR5 agonists may be useful in the treatment of obesity and T2D. Semi-synthetic BA-derivatives INT-777[69] and nonsteroidal TGR5 agonists[135,136] improve glucose homeostasis in preclinical models. Unfortunately, systemic exposure to TGR5 agonists increases gallbladder volume[135,136] and promotes pruritus.[137] The ideal molecule would be a topical intestinal agonist with limited local absorption to reach the basolateral membrane of L cells, but without systemic exposure to avoid cholecystomegaly. This would thus preserve GLP-1 secretion, but unfortunately also preclude BAT activation.

Farnesoid X Receptor

Due to their inhibitory effects on lipogenesis and hepatic fibrosis, FXR agonists, such as OCA, are in development for NASH treatment. Other FXR nonsteroidal agonists are GW4064, Px-104 (Phenex Pharmaceutical AG, Ludwigshafen, Germany), WAY-362450 (Wyeth, Collegeville, PA), EDP-305, and EP-024297 (Enanta Pharmaceuticals, Watertown, MA), fexaramine, LJN452 (Novartis Pharmaceuticals, Basel, Switzerland) and GS-9674 (Phenex-Gilead, Foster City, CA), some of which are in clinical development. As discussed, intestinal antagonism of FXR appears to improve metabolic control, whereas hepatic FXR activation may improve hepatic fibrosis. FXR inactivation triggered with BAS has beneficial effects on diabetes and hypercholesterolemia, but increases plasma triglycerides and hepatic steatosis, which precludes their use in patients with hypertriglyceridemia.

Fibroblast Growth Factor 19

Due its metabolic effects,[101] FGF19 is a candidate to treat NASH and obesity-related disorders, but it may also increase the risk of cancer. The recombinant FGF19 mimetic peptide NGM282, currently clinically tested in metabolic liver disease, does not induce cell proliferation. Surprisingly, antisense FGFR4[138] (ISIS) has also been shown to induce fat burning and energy expenditure in mice. However, potential species differences between FGF19 and FGF15 signal a call for caution when interpreting FGF19 studies in rodents.

Cytochrome P450 12α-Hydroxylase B1

Based on the beneficial metabolic phenotype of CYP8B1-deficiency,[113] inhibition of CYP8B1 by decreasing the 12α-hydroxylated to non-12α-hydroxylated BA ratio has potential therapeutic implications. However, CYP8B1 inhibition results in opposite changes in physicochemical properties of the BA pool in humans vs rodents. In addition, it induces the FXR antagonist MCA in rodents, whereas the FXR agonist CDCA is predominant in humans, cautioning the extrapolation of rodent studies to humans.

Apical Sodium–Dependent Bile Acid Transporter Inhibitors

The impairment of intestinal BA reuptake by inhibiting ASBT improves features of NAFLD and insulin sensitivity in HFD-fed mice.[139] Similar to BAS, ASBT inhibitors (ASBT-I) reduce intestinal BA absorption, decreasing hepatic BA supply, and thus FXR activation, resulting in de-repression of CYP7A1 and enhanced conversion of cholesterol to BA. In addition, shifting BA to the distal part of intestine may sustain TGR5-induced GLP-1 secretion as, in contrast to resins, free BA absorption is not impaired upon ASBT-I treatment. Further studies on ASBT-I should be performed to assess the efficacy and safety of this approach in humans or relevant models.[139]

NorUrsodesoxycholic Acid

Finally, the UDCA derivative, norUDCA, improves hepatic steatosis in mice.[140,141] NorUDCA does not activate FXR/TGR5, but may exert FXR antagonistic effects inhibiting intestinal FGF19, enhancing BA synthesis and decreasing plasma cholesterol, but possibly increasing lipogenesis.[142]

Conclusions and Perspectives

The high BA concentrations in enterohepatic tissues regulate metabolism in an inter-organ dialogue between the intestine, its microbiota, and the liver. In turn, the liver secretes BAs, hence modulating intestinal metabolism. In peripheral organs, BAs also contribute to metabolic homeostasis, even though their concentrations are lower in the systemic circulation. Thus, impaired BA metabolism likely contributes to the pathophysiology of metabolic diseases (obesity, T2D, and NASH). The bidirectional effects between intestinal microbiota and BAs suggest that dysbiosis and associated alterations in BA homeostasis may interactively contribute to the metabolic dysregulations seen in T2D, obesity, and NAFLD, as well as their remission upon bariatric surgery.

An important question that remains is whether FXR should be activated or inhibited in the intestine and/or liver to reverse metabolic abnormalities and NAFLD. Intestinal-specific FXR deactivation, either by natural/chemical antagonism or BA sequestration, prevents obesity, T2D, and NAFLD/NASH in rodents (Figure 4). Contradictorily, fexaramine, reportedly exerting exclusively intestinal FXR-specific actions, confers similar beneficial effects. A systematic comparison of the activity of FXR agonists and

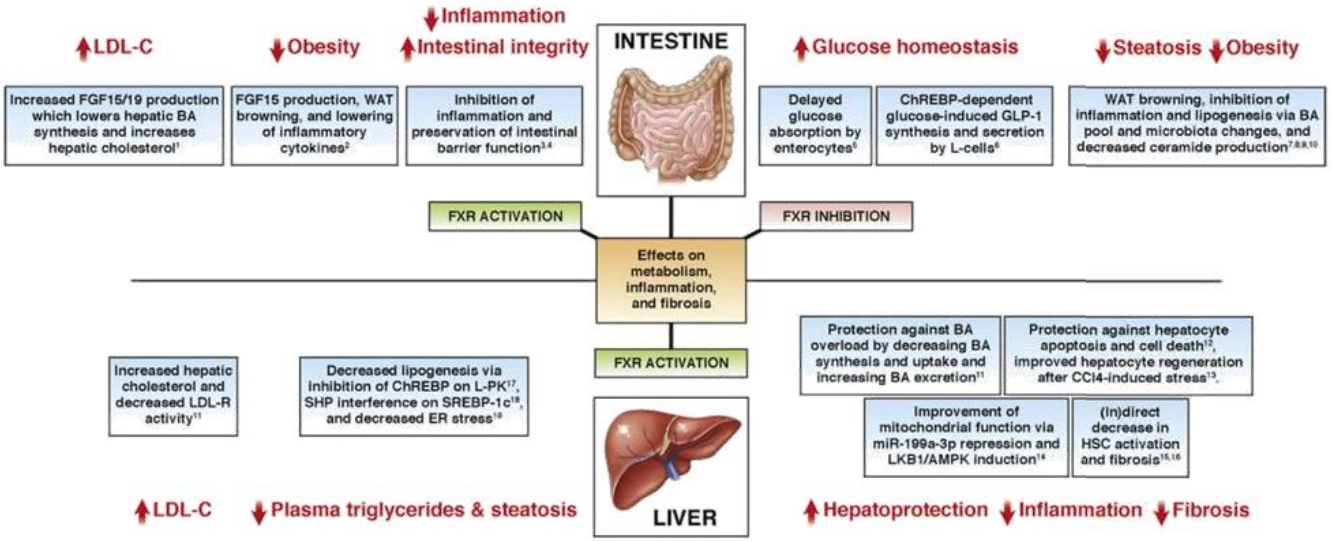


Figure 4. Differential effects of FXR inhibition vs activation in liver and intestine on metabolism, inflammation, and fibrosis. See text and Supplementary References 3 for details. AMPK, adenosine monophosphate–activated protein kinase; ChREBP, carbohydrate responsive element binding protein; ER, endoplasmic reticulum; HSC, hepatic stellate cell; LKB1, liver kinase B1; L-PK, liver pyruvate kinase; miR, micro RNA; SREBP-1c, sterol responsive element binding protein 1; WAT, white adipose tissue.

antagonists on the intestinal FXR signaling pathway could provide clues about their mechanism of action, as they clearly differ in their ability to activate subsets of FXR target genes and thus act as selective BA receptor modulators.[143] In addition, exploring their ability to affect the gut microbiota, and vice versa, may also identify reasons for their different biological activities.

In the liver, FXR exerts hepatoprotective activities, improving steatosis, inflammation, and fibrosis (Figure 4). An important unresolved question is whether, through treating with metabolically stable, highly active synthetic BA analogues, interfering permanently with the FXR signaling pathway, whose functions differ in fasting and fed conditions and BA synthesis, which is submitted to circadian fluctuations, might, in the long term, induce unwanted effects.

Based on existing preclinical data and clinical use of BAS, it appears that an orally administered inhibitor of intestinal FXR would be preferential to improve glucose and cholesterol metabolism, whereas a liver-targeted FXR agonist would improve liver function and fibrosis.

Caution should also be taken when translating data from preclinical murine models to humans because BA pool modulation affects the hydrophobicity index differently in mice vs humans, and mice produce the FXR antagonist TβMCA, which is absent in humans. The recent identification of the enzyme responsible for MCA synthesis in mice will allow the development of humanized-BA pool murine models. Finally, most beneficial effects of the FGF15/19 pathway have been observed by treating mice with supra-pharmacologic concentrations of human recombinant FGF19, which could, due to species-specific differences, result in erroneous extrapolations. Further studies in this exciting field will determine whether pharmacologic modulation of the novel BA metabolism targets (TGR5, FXR, ASBT, FGF19, and CYP8B1) will convey beneficial clinical effects in humans.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2017.01.055.

References

1. Lefebvre P, Cariou B, Lien F, et al. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147–191.
2. Vlahcevic ZR, Stravitz RT, Heuman DM, et al. Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat. Gastroenterology 1997;113:1949–1957.
3. Duane WC, Javitt NB. 27-hydroxycholesterol: production rates in normal human subjects. J Lipid Res 1999; 40:1194–1199.
4. Takahashi S, Fukami T, Masuo Y, et al. Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. J Lipid Res 2016;57:2130–2137.
5. Duez H, Veen JN van der, Duhem C, et al. Regulation of bile acid synthesis by the nuclear receptor Rev-erbalpha. Gastroenterology 2008;135:689–698.
6. Han S, Han SS, Zhang R, et al. Circadian control of bile acid synthesis by a KLF15-Fgf15 axis. Nat Commun 2015;6:7231.
7. Goodwin B, Jones SA, Price RR, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 2000; 6:517–526.
8. Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab 2005;2:217–225.
9. Ridlon JM, Harris SC, Bhowmik S, et al. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes 2016;7:22–39.
10. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006;47:241–259.
11. Steiner C, Holleboom AG, Karuna R, et al. Lipoprotein distribution and serum concentrations of 7α-hydroxy-4-cholesten-3-one and bile acids: effects of monogenic disturbances in high-density lipoprotein metabolism. Clin Sci 2012;122:385–400.
12. Devlin AS, Fischbach MA. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat Chem Biol 2015;11:685–690.
13. Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature 2012;487:104–108.
14. Joyce SA, MacSharry J, Casey PG, et al. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. Proc Natl Acad Sci U S A 2014;111:7421–7426.
15. Swann JR, Want EJ, Geier FM, et al. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci U S A 2011;108-(Suppl 1):4523–4530.
16. Sayin SI, Wahlström A, Felin J, et al. gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17:225–235.
17. Degirolamo C, Rainaldi S, Bovenga F, et al. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. Cell Rep 2014;7:12–18.
18. Wahlström A, Kovatcheva-Datchary P, Ståhlman M, et al. Induction of FXR signaling in germ-free mice colonized with a human microbiota. J Lipid Res 2017;58:412–419.
19. Boursier J, Diehl AM. Implication of gut microbiota in nonalcoholic fatty liver disease. PLoS Pathog 2015; 11:e1004559.
20. Haeusler RA, Astiarraga B, Camastra S, et al. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. Diabetes 2013; 62:4184–4191.
21. Lake AD, Novak P, Shipkova P, et al. Decreased hepatotoxic bile acid composition and altered synthesis in

progressive human nonalcoholic fatty liver disease. Toxicol Appl Pharmacol 2013;268:132–140.
22. Prinz P, Hofmann T, Ahnis A, et al. Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients. Front Neurosci 2015;9:199.
23. Mouzaki M, Wang AY, Bandsma R, et al. Bile acids and dysbiosis in non-alcoholic fatty liver disease. PLoS One 2016;11:e0151829.
24. Vrieze A, Out C, Fuentes S, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol 2014;60:824–831.
25. Jiang C, Xie C, Li F, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest 2015;125:386–402.
26. Stadlbauer V, Leber B, Lemesch S, et al. *Lactobacillus casei* Shirota supplementation does not restore gut microbiota composition and gut barrier in metabolic syndrome: a randomized pilot study. PLoS One 2015;10:e0141399.
27. Reijnders D, Goossens GH, Hermes GDA, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. Cell Metab 2016;24:63–74.
28. Lackey DE, Olefsky JM. Regulation of metabolism by the innate immune system. Nat Rev Endocrinol 2016;12:15–28.
29. Li T, Jahan A, Chiang JYL. Bile acids and cytokines inhibit the human cholesterol 7α-hydroxylase gene via the JNK/c-Jun pathway. Hepatology 2006;43:1202–1210.
30. Ferslew BC, Xie G, Johnston CK, et al. Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. Dig Dis Sci 2015;60:3318–3328.
31. Aranha MM, Cortez-Pinto H, Costa A, et al. Bile acid levels are increased in the liver of patients with steatohepatitis. Eur J Gastroenterol Hepatol 2008;20:519–525.
32. Spinelli V, Lalloyer F, Baud G, et al. Influence of Roux-en-Y gastric bypass on plasma bile acid profiles: a comparative study between rats, pigs and humans. Int J Obes 2016;40:1260–1267.
33. Jansen PLM, Werven J van, Aarts E, et al. Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery. Dig Dis Basel Switz 2011;29:48–51.
34. Pournaras DJ, Glicksman C, Vincent RP, et al. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. Endocrinology 2012;153:3613–3619.
35. Dutia R, Embrey M, O'Brien CS, et al. Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. Int J Obes 2015;39:806–813.
36. Simonen M, Dali-Youcef N, Kaminska D, et al. Conjugated bile acids associate with altered rates of glucose and lipid oxidation after Roux-en-Y gastric bypass. Obes Surg 2012;22:1473–1480.
37. Werling M, Vincent RP, Cross GF, et al. Enhanced fasting and post-prandial plasma bile acid responses after Roux-en-Y gastric bypass surgery. Scand J Gastroenterol 2013;48:1257–1264.
38. Steinert RE, Peterli R, Keller S, et al. Bile acids and gut peptide secretion after bariatric surgery: a 1-year prospective randomized pilot trial. Obesity 2013;21:E660–E668.
39. Kohli R, Bradley D, Setchell KD, et al. Weight loss induced by Roux-en-Y gastric bypass but not laparoscopic adjustable gastric banding increases circulating bile acids. J Clin Endocrinol Metab 2013;98:E708–E712.
40. Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. Int J Obes 2013;37:1553–1559.
41. Sachdev S, Wang Q, Billington C, et al. FGF 19 and bile acids increase following roux-en-Y gastric bypass but not after medical management in patients with type 2 diabetes. Obes Surg 2016;26:957–965.
42. Dirksen C, Jørgensen NB, Bojsen-Møller KN, et al. Gut hormones, early dumping and resting energy expenditure in patients with good and poor weight loss response after Roux-en-Y gastric bypass. Int J Obes 2013;37:1452–1459.
43. Gerhard GS, Styer AM, Wood GC, et al. A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. Diabetes Care 2013;36:1859–1864.
44. Haluzíková D, Lacinová Z, Kaválková P, et al. Laparoscopic sleeve gastrectomy differentially affects serum concentrations of FGF-19 and FGF-21 in morbidly obese subjects. Obesity 2013;21:1335–1342.
45. Goncalves D, Barataud A, De Vadder F, et al. Bile routing modification reproduces key features of gastric bypass in rat. Ann Surg 2015;262:1006–1015.
46. Bhutta HY, Rajpal N, White W, et al. Effect of Roux-en-Y gastric bypass surgery on bile acid metabolism in normal and obese diabetic rats. PLoS One 2015;10:e0122273.
47. Liou AP, Paziuk M, Luevano J-M, et al. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 2013;5:178ra41.
48. Ryan KK, Tremaroli V, Clemmensen C, et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 2014;509:183–188.
49. Patti M-E, Houten SM, Bianco AC, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity 2009;17:1671–1677.
50. Ding L, Sousa KM, Jin L, et al. Vertical sleeve gastrectomy activates GPBAR-1/TGR5 to sustain weight loss, improve fatty liver, and remit insulin resistance in mice. Hepatology 2016;64:760–773.
51. McGavigan AK, Garibay D, Henseler ZM, et al. TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. Gut 2017;66:226–234.
52. Wang H, Chen J, Hollister K, et al. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3:543–553.
53. Makishima M, Lu TT, Xie W, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002;296:1313–1316.

54. Staudinger JL, Goodwin B, Jones SA, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001; 98:3369–3374.
55. Kawamata Y, Fujii R, Hosoya M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003;278:9435–9440.
56. Nagahashi M, Yuza K, Hirose Y, et al. The roles of bile acids and sphingosine-1-phosphate signaling in the hepatobiliary diseases. J Lipid Res 2016;57:1636–1643.
57. Sheikh Abdul Kadir SH, Miragoli M, Abu-Hayyeh S, et al. Bile acid-induced arrhythmia is mediated by muscarinic M2 receptors in neonatal rat cardiomyocytes. PLoS One 2010;5:e9689.
58. Han S, Chiang JYL. Mechanism of vitamin D receptor inhibition of cholesterol 7alpha-hydroxylase gene transcription in human hepatocytes. Drug Metab Dispos Biol Fate Chem 2009;37:469–478.
59. Moore LB, Maglich JM, McKee DD, et al. Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. Mol Endocrinol 2002;16:977–986.
60. Lee JM, Wagner M, Xiao R, et al. Nutrient-sensing nuclear receptors coordinate autophagy. Nature 2014; 516:112–115.
61. Seok S, Fu T, Choi S-E, et al. Transcriptional regulation of autophagy by an FXR-CREB axis. Nature 2014; 516:108–111.
62. Berrabah W, Aumercier P, Gheeraert C, et al. Glucose sensing O-GlcNAcylation pathway regulates the nuclear bile acid receptor farnesoid X receptor (FXR). Hepatology 2014;59:2022–2033.
63. Kemper JK, Xiao Z, Ponugoti B, et al. FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. Cell Metab 2009;10:392–404.
64. Lien F, Berthier A, Bouchaert E, et al. Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. J Clin Invest 2014;124:1037–1051.
65. Gineste R, Sirvent A, Paumelle R, et al. Phosphorylation of farnesoid X receptor by protein kinase C promotes its transcriptional activity. Mol Endocrinol 2008;22: 2433–2447.
66. Hashiguchi T, Arakawa S, Takahashi S, et al. Phosphorylation of farnesoid X Receptor at serine 154 links ligand activation with degradation. Mol Endocrinol 2016; 30:1070–1080.
67. Kim D-H, Xiao Z, Kwon S, et al. A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. EMBO J 2015;34:184–199.
68. Reimann F, Habib AM, Tolhurst G, et al. Glucose sensing in L cells: a primary cell study. Cell Metab 2008;8: 532–539.
69. Thomas C, Gioiello A, Noriega L, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009;10:167–177.
70. Nagahashi M, Takabe K, Liu R, et al. Conjugated bile acid-activated S1P receptor 2 is a key regulator of sphingosine kinase 2 and hepatic gene expression. Hepatology 2015;61:1216–1226.
71. Cariou B, Harmelen K van, Duran-Sandoval D, et al. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. J Biol Chem 2006;281: 11039–11049.
72. Abdelkarim M, Caron S, Duhem C, et al. The farnesoid X receptor regulates adipocyte differentiation and function by promoting peroxisome proliferator-activated receptor-gamma and interfering with the Wnt/beta-catenin pathways. J Biol Chem 2010;285:36759–36767.
73. Watanabe M, Houten SM, Mataki C, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 2006;439:484–489.
74. Broeders EPM, Nascimento EBM, Havekes B, et al. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. Cell Metab 2015;22:418–426.
75. Parséus A, Sommer N, Sommer F, et al. Microbiota-induced obesity requires farnesoid X receptor. Gut 2017; 66:429–443.
76. Rizzo G, Disante M, Mencarelli A, et al. The farnesoid X receptor promotes adipocyte differentiation and regulates adipose cell function in vivo. Mol Pharmacol 2006; 70:1164–1173.
77. Fu L, John LM, Adams SH, et al. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. Endocrinology 2004;145: 2594–2603.
78. Fang S, Suh JM, Reilly SM, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med 2015;21:159–165.
79. Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004;113:1408–1418.
80. Bhatnagar S, Damron HA, Hillgartner FB. Fibroblast growth factor-19, a novel factor that inhibits hepatic fatty acid synthesis. J Biol Chem 2009;284:10023–10033.
81. Hirokane H, Nakahara M, Tachibana S, et al. Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4. J Biol Chem 2004;279:45685–45692.
82. Kast HR, Nguyen CM, Sinal CJ, et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 2001;15:1720–1728.
83. Claudel T, Inoue Y, Barbier O, et al. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. Gastroenterology 2003;125:544–555.
84. Sirvent A, Claudel T, Martin G, et al. The farnesoid X receptor induces very low density lipoprotein receptor gene expression. FEBS Lett 2004;566:173–177.
85. Chennamsetty I, Claudel T, Kostner KM, et al. FGF19 signaling cascade suppresses APOA gene expression. Arterioscler Thromb Vasc Biol 2012;32:1220–1227.
86. Claudel T, Sturm E, Duez H, et al. Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. J Clin Invest 2002;109:961–971.

87. Zhang Y, Yin L, Anderson J, et al. Identification of novel pathways that control farnesoid X receptor-mediated hypocholesterolemia. J Biol Chem 2010;285:3035–3043.
88. Gautier T, Haan W de, Grober J, et al. Farnesoid X receptor activation increases cholesteryl ester transfer protein expression in humans and transgenic mice. J Lipid Res 2013;54:2195–2205.
89. Wang Y, Jones PJH, Woollett LA, et al. Effects of chenodeoxycholic acid and deoxycholic acid on cholesterol absorption and metabolism in humans. Transl Res J Lab Clin Med 2006;148:37–45.
90. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet Lond Engl 2015;385:956–965.
91. Nilsson L-M, Abrahamsson A, Sahlin S, et al. Bile acids and lipoprotein metabolism: effects of cholestyramine and chenodeoxycholic acid on human hepatic mRNA expression. Biochem Biophys Res Commun 2007; 357:707–711.
92. Xu Y, Li F, Zalzala M, et al. Farnesoid X receptor activation increases reverse cholesterol transport by modulating bile acid composition and cholesterol absorption in mice. Hepatology 2016;64:1072–1085.
93. Ferrell JM, Boehme S, Li F, et al. Cholesterol 7α-hydroxylase-deficient mice are protected from high-fat/high-cholesterol diet-induced metabolic disorders. J Lipid Res 2016;57:1144–1154.
94. de Boer JF, Schonewille M, Boesjes M, et al. Intestinal farnesoid X receptor controls transintestinal cholesterol excretion in mice. Gastroenterology 2017;152: 1126–1138.e6.
95. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA 1984;251:351–364.
96. Zweers SJLB, Booij KAC, Komuta M, et al. The human gallbladder secretes fibroblast growth factor 19 into bile: towards defining the role of fibroblast growth factor 19 in the enterobiliary tract. Hepatology 2012;55:575–583.
97. Picard A, Soyer J, Berney X, et al. A genetic screen identifies hypothalamic Fgf15 as a regulator of glucagon secretion. Cell Rep 2016;17:1795–1806.
98. Dijk TH van, Grefhorst A, Oosterveer MH, et al. An increased flux through the glucose 6-phosphate pool in enterocytes delays glucose absorption in Fxr-/- mice. J Biol Chem 2009;284:10315–10323.
99. Duran-Sandoval D, Cariou B, Percevault F, et al. The farnesoid X receptor modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. J Biol Chem 2005;280:29971–29979.
100. Caron S, Huaman Samanez C, Dehondt H, et al. Farnesoid X receptor inhibits the transcriptional activity of carbohydrate response element binding protein in human hepatocytes. Mol Cell Biol 2013;33:2202–2211.
101. Kir S, Beddow SA, Samuel VT, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 2011;331:1621–1624.
102. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 2005;329:386–390.
103. Brighton CA, Rievaj J, Kuhre RE, et al. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. Endocrinology 2015;156:3961–3970.
104. Trabelsi M-S, Daoudi M, Prawitt J, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. Nat Commun 2015;6:7629.
105. Popescu IR, Helleboid-Chapman A, Lucas A, et al. The nuclear receptor FXR is expressed in pancreatic beta-cells and protects human islets from lipotoxicity. FEBS Lett 2010;584:2845–2851.
106. Kumar DP, Rajagopal S, Mahavadi S, et al. Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β cells. Biochem Biophys Res Commun 2012;427:600–605.
107. Kumar DP, Asgharpour A, Mirshahi F, et al. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet α cells to promote glucose homeostasis. J Biol Chem 2016;291:6626–6640.
108. Seyer P, Vallois D, Poitry-Yamate C, et al. Hepatic glucose sensing is required to preserve β cell glucose competence. J Clin Invest 2013;123:1662–1676.
109. Smushkin G, Sathananthan M, Piccinini F, et al. The effect of a bile acid sequestrant on glucose metabolism in subjects with type 2 diabetes. Diabetes 2013;62: 1094–1101.
110. Li F, Jiang C, Krausz KW, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun 2013; 4:2384.
111. Jiang C, Xie C, Lv Y, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. Nat Commun 2015;6:10166.
112. McGettigan BM, McMahan RH, Luo Y, et al. Sevelamer improves steatohepatitis, inhibits liver and intestinal farnesoid X receptor (FXR), and reverses innate immune dysregulation in a mouse model of non-alcoholic fatty liver disease. J Biol Chem 2016; 291:23058–23067.
113. Kaur A, Patankar JV, Haan W de, et al. Loss of Cyp8b1 improves glucose homeostasis by increasing GLP-1. Diabetes 2015;64:1168–1179.
114. Yamagata K, Daitoku H, Shimamoto Y, et al. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. J Biol Chem 2004; 279:23158–23165.
115. Ma K, Saha PK, Chan L, et al. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006;116:1102–1109.
116. Stayrook KR, Bramlett KS, Savkur RS, et al. Regulation of carbohydrate metabolism by the farnesoid X receptor. Endocrinology 2005;146:984–991.
117. Mudaliar S, Henry RR, Sanyal AJ, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology 2013;145: 574–582.e1.

118. Lewis ND, Patnaude LA, Pelletier J, et al. A GPBAR1 (TGR5) small molecule agonist shows specific inhibitory effects on myeloid cell activation in vitro and reduces experimental autoimmune encephalitis (EAE) in vivo. PLoS One 2014;9:e100883.
119. Keitel V, Donner M, Winandy S, et al. Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem Biophys Res Commun 2008;372:78–84.
120. Ichikawa R, Takayama T, Yoneno K, et al. Bile acids induce monocyte differentiation toward interleukin-12 hypo-producing dendritic cells via a TGR5-dependent pathway. Immunology 2012;136:153–162.
121. Guo C, Xie S, Chi Z, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity 2016;45:802–816.
122. Wang Y-D, Chen W-D, Yu D, et al. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor κ light-chain enhancer of activated B cells (NF-κB) in mice. Hepatology 2011; 54:1421–1432.
123. Pols TWH, Nomura M, Harach T, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab 2011; 14:747–757.
124. Liu H, Pathak P, Boehme S, et al. Cholesterol 7α-hydroxylase protects the liver from inflammation and fibrosis by maintaining cholesterol homeostasis. J Lipid Res 2016;57:1831–1844.
125. Gadaleta RM, Erpecum KJ van, Oldenburg B, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011;60:463–472.
126. Vavassori P, Mencarelli A, Renga B, et al. The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol 2009;183:6251–6261.
127. Inagaki T, Moschetta A, Lee Y-K, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006; 103:3920–3925.
128. Schiöth HB, Boström A, Murphy SK, et al. A targeted analysis reveals relevant shifts in the methylation and transcription of genes responsible for bile acid homeostasis and drug metabolism in non-alcoholic fatty liver disease. BMC Genomics 2016;17:462.
129. Verbeke L, Mannaerts I, Schierwagen R, et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. Sci Rep 2016;6:33453.
130. Fickert P, Fuchsbichler A, Moustafa T, et al. Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts. Am J Pathol 2009;175:2392–2405.
131. Feaver RE, Cole BK, Lawson MJ, et al. Development of an in vitro human liver system for interrogating nonalcoholic steatohepatitis. JCI Insight 2016;1:e90954.
132. Pencek R, Marmon T, Roth JD, et al. Effects of obeticholic acid on lipoprotein metabolism in healthy volunteers. Diabetes Obes Metab 2016;18:936–940.
133. Camilleri M, Gores GJ. Therapeutic targeting of bile acids. Am J Physiol Gastrointest Liver Physiol 2015; 309:G209–G215.
134. Schaap FG, Trauner M, Jansen PLM. Bile acid receptors as targets for drug development. Nat Rev Gastroenterol Hepatol 2014;11:55–67.
135. Duan H, Ning M, Zou Q, et al. Discovery of intestinal targeted TGR5 Agonists for the treatment of type 2 diabetes. J Med Chem 2015;58:3315–3328.
136. Briere DA, Ruan X, Cheng CC, et al. Novel small molecule agonist of TGR5 possesses anti-diabetic effects but causes gallbladder filling in mice. PLoS One 2015; 10:e0136873.
137. Alemi F, Kwon E, Poole DP, et al. The TGR5 receptor mediates bile acid-induced itch and analgesia. J Clin Invest 2013;123:1513–1530.
138. Ge H, Zhang J, Gong Y, et al. Fibroblast growth factor receptor 4 (FGFR4) deficiency improves insulin resistance and glucose metabolism under diet-induced obesity conditions. J Biol Chem 2014;289:30470–30480.
139. Rao A, Kosters A, Mells JE, et al. Inhibition of ileal bile acid uptake protects against nonalcoholic fatty liver disease in high-fat diet-fed mice. Sci Transl Med 2016; 8:357ra122.
140. Beraza N, Ofner-Ziegenfuss L, Ehedego H, et al. Norursodeoxycholic acid reverses hepatocyte-specific nemo-dependent steatohepatitis. Gut 2011;60:387–396.
141. Trauner M, Halilbasic E, Claudel T, et al. Potential of norursodeoxycholic acid in cholestatic and metabolic disorders. Dig Dis Basel Switz 2015;33:433–439.
142. Mueller M, Thorell A, Claudel T, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. J Hepatol 2015;62:1398–1404.
143. Downes M, Verdecia MA, Roecker AJ, et al. A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. Mol Cell 2003;11:1079–1092.

Received November 16, 2016. Accepted January 20, 2017.

Reprint requests
Address requests for reprints to: Bart Staels, PhD, Institut Pasteur de Lille, 1 Rue du Professeur Calmette, BP245, 59019 Lille, France. e-mail: bart.staels@pasteur-lille.fr; fax: (33) 320877360.

Acknowledgments
Bart Staels is a member of the Institut Universitaire de France and Oscar Chávez-Talavera is a participant of the Program of Combined Studies in Medicine (PECEM), Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico.
Oscar Chávez-Talavera and Anne Tailleux contributed equally to this work.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by grants from the European Genomic Institute for Diabetes (E.G.I.D., ANR-10-LABX-46) and the European Research Council (ERC Grant Immunobile, contract 694717).

Supplementary References

Supplementary References 1

1. Prinz P, Hofmann T, Ahnis A, et al. Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients. Front Neurosci 2015;9:199.
2. Sonne DP, Nierop FS van, Kulik W, et al. postprandial plasma concentrations of individual bile acids and FGF-19 in patients with type 2 diabetes. J Clin Endocrinol Metab 2016;101:3002–3009.
3. Haeusler RA, Astiarraga B, Camastra S, et al. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. Diabetes 2013;62:4184–4191.
4. Vincent RP, Omar S, Ghozlan S, et al. Higher circulating bile acid concentrations in obese patients with type 2 diabetes. Ann Clin Biochem 2013;50:360–364.
5. Wewalka M, Patti M-E, Barbato C, et al. Fasting serum taurine-conjugated bile acids are elevated in type 2 diabetes and do not change with intensification of insulin. J Clin Endocrinol Metab 2014;99:1442–1451.
6. Cariou B, Chetiveaux M, Zaïr Y, et al. Fasting plasma chenodeoxycholic acid and cholic acid concentrations are inversely correlated with insulin sensitivity in adults. Nutr Metab 2011;8:48.
7. Ferslew BC, Xie G, Johnston CK, et al. Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. Dig Dis Sci 2015;60:3318–3328.
8. Patti M-E, Houten SM, Bianco AC, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity (Silver Spring) 2009;17:1671–1677.
9. Nakatani H, Kasama K, Oshiro T, et al. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. Metabolism 2009;58:1400–1407.
10. Jansen PLM, Werven J van, Aarts E, et al. Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery. Dig Dis 2011;29:48–51.
11. Pournaras DJ, Glicksman C, Vincent RP, et al. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. Endocrinology 2012;153:3613–3619.
12. Simonen M, Dali-Youcef N, Kaminska D, et al. Conjugated bile acids associate with altered rates of glucose and lipid oxidation after Roux-en-Y gastric bypass. Obes Surg 2012;22:1473–1480.
13. Gerhard GS, Styer AM, Wood GC, et al. A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. Diabetes Care 2013;36:1859–1864.
14. Kohli R, Bradley D, Setchell KD, et al. Weight loss induced by Roux-en-Y gastric bypass but not laparoscopic adjustable gastric banding increases circulating bile acids. J Clin Endocrinol Metab 2013;98:E708–E712.
15. Albaugh VL, Flynn CR, Cai S, et al. Early increases in bile acids post roux-en-y gastric bypass are driven by insulin-sensitizing, secondary bile acids. J Clin Endocrinol Metab 2015;100:E1225, 1233.
16. Sachdev S, Wang Q, Billington C, et al. FGF 19 and bile acids increase following roux-en-Y gastric bypass but not after medical management in patients with type 2 diabetes. Obes Surg 2016;26:957–965.
17. Steinert RE, Peterli R, Keller S, et al. Bile acids and gut peptide secretion after bariatric surgery: a 1-year prospective randomized pilot trial. Obesity 2013;21:E660–E668.
18. Werling M, Vincent RP, Cross GF, et al. Enhanced fasting and post-prandial plasma bile acid responses after Roux-en-Y gastric bypass surgery. Scand J Gastroenterol 2013;48:1257–1264.
19. Dutia R, Embrey M, O'Brien CS, et al. Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. Int J Obes 2015;39:806–813.
20. Jørgensen NB, Dirksen C, Bojsen-Møller KN, et al. Improvements in glucose metabolism early after gastric bypass surgery are not explained by increases in total bile acids and fibroblast growth factor 19 concentrations. J Clin Endocrinol Metab 2015;100:E396–E406.
21. Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. Int J Obes 2013;37:1553–1559.
22. Dirksen C, Jørgensen NB, Bojsen-Møller KN, et al. Gut hormones, early dumping and resting energy expenditure in patients with good and poor weight loss response after Roux-en-Y gastric bypass. Int J Obes 2013;37:1452–1459.
23. Haluzíková D, Lacinová Z, Kaválková P, et al. Laparoscopic sleeve gastrectomy differentially affects serum concentrations of FGF-19 and FGF-21 in morbidly obese subjects. Obesity (Silver Spring) 2013;21:1335–1342.
24. Ferrannini E, Camastra S, Astiarraga B, et al. Increased bile acid synthesis and deconjugation after biliopancreatic diversion. Diabetes 2015;64:3377–3385.

Supplementary References 2

1. Lefebvre P, Cariou B, Lien F, et al. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147–191.
2. Pircher PC, Kitto JL, Petrowski ML, et al. Farnesoid X receptor regulates bile acid-amino acid conjugation. J Biol Chem 2003;278:27703–27711.
3. Ananthanarayanan M, Balasubramanian N, Makishima M, et al. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem 2001;276:28857–28865.
4. Boyer JL, Trauner M, Mennone A, et al. Upregulation of a basolateral FXR-dependent bile acid efflux transporter OSTalpha-OSTbeta in cholestasis in humans and rodents. Am J Physiol Gastrointest Liver Physiol 2006;290:G1124–G1130.
5. Kir S, Beddow SA, Samuel VT, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 2011;331:1621–1624.

6. Caron S, Huaman Samanez C, Dehondt H, et al. Farnesoid X receptor inhibits the transcriptional activity of carbohydrate response element binding protein in human hepatocytes. Mol Cell Biol 2013; 33:2202–2211.
7. Zhang L, Wang Y-D, Chen W-D, et al. Promotion of liver regeneration/repair by farnesoid X receptor in both liver and intestine in mice. Hepatology 2012;56:2336–2343.
8. Verbeke L, Mannaerts I, Schierwagen R, et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. Sci Rep 2016;6:33453.
9. Fickert P, Fuchsbichler A, Moustafa T, et al. Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts. Am J Pathol 2009;175:2392–2405.
10. Kumar DP, Asgharpour A, Mirshahi F, et al. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet α cells to promote glucose homeostasis. J Biol Chem 2016;291:6626–6640.
11. Popescu IR, Helleboid-Chapman A, Lucas A, et al. The nuclear receptor FXR is expressed in pancreatic beta-cells and protects human islets from lipotoxicity. FEBS Lett 2010;584:2845–2851.
12. Renga B, Mencarelli A, Vavassori P, et al. The bile acid sensor FXR regulates insulin transcription and secretion. Biochim Biophys Acta 2010;1802:363–372.
13. Kumar DP, Rajagopal S, Mahavadi S, et al. Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β cells. Biochem Biophys Res Commun 2012;427:600–605.
14. Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab 2005;2:217–225.
15. Neimark E, Chen F, Li X, et al. Bile acid-induced negative feedback regulation of the human ileal bile acid transporter. Hepatol 2004;40:149–156.
16. Dijk TH van, Grefhorst A, Oosterveer MH, et al. An increased flux through the glucose 6-phosphate pool in enterocytes delays glucose absorption in Fxr-/- mice. J Biol Chem 2009;284:10315–10323.
17. Gadaleta RM, Erpecum KJ van, Oldenburg B, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011;60:463–472.
18. Inagaki T, Moschetta A, Lee Y-K, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006; 103:3920–3925.
19. Trabelsi M-S, Daoudi M, Prawitt J, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. Nat Commun 2015;6:7629.
20. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 2005;329:386–390.
21. Watanabe M, Houten SM, Mataki C, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 2006;439:484–489.
22. Abdelkarim M, Caron S, Duhem C, et al. The farnesoid X receptor regulates adipocyte differentiation and function by promoting peroxisome proliferator-activated receptor-gamma and interfering with the Wnt/beta-catenin pathways. J Biol Chem 2010;285:36759–36767.
23. Pols TWH, Nomura M, Harach T, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab 2011;14: 747–757.
24. Guo C, Xie S, Chi Z, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity 2016;45:802–816.
25. Keitel V, Donner M, Winandy S, et al. Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem Biophys Res Commun 2008;372:78–84.
26. Ichikawa R, Takayama T, Yoneno K, et al. Bile acids induce monocyte differentiation toward interleukin-12 hypo-producing dendritic cells via a TGR5-dependent pathway. Immunology 2012;136:153–162.

Supplementary References 3

1. Zhou M, Learned RM, Rossi SJ, et al. Engineered fibroblast growth factor 19 reduces liver injury and resolves sclerosing cholangitis in Mdr2-deficient mice. Hepatology 2016;63:914–929.
2. Fang S, Suh JM, Reilly SM, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med 2015;21:159–165.
3. Gadaleta RM, Erpecum KJ van, Oldenburg B, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011;60:463–472.
4. Inagaki T, Moschetta A, Lee Y-K, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006; 103:3920–3925.
5. Dijk TH van, Grefhorst A, Oosterveer MH, et al. An increased flux through the glucose 6-phosphate pool in enterocytes delays glucose absorption in Fxr-/- mice. J Biol Chem 2009;284:10315–10323.
6. Trabelsi M-S, Daoudi M, Prawitt J, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. Nat Commun 2015;6:7629.
7. Jiang C, Xie C, Li F, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest 2015;125:386–402.
8. Jiang C, Xie C, Lv Y, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. Nat Commun 2015;6:10166.
9. Li F, Jiang C, Krausz KW, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun 2013; 4:2384.
10. Zhang L, Xie C, Nichols RG, et al. Farnesoid X receptor signaling shapes the gut microbiota and controls hepatic lipid metabolism. mSystems 2016;1.
11. Lefebvre P, Cariou B, Lien F, et al. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147–191.

12. Lian F, Wang Y, Xiao Y, et al. Activated farnesoid X receptor attenuates apoptosis and liver injury in autoimmune hepatitis. Mol Med Rep 2015;12: 5821–5827.
13. Meng Z, Wang Y, Wang L, et al. FXR regulates liver repair after CCl4-induced toxic injury. Mol Endocrinol 2010; 24:886–897.
14. Lee CG, Kim YW, Kim EH, et al. Farnesoid X receptor protects hepatocytes from injury by repressing miR-199a-3p, which increases levels of LKB1. Gastroenterology 2012;142:1206–1217.e7.
15. Verbeke L, Mannaerts I, Schierwagen R, et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. Sci Rep 2016;6:33453.
16. Fickert P, Fuchsbichler A, Moustafa T, et al. Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts. Am J Pathol 2009;175:2392–2405.
17. Caron S, Huaman Samanez C, Dehondt H, et al. Farnesoid X receptor inhibits the transcriptional activity of carbohydrate response element binding protein in human hepatocytes. Mol Cell Biol 2013;33:2202–2211.
18. Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004;113:1408–1418.
19. Xiong X, Wang X, Lu Y, et al. Hepatic steatosis exacerbated by endoplasmic reticulum stress-mediated downregulation of FXR in aging mice. J Hepatol 2014;60:847–854.

PART I

BILE ACIDS IN THE CONTEXT OF OBESITY, INSULIN RESISTANCE, TYPE 2 DIABETES, AND NAFLD.

The introduction of this chapter consists on a review that I wrote with the support of Joel Haas, Anne Tailleux and Bart Staels (***Chávez-Talavera et al. Curr Opin Lipidol***. ***2019. Manuscript number 2, page 36****)*. In this review, we summarized and discussed the currently available clinical studies analyzing bile acids in patients with obesity, insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD). The different studies are summarized in a table, in which the reader is able to see the high heterogeneity between the metabolic parameters of the studied populations chosen for the studies, which might explain the highly discordant reported results. Such diseases are difficult to be studied separately, since they are often co-morbid and their respective severity can vary from patient to patient. The broad severity spectrum of these metabolic diseases could be responsible for particular bile acid metabolism alterations present only at certain stages of the natural history of each disease. Therefore, selection of the clinico-biological status of the studied populations might be crucial to understand the conflicting results and allow their appropriate interpretation.

Although current literature does not clearly support a causal role, the articles analyzed in our review show that bile acid alterations are associated with the metabolic syndrome components. To be able to study a particular metabolic context, further clinical studies should consider matching body mass index (BMI), insulin resistance (IR) or NAFLD stages across the groups, so that the compared groups differ amongst them only in the studied metabolic context.

Based on the bile acid alterations present in patients with obesity and insulin resistance that we summarized in (***Chávez-Talavera et al. Curr Opin Lipidol***. ***2019. Manuscript number 2, page 36****)*, we wondered whether bile acids have a predictive association with the conversion from prediabetes to new-onset type 2 diabetes in a prospective cohort study, in a collaboration with the team of Pr. Bertrand Cariou from the UMR 1087, l'Institut du Thorax, University of Nantes. To answer this scientific question, we used the IT-DIAB cohort, which is a 5-year prospective, observational study designed to identify new biomarkers of type 2 diabetes risk in a population with prediabetes. During a one-week stay in our collaborator's laboratory, I participated to the selection of the patients. Then, I was responsible for the measurement of bile acid and C4 concentrations in plasma, with the precious help of the Mass Spectrometry Department (PSM-GRITA) of the Pharmacy Faculty at the University of Lille, and I also actively participated to the main analysis of the data along with Matthieu

Wargny at the University of Nantes. I drafted the manuscript with the close supervision of Anne Tailleux, Bart Staels and Bertrand Cariou, and therefore I am co-first author of this publication.

Briefly, 205 patients with prediabetes were included in this study, and were followed each year during 5 years. Baseline fasting plasma bile acids and C4 concentrations were quantified and the association between bile acids and new onset type 2 diabetes was determined using Cox proportional hazards models. In the longitudinal analyses, plasma bile acid species were not independently associated with the conversion to type 2 diabetes after adjustment with classical type 2 diabetes risk factors. However, total HCA and the total HCA/total CDCA ratio (reflecting hepatic 6α-hydroxylation rate) negatively correlated with BMI and HOMA-IR, suggesting that this often neglected bile acid species could be linked with metabolic homeostasis (***Chávez-Talavera. et al., Article submitted. Manuscript number 3, page 48***).

For the final part of this chapter, I could contribute to the technical standardization and validation of C4 measurements by our team, which at this time was not completed, and the scientific discussion of the work published by ***Legry et al. JCEM 2018 (Manuscript number 4, page 74).*** As we discuss in our review (**Chávez-Talavera *et al.* Curr Opinion LIpidol 2019. Manuscript number 2, page 36**), many clinical studies have reported bile acid alterations in the context of NAFLD, regardless of the metabolic status of the studied populations. We know also that bile acid alterations are associated with the metabolic co-morbidities of NAFLD. Therefore, the association between bile acid metabolism alterations and the necro-inflammatory histological lesions of NASH was studied and fasting plasma bile acid and hepatic bile acid metabolism transcriptomic analyses were compared between NASH patients and no-NASH patients matched by BMI and IR. The originality of this study is that the patients were carefully matched so that they only vary on the NASH status; the results show that there are no differences in hepatic bile acid metabolism gene expression nor in the plasma concentrations of C4 and FGF19, a marker of intestinal FXR activation. Moreover, plasma bile acids were not correlated with the hepatic lesions of NASH but were correlated with glucose homeostasis parameters, suggesting that bile acid alterations are related with the metabolic phenotype associated with NASH, rather than with the histological lesions *per se (**Legry et al. JCEM 2018) (Manuscript number 4, page 74)***.

MANUSCRIPT NUMBER 2

Chávez-Talavera & Haas *et al.* Bile Acid Alterations in Nonalcoholic Fatty Liver Disease, Obesity, Insulin Resistance and Type 2 Diabetes: What Do the Human Studies Tell?" Current Opinion in Lipidology, March. 2019.

Bile acid alterations in nonalcoholic fatty liver disease, obesity, insulin resistance and type 2 diabetes: what do the human studies tell?

Oscar Chávez-Talavera, Joel Haas*, Guillaume Grzych, Anne Tailleux†, and Bart Staels†*

Purpose of review
The purpose of this review is to discuss the influence of obesity, insulin resistance, type 2 diabetes (T2D), and nonalcoholic fatty liver disease (NAFLD) on bile acid metabolism and to analyze whether these findings reinforce current beliefs about the role of bile acids in the pathophysiology of these diseases.

Recent findings
Discordant results on plasma bile acid alterations in NAFLD patients have been reported. Obesity, insulin resistance, and T2D, common comorbidities of NAFLD, have been associated with bile acid changes, but the individual bile acid species variations differ between studies (summarized in this review), perhaps because of clinicobiological differences between the studied patient populations and the heterogeneity of statistical analyses applied.

Summary
The regulatory role of bile acids in metabolic and cellular homeostasis renders bile acids attractive candidates as players in the pathophysiology of NAFLD. However, considering the complex relationship between NAFLD, obesity, insulin resistance and T2D, it is difficult to establish clear and independent associations between bile acid alterations and these individual diseases. Though bile acid alterations may not drive NAFLD progression, signaling pathways activated by bile acids remain potent therapeutic targets for its treatment. Further studies with appropriate matching or adjustment for potential confounding factors are necessary to determine which pathophysiological conditions drive the alterations in bile acid metabolism.

Keywords
bile acids, insulin resistance, metabolic syndrome, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, obesity, type 2 diabetes

INTRODUCTION

The increasing global prevalence of the metabolic syndrome (MetS), which groups obesity, hyperglycemia, dyslipidemia, and hypertension, is a major worldwide public health problem. Nonalcoholic fatty liver disease (NAFLD) is often considered the hepatic manifestation of MetS and is closely related to many of its features. NAFLD comprises a hepatic spectrum ranging from simple hepatic steatosis or nonalcoholic fatty liver (NAFL), characterized by abnormal triglyceride accumulation in hepatocytes, which can progress to nonalcoholic steatohepatitis (NASH). While NAFL is generally considered benign, NASH combines NAFL with elements of hepatic tissue injury including evidence of inflammation and hepatocyte ballooning with or without fibrosis. NAFLD is an independent risk factor for cirrhosis, hepatocellular carcinoma, cardiovascular disease, and mortality. Importantly, NAFLD is strongly and bidirectionally associated with obesity, insulin resistance, and type 2 diabetes (T2D) [1▪▪,2▪,3].

There are several unmet research needs in the field of NAFLD. The natural history of NAFLD is not entirely understood and no pharmacological therapies exist for its treatment. For example, NAFL can be diagnosed with noninvasive imaging techniques,

University of Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011 - EGID, F-59000 Lille, France

Correspondence to Bart Staels, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP245, 59019 Lille, France. Tel: +33320877388; fax: +33320877360; e-mail: bart.staels@pasteur-lille.fr

*O.C.T. and J.H.: cofirst authors.

†Anne Tailleux and Bart Staels: colast authors.

Curr Opin Lipidol 2019, 30:244–254
DOI:10.1097/MOL.0000000000000597

KEY POINTS

- Bile acid changes have been associated with obesity, insulin resistance, and T2D, common comorbidities of NAFLD.
- Discordant results on plasma bile acid alterations in NAFLD patients have been reported.
- The intricate relationship between NAFLD, obesity, insulin resistance, and T2D renders difficult the establishment of clear and independent associations between bile acid alterations and these individual diseases.
- Further studies with appropriate matching or adjustment for potential confounding factors are necessary to determine which of these diseases drive the alterations in bile acid metabolism in NAFLD.
- Bile acid signaling pathways remain attractive candidates for the treatment of NAFLD, but whether bile acids are a driving force in the pathogenesis and progression of NAFLD or just innocuous bystanders remains undetermined.

but NASH diagnosis currently requires liver biopsy for histological evaluation, an intervention not devoid of risk, and highly susceptible to interobserver variations [4▪]. It is thus urgent to find circulating biomarkers to better identify those patients to be submitted to biopsy for NASH screening and monitoring NAFLD progression and staging for assessment of treatment efficacy. Recently, bile acids have emerged as potential candidates, and new emerging therapies for NAFLD target bile acid signaling pathways. Moreover, some evidence suggests that bile acid alterations could be associated with NAFLD.

BILE ACID PHYSIOLOGY

Bile acids are cholesterol-derived molecules synthesized exclusively in hepatocytes via two pathways. The classical pathway produces the majority of bile acids. It is initiated by cytochrome P450 (CYP) cholesterol 7α-hydroxylase (CYP7A1), whose activity correlates with plasma levels of 7α-hydroxy-4-cholesten-3-one (C4). The alternative pathway, initiated by CYP 27α-hydroxylase (CYP27A1), synthesizes the remaining minor bile acid fraction. These pathways produce the primary bile acids cholic acid (CA), chenodeoxycholic acid (CDCA), and hyocholic acid (HCA) in humans. Notably, CA is a 12α-hydroxylated bile acid, synthetized by the CYP 12α-hydroxylase (CYP8B1), which is indirectly regulated by insulin signaling via forkhead box protein O1 (FoxO1). Thus, the 12α-hydroxylated:non-12α-hydroxylated bile acid ratio reflects the activity of CYP8B1 and is a marker of hepatic insulin sensitivity of the bile acid synthesis pathway. Upon synthesis, bile acids are conjugated to glycine or taurine (the proportion being essentially determined by dietary intake), secreted into bile, and released in the duodenum postprandially to facilitate dietary fat solubilization and absorption. In the gut, microbiota deconjugate and transform primary bile acids into secondary bile acids:CA into deoxycholic acid (also a 12α-hydroxylated bile acid), CDCA into ursodeoxycholic acid (UDCA) and lithocholic acid (LCA), and HCA into hyodeoxycholic acid. Approximately 95% of bile acids are recaptured in the ileum and return to the liver via the portal vein to be reconjugated and resecreted into bile. A minor fraction of portal bile acids escapes hepatic reuptake and reach the systemic circulation, hence potentially allowing signaling actions in peripheral organs [5▪]. However, it should be noted that circulating plasma bile acids do not necessarily reflect the composition of the portal, intrahepatic, biliary, or intestinal bile acid pools.

Apart from their role in dietary lipid solubilization, bile acids are natural ligands of the farnesoid X receptor (FXR) and the Takeda G-protein-coupled receptor (TGR5), through which bile acids regulate their own synthesis, as well as glucose, lipid and energy homeostasis, inflammation, hepatic fibrosis, and modulate reparative activity (reviewed in [5▪]). Importantly, not all bile acids activate FXR (CDCA > DCA > CA > LCA) and TGR5 (LCA > DDCA > CDCA > CA) with the same affinity or potency. Hence, qualitative alterations of the bile acid pool composition, in addition to quantitative changes in total bile acids, could modulate the activity of their receptors and hence impact metabolism.

Bile acid-FXR signaling in enterocytes stimulates the secretion of fibroblast growth factor 19 (FGF19) into portal venous blood, which then activates FGFR4/β-Klotho in the liver. Through this mechanism, FGF19 suppresses CYP7A1, thereby decreasing bile acid synthesis via the classical pathway, and also regulates hepatic carbohydrate and lipid metabolism. Interestingly, plasma FGF19 (a biomarker of intestinal FXR activation) is decreased in the metabolic syndrome, suggesting that it could be implicated in the pathogenesis of NAFLD [6,7]. Considering many interactions between diet, insulin signaling, and bile acid metabolism, it appears logical that alterations in circulating bile acids could be markers of, or even actors in, the pathophysiology of the metabolic processes that they modulate.

BILE ACIDS IN OBESITY, INSULIN RESISTANCE, AND TYPE 2 DIABETES

The effect of obesity on bile acid metabolism is not entirely clear. BMI has been reported to positively

correlate with fasting bile acids [8] but negatively with postprandial bile acids [9]. Other studies have reported either no changes [10–12] or increased concentrations of CA [13] in fasting plasma bile acids, and no changes [10] or decreased glyco-conjugated bile acids [11] in postprandial plasma of obese individuals. There is also evidence that the normal meal-induced bile acid increase in plasma bile acids is suppressed with obesity [12,14]. Similarly, insulin infusion during an euglycemic hyperinsulinemic clamp decreased serum bile acids in lean but not in obese patients [12]. Fasting plasma C4 levels were higher in obese patients in two different studies [12,15▪▪], suggesting increased bile acid biosynthesis. Expression of the canalicular (BSEP) and sinusoidal (NTCP) bile acid transporter mRNA levels were negatively correlated with BMI [12]. Although there is a large heterogeneity between the reported results, possibly because of the known large inter and intraindividual variability in bile acids and the diurnal variations of bile acids in humans [15▪▪], overall fasting plasma bile acids appear elevated, but the postprandial increase is lower in obese individuals.

A parameter confounding these studies may be related to differences in the level of insulin resistance among the studied patient populations. Studies analyzing bile acid changes in insulin resistant, but not diabetic patients have reported higher fasting total and 12α-hydroxylated bile acids [16▪]. Mechanistic studies in insulin-resistant mice suggest that impaired insulin-mediated repression of FoxO1 results in higher 12α-hydroxylase CYP8B1 expression [17]. Moreover, insulin resistance was associated with increased total bile acid concentrations [18,19], fasting tauro-conjugated bile acids [20], CDCA, CA, DCA [13], and glyco-chenodeoxycholic acid (GCDCA) [21].

Some studies in overtly T2D patients have reported no changes in fasting [9,22] or postprandial [9,23] bile acids compared with controls, whereas other studies have reported either increased fasting [7,16▪,24] and postprandial [24] total bile acid concentrations, increased fasting DCA [9,13,25], tauro-chenodeoxycholic acid, tauro-deoxycholic acid, and tauro-ursodeoxycholic acid (TUDCA) [20], or decreased CA and HCA [25]. Bile acid levels were not modified by intensive insulin treatment in diabetic patients [20].

The mechanisms underlying the quantitative and qualitative modifications in bile acids in the metabolic syndrome are not fully elucidated. Apart from the previously described alterations of the insulin receptor–protein kinase b (Akt)–FoxO1 signaling pathway impacting bile acid synthesis in insulin resistance, dysbiosis (occurring in metabolic disorders) could modify bile acid deconjugation, secondary bile acid synthesis, and modulate the bile acid pool composition [5▪]. Another mechanism implies the enterohepatic cross-talk via intestinally FXR-induced FGF19. Indeed, FGF19 levels correlate positively with total bile acid concentrations [10,24] and negatively with plasma C4 [10,26]. Interestingly, FGF19 is lower in T2D and obese patients and correlates negatively with BMI [10,27] and glycated hemoglobin (HbA1c) [27]. However, these changes in FGF19 in T2D patients [9,24] or its correlation with MetS components [20] are not universally observed. Importantly, differences in control populations and the degree of insulin resistance or T2D severity are potential confounding factors between these studies and are not always well reported nor well characterized.

In conclusion, although not all reported data are entirely consistent, it is clear that bile acid metabolism alterations occur in obese and/or insulin-resistant humans (summarized in Table 1). However, it remains undetermined whether bile acids are innocent bystanders or if they participate to the pathophysiology of these diseases.

BILE ACIDS AND NONALCOHOLIC FATTY LIVER DISEASE

Given that bile acid accumulation in hepatocytes is cytotoxic in intrahepatic cholestatic liver diseases, it is conceivable that bile acids could be altered in NAFLD also and that their accumulation in NASH patients could favor progression of the disease. Furthermore, hepatic necroinflammatory lesions in NAFLD may alter the anatomy of liver zonation. Since the liver is the sole site of bile acid synthesis and as bile acid synthesis is zonated in physiological conditions, altered bile acid metabolism could occur in a setting of pathologically altered liver zonation. Indeed, treatment of NASH with the FXR agonist obeticholic acid [28▪▪] or with the FGF19 analog NGM282 [29▪▪] improved several NAFLD histological features. Therefore, several groups have studied whether NAFLD patients present bile acid profile alterations (summarized in Table 2).

Fasting plasma bile acids were reported to be higher in NASH patients because of increased primary [30▪▪] and conjugated bile acid species [30▪▪,31,32]. Similar changes were also observed in NAFL patients [31]. Moreover, histological lesions of NASH have been associated with bile acid alterations [i.e. associations of high plasma glyco-cholic acid (GCA) [30▪▪] or CA [33▪▪] concentrations with lobular inflammation, low TUDCA, and high tauro-lithocholic acid concentrations with portal inflammation [30▪▪], high

Table 1. Clinical studies assessing bile acids in obesity, insulin resistance, and type 2 diabetes

Topic	Reference	Nutritional state	n	BMI (kg/m^2)	HOMA-IR	FPG (mg/l)	HbA1c (%)	Results
Obesity – bile acids	[11]	Fasting Meal test (30, 60, 90, 120, 150, 180 min)	Control: 12 Obese: 7	Control: 23.2 (2.8) Obese: 47.2 (7.2)[a]	ND	ND	ND	Fasting: No ≠ in BAs Postprandial: ↓glyco-bile acids
Obesity – bile acids	[14]	Fasting Meal test (−15, 0, 15, 30, 60, 90, 120, 150, 180 min)	Control: 8 Obese: 5	Control: 21.7 ± 1.6 Obese: 47.7 ± 7.4	ND	ND	ND	Fasting: ↓tauro-bile acids in obese versus controls Meal test: ↓meal-induced conjugated bile acid increase in obese versus controls
Obesity – bile acids	[8]	Fasting	Anorexia: 15 Control: 15 Obesity I: 15 Obesity II: 14 Obesity III: 15	Anorexia nervosa: 13.1 (8.9–15.6)[a] Control: 22.1 (20.3–25) Obesity I: 37.6 (30.1–39.8)[a] Obesity II: 45.6 (41.1–49.7)[a] Obesity III: 67.1 (59–84.6)[a]	ND	ND	ND	Fasting: Total bile acids positively correlated with BMI
Obesity – bile acids	[12]	Fasting Meal test (60, 120, 180 min) Euglycemic hyperinsulinemic clamp	Control: 11 Obese: 32	Control: 23.9 ± 0.7 Obese: 44 ± 1.6[a]	M/I Control: 103 (63) Obese: 33 (26)[a]	Control: 90 ± 1.8 Obese: 91.8 ± 1.8	ND	Fasting: ↑C4 and ↑7,12-diHCO in obese versus controls. C4 and 7,12-diHCO positively correlated with BMI and negatively correlated with insulin sensitivity Meal test: ↓meal-induced bile acid increase despite ↑total bile acid concentrations in obese versus controls Insulin infusion: ↓total bile acids in controls whereas ↓free but not conjugated bile acids in obese patients Other results: BMI negatively correlated with NTCP and BSEP hepatic gene expression
Insulin resistance – bile acids	[19]	Fasting	Control: 40 Overweight and obese: 40	Control: 22.3 ± 1.6 Overweight and obese: 32.6 ± 3.7	Control: 1.8 ± 1.2 Overweight and obese: 3.5 ± 2.2	ND	ND	Fasting: No bile acid ≠ between groups Total bile acids positively associated with HOMA-IR in pooled patients, stronger association in overweight and obese patients
Insulin resistance – bile acids	[21]	Fasting	Heterogeneous patients: 997	26.6 ± 5.3	1.04 ± 0.83	96 ± 19	ND	Fasting: GCDCA associated with HOMA-IR
T2D – bile acids	[23]	Fasting Meal test (0, 30, 60, 90, 120, 150, 180, 210, 240 min)	Control: 12 T2D: 13	Controls: 41 (38.4–43.4) T2D: 44 (40.8–45.5)	Control: 2.6 (2.2–2.8) T2D: 6.2 (4.4–8)[a]	Control: 95.4 (90–104.4) T2D: 144 (138.6–158.4)[a]	ND	Fasting and postprandial: No ≠ in total bile acids in obese nondiabetic patients versus obese T2D patients
T2D – bile acids	[9]	Postprandial (120 min)	Control: 12 T2D: 16	Control: 28.9 (25.2–32) T2D: 30.6 (25.2–35.5)	Control: 1.6 (1.1–4.8) T2D: 6.2 (2.1–16.6)[a]	Control: 91.8 (75.6–102.6) T2D: 156.6 (113.4–279)[a]	Control: 5.5 (5.1–6.1) T2D: 8.6 (6.3–10.6)[a]	Fasting and postprandial: no ≠ in total bile acids in fasting and postprandial bile acids in T2D versus controls ↑DCA pool size in T2D versus controls Postprandial bile acids negatively correlated with BMI
T2D – bile acids	[22]	Fasting Postprandial (30, 60, 90, 120 min)	Control: 12 T2D: 12	Control: 38 (35–38) T2D: 36 (32–37)	ND	Control: 88.2 (86.4–99) T2D: 131.4 (122.4–147.6)[a]	ND	Fasting: no ≠ in T2D versus controls Postprandial: ↑total bile acids in T2D versus controls due to glyco-bile acids

Table 1 (*Continued*)

Topic	Reference	Nutritional state	*n*	BMI (kg/m^2)	HOMA-IR	FPG (mg/l)	HbA1c (%)	Results
T2D – bile acids	[25]	Fasting	Control: 60 T2D: 40	Control: 28.31 ± 3.4 T2D: 30.01 ± 3.6[a]	ND	ND	Controls: 5.29 ± 0.37 T2D: 5.95 ± 0.72[a]	Fasting: ↓CA, ↑DCA, ↓HCA in T2D versus controls
Insulin resistance, T2D – bile acids	[16▪]	Fasting Euglycemic hyperinsulinemic clamp	Control: 200 (classified into quartiles based on insulin resistance) T2D: 35	Control: 25 ± 3.5 T2D: 38.3 ± 12.3[a]	M/I Control: 134 ± 88 T2D: 36 ± 19[a]	Control: 90 ± 9 T2D: 174.6 ± 57.6[a]	ND	↑12α-Hydroxy:non-12α-hydroxy-bile acid ratio in nondiabetic insulin resistance patients associated with ↓insulin sensitivity and ↑plasma Takeda Gs. ↑Total bile acids in T2D without ≠ in 12α-hydroxy:non-12α-hydroxy-bile acid ratio
Insulin resistance, T2D – bile acids	[18]	Fasting	Control: 967 Insulin resistance: 704 T2D: 254 T2D-insulin resistance: 224	Control: 24.4 ± 2.96 Insulin resistance: 27.48 ± 3.23[a] T2D: 24.79 ± 3.07 T2D-insulin resistance: 27.85 ± 3.24[b]	ND	Control: 90.72 ± 9.54 Insulin resistance: 97.2 ± 10.26[a] T2D: 117.36 ± 32.76[a] T2D-insulin resistance: 140.94 ± 46.44[a,b]	ND	Fasting: Insulin resistance (HOMA-IR > 2.7) positively associated with hyperbilacidemia (total bile acids > 10 nM) independently of diabetes status
Obesity, insulin resistance, T2D – bile acids	[13]	Fasting	Control: 14 Obese: 22 T2D: 20	Control: 23.85 (21.2–32.4) Obese: 33.6 (27.9–38.5)[a,b] T2D: 31.85 (22.8–39.1)[a]	Control: 1.33 (0.6–3.03) Obese: 3.15 (0.85–10.79) T2D: 3.85 (0.66–16.38)[a]	Control: 90.54 (81–105.3) Obese: 99 (77.4–122.4)[a,b] T2D: 132.66 (91.98–225)[a]	Control: 5.40 (5–5.9) Obese: 5.45 (4.8–6.5)[b] T2D: 7.4 (5.8–11.7)[a]	Fasting: ↑total bile acids, ↑CA in obese versus controls. ↑DCA in T2D versus controls. CDCA and CA negatively correlated glucose infusion rate. CDCA, DCA, and CA positively correlated with HOMA-IR
MetS, T2D – bile acids	[15▪▪]	Fasting	Control: 49 MetS: 50 T2D: 50	Control: 27.4 (19.1–42.1) MetS: 29.6 (21.8–37.7)[a,b] T2D: 31.2 (20–51.8)[a]	Control: 2.11 (0.21–8.96) MetS: 2.95 (0.87–26.31) T2D: 6.35 (1.11–47.14)	Control: 96.84 (75.96–112.86) MetS: 100.44 (82.98–120.96)[b] T2D: 144.9 (92.88–345.6)[a]	Control: 5.6 (4.9–6.1) MetS: 5.8 (5.3–6.4)[a,b] T2D: 7.4 (5.9–13.6)[a]	Fasting: No independent association of bile acids with MetS and T2D. ↑C4 in T2D and MetS patients confounded by positive correlation of C4 with BMI and Takeda G
Obesity – bile acids – FGF19	[10]	Fasting OFTT (2, 4, 6 h)	Control: 16 Overweight: 14 Obese: 12	Control: 24.2 (21.8–26.6) Overweight: 28.3 (26.3–29.2)[a] Obese: 35.3 (32.7–39)[a]	ND	ND	ND	Fasting: No ≠ in bile acids or C4 between groups. ↓FGF19 in obese versus controls. FGF19 positively correlated with bile acids only in controls and negatively correlated with BMI in all patients OFTT: No ≠ in bile acids or C4, ↓FGF19 in overweight versus controls. FGF19 negatively correlated to C4
Insulin resistance, T2D – bile acids – FGF19	[20]	Fasting Postglucose load (120 min)	Control: 62 IGT: 25 T2D: 12	Control: 28.6 ± 5.8 IGT: 31 ± 8.6 T2D: 32.3 ± 8.7	Control: 2.2 ± 1.4 IGT: 3.3 ± 2.1[b] T2D: 6.7 ± 6.7[a]	Control: 93 ± 7 IGT: 96 ± 8 T2D: 115 ± 17	Control: 5.3 ± 0.3 IGT: 5.5 ± 0.3 T2D: 6.2 ± 0.8[a,b]	Fasting: ↑tauro-bile acids in T2D versus controls but not in IGT Postglucose load: ↑tauro-MCA in IGT versus controls Tauro-bile acids positively correlated with FPG, fasting insulin, HOMA-IR, HbA1c and postglucose load glycemia, but negatively correlated with oral disposition index bile acids not correlated with sex, age, BMI, Takeda G or cholesterol FGF19 not correlated with bile acids, BMI, age, lipids, HbA1c neither on fasting nor postglucose load No ≠ in bile acids upon insulin intensification

Table 1 (*Continued*)

Topic	Reference	Nutritional state	n	BMI (kg/m^2)	HOMA-IR	FPG (mg/l)	HbA1c (%)	Results
T2D – bile acids – FGF19	[24]	Fasting OGTT Low-fat, medium-fat, and high-fat meal tests (−20, −10, 0, 15, 30, 45, 60, 90, 120, 180, 240 min)	Control: 15 T2D: 15	Control: 27.9 ± 2 T2D: 28 ± 2.2	ND	ND	Control: 5.2 ± 0.2 T2D: 7.5 ± 1.4	Fasting: ↑total bile acids in T2D versus controls Postprandial: ↑total bile acids in OGTT, low-fat and medium-fat meals but not in high fat meal in T2D versus controls. Total bile acids positively correlated with meal fat content No ≠ in FGF19 between groups. FGF19 correlated positively with total bile acids but negatively with C peptide in T2D and controls. FGF19 negatively correlated with plasma glucose in controls but not in T2D.
T2D – FGF19	[27]	Fasting	Control: 12 T2D: 26	Control: 22.6 (21.4–23.9) T2D: 28.9 (26.2–31.3)[a]	ND	Control: 86.58 (84.24–89.82) T2D: 124.74 (106.74–156.78)[a]	Control: 5 (4.5–5) T2D: 6 (6–8)[a]	Fasting: FGF19 negatively correlated with BMI and HbA1c
Insulin resistance, T2D – FGF19	[6]	Fasting	Control: 81 IGT: 93 IFG: 91 T2D: 104	Control: 22.2 ± 2.2 IGT: 25.2 ± 3.7[a] IFG: 24.5 ± 2.6[a] T2D: 25.4 ± 3.6[a]	Control: 1.24 (0.89–1.61) IGT: 1.65 (1.19–2.7)[a] IFG: 2.04 (1.24–3.27)[a] T2D: 2.81 (1.77–4.04)[a]	Control: 92 (84.96–97.56) IGT: 97 (88.56–102.06) IFG: 114 (111.06–117)[a] T2D: 137 (118.8–165.06)[a]	Control: 5.5 (5.3–5.7) IGT: 5.8 (5.5–5.9)[a] IFG: 5.7 (5.5–6)[a] T2D: 6.7 (6–7.8)[a]	Fasting: ↓FGF19 in IFG and T2D versus controls FGF19 negatively associated with FPG
Insulin resistance, T2D – bile acids, FGF19	[7]	Fasting	Control: 125 T2D: 181	ND	ND	ND	ND	Fasting: ↑total bile acids, ↓FGF19 in T2D versus controls

Data extracted from the articles and presented as means ± SD, means ± SEM, medians (Q1–Q3), medians (IQR), or medians (range). BA, bile acid; BSEP, bile salt export pump; *7, 12-diHCO*, 7α-12α-dihydroxy-4-cholesten-3-one; *C4*, 7α-hydroxy-4-cholesten-3-one; *FPG*, fasting plasma glycemia; GCDCA, glyco-chenodeoxycholic acid; *HbA1c*, glycated hemoglobin; *HOMA-IR*, homeostatic model assessment for insulin resistance; *IFG*, impaired fasting glucose; *IGT*, impaired glucose tolerance; *MCA*, muricholic acid; *M/I*, insulin sensitivity index; *MetS*, metabolic syndrome; *OFTT*, oral fat tolerance test; OGTT, oral glucose tolerance test.
[a]Statistically different from control.
[b]Statistically different from T2D.

Table 2. Clinical studies assessing bile acids in NAFLD.

Reference	Medium for bile acid analysis	Nutritional state	*N*	BMI (kg/m^2)	HOMA-IR	FPG (mg/dl)	HbA1c (%)	Results
[40]	Plasma	ND	Control: 38 NASH: 36	ND	ND	ND	ND	Bile acids: ↑GCA ↑TCA ↑TCDCA in NASH versus controls Other: ↑FGF21 in NASH versus controls
[31]	Plasma	Fasting	Control: 25 NAFL: 11 NASH: 24	Control: 24.5 ± 2.6 NAFL: 34 ± 4[a] NASH: 34.8 ± 4.7[a]	Control: 0.96 ± 0.4 NAFL: 2.6 ± 1.1[a] NASH: 3.26 ± 1.6[a]	Control: 84.6 ± 9 NAFL: 88.2 ± 10.8 NASH: 95.4 ± 16.2[a]	ND	Bile acids: ↑GCA ↑TCA ↑GCDCA in NASH versus controls ↑TCA in NAFL versus controls
[34]	Plasma	ND	Control: 10 NAFL: 39 NASH: 59	Control: 22.4 ± 2.46 NAFL: 51.3 ± 1.5 NASH: 53.5 ± 1.3	ND	Control: ND NAFL: 95.5 ± 8.5 NASH: 122.9 ± 11[a]	Control: ND NAFL: 5.54 ± 0.16 NASH: 16.92 ± 4.59[a]	Bile acids: total bile acids positively correlated with NAS Other: no ≠ in C4 between NAFL, NASH and controls. Serum adiponectin negatively correlated with serum bile acids and NAS Hepatic mRNA levels: ↓NTCP, ↓BSEP, ↓EPHX1 in NASH versus controls
[32]	Serum, urine	Fasting, postprandial	Control: 15 NASH: 7	Control: 25 ± 2.7 NASH: 32 ± 5.2[a]	Control: 1.6 ± 0.6 NASH: 13 ± 8.7[a]	Control: 86 ± 8 NASH: 124 ± 16[a]	ND	Bile acids: fasting plasma: ↑conjugated bile acids in NASH versus controls. Postprandial plasma: ↑total bile acids in NASH versus controls. Postprandial urine: ↑GCA ↑TCA ↑GCDCA ↑DCA in NASH versus controls Other: MCA are majority bile acid species in urine
[38]	Serum	ND	Control: 11 NASH: 16	Control: 19.2 ± 3.4 NASH: 33.8 ± 7.7[a]	Control: ND NASH: 4.28 ± 2.83	ND	ND	Bile acids: ↑total bile acids ↑CA ↑CDCA ↑DCA ↑UDCA in NASH versus controls ↑secondary/primary BA ratio, ↓CDCA ↑DCA proportions in NASH versus controls Other: ↓FGF19 in NASH versus control. Microbiota: ↑glycine and taurine-metabolizing bacteria Hepatic mRNA levels: ↑CYP7A1 ↑CYP27A1 ↑CYP8B1 ↑BACS ↑NTCP ↑OATP1B1 ↑OATP1B3 ↑HNF4A ↑FGFR4 ↑KLB in NASH versus controls
[30▪▪]	Plasma	Fasting	Control: 24 NAFL: 25 NASH: 37	Control: 27.3 ± 5.8 NAFL: 32.6 ± 5.4 NASH: 34.4 ± 4.2[a]	ND	ND	ND	Bile acids: ↑Primary BAs ↑UDCA in NASH versus Controls. ↑GCA ↑TCA in NASH versus NAFL. ↑TCA ↓CDCA in NAFLD associated with severe steatosis. ↑GCA associated with severe lobular inflammation. ↓TUDCA and ↑TLCA associated with portal inflammation. ↑GCA, ↑TCA, ↑GCDCA, ↑DCA, ↑GLCA associated with hepatocyte ballooning. ↑Conjugated CA associated with NAS > 4 Other: No ≠ in C4 or FGF19 Hepatic mRNA levels: ↑CYP7A1 in NASH versus controls and NAFL
[39▪]	Plasma	Fasting	Control: 26 NASH: 32	Control: 39.4 ± 5.9 NASH: 40.2 ± 5.8	Control: 3.25 ± 2.05 NASH: 4.05 ± 2.65	Control: 82.3 ± 11.8 NASH: 85.8 ± 8.4	ND	Bile acids: no ≠ in bile acids in NASH versus controls. No correlation of bile acids and necroinflammatory lesions of NASH Other: no ≠ in C4 Hepatic mRNA levels: ↓PPARA ↓CYP3A4 ↓BSEP ↑CYP7A1 ↑ATP8B1 in NASH versus controls

Table 2 (*Continued*)

Reference	Medium for bile acid analysis	Nutritional state	*N*	BMI (kg/m^2)	HOMA-IR	FPG (mg/dl)	HbA1c (%)	Results
[33▪▪]	Plasma	Fasting	Cohort 1 Non-NAFLD by MRI: 120 NAFLD by MRI: 36 Cohort 2 Biopsy-proven NAFLD: 156	Cohort 1 Non-NAFLD by MRI: 25 ± 5.2 NAFLD by MRI: 31.8 ± 5.9[a] Cohort 2 Biopsy-proven NAFLD: 32 ± 6	Cohort 1 Non-NAFLD by MRI: 1.7 ± 1 NAFLD by MRI: 6.6 ± 11.4[a] Cohort 2 Biopsy-proven NAFLD: 8.5 ± 17.3	Cohort 1 Non-NAFLD by MRI: 88.4 ± 9.6 NAFLD by MRI: 102.5 ± 27.5[a] Cohort 2 Biopsy-proven NAFLD: 106.2 ± 33.1	Cohort 1 Non-NAFLD by MRI: 5.7 ± 0.4 NAFLD by MRI: 6.1 ± 0.7[a] Cohort 2 Biopsy-proven NAFLD: 6 ± 0.9	Cohort 1: bile acids: No ≠ in bile acid concentrations in NAFLD versus non-NAFLD. ↑CA ↑CDCA ↓GHCA proportions in NAFLD versus non-NAFLD Cohort 2: bile acids: No ≠ in bile acid concentrations among NAFLD patients classified on NAFL versus NASH. ↑Bile acids associated with ↑fibrosis stages. Lobular inflammation positively correlated with free CA. Ballooning positively correlated with free CA and CDCA but negatively correlated with conjugated CA and DCA. NAS positively correlated with free CA and CDCA but negatively correlated with conjugated CA
[37]	Liver	ND	Control: 8 NASH: 15	Control: ND NASH 26.6 ± 2.5	ND	ND	ND	Bile acids: ↑CDCA ↑DCA in NASH versus controls. ↑DCA in NASH versus controls. CA correlated with inflammation and CDCA with fibrosis in NASH
[36]	Liver	ND	Control: 17 NAFL: 4 NASH: 16	ND	ND	ND	ND	Bile acids: ↓CA ↑TCA ↑GCDCA ↓GDCA ↑TDCA in NASH versus controls. ↓GDCA ↑TDCA ↑GCDCA in NASH versus NAFL Other: CA, DCA, CDCA, and GDCA negatively correlated with CYP7B1 gene expression. GDCA positively correlated with CYP8B1 gene expression Hepatic mRNA levels: ↓CYP27A1 ↑CYP7B1 ↓HSD3B7 ↓CYP8B1 ↑BAAT in NASH versus controls. Protein levels: ↑CYP7B1 in NASH versus controls and NAFL
[35]	Feces	ND	Control: 25 NAFL: 12 NASH: 16	Control: 27 (20–35) NAFL: 28 (25–44) NASH: 33 (24–50)[a]	Control: 1.3 (0.5–7.6) NAFL: 1.6 (0.6–15.6) NASH: 4.3 (1.2–40)[a]	Control: 88.2 (72–117) NAFL: 95.4 (82.2–205.2) NASH: 102.6 (73.8–135)	Control: 5.1 (4.4–7.2) NAFL: 5.3 (0.1–5.8)[a] NASH: 5.9 (5.3–7.3)[a]	Bile acids: in feces: ↑Total bile acids, ↑CA, ↑CDCA in NASH versus controls. ↑Conjugated LCA in NASH versus NAFL. Primary fecal bile acids positively correlated with ALT, NAS and serum Takeda G Other: ↑C4 in NASH versus controls. Gut microbiota: ↓*Bacteroidetes*, *Clostridium leptum* negatively correlated with CA and LCA

Data extracted from the articles and presented either as mean ± SD, means ± SEM, or median (range). BA, bile acid; BACS, bile acid Co-A synthase; BAAT, bile acid-CoA:amino acid N-acyltransferase; BSEP, bile salt export pump; CA, cholic acid; DCA, deoxycholic acid; FGF21, fibroblast growth factor 21; *FPG*, Fasting plasma glycemia – data reported in sources as mmol/l was transformed to mg/dl by multiplying the reported value by 18; GCA, glyco-cholic acid; GCDCA, glyco-chenodeoxycholic acid; GDCA, glyco-deoxycholic acid; GHCA, glyco-hyocholic acid; GLCA, glyco-lithocholic acid; *HbA1c*, glycated hemoglobin; *HOMA-IR*, homeostatic model assessment for insulin resistance; LCA, lithocholic acid; MCA, muricholic acid; *NAS*, NAFLD activity score; TCDCA, tauro-chenodeoxycholic acid; TDCA, tauro-deoxycholic acid; TLCA, tauro-lithocholic acid; TUDCA, tauro-ursodeoxycholic acid; UDCA, ursodeoxycholic acid.
[a]NASH statistically different from controls.

CA, CDCA [33▪▪], GCA, TCA, GCDCA, DCA, and glyco-lithocholic acid [30▪▪] concentrations with hepatocyte ballooning have been reported, as well as elevated total bile acids [34], CA, CDCA [33▪▪], and both increased [30▪▪] or decreased [33▪▪] conjugated CA concentrations have been associated with high NAFLD activity score (NAS)]. Additionally, increased postprandial total bile acids have been reported in plasma and in urine [32], as well as increased fecal bile acid loss, which positively correlated with NAS and associated with decreased proportions of the gut microbiota phylum *Bacteroidetes* [35]. Furthermore, hepatic tissue-extracted bile acids were reported to be altered in NASH and NAFL patients resulting in qualitative changes in hepatic bile acid content, although inconsistent results were obtained between both studies [36,37]. Such results should be taken with caution, as bile acids were extracted from whole hepatic tissue, preventing discrimination between blood, biliary, and intrahepatocyte bile acid concentrations.

The expression of hepatic bile acid metabolism genes has also been studied in livers from NASH patients. Both increased [38] and decreased [36] CYP27A1 mRNA levels were reported, in addition to increased CYP7B1 gene and protein levels [36]. These results suggest possible modifications in the alternative bile acid synthesis pathway. Furthermore, increased expression of the gene encoding CYP7A1 [30▪▪,38,39▪] suggests that the classical bile acid synthesis pathway may also be activated. FGF19 levels, which regulate bile acid synthesis, have been reported as decreased [38] or unchanged [30▪▪] in NASH patients. Plasma C4 levels are, however, unchanged in NASH patients versus controls [30▪▪,34,39▪]. A summary of the hepatic gene expression changes reported in NASH patients is provided in Table 2.

We recently compared bile acid metabolism parameters in NASH patients matched for BMI and insulin resistance with obese individuals without NASH. No qualitative or quantitative differences in fasting plasma bile acids were found in NASH patients, with only GCA weakly correlating with steatosis ($r = 0.29$, $p = 0.03$). However, reanalysis by severity of insulin resistance revealed a tendency toward previously reported associations in fasting plasma bile acids (Table 1). These findings suggest that bile acid metabolism correlates with the metabolic comorbidities of NAFLD, rather than with the histopathological parameters of NASH itself [39▪]. It will be important in future studies to carefully assess NASH independently of common comorbidities to determine which changes, if any, in bile acid metabolism are specifically related to histological changes in NASH.

ARE BILE ACID CHANGES ASSOCIATED WITH NONALCOHOLIC FATTY LIVER DISEASE INDEPENDENTLY FROM OBESITY, INSULIN RESISTANCE, AND TYPE 2 DIABETES?

Assessment of the clinicobiological status of the studied populations appears essential to understand discordant results and allow their appropriate interpretation. In studies lacking information on the nutritional status [34,38,40] and biochemical markers of glucose metabolism as fasting plasma glycemia, homeostatic model assessment for insulin resistance (HOMA-IR), or HbA1c [30▪▪,33▪▪,34,38,40], it cannot be excluded that differences between the compared groups, other than histological parameters of NAFLD, influence the interpretation of the results. Many studies that provided the clinicobiological characteristics of the study groups are flawed by the comparison of NASH or NAFL patients with control groups presenting significantly lower BMI [30▪▪,31,32,34,38], HbA1c [34], insulin resistance, or fasting glycemia [31,32]. As discussed above, these metabolic comorbidities of NAFLD are correlated with bile acid alterations. It is thus difficult to conclude whether bile acid alterations are associated per se with NAFLD or with the associated insulin resistance. Appropriate statistical adjustment or patient matching could help to determine whether the associations are independent from potential confounding factors.

CONCLUSION

Although bile acid signaling targets are attractive candidates for the treatment of NAFLD, it remains undetermined whether bile acids are a driving force in the pathogenesis and progression of NAFLD or just innocuous bystanders. The current literature does not clearly support a causal role. Moreover, the large interindividual variation and diurnal bile acid changes in peripheral blood suggest that they are likely unsuitable as biomarkers for the diagnosis or leading to the decision of biopsy taking in NAFLD patients. However, this does not exclude that targeting bile acid signaling remains an attractive therapeutic target for NASH treatment. Further clinical studies assessing plasma bile acid profiles should carefully consider matching BMI and insulin resistance profiles across controls, NAFL, and NASH patients to more clearly assess the connection

between alterations in bile acid metabolism and these parameters of the metabolic syndrome.

Acknowledgements

None.

Financial support and sponsorship

The work was supported by Agence Nationale pour la Recherche (ANR-10-LABX-46 and ANR-10-INBS-08; ProFI project, 'Infrastructures Nationales en Biologie et Santé'; 'Investissements d'Avenir' call) and by Société Francophone du diabète. OCT received a Ph.D. fellowship from the French Ministry of Research. B.S. is a recipient of an advanced ERC grant (694717).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Diehl AM, Day C. Cause, pathogenesis, and treatment of nonalcoholic ■■ steatohepatitis. N Engl J Med 2017; 377:2063–2072.

This review summarizes clinical essentials on NASH.

2. Haas JT, Francque S, Staels B. Pathophysiology and mechanisms of non- ■ alcoholic fatty liver disease. Annu Rev Physiol 2016; 78:181–205.

This review summarizes basics of the pathophysiology of NAFLD.

3. Younossi Z, Henry L. Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. Gastroenterology 2016; 150:1778–1785.
4. Younossi ZM, Loomba R, Anstee QM, *et al.* Diagnostic modalities for non- ■ alcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. Hepatology (Baltimore, MD) 2018; 68:349–360.

This review presents a critical overview of the currently available diagnostic techniques for NAFLD.

5. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of ■ metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia and NAFLD. Gastroenterology 2017; 152:1679.e3–1694.e3.

This review describes the role of bile acids in the regulation of metabolism in physiology and in the metabolic syndrome.

6. Fang Q, Li H, Song Q, *et al.* Serum fibroblast growth factor 19 levels are decreased in Chinese subjects with impaired fasting glucose and inversely associated with fasting plasma glucose levels. Diabetes Care 2013; 36:2810–2814.
7. Gerhard GS, Styer AM, Wood GC, *et al.* A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. Diabetes Care 2013; 36:1859–1864.
8. Prinz P, Hofmann T, Ahnis A, *et al.* Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients. Front Neurosci 2015; 9: 199.
9. Brufau G, Stellaard F, Prado K, *et al.* Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. Hepatology (Baltimore, MD) 2010; 52:1455–1464.
10. Friedrich D, Marschall HU, Lammert F. Response of fibroblast growth factor 19 and bile acid synthesis after a body weight-adjusted oral fat tolerance test in overweight and obese NAFLD patients: a nonrandomized controlled pilot trial. BMC Gastroenterol 2018; 18:76.
11. Glicksman C, Pournaras DJ, Wright M, *et al.* Postprandial plasma bile acid responses in normal weight and obese subjects. Ann Clin Biochem 2010; 47:482–484.
12. Haeusler RA, Camastra S, Nannipieri M, *et al.* Increased bile acid synthesis and impaired bile acid transport in human obesity. J Clin Endocrinol Metab 2016; 101:1935–1944.
13. Cariou B, Chetiveaux M, Zaïr Y, *et al.* Fasting plasma chenodeoxycholic acid and cholic acid concentrations are inversely correlated with insulin sensitivity in adults. Nutr Metab 2011; 8:48.
14. Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. Int J Obes 2013; 37:1553–1559.
15. Steiner C, Othman A, Saely CH, *et al.* Bile acid metabolites in serum: ■■ intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. PLoS One 2011; 6:e25006.

This article describes inter and intraindividual bile acid variations, suggesting that bile acids are not good biomarkers for the diagnosis and staging of noncholestatic metabolic diseases.

16. Haeusler RA, Astiarraga B, Camastra S, *et al.* Human insulin resistance is ■ associated with increased plasma levels of 12α-hydroxylated bile acids. Diabetes 2013; 62:4184–4191.

This article links insulin sensitivity and bile acid metabolism, showing increased 12a hydroxylation and bile acid synthesis in obese and insulin resistant patients.

17. Haeusler RA, Pratt-Hyatt M, Welch CL, *et al.* Impaired generation of 12-hydroxylated bile acids links hepatic insulin signaling with dyslipidemia. Cell Metab 2012; 15:65–74.
18. Sun W, Zhang D, Wang Z, *et al.* Insulin resistance is associated with total bile acid level in type 2 diabetic and nondiabetic population: a cross-sectional study. Medicine (Baltimore) 2016; 95:e2778.
19. Ginos BN, Navarro SL, Schwarz Y, *et al.* Circulating bile acids in healthy adults respond differently to a dietary pattern characterized by whole grains, legumes and fruits and vegetables compared to a diet high in refined grains and added sugars: a randomized, controlled, crossover feeding study. Metabolism 2018; 83:197–204.
20. Wewalka M, Patti ME, Barbato C, *et al.* Fasting serum taurine-conjugated bile acids are elevated in type 2 diabetes and do not change with intensification of insulin. J Clin Endocrinol Metab 2014; 99:1442–1451.
21. Kimberly WT. O'Sullivan JF, Nath AK, *et al.* Metabolite profiling identifies anandamide as a biomarker of nonalcoholic steatohepatitis. JCI Insight 2017; 2:e92989.
22. Vincent RP, Omar S, Ghozlan S, *et al.* Higher circulating bile acid concentrations in obese patients with type 2 diabetes. Ann Clin Biochem 2013; 50:360–364.
23. Jørgensen NB, Dirksen C, Bojsen-Møller KN, *et al.* Improvements in glucose metabolism early after gastric bypass surgery are not explained by increases in total bile acids and fibroblast growth factor 19 concentrations. J Clin Endocrinol Metab 2015; 100:E396–E406.
24. Sonne DP, van Nierop FS, Kulik W, *et al.* Postprandial plasma concentrations of individual bile acids and FGF-19 in patients with type 2 diabetes. J Clin Endocrinol Metab 2016; 101:3002–3009.
25. Suhre K, Meisinger C, Döring A, *et al.* Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS One 2010; 5:e13953.
26. Lundåsen T, Gälman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. J Intern Med 2006; 260:530–536.
27. Barutcuoglu B, Basol G, Cakir Y, *et al.* Fibroblast growth factor-19 levels in type 2 diabetic patients with metabolic syndrome. Ann Clin Lab Sci 2011; 41:390–396.
28. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, *et al.* Farnesoid X nuclear ■■ receptor ligand obeticholic acid for noncirrhotic, nonalcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet Lond Engl 2015; 385:956–965.

This phase 2 trial shows the effectiveness of obeticholic acid for the treatment of NASH, despite elevated LDL-C as an adverse effect.

29. Harrison SA, Rinella ME, Abdelmalek MF, *et al.* NGM282 for treatment of ■■ nonalcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Lond Engl 2018; 391:1174–1185.

This phase 2 trial reports improved hepatic steatosis in NAFLD patients treated with the FGF19 analog NGM282, showing well-tolerated profiles.

30. Puri P, Daita K, Joyce A, *et al.* The presence and severity of nonalcoholic ■■ steatohepatitis is associated with specific changes in circulating bile acids. Hepatology (Baltimore, MD) 2017; 67:534–548.

This article reports circulating bile acids and hepatic bile acid metabolism alterations in NASH patients, and correlation of hepatic necroinflammatory lesions of NASH with plasma bile acid alterations.

31. Kalhan SC, Guo L, Edmison J, *et al.* Plasma metabolomic profile in nonalcoholic fatty liver disease. Metabolism 2011; 60:404–413.
32. Ferslew BC, Xie G, Johnston CK, *et al.* Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. Dig Dis Sci 2015; 60: 3318–3328.
33. Caussy C, Hsu C, Singh S, *et al.* Serum bile acid patterns are associated with ■■ the presence of NAFLD in twins, and dose-dependent changes with increase in fibrosis stage in patients with biopsy-proven NAFLD. Aliment Pharmacol Ther 2019; 49:183–193.

This article reports circulating bile acid alterations in NAFLD patients, and correlation of hepatic necroinflammatory lesions of NASH with plasma bile acid alterations.

34. Bechmann LP, Kocabayoglu P, Sowa JP, *et al.* Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. Hepatology (Baltimore, MD) 2013; 57:1394–1406.
35. Mouzaki M, Wang AY, Bandsma R, *et al.* Bile acids and dysbiosis in non-alcoholic fatty liver disease. PLoS One 2016; 11:e0151829.
36. Lake AD, Novak P, Shipkova P, *et al.* Decreased hepatotoxic bile acid composition and altered synthesis in progressive human nonalcoholic fatty liver disease. Toxicol Appl Pharmacol 2013; 268:132–140.
37. Aranha MM, Cortez-Pinto H, Costa A, *et al.* Bile acid levels are increased in the liver of patients with steatohepatitis. Eur J Gastroenterol Hepatol 2008; 20:519–525.
38. Jiao N, Baker SS, Chapa-Rodriguez A, *et al.* Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. Gut 2018; 67:1881–1891.
39. ▪ Legry V, Francque S, Haas JT, *et al.* Bile acid alterations are associated with insulin resistance, but not with NASH in obese subjects. J Clin Endocrinol Metab 2017; 102:3783–3794.

This is the only study reporting no independent association between circulating bile acids or bile acid metabolism and NASH, in BMI and insulin resistance-matched patients.

40. Dasarathy S, Yang Y, McCullough AJ, *et al.* Elevated hepatic fatty acid oxidation, high plasma fibroblast growth factor 21, and fasting bile acids in nonalcoholic steatohepatitis. Eur J Gastroenterol Hepatol 2011; 23:382–388.

MANUSCRIPT NUMBER 3

Chávez-Talavera & Wargny *et al.* Bile acids associate with glucose metabolism, but do not predict conversion to diabetes. Article submitted.

Bile acids associate with glucose metabolism, but do not predict conversion to diabetes.

Oscar Chávez-Talavera[*,1], Matthieu Wargny[*,2,3], Matthieu Pichelin[2,3], Amandine Descat[4], Emmanuelle Vallez[1], Mostafa Kouach[4], Edith Bigot-Corbel[5], Marielle Joliveau[2], Jean-Francois Goossens[4], Cédric Le May[3], Samy Hadjadj[2,3], Rémy Hanf[6], Anne Tailleux[#,1], Bart Staels[#,1], Bertrand Cariou[$,#,2,3]

[*] Co-first authors
[#] Co-last authors

[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011- EGID, F-59000 Lille, France.
[2]L'institut du thorax, Department of Endocrinology, CIC 1413 INSERM, CHU Nantes, Nantes, France
[3]L'institut du thorax, INSERM, CNRS, Univ. Nantes, CHU Nantes, Nantes, France
[4]Plateau de Spectrométrie de Masse, PSM-GRITA EA 7365, Faculté de Pharmacie, F-59000 Lille, France.
[5]Department of Biochemistry, CHU de Nantes, Nantes, France.
[6]Genfit SA, Loos, France.

[$] **Corresponding author:** Bertrand Cariou, Clinique d'Endocrinologie, Hôpital Guillaume & René Laennec, Boulevard Jacques Monod, Saint-Herblain, 44093 Nantes Cedex 1, France. Tel: 00 33 2 53 48 27 07; Fax: 00 33 2 53 48 27 08; e-mail: bertrand.cariou@univ-nantes.fr ORCID: 0000-0002-1580-8040

Word count: 3464 words

ABSTRACT

Objective: Bile acids (BAs) are signaling molecules controlling lipid and glucose metabolism. Since BA alterations are associated with obesity and insulin resistance, plasma BAs have been considered candidates to predict type 2 diabetes risk. We aimed to determine (1) the association of BAs with glucose homeostasis parameters and (2) their predictive association with the conversion from prediabetes to new-onset diabetes (NOD) in a prospective cohort study.

Design: 205 patients with impaired fasting glucose were followed each year during 5 years in the IT-DIAB cohort study. Twenty-one BA species and 7α-hydroxy-4-cholesten-3-one (C4), a marker of BA synthesis, were quantified by LC/MS-MS in plasma from fasted patients at baseline. Correlations between plasma BA species and metabolic parameters at baseline were assessed by Spearman's analyses and the association between BAs and NOD was determined using Cox proportional hazards models.

Results: Among the analyzed BA species, only HCA and the total HCA/total CDCA ratio negatively correlated with BMI and HOMA-IR. The total HCA/total CDCA ratio also correlated negatively with HbA_{1C}. However, plasma BA species were not independently associated with the conversion to NOD after adjustment with classical type 2 diabetes risk factors. The total HCA/total CDCA ratio, reflecting hepatic BA 6α-hydroxylation activity, was significantly associated with NOD, but this association was confounded by BMI and HOMA-IR.

Conclusions: Fasting plasma BAs are not useful clinical biomarkers for predicting NOD in patients with prediabetes. However, an unexpected association between 6α-hydroxylated BAs and glucose parameters was found, suggesting a role for this pathway in metabolic homeostasis.

Keywords: Bile acids, prediabetes, type 2 diabetes, hyocholic acid, C4

Abbreviations: 12α-hydroxylated (12α-OH); 6α-hydroxylated (6α-OH); 7α-hydroxy-4-cholesten-3-one (C4); Agence Nationale pour la Recherche (ANR); Bile acids (BAs); body mass index (BMI); chenodeoxycholic acid (CDCA); cholesterol associated with high density lipoproteins (HDL-c); cholesterol associated with low density lipoproteins (LDL-c); cholic acid (CA); CYP cholesterol 27α-hydroxylase (CYP27A1); CYP cholesterol 7α-hydroxylase (CYP7A1); cytochrome P450 (CYP); deoxycholic acid (DCA) electrochemiluminescent enzyme immunoassay (ECLIA); farnesoid X Receptor (FXR); fasting plasma glucose (FPG); glucagon-like peptide-1 (GLP-1); glycated hemoglobin (HbA_{1C}); hazard ratio (HR); high

molecular weight (HMW); Homeostasis model assessment of insulin resistance (HOMA-IR); hyocholic acid (HCA); hyocholic acid (HCA); hyodeoxycholic acid (HDCA); insulin resistance (IR); interquartile range (IQR); liquid chromatography tandem mass spectrometry (LC-MS/MS); methanol (MeOH); new onset diabetes (NOD); non-diabetic group (ND); Relationship between Insulin Sensitivity and Cardiovascular disease cohort (RISC); SFSTP (Société Française des Sciences et Techniques Pharmaceutiques); standard deviation (SD); Takeda G protein coupled Receptor 5 (TGR5); tandem mass spectrometry (MS/MS); total cholesterol (TC); triglycerides (TG); type 2 diabetes (T2D); ursodeoxycholic acid (UDCA).

SUMMARY BOX

What is already known about this subject?

- Bile acids are signaling molecules modulating metabolic homeostasis.
- Plasma BA changes are associated with obesity, insulin resistance and type 2 diabetes.
- It has been hypothesized for years that BAs could predict new onset type 2 diabetes (NOD).
- The predicting value of plasma BAs for the risk of NOD has not been assessed in longitudinal analyses yet.

What are the new findings?

- Total HCA and total HCA/total CDCA ratio negatively correlated with BMI and HOMA-IR.
- Most plasma BA species and ratios were not associated with NOD in longitudinal analyses, except for the total HCA/total CDCA ratio. This association, however, was no longer maintained after adjustment for classical type 2 diabetes risk factors.

How might this impact on clinical practice in the foreseeable future?

- The novel association of 6α-hydroxylated BAs and glucose homeostasis opens a new research question and warrants further study on 6α-hydroxylated BAs and metabolic disorders.

- Fasting plasma BAs are not useful clinical biomarkers for predicting type 2 diabetes.

INTRODUCTION

Bile acids (BAs) are cholesterol-derived molecules synthesized in perivenous hepatocytes *via* two pathways [1]. In adult humans, the majority of BAs are synthesized *via* the classical pathway. Its rate-limiting enzyme is the cytochrome P450 (CYP) cholesterol 7α-hydroxylase (CYP7A1). Plasma concentration of its metabolite 7α-hydroxy-4-cholesten-3-one (C4) is a circulating biomarker of the hepatic classical BA synthesis pathway. A minor fraction of BAs is synthesized in the adult liver by the alternative pathway, initiated by CYP sterol 27α-hydroxylase (CYP27A1). These pathways produce the primary BAs cholic acid (CA), chenodeoxycholic acid (CDCA) and hyocholic acid (HCA) (**Figure 1**). CA differs from CDCA and HCA through its 12α-hydroxylation (12α-OH) by the hepatic 12α-hydroxylase CYP8B1. BAs are subsequently conjugated to glycine or taurine and secreted through the hepatocyte's canalicular membrane into the bile to promote dietary lipid solubilization and absorption. By the action of the gut microbiota, conjugated BAs are deconjugated *via* bile salt hydrolases (BSH) and dehydroxylated and/or epimerized *via* hydroxysteroid dehydrogenases, to be transformed from primary BAs into secondary BAs: CA into DCA (also a 12α-OH BA), CDCA into UDCA and LCA, and HCA into HDCA [2]. Most BAs are recaptured in the ileum, and the remaining BAs are lost in the feces. Reabsorbed BAs return, *via* the portal blood, to the liver, where the majority of BAs are actively taken up by BA transporters located in the sinusoidal membrane of hepatocytes. Within hepatocytes, BAs are re-conjugated and re-secreted into bile, completing their entero-hepatic circulation. However, a minor fraction of venous portal BAs escapes hepatic reuptake and spill over in the systemic circulation, hence reaching peripheral organs.

Beside their physicochemical role in dietary lipid solubilization and absorption, BAs have emerged as signaling molecules that bind receptors, such as the Farnesoid X Receptor (FXR) and the Takeda G protein coupled Receptor 5 (TGR5), to initiate signaling pathways. As such, BAs not only modulate their own synthesis in the liver, but also lipid and energy homeostasis and inflammation [3,4]. Both preclinical and, mostly observational, human studies have demonstrated that BAs and their receptors are involved in the regulation of glucose metabolism by controlling, among others, insulin signaling, hepatic glucose production, glucose utilization and the secretion of glucagon-like peptide-1 (GLP-1) (reviewed in [3,5,6]).

Plasma BA profiles are remodeled under several pathological conditions. Since FXR and TGR5 are activated by different BA species, changes in BA pool composition or size potentially modulate the metabolic pathways regulated by these receptors. Quantitative and/or qualitative BA alterations have been reported in obesity, insulin resistance (IR) and

type 2 diabetes (T2D). These features are strongly and bidirectionally associated with non-alcoholic fatty liver disease (NAFLD). BA alterations have been associated therefore with NAFLD, but the current scientific evidence does not clearly support a causal role (reviewed in [7]). We previously found an inverse relationship between plasma CDCA and CA concentrations and insulin sensitivity assessed during a hyperinsulinemic euglycemic clamp in a variety of subjects, including healthy volunteers, obese and T2D patients [8]. In addition, Haeusler *et al.* have reported that the ratio of 12α-OH/non-12α-OH BAs, reflecting CYP8B1 12α-hydroxylase activity, is regulated by insulin receptor signaling *via* Akt-FoxO1[9,10], hence being associated with hepatic insulin sensitivity in humans and mice [9,10]. Importantly, several studies in humans have reported that plasma BA levels or the 12α-OH/non-12α-OH ratio increase after Roux-en-Y gastric bypass, biliopancreatic diversion and sleeve gastrectomy, but not after gastric banding [11,12]. While studies in either FXR- [13,14] and TGR5-deficient [15] mice support a critical role for BAs in the metabolic improvements after bariatric surgery, a causal role of BA changes in mediating the beneficial effects of bariatric surgery in humans remains debates [16].

These data establish that BA metabolism alterations occur in obesity and/or IR in humans. Whether BAs play a causal role in the pathophysiology of the metabolic syndrome and progression to T2D is unknown. Although BAs have been proposed as biomarkers of IR and T2D risk, their clinical utility has not been evaluated in a longitudinal study yet. To address this question, we determined, for the first time, the predictive association of fasting plasma BAs with the conversion from prediabetes to new onset T2D (NOD) in a 5-year prospective cohort study (IT-DIAB study). Additionally, we investigated the correlations between circulating BA species and glycemic parameters in individuals with prediabetes.

METHODS

Study population

The study population belongs to the IT-DIAB study (NCT01218061). Briefly, the IT-DIAB study is a 5-year prospective, observational study designed to identify new biomarkers of T2D risk in a population with prediabetes. The population was recruited in the occupational centers of three French cities: Nantes, Saint-Nazaire and Lille. The patient samples from Lille were not available for BA measurements and were therefore not included in the present analysis. The institutional ethics committee approved the protocol, and all the reported investigations have been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008. All patients underwent a baseline visit between June 2010 and February 2013, including a medical interview, signing of the informed consent and self-administered questionnaire of diabetes risk score, physical examination (including body

weight, height, waist and hip circumference measurements) and blood sampling. The diabetes risk score and the fatty liver index (FLI) were computed as previously described [17,18]. Patients without history of diabetes and with impaired fasting glucose (FPG) (*i.e.* fasting plasma glucose (FPG) between 110 and 125 mg/dL) were eligible for the IT-DIAB study. The main non-inclusion criteria were a history of treatment with oral anti-diabetic agents or insulin (with the exception of gestational diabetes), severe coagulation disorder or thrombocytopenia (platelets levels < 100,000/mm^3), severe renal insufficiency (defined using MDRD equation as eGFR <30mL/min.1.73m^2), severe liver impairment (prothrombin ratio <50%), severe psychiatric disorders, alcohol abuse (estimated >30 g/day), patient's opposition or inability to participate at least 5 years in the study.

For the present analysis, we also secondarily excluded the population in which plasma samples was not available for BA measurements (n=7) and/or without follow-up visit (n=24), with concomitant statin (n=68) and/or fibrate (n=11) therapy, and/or with baseline HbA_{1C} ≥6.5% (n=16) or missing HbA_{1C} (n=1). Ultimately, 205 patients with at least one follow-up visit were considered for the present analysis as shown in the flow chart (**Supplemental figure 1).**

Follow-up and conversion to new onset diabetes (NOD)

The end of the follow-up occurred at the fifth yearly visit, or prematurely if the patient met one of the following criteria: patient's withdrawal or loss to follow-up, inappropriate prescription of anti-diabetic agent, bariatric surgery or death. NOD was defined by a FPG value ≥126 mg/dL or a plasma glucose ≥200 mg/dL after 2-hour OGTT.

Biochemical analyses

During the baseline visit, peripheral venous blood samples were obtained in the morning after overnight fasting for biological analysis and the constitution of a biocollection. Standard biological analyses included FPG and HbA_{1C}. Frozen heparinized plasma was used for insulin measurement by electrochemiluminescent enzyme immunoassay (ECLIA) using the Cobas e automated clinical analyzer system (Roche Diagnostics, Meylan, France). Plasma high molecular weight (HMW) adiponectin levels were measured by ECLIA on an automated clinical analyzer system Lumipulse G600 (Fujirebio, Les Ulis, France). Homeostasis model assessment of insulin resistance (HOMA-IR) was defined according to the equation proposed by Matthews *et al.* [19].

Plasma bile acids and C4 quantification

All measurements were performed on morning-collected fasting EDTA-plasma samples. Twenty-one BA species were quantified by liquid chromatography tandem mass

spectrometry (LC-MS/MS) as described previously [20]. Briefly, 50 µL plasma were mixed with 25 µL internal standard solution containing 5 deutered BAs (D4-CA, D4-GCA, D4-TCA, D4-CDCA, D4-GCDCA) at 0.5 µM in methanol (MeOH) and 225 µL cold MeOH, vortexed for 30 seconds and stored for 20 min at -20°C. After centrifugation at 10,000 g for 10 min at +4°C, the supernatant was evaporated. The pellet was then dissolved in MeOH/water (v/v) and injected in the LC-MS/MS system for BAs separation and quantification. BAs were separated on a Symmetry C18 Luna column (250 mm× 2.1 mm, particle size 5 µm; Phenomenex, Le Pecq, France). The oven temperature was set at 30°C. Solvent A was 20 mM ammonium acetate, adjusted to pH 8 and solvent B was 70% acetonitrile/30 % MeOH. Solvents were delivered at a total flow rate of 500 $\mu L.min^{-1}$. After 5 min plateau establishment with 35% B, the gradient profile was changed linearly from 35 % B to 90% B in 30 min, followed by a 2 min plateau with 90% B. Column re-equilibration was performed for 4 min. The injection cycle was 41 min. The quantification of 21 different BAs was then performed by high-performance liquid chromatography (UFLC-XR device; Shimadzu, Kyoto, Japan) coupled to tandem mass spectrometry (MS/MS) (QTRAP 5500 hybrid system, equipped with a Turbo VTM ion source; Sciex, Foster City, CA, USA). Instrument control, data acquisition and processing were performed using the associated Analyst 1.5.2 software (Sciex, Concord, ON, Canada). The standard curves range from 1.5 nM to 2000 nM (TCDCA, GDCA, GCDCA, TDCA, GLCA, TUDCA, GCA, TLCA and GUDCA), 3 nM to 2000 nM (for TCA, THCA, GHCA GHDCA and THDCA) and 15 nM to 2000 nM (CA, UDCA, LCA, DCA, CDCA, HDCA and HCA).

Plasma 7alpha-hydroxy-4-cholesten-3-one (C4) was determined as previously described [20]. Briefly, 100 µL of plasma were mixed with 500 µL of purified water, 50 µL of the 0.06 µM D7-C4 internal standard solution and 60 µL of 1M hydrochloric acid. Solid-phase extraction was performed using cartridges SPE (Bound Elut C18 200 mg) pre-conditioned with MeOH. The analyte was eluted with MeOH and the eluted solution, evaporated. The residue was dissolved in 80 µL MeOH and mixed with 10 µL ammonium acetate buffer at 10 mM, pH 6.5, upon which the sample was centrifuged at 13 400 g and 10°C for 10 min. The supernatant was evaporated. The pellet was then dissolved in 100 µL of MeOH/water (v/v) and 2 µL injected into the LC-MS/MS system. The isolation of C4 was carried out on a Kinetex Coreshell C18 column (100x2.1 mm, 5 µm Phenomenex, Le Pecq, France). The oven temperature was 30°C. Solvent A was formic acid 0.1% and solvent B was acetonitrile. Solvents were delivered at a total flow rate of 500 µL/min. After a 2 min plateau with 75% B, the gradient profile was changed from 75% B to 95% B linearly in 5 min, followed by a 2 min plateau with 95% B. The column was re-equilibrated for 3 min. The injection cycle was 12 min. MS analysis was carried out in positive ionization mode. The ion source parameters were set as follows: ion spray voltage, 5500V; nebulizer gas (air) and curtain gas (nitrogen)

flows, 50 and 20 psi, respectively; source temperature, 650°C with auxiliary gas flow (air), 50 psi; declustering potential, 100V; collision cell exit potential, 15V; collision energy, 31V. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 with a dwell time of 100 ms in each transition. Instrument control, data acquisition and processing were performed using the associated Analyst 1.5.2 software (AB Sciex, Concord, ON, Canada). The standard curve ranges from 1 nM to 200 nM. All the analytical methods were validated using the SFSTP (Société Française des Sciences et Techniques Pharmaceutiques) guidelines.

Statistical analyses

Categorical variables are presented using population size (%) and related between-group comparisons were tested using Fisher's exact test. Quantitative variables are presented using mean ± standard deviation (SD) or median (interquartile range, IQR) in case of skewed distribution, with appropriate comparison tests for non-paired series (respectively, Student's T-test or Wilcoxon rank-sum test). The association between two quantitative variables is presented using the Spearman's R correlation coefficient.

For the longitudinal study, Kaplan-Meier survival curves were drawn using conversion to NOD as the event of interest, and the terciles of the distribution of different explanatory variables to define the studied groups. The association between the different BAs and the conversion to NOD was also studied using the Cox model based on the proportional hazard hypothesis, before and after adjustment on the classical risk factors: age, BMI, FPG and HbA_{1C}.

A p value < 0.05 was deemed statistically significant. All analyses were performed using R software version 3.5.1 (The R Foundation for Statistical Computing, R Core Team, Vienna, 2018), with the RStudio interface.

RESULTS

Study participants

The baseline clinical-biological parameters of the analytical study population of IT-DIAB are shown **Table 1**. In accordance with inclusion criteria, all participants had prediabetes at baseline with IFG (mean FPG ± SD: 116 ± 4 mg/dL) and a median HbA_{1C} at 5.8% (39.9 mmol/mol). The participants were middle aged (mean age ± SD: 56.0 ± 9.9 years), overweight (mean BMI ± SD: 29.4 ± 6.3 kg/m^2), with 68.8% males. Participants had insulin resistance with a mean ± SD HOMA-IR at 3.82 ± 2.74 and NAFLD with median FLI > 60% (median, [IQR]: 62.4 [36.0-83.4])[18].

Plasma bile acid species and conversion to type 2 diabetes

To determine the potential clinical relevance of plasma BAs as biomarkers of T2D risk, we analyzed whether baseline BAs predict the risk of conversion to NOD in this population with prediabetes after a median follow-up of 59.6 months (min: 0.7 - max: 73.1). Interestingly, Kaplan-Meier survival curves show that patients upper the third tertile of the ratio of total HCA/total CDCA displayed a significantly lower risk of conversion to NOD (p=0.0039) (**Figure 2F**). In the univariate Cox model, the total HCA/total CDCA ratio was significantly associated with NOD conversion (HR: 0.77 [0.60; 0.98]; p=0.035), but the association was no longer significant after adjustment (**Table 2**). By contrast, none of the other plasma BA parameters (total plasma BAs (including total free, total conjugated, total primary and total secondary BAs), total CA, total CDCA, total DCA concentrations) nor the 12α-OH/non-12α-OH BA ratio were associated with the risk of NOD, both in univariate and multivariate Cox models (**Figure 2B, 2C**). Particularly, plasma total HCA and C4 concentrations also failed to be significantly associated with the conversion to NOD (HR: 0.83 [0.64; 1.07] and 1.19 [0.95; 1.50], respectively) (**Figure 2D, 2E**). However, well-known risk factors for diabetes (BMI, FPG, HbA_{1C} and HOMA-IR) **(Table 2)** were significantly associated with NOD conversion in both univariate and multivariate Cox models (**Figure 2A**).

Cross-sectional associations between plasma bile acid species and metabolic parameters at baseline

To further investigate the link between BA metabolism and glucose homeostasis, we next assessed the correlations between plasma BA species and the metabolic parameters in the IT-DIAB participants at baseline (**Table 3**; significant correlations are plotted in **Supplemental Figure 2**).

Total concentration of the 6α-hydroxylated HCA negatively correlated with BMI, HOMA-IR and FLI, suggesting that 6α-hydroxylation could be implicated in the pathophysiology of obesity, insulin resistance and NAFLD. These correlations were even more significant for the total HCA/total CDCA ratio, reflecting the rate of hepatic 6α-hydroxylation of CDCA to form HCA, which in addition negatively correlated with HbA_{1C} (**Table 3**). Inversely, plasma C4 levels were significantly and positively associated with BMI, HOMA-IR and FLI. There was also a strong inverse correlation between C4 and total HCA (R=-0.33; p<0.0001) (**Supplemental Figure 3**), indicating the existence of a regulated balance of the classical and alternative pathways in prediabetes. The correlations between the metabolic parameters and the individual BA species are presented in the **Supplemental Table 2**.

CONCLUSIONS

This is, to the best of our knowledge, the first longitudinal study assessing the clinical relevance of plasma BAs as biomarkers of T2D risk in a population with prediabetes. Our results indicate that peripheral BAs do not predict the transition from prediabetes to NOD. Indeed, in our cohort, baseline plasma concentrations of the different BA species were not independently associated with the risk of NOD after a 5-year longitudinal follow-up. In addition, qualitative BA pool parameters, such as the 12α-OH/non-12α-OH (previously reported to reflect hepatic insulin resistance [9]) or conjugated/free BA ratios, did not predict NOD conversion.

It is noteworthy that the total HCA/total CDCA ratio, reflecting the rate of hepatic 6α-hydroxylation of CDCA to form HCA, predicted the risk of NOD in univariate survival analyses. However, the predicting value of the total HCA/total CDCA ratio did not resist to adjustment for classical T2D risk factors (age, BMI, FPG and HbA_{1C}), indicating that these BAs rather co-segregate with metabolic alterations in prediabetes. Accordingly, a negative correlation of baseline total HCA concentrations was observed with BMI, insulin resistance (*i.e.* HOMA-IR), and FLI. HOMA-IR and BMI associate with FLI, as expected, since FLI is a good predictor of NAFLD, and since NAFLD is a common co-morbidity of the metabolic syndrome. Therefore, our results show that although BAs do not predict NOD, 6α-hydroxylated (6α-OH) BAs negatively correlate with metabolic alterations predisposing to T2D, suggesting a potential pathophysiological link between glucose metabolism and hepatic 6α-hydroxylation.

The synthesis routes of the 6α-OH HCA (also known as γ-muricholic acid) differ between species. In humans, hepatic 6α-hydroxylation of CDCA *via* CYP3A4[21] produces HCA. Due to its minor concentrations, HCA is often neglected in human plasma BA analyses notwithstanding its high concentrations in human fetal bile [22]. The total HCA/total CDCA ratio can be considered a marker of 6α-hydroxylation activity in human liver. Our data revealed a negative correlation of BMI and HOMA-IR with total HCA (**Table 3**), due to conjugated HCA species, in line with previous reports[23,24], thus identifying a potential role of hepatic 6α-hydroxylation activity in glucose homeostasis. In line, previous studies reported an association between a polymorphism in *CYP3A4* and the risk of T2D in a Japanese population[25] and reduced expression and activity of CYP3A4 in livers from diabetic donors[26,27]. While the underlying molecular mechanisms remain to be elucidated, these data strongly suggest a link between HCA synthesis, CYP3A4 activity (the enzyme catalyzing the synthesis of HCA and HDCA from CDCA and LCA, respectively) and metabolic homeostasis. Interestingly, HCA and its secondary intestinal microbiota-derived HDCA species are the major BA species in pigs [28], rendering this animal model suitable for the study of these BA species. In contrast, HCA concentrations are minor in rodents due to

the predominant transformation of CDCA into α-muricholate, rendering mice and rats less appropriate to study 6α-OH BAs and less relevant for human physiology.

While plasma C4, a marker of the rate of the classical BA synthesis pathway, failed to be significantly associated with the risk of NOD, it was positively correlated with both BMI and HOMA-IR, a signature of metabolic syndrome, as previously described[29,30]. Conversely, plasma C4 levels were negatively correlated with adiponectin reinforcing the hypothesis for a link between the classical BA synthesis pathway and insulin resistance. Interestingly, C4 was positively associated with FLI, further confirming the role of BAs in hepatic fat metabolism. In line with this observation, inhibiting BA synthesis with the FXR agonist obeticholic acid has recently demonstrated its clinical efficacy in lowering steatosis in a randomized controlled trial [31]. C4 and total HCA were negatively correlated, suggesting that metabolic syndrome simultaneously drives increased BA synthesis *via* the classical pathway while inhibiting the 6α-hydroxylation of CDCA.

In the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) cohort, the 12α-OH/non-12α-OH BA ratio was associated with insulin sensitivity [10]. Surprisingly, the 12α-OH/non-12α-OH BA ratio was neither associated with HOMA-IR nor with the risk of NOD in the IT-DIAB study. This divergence could be due to the differences in the range of insulin resistance between the patients from IT-DIAB and those in the previously published studies, since our study included only individuals with prediabetes (*i.e.* with IFG), whereas the RISC cohort included a broader range of patients. Furthermore, we assessed insulin resistance by calculating HOMA-IR, whereas it was assessed during hyperinsulinemic euglycemic clamps in the RISC cohort.

The main strengths of our study are the prospective design and the duration of the follow-up, with substantial numbers of patients converting to NOD, and the quality and the frequency of the data collection, including a consultation with a physician and biological follow-up at least on a yearly basis, allowing timely detection of the primary outcome. Also, complete and precise assessment of the BA profiles with 21 species, as well as C4, was obtained. Our study presents some limitations however. Only fasting plasma BA samples were measured and post-prandial samples are lacking. The observational design of the study allows us to conclude on associations, but not on causality. Finally, the exploratory correlations analysis did not take into account the α-risk inflation, and the statistical significance of the results should be considered with caution.

In conclusion, while some quantitative and qualitative BA alterations are associated with metabolic parameters, most plasma BA species are not useful biomarkers to predict NOD in patients with prediabetes. The role of the 6α-hydroxylation pathway, with CYP3A4 activity and HCA synthesis, in the pathophysiology of T2D is intriguing and requires further confirmation. The total HCA/total CDCA ratio, as a surrogate of hepatic 6α-hydroxylation,

could be a good biomarker of metabolic complications such as T2D even though it does not useful to predict NOD.

ACKNOWLEDGEMENTS

We thank Marina Blanchard and Béatrice Lamour (CIC Endocrino-Nutrition, 1413 INSERM, CHU Nantes) for their help in data collection, as well as Tanguy Roman and his team for their precious help in data management. We also thank the biological resource center for biobanking (CHU Nantes, Hôtel Dieu, Centre de ressources biologiques (CRB), Nantes, F-44093, France (BRIF: BB-0033-00040)). We thank Paul Emile Hecquet for excellent technical assistance.

Duality of interest

BC has received research funding from Amgen, Pfizer and Sanofi and Regeneron Pharmaceuticals Inc. and has served on scientific advisory boards and received honoraria or consulting fees from Abbott, Akcea, Amgen, AstraZeneca, Genfit, Pierre Fabre, Eli Lilly and Company, MSD Merck & Co., Novo Nordisk, Regeneron, Sanofi and Servier, outside the submitted work. **SH** personnally or collectively received research grants, honoraria and fees from the following companies in the last 3 years: Astra Zeneca, Bayer, Boehringer Ingelheim, Dinno Santé, Eli Lilly, LVL, Johnson & Johnson, Medtronic, MSD, Novo Nordisk, Novartis, Pierre Fabre Santé, Sanofi, Servier, Valbiotis; outside the submitted work. **RH** is employed by Genfit SA. The other authors have no relevant conflicts of interest to disclose.

Funding

IT-DIAB was funded by an ISI grant from OSEO-API, France to Genfit (coordinator of IT-Diab consortium) and CHU de Nantes, France. This work was also supported by Agence Nationale pour la Recherche (ANR-10-LABX-46 and ANR-10-INBS-08; ProFI project, “Infrastructures Nationales en Biologie et Santé”; “Investissements d'Avenir” call), by a grant from Société Francophone du Diabète (SFD) and by an internal grant of CHU Nantes (RC17_0470). OCT received a PhD fellowship from the French Ministry of Research. BS is a recipient of an Advanced ERC Grant (694717).

Contributions

BS and BC designed the study. MW performed the statistical analysis. MW, MP and MJ collected the data. OCT, AD, EV, MK, EBC and JFG performed the LC-MS and biochemical analyses. MW, MP, CLM, RH and SH contributed to the discussion and reviewed the manuscript. OCT, MW, AT, BS and BC wrote the first draft, edited and reviewed the manuscript. MW and BC are investigators of the IT-DIAB study. All the authors have read and approved the final version of the manuscript. BC is the guarantor of this study.

References

1 Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003;**72**:137–74. doi:10.1146/annurev.biochem.72.121801.161712

2 Hofmann AF, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci CMLS* 2008;**65**:2461–83. doi:10.1007/s00018-008-7568-6

3 Chávez-Talavera O, Tailleux A, Lefebvre P, *et al.* Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia and NAFLD. *Gastroenterology* Published Online First: 15 February 2017. doi:10.1053/j.gastro.2017.01.055

4 Lefebvre P, Cariou B, Lien F, *et al.* Role of bile acids and bile acid receptors in metabolic regulation. *Physiol Rev* 2009;**89**:147–91. doi:10.1152/physrev.00010.2008

5 van Nierop FS, Scheltema MJ, Eggink HM, *et al.* Clinical relevance of the bile acid receptor TGR5 in metabolism. *Lancet Diabetes Endocrinol* Published Online First: 14 September 2016. doi:10.1016/S2213-8587(16)30155-3

6 Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap--bile acids in metabolic control. *Nat Rev Endocrinol* 2014;**10**:488–98. doi:10.1038/nrendo.2014.60

7 Chávez-Talavera O, Haas J, Grzych G, *et al.* Bile acid alterations in nonalcoholic fatty liver disease, obesity, insulin resistance and type 2 diabetes: what do the human studies tell? *Curr Opin Lipidol* Published Online First: 18 March 2019. doi:10.1097/MOL.0000000000000597

8 Cariou B, Chetiveaux M, Zaïr Y, *et al.* Fasting plasma chenodeoxycholic acid and cholic acid concentrations are inversely correlated with insulin sensitivity in adults. *Nutr Metab* 2011;**8**:48. doi:10.1186/1743-7075-8-48

9 Haeusler RA, Pratt-Hyatt M, Welch CL, *et al.* Impaired generation of 12-hydroxylated bile acids links hepatic insulin signaling with dyslipidemia. *Cell Metab* 2012;**15**:65–74. doi:10.1016/j.cmet.2011.11.010

10 Haeusler RA, Astiarraga B, Camastra S, *et al.* Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. *Diabetes* 2013;**62**:4184–91. doi:10.2337/db13-0639

11 Albaugh VL, Banan B, Ajouz H, *et al.* Bile acids and bariatric surgery. *Mol Aspects Med* 2017;**56**:75–89. doi:10.1016/j.mam.2017.04.001

12 Dutia R, Embrey M, O'Brien CS, *et al.* Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. *Int J Obes 2005* 2015;**39**:806–13. doi:10.1038/ijo.2015.1

13 Ryan KK, Tremaroli V, Clemmensen C, *et al.* FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014;**509**:183–8. doi:10.1038/nature13135

14 Albaugh VL, Banan B, Antoun J, *et al.* Role of Bile Acids and GLP-1 in Mediating the Metabolic Improvements of Bariatric Surgery. *Gastroenterology* 2019;**156**:1041-1051.e4. doi:10.1053/j.gastro.2018.11.017

15 McGavigan AK, Garibay D, Henseler ZM, *et al.* TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut* 2015;**[Epub ahead of print]**:gutjnl-2015-309871. doi:10.1136/gutjnl-2015-309871

16 Jørgensen NB, Dirksen C, Bojsen-Møller KN, *et al.* Improvements in glucose metabolism early after gastric bypass surgery are not explained by increases in total bile acids and fibroblast growth factor 19 concentrations. *J Clin Endocrinol Metab* 2015;**100**:E396-406. doi:10.1210/jc.2014-1658

17 Lindström J, Tuomilehto J. The diabetes risk score: a practical tool to predict type 2 diabetes risk. *Diabetes Care* 2003;**26**:725–31.

18 Bedogni G, Bellentani S, Miglioli L, *et al.* The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;**6**:33. doi:10.1186/1471-230X-6-33

19 Matthews DR, Hosker JP, Rudenski AS, *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–9.

20 Legry V, Francque S, Haas JT, *et al.* Bile acid alterations are associated with insulin resistance, but not with NASH in obese subjects. *J Clin Endocrinol Metab* Published Online First: 4 August 2017. doi:10.1210/jc.2017-01397

21 Deo AK, Bandiera SM. Identification of human hepatic cytochrome p450 enzymes involved in the biotransformation of cholic and chenodeoxycholic acid. *Drug Metab Dispos Biol Fate Chem* 2008;**36**:1983–91. doi:10.1124/dmd.108.022194

22 Setchell KD, Dumaswala R, Colombo C, *et al.* Hepatic bile acid metabolism during early development revealed from the analysis of human fetal gallbladder bile. *J Biol Chem* 1988;**263**:16637–44.

23 Wewalka M, Patti M-E, Barbato C, *et al.* Fasting serum taurine-conjugated bile acids are elevated in type 2 diabetes and do not change with intensification of insulin. *J Clin Endocrinol Metab* 2014;**99**:1442–51. doi:10.1210/jc.2013-3367

24 Suhre K, Meisinger C, Döring A, *et al.* Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. *PloS One* 2010;**5**:e13953. doi:10.1371/journal.pone.0013953

25 Yamada Y, Matsuo H, Watanabe S, *et al.* Association of a polymorphism of CYP3A4 with type 2 diabetes mellitus. *Int J Mol Med* 2007;**20**:703–7.

26 Dostalek M, Court MH, Yan B, *et al.* Significantly reduced cytochrome P450 3A4 expression and activity in liver from humans with diabetes mellitus. *Br J Pharmacol* 2011;**163**:937–47. doi:10.1111/j.1476-5381.2011.01270.x

27 Jamwal R, de la Monte SM, Ogasawara K, *et al.* Nonalcoholic Fatty Liver Disease and Diabetes Are Associated with Decreased CYP3A4 Protein Expression and Activity in Human Liver. *Mol Pharm* 2018;**15**:2621–32. doi:10.1021/acs.molpharmaceut.8b00159

28 Spinelli V, Lalloyer F, Baud G, *et al.* Influence of Roux-en-Y gastric bypass on plasma bile acid profiles: a comparative study between rats, pigs and humans. *Int J Obes 2005* 2016;**40**:1260–7. doi:10.1038/ijo.2016.46

29 Haeusler RA, Camastra S, Nannipieri M, *et al.* Increased Bile Acid Synthesis and Impaired Bile Acid Transport in Human Obesity. *J Clin Endocrinol Metab* 2016;**101**:1935–44. doi:10.1210/jc.2015-2583

30 Steiner C, Othman A, Saely CH, *et al.* Bile acid metabolites in serum: intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. *PloS One* 2011;**6**:e25006. doi:10.1371/journal.pone.0025006

31 Neuschwander-Tetri BA, Loomba R, Sanyal AJ, *et al.* Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet Lond Engl* 2015;**385**:956–65. doi:10.1016/S0140-6736(14)61933-4

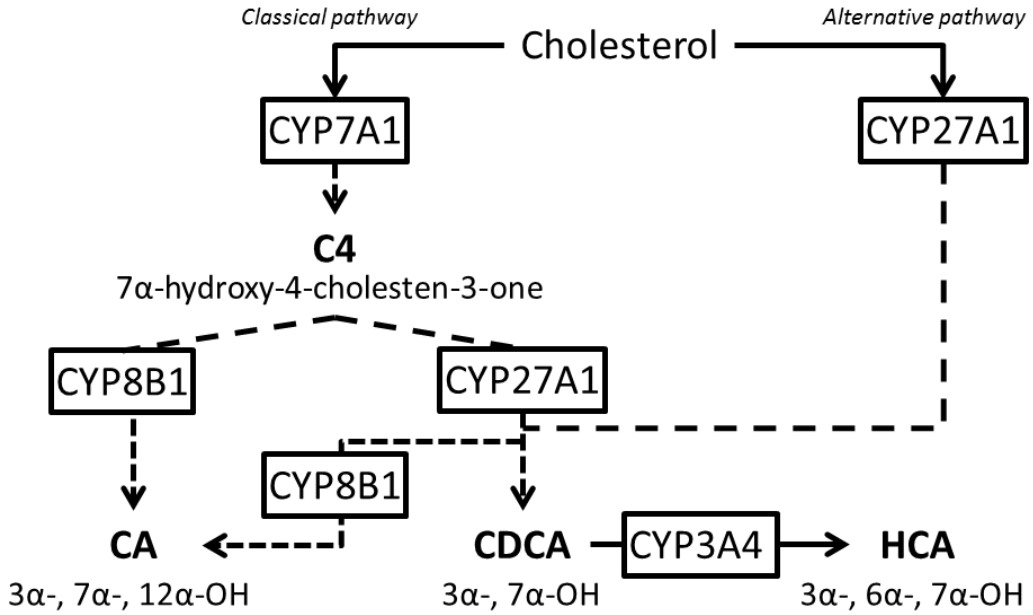


Figure 1. Primary bile acid synthesis pathways.

Legend: Primary BAs are synthesized from cholesterol within hepatocytes by two pathways. The classical pathway synthesizes most of BAs and its rate-limiting enzyme is the CYP7A1, whose synthesis rate biomarker is the metabolite C4. The alternative pathway's rate-limiting enzyme is the CYP27A1. The end products of these pathways are the primary BAs: CA is a 3α-, 7α-, 12α-OH BA whose synthesis is mediated by the 12α-hydroxylase CYP8B1. CDCA is 3α-, 7α-OH and is produced via both the classical and the alternative BA synthesis pathways via the enzyme CYP27A1. HCA is 3α-, 6α-, 7α-OH and is synthesized upon 6α-hydroxylation of CDCA via the CYP3A4.

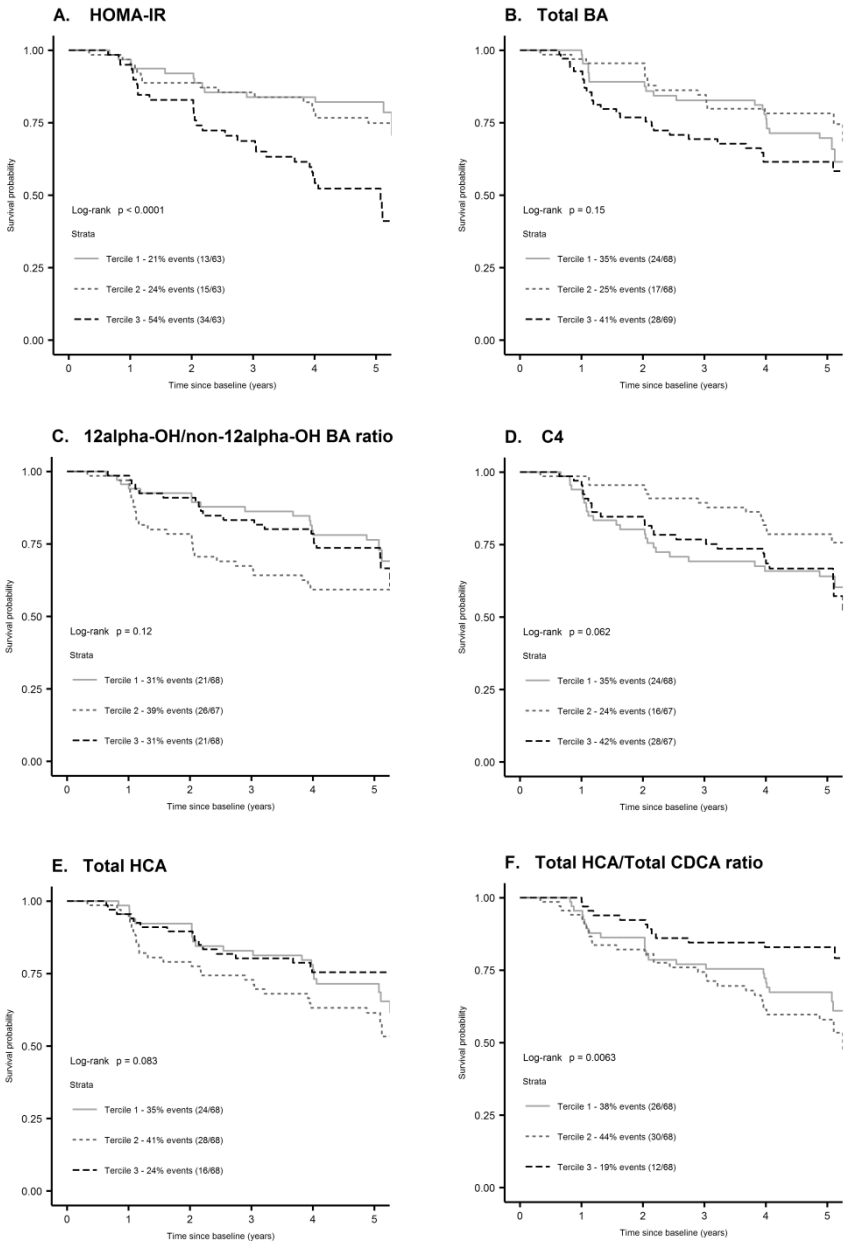


Figure 2. Survival curves for conversion from prediabetes to type 2 diabetes in the IT-DIAB study.

Legend: Survival curves for conversion to new onset diabetes according to HOMA-IR (A), total bile acids (B), 12αOH/non12αOH BA ratio (C), C4 (D), total HCA (E) and total HCA/total CDCA ratio (F)**.** Kaplan-Meier's survival curves, each parameter being presented by groups created using tertiles as thresholds (T1 < first tertile < T2 < second tertile < T3). BA clusters are defined in the **Supplemental table 1**.

Table 1

	N=205	Available data
Clinical characteristics		
Age (y)	56.0 ± 9.9	205/205
Sex (female)	64 (31.2 %)	205/205
BMI (kg/m²)	29.4 ± 6.3	205/205
Diabetes risk score	13.9 ± 4.7	205/205
Waist circumference (cm)	97.3 ± 14.2	203/205
Hip circumference (cm)	104.8 ± 13.3	201/205
Waist:hip ratio	0.93 ± 0.08	201/205
Lipid profile		
Total cholesterol (mg/dL)	225.3 ± 38.5	205/205
Triglycerides (mg/dL)	133.9 ± 81.7	204/205
LDL-c (mg/dL)	144.6 ± 35.1	201/205
HDL-c (mg/dL)	53.7 ± 15.7	204/205
Non-HDL-c (mg/dL)	171.2 ± 41.0	204/205
Glucose homeostasis		
FPG (mg/dL)	116 ± 4	205/205
HbA_{1c} (%)	5.8 (5.5-6.0)	205/205
HbA_{1c} (mmol/mol)	39.9 (36.6-42.1)	205/205
Insulin (mUI/L)	10.6 (7.2-16.4)	189/205
HOMA-IR	3.82 ± 2.74	189/205
Adiponectin (µg/L)	3.94 ± 2.27	190/205
Fatty liver index	62.4 (36.0-83.4)	199/205

Table 1. Baseline characteristics of the studied population.

Legend: Data are presented as population size (%), mean ± SD or median (25^{th} – 75^{th} percentile), according to their distribution.

Body mass index (BMI); FPG: fasting plasma glucose; Homeostasis model assessment of insulin resistance (HOMA-IR).

Table 2

	HR ($_{95\%}$CI)	
	Not adjusted	**Adjusted**
Adjustment factors		
Age (+1 SD)	1.14 (0.88; 1.48)	
BMI (+1 SD)	**1.32 (1.08; 1.61)****	
FPG (+1 SD)	**1.62 (1.28; 2.06)*****	
HbA_{1c} (+1 SD)	**1.66 (1.26; 2.19)*****	
Bile acid clusters tested‡		
Total BAs (+1 SD)	0.99 (0.78; 1.26)	0.93 (0.71; 1.22)
Total free BAs (+1 SD)	0.99 (0.78; 1.26)	0.96 (0.74; 1.24)
Total glyco-conjugated BAs (+1 SD)	1.05 (0.84; 1.33)	1.00 (0.78; 1.27)
Total tauro-conjugated BAs (+1 SD)	0.94 (0.73; 1.22)	0.89 (0.67; 1.18)
Total CA (+1 SD)	0.97 (0.76; 1.23)	0.93 (0.70; 1.23)
Total CDCA (+1 SD)	0.98 (0.77; 1.25)	0.90 (0.70; 1.17)
Total DCA (+1 SD)	1.07 (0.84; 1.36)	1.00 (0.79; 1.26)
Total LCA (+1 SD)	0.96 (0.75; 1.22)	0.98 (0.75; 1.28)
Total UDCA (+1 SD)	1.05 (0.85; 1.30)	1.09 (0.88; 1.36)
Total HCA (+1 SD)	0.84 (0.65; 1.08)	0.89 (0.68; 1.16)
Total HDCA (+1 SD)	0.72 (0.40; 1.31)	0.73 (0.36; 1.49)
Primary/Secondary BA ratio (+1 SD)	0.86 (0.66; 1.11)	0.81 (0.62; 1.07)
Conjugated/Free BA ratio (+1 SD)	0.92 (0.71; 1.19)	0.91 (0.70; 1.18)
12αOH/non-12αOH BA ratio (+1 SD)	1.01 (0.80; 1.27)	1.01 (0.80; 1.27)
Total HCA/Total CDCA ratio (+1 SD)	**0.78 (0.61; 1.00)***	0.88 (0.67; 1.16)
C4 (+1 SD)	1.17 (0.93; 1.48)	1.08 (0.84; 1.37)
HOMA-IR (+1 SD)	**1.41 (1.17; 1.69)*****	**1.33 (1.03; 1.72)****

Table 2. Association between bile acid clusters at baseline and the conversion to new onset diabetes during the 5-year follow-up.

Legend: Cox proportional hazards models before and after adjustment on baseline values for age, BMI, fasting plasma glucose and HbA_{1C}. No significant interaction was found between sex and the other parameters of interest. The multivariate model with HOMA-IR was not adjusted on baseline FPG because of obvious co-linearity. ‡ To better fit with the model, all BA values were transformed using square root function except for total HDCA. Significant values displayed as: *p<0.05, **p<0.01 or ***p<0.001. BA clusters are defined in the **Supplemental Table 1**. BAs: bile acids; C4: 7α-hydroxy-4-cholesten-3-one; FPG: fasting plasma glucose; HOMA-IR: Homeostasis model assessment for insulin resistance; Data shown as HR (95% CI): Hazard-Ratio with 95% confidence interval.

Table 3

	Spearman's correlation coefficient					
	BMI	**WHR**	**FPG**	**HbA_{1c}**	**HOMA-IR**	**FLI**
Total BAs	0.01	0.07	0.05	0.04	0.09	0.08
Total free BAs	0.04	**0.15***	0.07	0.02	0.09	0.13
Total glyco-conjugated BAs	-0.02	0.03	0.07	0.01	0.07	0.07
Total tauro-conjugated BAs	0.04	-0.12	0.09	0.02	**0.18***	0.07
Total CA	0.05	0.04	0.07	0.05	0.12	0.05
Total CDCA	0.03	0.06	0.03	0.09	0.11	0.07
Total DCA	0.0	0.06	0.12	-0.03	0.05	0.05
Total LCA	-0.10	-0.01	0.08	-0.10	0.03	-0.09
Total UDCA	0.01	0.12	0.01	-0.02	0.04	0.09
Total HCA	**-0.20****	-0.01	0	-0.11	**-0.16***	**-0.15***
Total HDCA	-0.06	0.05	0.06	-0.06	-0.04	0.01
Primary/Secondary BA ratio	0.01	-0.06	-0.04	0.10	0.07	-0.07
Conjugated/Free BA ratio	-0.07	**-0.15***	0.01	-0.02	-0.03	-0.09
12αOH/non-12αOH BA ratio	0.03	-0.01	0.07	-0.06	-0.06	0.03
Total HCA/Total CDCA ratio	**-0.27*****	-0.06	-0.05	**-0.20****	**-0.30*****	**-0.25*****
C4	**0.32*****	0.14	-0.05	0.11	**0.34*****	**0.35*****

Table 3. Correlation between bile acids and the clinical-biological parameters of the studied population at baseline.

Legend: Spearman's correlation coefficient (R) is presented for each pair of parameters. Significant values are presented in bold: *p<0.05, **p<0.01 or ***p<0.001. BA clusters are defined in the Supplemental Table 1. BAs: bile acids; BMI: body mass index; C4: 7α-hydroxy-4-cholesten-3-one; FLI: Fatty Liver Index; FPG: fasting plasma glucose; HOMA-IR: homeostasis model assessment for insulin resistance; WHR: waist-hip ratio.

Supplemental table 1

Bile acid cluster	Acronym and definition
Free cholic acid	CA
Free chenodeoxycholic acid	CDCA
Free hyocholic acid	HCA
Free deoxycholic acid	DCA
Free lithocholic acid	LCA
Free ursodeoxycholic acid	UDCA
Free hyodeoxycholic acid	HDCA
Total free BAs	CA+ CDCA + HCA + DCA + LCA + UDCA + HDCA
Glyco-cholic acid	GCA
Glyco-chenodeoxycholic acid	GCDCA
Glyco-hyocholic acid	GHCA
Glyco-deoxycholic acid	GDCA
Glyco-lithocholic acid	GLCA
Glyco-ursodeoxycholic acid	GUDCA
Glyco-hyodeoxycholic acid	GHDCA
Total glyco-conjugated BAs	GCA + GCDCA + GHCA + GDCA + GLCA + GUDCA + GHDCA
Tauro-cholic acid	TCA
Tauro-chenodeoxycholic acid	TCDCA
Tauro-hyocholic acid	THCA
Tauro-deoxycholic acid	TDCA
Tauro-lithocholic acid	TLCA
Tauro-ursodeoxycholic acid	TUDCA
Tauro-hyodeoxycholic acid	THDCA
Total tauro-conjugated BAs	TCA + TCDCA + THCA + TDCA + TLCA + TUDCA + THDCA
Total Conjugated BAs	Total Glyco-BAs + Total Tauro BAs
Total BAs	Total Free BAs + Total Conjugated BAs
Total CA	CA + GCA + TCA
Total CDCA	CDCA + GCDCA + TCDCA
Total HCA	HCA + GHCA + THCA
Total DCA	DCA + GDCA + THCA
Total LCA	LCA + GLCA + TLCA
Total UDCA	UDCA + GUDCA + TUDCA
Total HDCA	HDCA + GHDCA + THDCA
Total primary BAs	Total CA + Total CDCA + Total HCA
Total secondary BAs	Total DCA + Total LCA + Total UDCA + Total HDCA
Conjugated/Free BA ratio	Total Conjugated BAs / Total Free BAs
Primary/secondary BA ratio	Total primary BAs / Total secondary BAs
12α-hydroxylated BAs	Total CA + Total DCA
Non-12α-hydroxylated BAs	Total CDCA + Total HCA + Total LCA + Total UDCA + Total HDCA
12α-hydroxylated/non-12α-hydroxylated BA ratio	12α-OH BAs / Non-12α-OH BAs

Supplemental Table 1. Definition of the bile acid clusters.

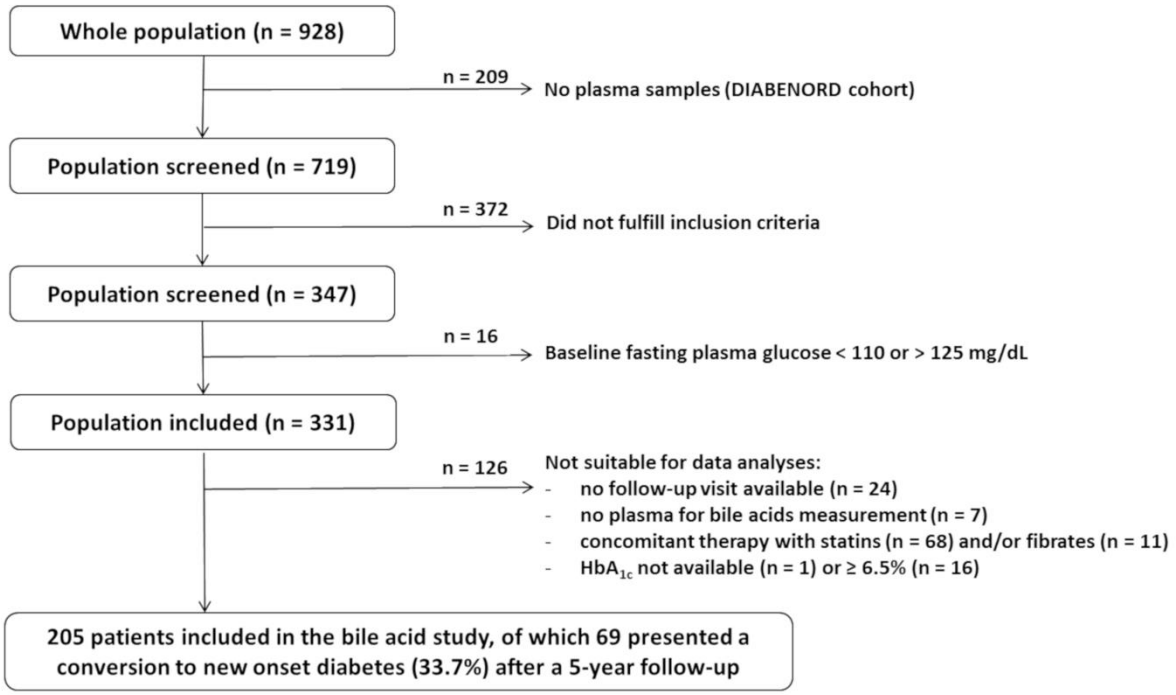
Legend: BAs: bile acids.

Supplemental table 2

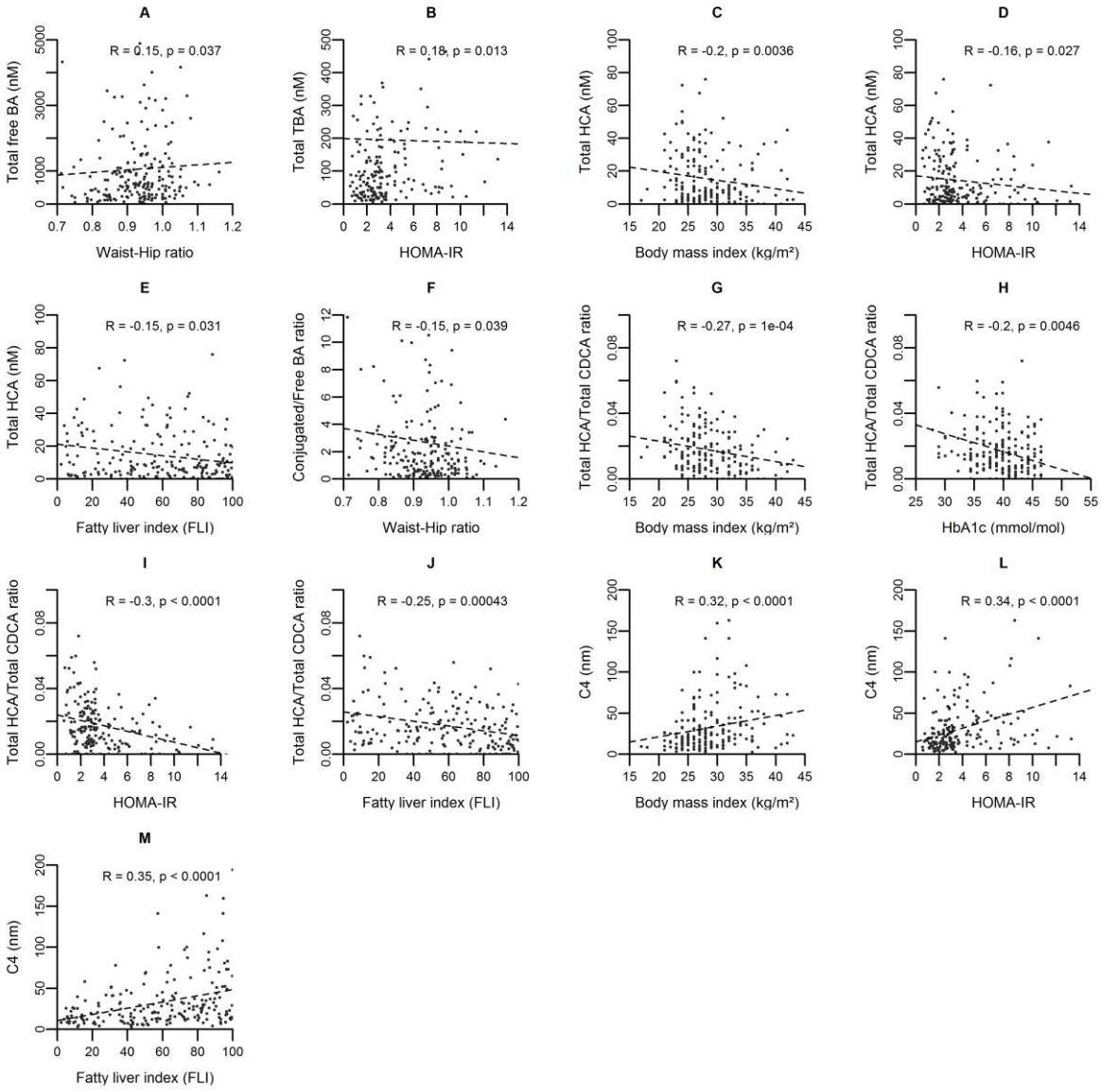
	Spearman's correlation coefficient					
	BMI	**WHR**	**FPG**	**HbA1c**	**HOMA-IR**	**FLI**
CA	0.02	0.08	0.11	0.06	0.09	0.04
GCA	0.04	0	0.04	-0.03	**0.17***	0.08
TCA	0.08	-0.10	0.08	-0.01	**0.22****	0.08
CDCA	0.04	0.10	0.05	0.10	0.10	0.11
GCDCA	-0.02	0.05	0.06	0.03	0.07	0.07
TCDCA	0.02	-0.12	0.08	0.06	**0.16***	0.03
DCA	0.04	0.11	0.07	0	0.05	0.09
GDCA	-0.02	0	**0.14***	-0.06	0.04	0.04
TDCA	0.05	-0.06	0.13	-0.05	0.13	0.13
UDCA	0.06	0.12	0.02	-0.04	0.09	0.12
GUDCA	-0.03	0.09	0.02	0.01	0.01	0.06
TUDCA	-0.04	-0.07	0.02	-0.04	0.04	0
HCA	-0.09	0.03	0	-0.10	-0.06	-0.05
GHCA	**-0.22****	-0.07	0.04	-0.08	**-0.18***	**-0.18***
THCA	**-0.16***	**-0.14***	0.11	0	-0.06	**-0.20****
HDCA	-0.10	-0.03	0.01	-0.08	-0.07	-0.03
GHDCA	-0.03	**0.15***	0.06	-0.04	0.04	0.03
THDCA	0.04	0.02	0.11	0.07	0.03	0.02

Supplemental Table 2. Correlation between bile acids and metabolic parameters at baseline, details for free, gluco-conjugated and tauro-conjugated bile acid species.

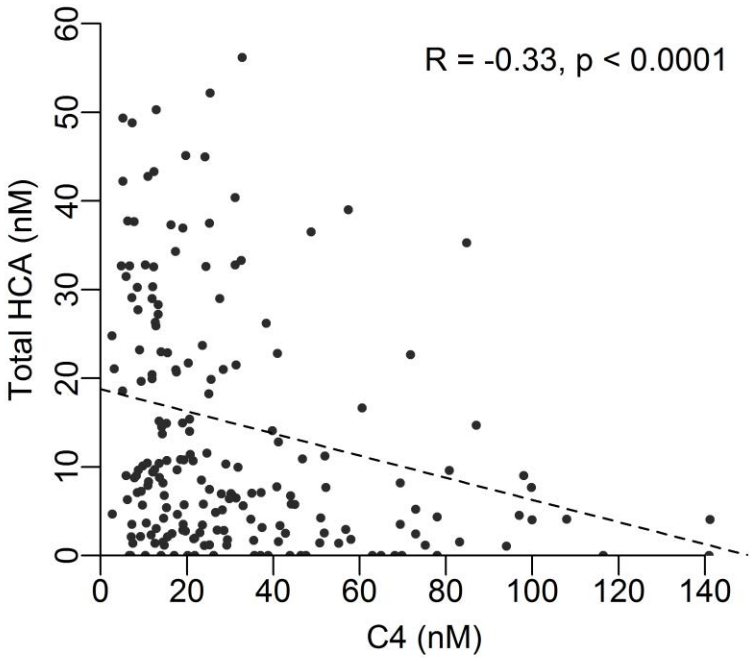
Legend: Spearman's correlation coefficient is presented for each pair of parameters, and only significant p values displayed as: *p<0.05, **p<0.01 or ***p<0.001. BA clusters are defined in the Supplemental Table 1. BAs: bile acids; C4: 7α-hydroxy-4-cholesten-3-one; FPG: fasting plasma glucose; G-: Gluco-conjugated; FLI: Fatty liver index; HOMA-IR: Homeostasis model assessment for insulin resistance; HR (95% CI): Hazard-Ratio with 95% confidence interval; T-: tauro-conjugated; TC: total cholesterol; TG: triglycerides; WHR: waist-hip ratio.



Supplemental Figure 1. Flow-chart of the selection of the patients for the bile acid study of the IT-DIAB cohort.



Supplemental Figure 2. Dot plots of all the significant correlations between BAs and clinic-biological parameters in Table 2.



Supplemental Figure 3. Correlation between HCA and C4 (7α-hydroxy-4-cholesten-3-one) at baseline.

MANUSCRIPT NUMBER 4

Legry *et al.* Bile acid alterations are associated with insulin resistance, but not with NASH in obese subjects. J Clin Endocrinol Metab doi:10.1210/jc.2017-01397

Bile Acid Alterations Are Associated With Insulin Resistance, but Not With NASH, in Obese Subjects

Vanessa Legry,[1] Sven Francque,[2,3] Joel T. Haas,[1] An Verrijken,[3,4] Sandrine Caron,[1] Oscar Chávez-Talavera,[1] Emmanuelle Vallez,[1] Luisa Vonghia,[2,3] Eveline Dirinck,[4] Ann Verhaegen,[4] Mostafa Kouach,[5] Sophie Lestavel,[1] Philippe Lefebvre,[1] Luc Van Gaal,[3,4] Anne Tailleux,[1] Réjane Paumelle,[1]* and Bart Staels[1]*

[1]Université Lille, INSERM, Centre Hospitalier Universitaire de Lille, Institut Pasteur de Lille, U1011-European Genomic Institute for Diabetes, F-59000 Lille, France; [2]Department of Gastroenterology and Hepatology, Antwerp University Hospital, 2650 Edegem/Antwerp, Belgium; [3]Laboratory of Experimental Medicine and Pediatrics, Faculty of Medicine and Health Sciences, University of Antwerp, 2610 Wilrijk/Antwerp, Belgium; [4]Department of Endocrinology, Diabetes and Metabolism, Antwerp University Hospital, 2650 Egedem/Antwerp, Belgium; and [5]Plateau de Spectrométrie de Masse-Groupe de Recherche sur les formes Injectables et les Technologies Associées, (PSM-GRITA), Faculté de Pharmacie, F-59000 Lille, France

Context: Bile acids (BAs) are signaling molecules controlling energy homeostasis that can be both toxic and protective for the liver. BA alterations have been reported in obesity, insulin resistance (IR), and nonalcoholic steatohepatitis (NASH). However, whether BA alterations contribute to NASH independently of the metabolic status is unclear.

Objective: To assess BA alterations associated with NASH independently of body mass index and IR.

Design and Setting: Patients visiting the obesity clinic of the Antwerp University Hospital (a tertiary referral facility) were recruited from 2006 to 2014.

Patients: Obese patients with biopsy-proven NASH (n = 32) and healthy livers (n = 26) were matched on body mass index and homeostasis model assessment of IR.

Main Outcome Measures: Transcriptomic analyses were performed on liver biopsies. Plasma concentrations of 21 BA species and 7α-hydroxy-4-cholesten-3-one, a marker of BA synthesis, were determined by liquid chromatography–tandem mass spectrometry. Plasma fibroblast growth factor 19 was measured by enzyme-linked immunosorbent assay.

Results: Plasma BA concentrations did not correlate with any hepatic lesions, whereas, as previously reported, primary BA strongly correlated with IR. Transcriptomic analyses showed unaltered hepatic BA metabolism in NASH patients. In line, plasma 7α-hydroxy-4-cholesten-3-one was unchanged in NASH. Moreover, no sign of hepatic BA accumulation or activation of BA receptors—farnesoid X, pregnane X, and vitamin D receptors—was found. Finally, plasma fibroblast growth factor 19, secondary-to-primary BA, and free-to-conjugated BA ratios were similar, suggesting unaltered intestinal BA metabolism and signaling.

Conclusions: In obese patients, BA alterations are related to the metabolic phenotype associated with NASH, especially IR, but not liver necroinflammation. (***J Clin Endocrinol Metab*** **102: 3783–3794, 2017)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 20 June 2017. Accepted 31 July 2017.
First Published Online 4 August 2017

*These authors contributed equally to this study.

Abbreviations: AI, activity index; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BA, bile acid; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; C4, 7α-hydroxy-4-cholesten-3-one; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; FPG, fasting plasma glucose; FXR, farnesoid X receptor; GGT, γ-glutamyl transpeptidase; GPBAR1, G protein–coupled bile acid receptor 1; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OCA, obeticholic acid; PNPLA3, phospholipase domain–containing protein 3; PXR, pregnane X receptor; TGR5, Takeda G protein–coupled receptor 5; T2D, type 2 diabetes; VDR, vitamin D receptor.

Bile acids (BAs) are amphipathic molecules that facilitate absorption of dietary fat and lipophilic vitamins in the small intestine. However, owing to their detergent properties, BAs also are potentially harmful when accumulating, as seen in cholestatic liver diseases. BA overload induces hepatotoxicity by activation of inflammatory, oxidative stress, and necrotic cell death pathways (1, 2). Therefore, BA pool size and metabolism are under tight negative retrocontrol *via* activation of nuclear receptors, such as the farnesoid X (FXR), pregnane X (PXR), and vitamin D (VDR) receptors. BAs also activate cell surface receptors, such as the G protein–coupled BA receptor-1 [GPBAR1/Takeda G protein–coupled receptor 5 (TGR5)] and sphingosine-1-phosphate receptor 2 (3).

Chenodeoxycholic acid (CDCA) and cholic acid (CA) are the major primary BAs synthesized from cholesterol in the liver (4). Before secretion into bile, BAs are conjugated (mainly to glycine, less to taurine in humans), decreasing their hydrophobicity. Primary BAs are converted in the intestine into more hydrophobic secondary BAs, deoxycholic acid (DCA) and small amounts of lithocholic acid, hyodeoxycholic acid, and ursodeoxycholic acid (UDCA). Most BAs are reabsorbed in the distal intestine and transported back to the liver *via* the portal circulation. A small proportion of BA escapes this enterohepatic cycle and reaches peripheral organs *via* the systemic circulation.

BAs also act as signaling molecules controlling glucose, lipid, and energy homeostasis, notably *via* activating FXR and TGR5 (3). In humans, interruption of enterohepatic BA circulation using BA sequestrants improves both lipid and glucose metabolism through mechanisms involving increased L-cell glucagon-like peptide-1 (GLP-1) production and enhancement of splanchnic glucose utilization (5, 6).

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease and strongly associates with obesity and insulin resistance (IR). NAFLD encompasses a spectrum ranging from isolated steatosis to nonalcoholic steatohepatitis (NASH), characterized by steatosis, necroinflammatory changes (ballooned hepatocytes and lobular inflammation), and varying degrees of liver fibrosis (7). IR is a common feature in individuals with NAFLD and, reciprocally, >70% of type 2 diabetic (T2D) patients have fatty liver and rapidly progress to NASH (8). NASH pathogenesis is still unclear and appears multifactorial, resulting from several deleterious events occurring in parallel and involving the interaction of multiple organs, with the gut/liver axis playing a crucial role (9). There is also a genetic basis in NASH and, interestingly, the phospholipase domain–containing protein 3 (PNPLA3) I148M polymorphism associates with the severity of necroinflammatory changes independently of metabolic factors (10).

Obesity, T2D, and NAFLD are associated with dysbiosis (11). The intestinal microbiota modulates the development of metabolic diseases in part through BAs, because the microbiota deconjugates and converts primary into secondary BAs. Whereas it is unclear whether systemic total BA concentrations are altered in obesity (12, 13), they are elevated in T2D patients (3, 14). Moreover, the peripheral blood BA pool composition is altered in IR due to impaired insulin-mediated 12α-hydroxylase (CYP8B1) downregulation, resulting in increased 12α-hydroxylated BA (CA, DCA, and their conjugated forms) (15). Bariatric surgery, especially Roux-en-Y gastric bypass, which not only reduces body weight but also reverses T2D and NAFLD, increases peripheral blood BA levels, and BA changes might be involved in NAFLD reversion after bariatric surgery (3, 16).

However, whether alterations in BA metabolism play a role in the pathogenesis of NASH is unclear. Recent publications reported increased hepatic BA concentrations accompanied with altered synthesis in NASH patients (17, 18), suggesting the existence of a mild cholestatic injury in NASH. However, liver tissue BA composition and concentration analysis does not allow discrimination of intracellular, ductular, or blood origins. Changes in BA profile in plasma and feces were also described, notably increased total and primary BAs in NASH patients (19–23). However, these studies compared NASH patients to controls with lower body weight, fasting plasma glucose (FPG) (19), and/or IR (20–23). Because body mass index (BMI) and the insulin sensitivity status of the patients were not accounted for in these studies, it is unclear whether NASH and its histological components *per se* are associated with alterations in BA metabolism.

Therefore, we investigated BA metabolism in a cohort of drug-naive obese patients extensively phenotyped for metabolic parameters and biopsy-proven NASH. Plasma BA profiling coupled with transcriptomic analysis of BA metabolism and signaling in liver biopsies were compared between NASH patients and BMI- and IR-matched control (no-NASH) patients.

Materials and Methods

Description of the patients

Overweight (BMI of ≥25 to <30 kg/m^2) or obese (BMI of ≥30 kg/m^2) patients visiting the obesity clinic of the Antwerp University Hospital (a tertiary referral facility) were recruited from October 2006 to May 2014 (24). Patients were prospectively screened for the presence of NAFLD, and when NAFLD was suspected from abnormal blood biochemistry assays [alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), γ-glutamyl transpeptidase (GGT)] or ultrasound features, a liver biopsy was subsequently proposed. Exclusion criteria were alcohol

consumption, previous bariatric surgery, liver diseases other than NAFLD, diagnosed T2D (as diabetic patients constitute another specific risk group for NAFLD, and as long-term diabetes and its treatments potentially influence the presence and severity of NAFLD, this was considered a potential confounder and therefore excluded; patients who were *de novo* diagnosed with T2D as a result of their work-up were, however, included in the study), and antidiabetic, lipid-lowering, or antibiotic treatments. The study protocol is part of the HEPADIP protocol (Belgian registration number B30020071389); it was approved by the Ethical Committee of the Antwerp University Hospital (file 6/25/125), and required written informed consent of the patient.

Clinical assessment and biological measurements

Fasting blood was collected in the morning. Plasma glucose, insulin, triglyceride, total, high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C) cholesterol concentrations and liver enzymes (ALAT, ASAT, GGT, alkaline phosphatase) were measured as described (10). IR was estimated using homeostasis model assessment (HOMA-IR) calculated as [insulin (mU/L) × glucose (mmol/L)]/22.5.

Histological assessment of liver biopsies

Hematoxylin and eosin, Sirius red, reticulin stain, and Perls' iron stains were routinely performed on all liver biopsies and analyzed by two experienced pathologists blinded to clinical data. The histological features of NAFLD (steatosis, ballooning, lobular inflammation, and fibrosis) were assessed using the NASH Clinical Research Network Scoring System (25). NASH diagnosis was defined according to recent guidelines requiring the combined presence of steatosis, ballooning, and lobular inflammation (26, 27). The NAFLD activity score was also calculated. In line with recent insights on the differential role of steatosis and activity of disease, an activity index (AI) was also calculated as the sum of ballooning (range, 0 to 2) and lobular inflammation (range, 0 to 3), hence ranging from 0 to 5 (25, 28).

Selection and matching of patients for BA metabolism analysis

Among the whole cohort, 152 patients had liver biopsies, and a complete clinical and biological dataset is available for further analyses. For the present study, patients with cholecystectomy or cholelithiasis were excluded. Furthermore, to dissect influences of NASH and metabolic features, two clearly distinct histological phenotypes were selected for comparison and matched on BMI and HOMA-IR. Selection of the NASH patients was hence based on the unequivocal presence of NASH. For the no-NASH group, patients with normal liver or minor lesions largely insufficient for the diagnosis of NASH, as well as absence of fibrosis, were selected. Cases with borderline lesions (n = 4) were not included in the present study. Then, NASH patients were matched with no-NASH patients on quartiles of BMI ($Q1 < 35.6$, $Q2 < 39.5$, $Q3 < 43.2$, $Q4 \geq 43.2$ kg/m^2) and HOMA-IR ($Q1 < 2.38$, $Q2 < 3.49$, $Q3 < 4.89$, $Q4 \geq 4.89$). Patients that could not be matched were excluded. This resulted in 32 NASH and 26 no-NASH patients, representative of the whole cohort (Supplemental Table 1).

Transcriptomic analysis of liver biopsies

Total RNA was prepared and transcriptomic analysis with Affymetrix GeneChip arrays (HuGene 2.0 ST) was performed as described (24, 29). Signals were normalized by the Robust Multiarray Average method, and the baseline was adjusted to the median of all samples. Proprietary.CEL files were imported into Partek Genomics Suite 6.6 for analyses. Using the linear models for microarray data, 31,135 transcripts were tested for differential expression between NASH (n = 32) and no-NASH (n = 26) patients. Pathway analysis, including KEGG pathway and gene ontology term enrichment, was performed on transcripts coding for proteins that exhibit a significant >20% differential expression level between NASH and no-NASH patients.

Subsequently, all genes involved in BA metabolism were further analyzed based on the KEGG BA synthesis and bile secretion pathways (www.kegg.jp) and literature (4, 18, 30), resulting in 87 genes. A heat map was generated using the heatmap.2 function in the gplots package (R project) including these 87 genes. Hierarchical clustering was performed to group patients by Euclidean distance. Clustering was not performed on genes.

Plasma measurements of BA, 7α-hydroxy-4-cholesten-3-one, and fibroblast growth factor 19

All measurements were performed on fasting EDTA plasma collected in the morning. Twenty-one BA species were quantified by liquid chromatography–tandem mass spectrometry as previously described (31). BA concentrations are presented in Supplemental Table 2, except for UDCA and its conjugated forms that were only detected as trace amounts in these samples.

Plasma 7α-hydroxy-4-cholesten-3-one (C4) was determined as described (32). Briefly, 100 μL of plasma sample was mixed with 500 μL of purified water, 50 μL of the 0.06 μM D7-C4 internal standard solution, and 60 μL of 1 M hydrochloric acid. Solid-phase extraction was performed using solid phase extraction cartridges (Bound Elut-C18, 200 mg) preconditioned with 2 mL of methanol followed by 2 mL of purified water, upon which the sample was loaded and allowed to pass through it by gravity. The cartridge was washed, the analyte was eluted with MeOH, and the eluted solution was evaporated. The residue was dissolved in 80 μL of methanol and mixed with 10 μL of ammonium acetate buffer (10 mM, pH 6.5), upon which the sample was centrifuged at 13,400 × *g* and 10°C for 10 minutes. The supernatant was evaporated. The pellet was then dissolved in 100 μL of MeOH/water (volume-to-volume ratio) and 2 μL was injected into the liquid chromatography–tandem mass spectrometry system (31). The separation of C4 was carried out on a Kinetex Core-Shell C18 column (100 × 2.1 mm, 5 μm) from Phenomenex. The oven temperature was 30°C. Solvent A was water containing 0.1% formic acid, and solvent B was acetonitrile. Solvents were delivered at a total flow rate of 500 μL/min. After a 2-minute plateau with 75% solvent B, the gradient profile was from 75% solvent B to 95% solvent B linearly in 5 minutes, followed by a 2-minute plateau with 95% solvent B. The column was re-equilibrated for 3 minutes. The injection cycle was 12 minutes. Mass spectrometry analysis was carried out in positive ionization mode. The ion source parameters were set as follows: ion spray voltage, 5500 V; nebulizer gas (air) and curtain gas (nitrogen) flows, 50 and 20 psi, respectively; source temperature, 650°C with auxiliary gas flow (air), 50 psi; declustering potential, 100 V; collision cell exit potential, 15 V; collision energy, 31 V. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 with a dwell time of 100 ms in each

transition. The methods allow the quantification of C4 within the range of 1 to 200 nM, with interday and intraday precisions < 15%. All of the analytical methods were validated using the Société Française des Sciences et Techniques Pharmaceutiques guidelines.

Plasma fibroblast growth factor 19 (FGF19) concentrations were measured using a human FGF19 Quantikine enzyme-linked immunosorbent assay kit DF1900 (R&D Systems).

Statistical analyses

Statistical analyses were performed using the SAS statistical software, version 9.4 (SAS Institute, Cary, NC). Intergroup comparisons of quantitative variables were performed using the general linear model. To obtain normal data distributions, log transformation of some variables was applied in all samples, as indicated in the table legends. For plasma BA, C4, and FGF19 analyses, nonparametric tests were performed. *P* values for the comparison of distribution (sex, histological grades) between subject groups were assessed using the χ^2 test or Fisher's exact test, as indicated in the table legends. Multivariable analyses were performed to compare hepatic gene expression between groups, adjusted on confounding factors, including sex and the PNPLA3 I148M (rs738409) genotype (10). Statistical significance was considered when $P < 0.05$.

Results

Characteristics of the studied patients

To analyze BA alterations specifically associated with NASH, independent of obesity or IR, patients with well-established NASH and patients with no-NASH were matched on BMI and HOMA-IR, resulting in 32 NASH and 26 no-NASH patients (Supplemental Table 1; Table 1). The no-NASH patients had a liver with little or no steatosis and hepatic injury (Fig. 1). In contrast, besides steatosis, NASH patients presented clear signs of disease activity with inflammatory infiltrates (mostly grades 1 and 2) and prominent ballooning (grade 2 in 50% of patients). Approximately 50% of NASH patients presented some degree of fibrosis up to F3 (Supplemental Table 1).

Overall, all 58 subjects were severely obese (BMI of 39.8 ± 5.8 kg/m^2) and presented several features of the metabolic syndrome, that is, mild hypertriglyceridemia (157 ± 73 mg/dL) and relatively low plasma HDL-C levels (47 ± 13 mg/dL). FPG (84 ± 10 mg/dL) was in the normal range, whereas 2-hour postoral glucose levels were slightly elevated (145 ± 35 mg/dL), indicative of impaired glucose homeostasis. As could be expected because of the matching on BMI and HOMA-IR, NASH and no-NASH patients displayed no significant difference in terms of metabolic parameters, except a lower plasma HDL-C level in NASH patients (Table 1).

Systemic BA profile is unchanged in NASH patients

First, correlations between each hepatic histological feature and each plasma BA species concentration were analyzed in the 58 studied patients. No correlation, except glycocholic acid with steatosis ($r = 0.29$, $P = 0.03$), was found (Fig. 2A). Furthermore, comparison of plasma BA species concentrations between NASH and no-NASH patients did not show any significant difference (Fig. 2B). Total, primary, secondary, free, or conjugated BAs were unchanged with NASH (Supplemental Table 2). BA profile composition was also similar between NASH and no-NASH patients (Fig. 2C). In multivariable analyses,

Table 1. Clinical, Biological, and Histological Characteristics of the Studied Subjects With and Without NASH

	No NASH (n = 26)	NASH (n = 32)	*P* Value
Age, y	40.5 ± 11.7	41.3 ± 11.9	0.80
Sex, males/females[a]	3/23	13/19	0.01
BMI, kg/m^2	39.4 ± 5.9	40.2 ± 5.8	0.60
Plasma glucose, mg/dL[b]	82.3 ± 11.8	85.8 ± 8.4	0.13
Plasma insulin, μU/mL[b]	15.7 ± 9.1	18.9 ± 11.4	0.16
HOMA-IR[b]	3.25 ± 2.05	4.05 ± 2.65	0.11
Plasma TG, mg/dL[b]	153 ± 68	160 ± 78	0.70
Plasma cholesterol, mg/dL	212 ± 58	197 ± 35	0.22
Plasma HDL-C, mg/dL	51.8 ± 14.9	43.6 ± 9.1	0.01
Plasma LDL-C, mg/dL	130 ± 50	121 ± 31	0.44
ALAT, U/L[b]	27.3 ± 15.8	36.5 ± 22.7	0.08
ASAT, U/L[b]	21.3 ± 11.9	24.6 ± 17.5	0.32
GGT, U/L[b]	40.6 ± 22.0	40.6 ± 26.4	0.86
ALP, U/L	80.0 ± 23.5	74.9 ± 17.3	0.35
Total bilirubin, mg/dL	0.49 ± 0.20	0.56 ± 0.17	0.19
NAS[c]	1 (0–2)	5 (3–8)	<0.0001

Data are means ± standard deviation, except for NAS [median (minimum–maximum)].

Abbreviations: ALP, alkaline phosphatase; NAS, NAFLD activity score.

[a] *P* value from Fisher's exact test.

[b] *P* values on log-transformed data.

[c] *P* value from nonparametric Mann-Whitney test.

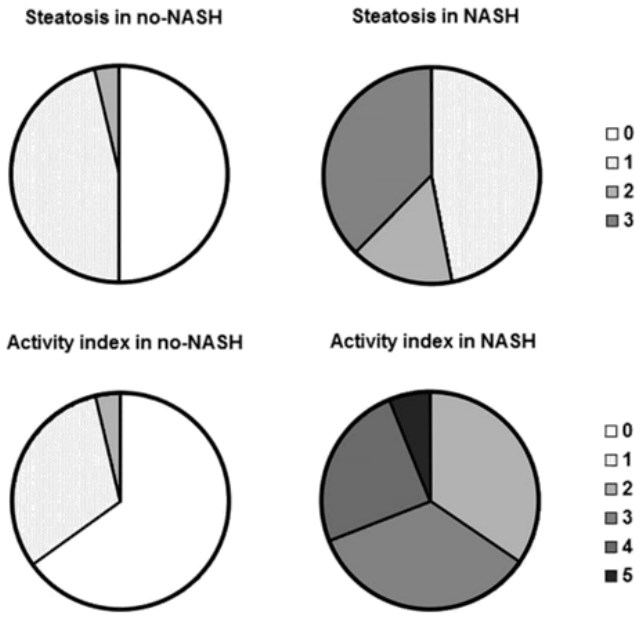


Figure 1. Histological characteristics of the studied patients. Pie charts show steatosis and AI (ballooning plus inflammation) ranges in NASH (n = 32) and no-NASH (n = 26) patients.

no significant interactions between NASH and HOMA-IR, BMI, sex, or PNPLA3 I148M polymorphism were found on plasma BA concentrations (not shown), indicating that these parameters did not mask associations between NASH and BA alterations. Therefore, when matched for BMI and IR, peripheral plasma BAs are not affected by NASH.

Hepatic gene expression analysis reveals limited BA metabolism and signaling changes in NASH

Because previous reports described altered hepatic expression of genes involved in BA synthesis and transport in NASH patients (18, 19), gene expression profiles were compared by microarray analyses performed on liver biopsies of all NASH and no-NASH patients. Differential expression analysis revealed 713 transcripts dysregulated in NASH. Pathway enrichment analysis showed clear activation of inflammatory response in NASH patients (Supplemental Fig. 1). However, no BA pathways (defined by KEGG or gene ontology term) were enriched in NASH.

To analyze in more detail whether changes in hepatic BA metabolism genes may exist, a literature search of all genes involved in BA metabolism (synthesis, transport, conjugation/detoxification, bile secretion, regulation of BA metabolism, and BA-activated signaling pathways) resulted in the identification of 87 genes (Fig. 3). Unsupervised hierarchical clustering showed that NASH status was not strongly associated with changes in expression of these genes (Fig. 3A). Only a few BA metabolism genes were significantly dysregulated in NASH (Fig. 3B): among them, as reported (24), *PPARA* was lower, along with *CYP3A4* and *ABCB11* (also known as BSEP), whereas *CYP7A1* and *ATP8B1* were higher in NASH patients. Most of these changes were observed when stratifying the patients on steatosis grade (Fig. 3C), but not when stratified on an AI, except for *PPARA* (Fig. 3D).

Because the rate-limiting enzyme of BA synthesis, 7α-hydroxylase (CYP7A1), messenger RNA levels were increased, we measured plasma C4 concentrations, a surrogate marker of hepatic BA synthesis (33). C4 concentrations did not differ between NASH and no-NASH patients (Fig. 3E), indicating that the increased *CYP7A1* gene expression did not translate into increased BA synthesis.

As it has been suggested that hydrophobic BA (CDCA, DCA) could accumulate in the liver of NASH patients and participate in liver injury (17, 34, 35), indirect markers of hepatic BA accumulation and injury were examined. Liver enzymes and metabolites known to increase with cholestatic injury were not elevated in NASH patients (Table 1). Moreover, upon intrahepatic BA overload, the BA receptors FXR, PXR, and VDR initiate protective mechanisms to downregulate BA uptake and synthesis and upregulate BA detoxification and systemic efflux (4). In this study, hepatic expression of well-known target genes of FXR, PXR, and VDR revealed increased *CYP7A1* in NASH (Supplemental Fig. 2) whereas its expression is typically repressed by these BA receptors (4). Also, *CYP3A4*, which is induced upon PXR or VDR activation, and the FXR target gene *ABCB11* (BSEP) (4) were downregulated in NASH *vs* no-NASH patients (Supplemental Fig. 2). Expression of the membrane BA receptor *GPBAR1* (TGR5), which mediates anti-inflammatory responses upon BA activation in Kupffer cells (36), was also unchanged (not shown). Taken together, these results indicate the absence of major changes in hepatic BA metabolism and intrahepatic BA accumulation in NASH patients compared with BMI- and HOMA-IR–matched controls.

Intestinal BA metabolism is unchanged in NASH patients

Because alterations in fecal BA content associated with dysbiosis were reported in NAFLD patients (23), we also investigated parameters of intestinal BA metabolism and signaling. Because neither intestinal gene expression nor BA profiles could be measured, plasma levels of FGF19, a marker of intestinal FXR activation (37), were measured. No difference was found between patients with and without NASH (62.0 ± 53.5 *vs* 54.7 ± 41.1 pg/mL, P = 0.37). Moreover, the ratios of free/conjugated BAs (1.11 ± 0.96 *vs* 1.27 ± 1.14, P = 0.56) and secondary/primary BAs (0.80 ± 0.48 *vs* 0.80 ± 0.78, P = 0.99), markers of

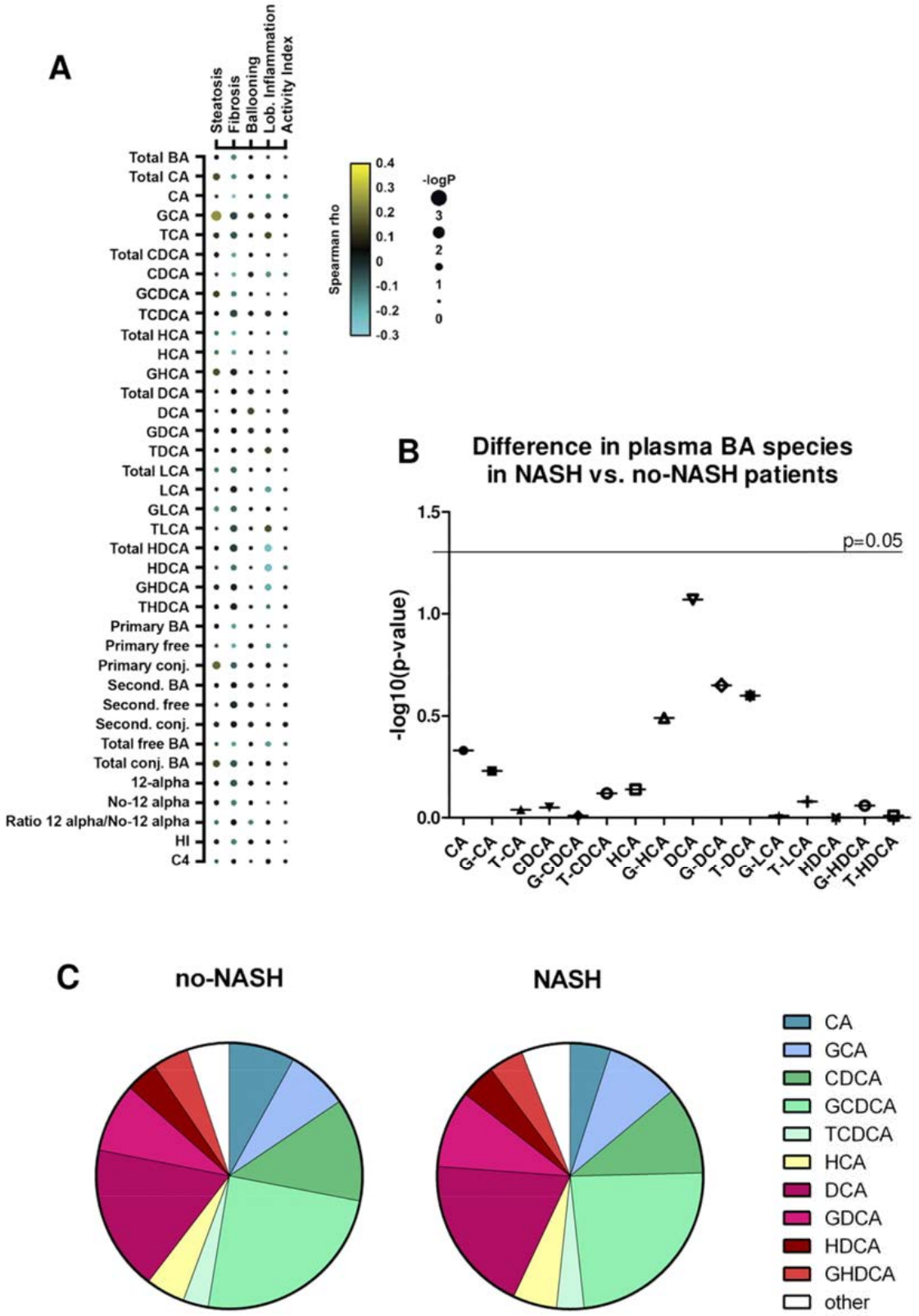


Figure 2. Plasma BA profiles are not associated with NASH. (A) Spearman correlations between plasma BA and hepatic histological features in the 58 patients. Colors reflect the Spearman ρ values (yellow for positive, blue for inverse correlations), and dot sizes reflect the *P* values. (B) Comparison of each plasma BA species between NASH (n = 32) and no-NASH (n = 26) patients. All *P* values are below the significance level of 0.05. (C) Comparison of plasma BA pool composition (expressed as percentage of the total BA pool) between NASH (n = 32) and no-NASH (n = 26) patients. Only BA species > 3% of the pool are depicted. GCA, glycocholic acid; GCDCA, glyco-CDCA; GDCA, glyco-DCA; GHDCA, glyco-HDCA; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; TCDCA, tauro-CDCA.

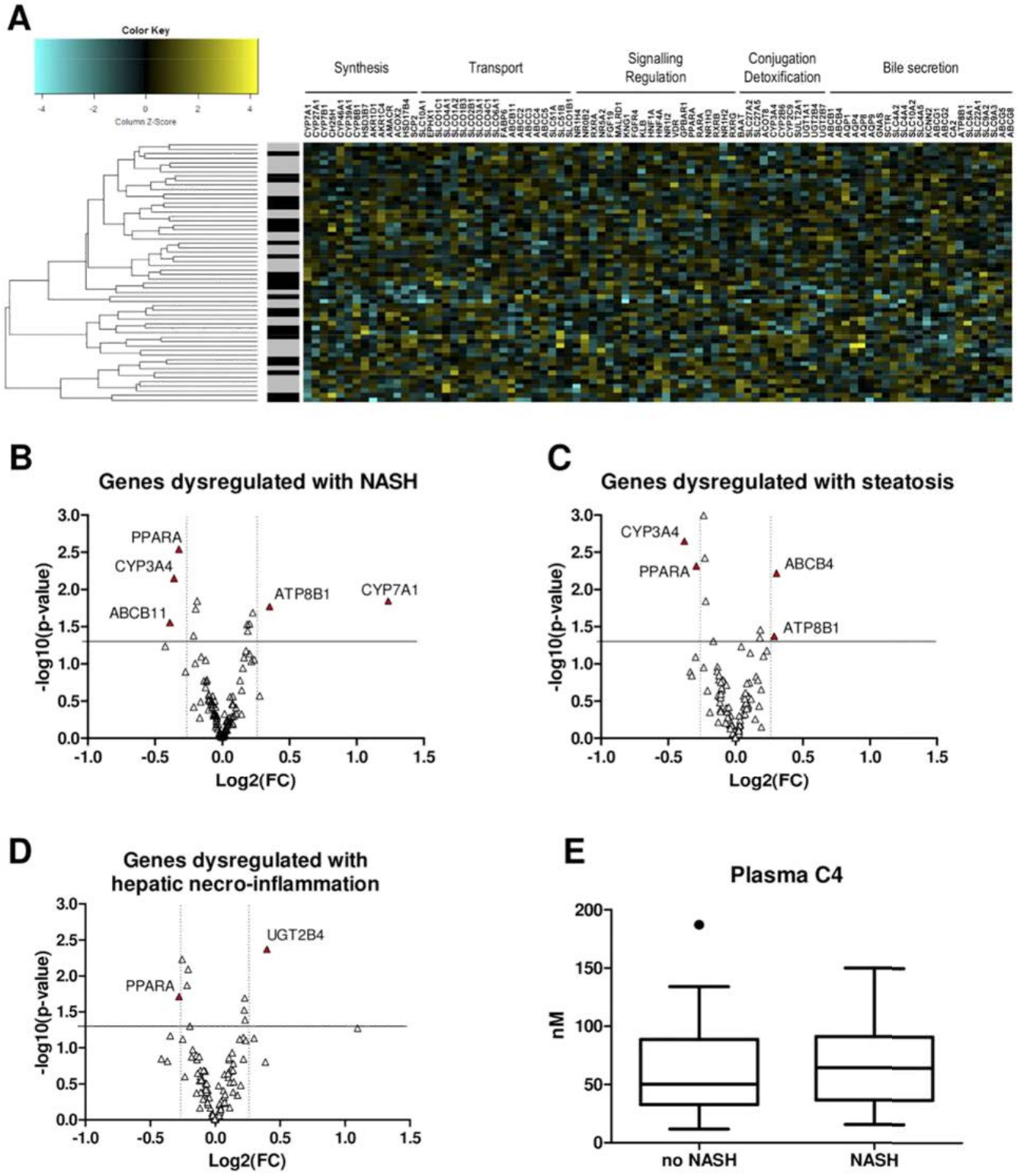


Figure 3. Expression of hepatic genes involved in BA metabolism is not altered in NASH patients. (A) Heat map showing hepatic expression of 87 genes involved in BA metabolism in NASH (black, n = 32) and no-NASH (gray, n = 26) patients. Colors reflect the *z* score for each gene: yellow and blue correspond to increased and decreased gene expression, respectively. Black indicates the median expression. (B–D) Volcano plots showing the most dysregulated genes involved in BA metabolism between NASH (n = 32) and no-NASH (n = 26) patients (B), between patients with (grade > 1, n = 18) and without (grade ≤ 1, n = 40) steatosis (C), and between patients with (AI ≥ 2, n = 33) and without (AI < 2, n = 25) hepatic necroinflammation (D). Red indicates the significantly dysregulated genes with an absolute fold change ≥ 1.2. (E) Plasma C4 concentrations as a surrogate marker of hepatic 7α-hydroxylase activity in NASH (n = 32) and no-NASH (n = 26) patients.

microbiota deconjugation and conversion activities, respectively, were also unchanged in NASH patients.

BA alterations are associated with glucose homeostasis independently of NASH

Considering the lack of association between BA alterations and NASH, we finally assessed whether differences in metabolic status correlate with BA metabolism considering the whole (NASH and no-NASH) studied population. Therefore, metabolic parameters were correlated with each plasma BA species concentration (Fig. 4A). BMI positively correlated with plasma CA ($r = 0.36$, $P = 0.006$) and CDCA ($r = 0.31$, $P = 0.02$). CA and CDCA also strongly correlated with fasting plasma insulin (CA, $r = 0.42$, $P = 0.0009$; CDCA, $r = 0.39$, $P = 0.003$) and HOMA-IR (CA, $r = 0.45$, $P = 0.0004$; CDCA, $r = 0.42$, $P = 0.001$). Additionally, plasma hyocholic acid correlated with FPG ($r = 0.39$, $P = 0.003$). In accordance with Haeusler *et al.* (15), 12α-hydroxylated BAs (CA, DCA, and their conjugated forms) tended to correlate with fasting plasma insulin ($r = 0.14$, $P = 0.08$).

Given the strong correlations between parameters of glucose homeostasis and plasma BA, the 58 subjects were stratified according to IR, and highest *vs* lowest quartiles of HOMA-IR were compared (for clinical and liver histology parameters, see Supplemental Table 3). Unlike in NASH, several changes in systemic BA profile occurred with IR, with notably increased primary (free) BA concentrations (Fig. 4B and 4C). Also, the total plasma BA concentration tended to increase in IR patients, which was not the case in NASH patients (Supplemental Table 2). Overall, these results show that BA metabolism is correlated with metabolic conditions, especially IR, but not with hepatic lesions.

Discussion

BA metabolism is altered in metabolic disorders associated with NAFLD (3). Because BA overload causes hepatotoxicity in some chronic liver diseases (cholestasis), it is tempting to speculate that BA alterations could participate in NASH pathogenesis, both through their impact on metabolic control and their physicochemical properties potentially causing liver injury. We therefore designed a study to assess BA changes in NASH, independently of confounding metabolic parameters. To our knowledge, this is the first study comparing BA metabolism and associated signaling pathways between patients with and without NASH matched on BMI and IR levels. Another advantage of this cohort is the absence of antidiabetic or lipid-lowering medications, which can interfere with BA metabolism (38). Our results revealed that neither circulating BA species nor hepatic or intestinal BA metabolism/signaling was altered in NASH. The absence of BA alterations in relationship to NASH suggests that alterations in BA metabolism do not participate in NAFL progression to NASH.

Increased plasma total BA has been reported in NASH patients (19, 21) as well as in IR or T2D patients (14, 39). Notably, plasma primary BA increased in NASH patients (20–22) as well as in T2D patients (15, 39). These BA changes could be attributed to increased hepatic BA synthesis in NASH (23) and T2D (40) patients. Importantly, in all of these studies (19–23), NASH patients had higher IR than did controls, suggesting that these BA changes could be confounded by the metabolic status. In the present study, although glucose homeostasis was merely assessed by HOMA-IR, we also found that plasma primary BAs increase with IR (but not with NASH). Given the number of patients and variability of measurements, our study was at least as statistically powerful as the previous reports. Therefore, our study design, comparing NASH patients to BMI- and HOMA-IR–matched controls, suggests that previous results showing increased primary and total BAs are due to IR and not NASH (19–23). Our results also indicate that hepatic BA synthesis is not increased in NASH, which is in accordance with the study of Min *et al.* (41) showing cholesterol accumulation in NASH livers without upregulation of its catabolism.

In our study, we did not find any sign of cholestatic injury or activation of BA receptors. In line, UDCA, which is widely used for the treatment of cholestatic liver diseases, and its tauro-conjugated form failed to show beneficial effects on NASH (42, 43). This suggests that the mechanisms leading to liver injury and inflammation are different between cholestatic liver diseases and NASH. UDCA, which does not activate FXR, is a hydrophilic BA protecting cellular membranes and preventing cytolysis, endoplasmic reticulum stress, and apoptosis. UDCA also decreases BA uptake and synthesis, stimulates basolateral export pumps, and improves renal excretion (44). Taken together, this suggests that cholestatic injury is not involved in NASH pathogenesis and that the UDCA's mechanisms of protection are inefficacious in NASH.

BA sequestrants are used to treat cholestatic pruritus as well as hypercholesterolemia and T2D. They prevent intestinal BA absorption, interrupt the enterohepatic cycle, and result in enhanced hepatic cholesterol conversion to BA to maintain the BA pool. Colesevelam treatment decreases LDL-C and reduces FPG and hemoglobin-A1c in T2D patients (5). However, colesevelam did not improve NASH and even slightly worsened steatosis (45), again suggesting that interfering with BA metabolism *per se* is not an efficient therapeutic option for NASH.

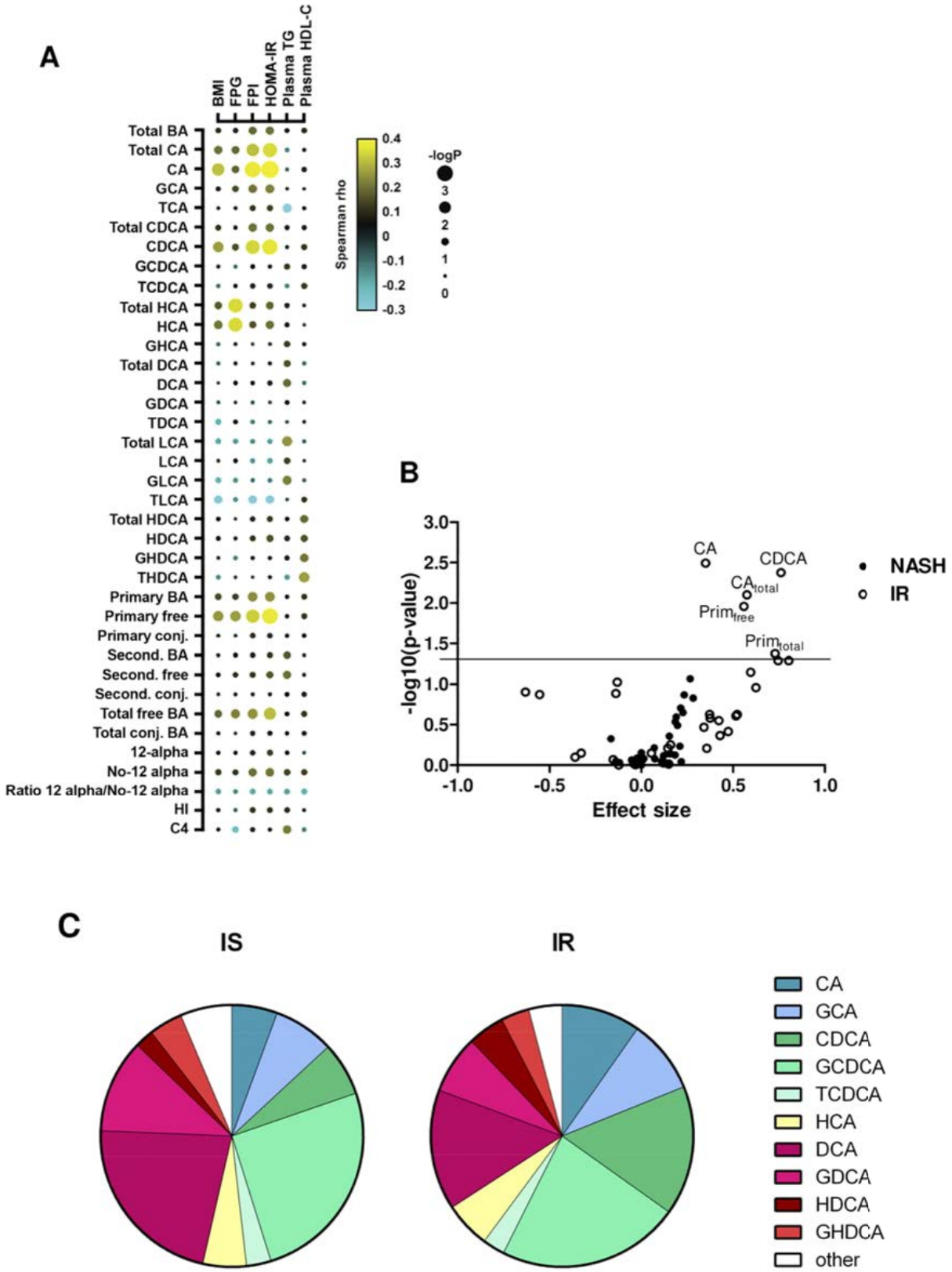


Figure 4. Plasma BA profile alterations are associated with metabolic parameters. (A) Spearman correlations between plasma BA and metabolic parameters in the 58 patients. Colors reflect the Spearman ρ values (yellow for positive, blue for inverse correlations) and dot sizes reflect the *P* values. (B) Plasma BA differences between IR (highest quartile of HOMA-IR, n = 15) and insulin sensitive (lowest quartile of HOMA-IR, n = 15) (IR, open dots) and between NASH (n = 32) and no-NASH (n = 26) patients (black dots), with *P* values plotted against estimation of effect size [Cohen's $d = (\text{mean}_{\text{IR or NASH}} - \text{mean}_{\text{IS or no-NASH}})/\text{standard deviation}_{\text{pooled}}$). (C) Comparison of plasma BA pool composition (expressed as percentage of the total BA pool) species between insulin-resistant (IR) and insulin-sensitive (IS) patients. Only BA species > 3% of the pool are depicted.

Interestingly, the FXR agonist obeticholic acid (OCA) is currently in phase 3 clinical development in NASH patients. Phase 2b trial results showed that OCA improves all hepatic histological features, including fibrosis, in NASH patients (46). OCA reduces hepatic inflammation and fibrosis *via* inhibition of Kupffer cell and hepatic stellate cell activation in experimental models (47). Intriguingly, OCA exerts beneficial hepatoprotective effects despite unfavorable metabolic effects, that is, worsening plasma lipid profile (increased LDL-C, decreased HDL-C) and IR (46). In the liver, FXR is found in two distinct genomic transcriptional regulatory modules, one involved in cellular maintenance and hepatoprotection, and the other in liver-specific metabolic functions, especially glucose, lipid, and BA metabolism (48). Therefore, it is tempting to speculate that the histological improvement of fibrosing NASH by FXR activation is mediated through the former module of metabolism-independent effects rather than through changes in BA metabolism. Finally, recent studies showed that intestinal FXR activation promotes NAFLD and that its antagonism improves metabolic control and NAFLD in mouse models of obesity (49, 50). However, intestinal FXR activation by OCA also prevents gut barrier dysfunction and attenuates intestinal inflammation in mouse models of cholestasis and cirrhosis (51, 52). Therefore, whether intestinal FXR mediates OCA's beneficial effects in NASH remains to be determined.

To conclude, our study shows that in obese patients, the hepatic necroinflammatory lesions observed in NASH are not associated with alterations in BA metabolism and signaling. BA alterations rather reflect the metabolic phenotype associated with NASH.

Acknowledgments

We thank Jean-François Goossens and Amandine Descat (Plateau de Spectrométrie de Masse, PSM-GRITA, Faculté de Pharmacie, Lille, France) for the metabolomic analyses, Bruno Derudas, Céline Gheeraert, and Hélène Dehondt (Université Lille, INSERM, Centre Hospitalier Universitaire de Lille, Institut Pasteur de Lille, U1011-EGID, Lille, France) for the transcriptomic analyses, and Jean Dallongeville (INSERM, U744, Université Lille Nord de France, Institut Pasteur de Lille, Lille, France) for precious advice in statistical analyses.

Financial Support: V.L. received grants from the Conseil Régional Nord Pas-de-Calais and the Société Francophone du Diabète. S.F. has a senior clinical research mandate from the Fund for Scientific Research (FWO) Flanders (1802154N). This work was supported by European Genomic Institute for Diabetes Grant ANR-10-LABX-46, European Commission projects HEPADIP (Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome) (Contract LSHM-CT-2005-018734) and RESOLVE (Contract FP7-305707), and by the European Research Council (ERC Grant Immunobile, Contract 694717).

Author Contributions: V.L. contributed to study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, and drafting of the manuscript. S.F. contributed to study concept and design and critical revision of the manuscript for important intellectual content. J.T.H. provided analysis and interpretation of data and statistical analysis. A.V. contributed to study concept and design and acquisition of data. S.C. provided administrative, technical, and material support. O.C.-T., E.V., L.V., E.D., A.V., and M.K. acquired data. S.L. provided analysis and interpretation of data. P.L. provided critical revision of the manuscript for important intellectual content. L.V.G. contributed to study concept and design. A.T. provided critical revision of the manuscript for important intellectual content. R.P. contributed to study supervision and critical revision of the manuscript for important intellectual content. B.S. provided study supervision and critical revision of the manuscript for important intellectual content.

Correspondence and Reprint Requests: Bart Staels, PhD, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP245, 59019 Lille, France. E-mail: bart.staels@pasteur-lille.fr.

Disclosure Summary: The authors have nothing to disclose.

References

1. Allen K, Jaeschke H, Copple BL. Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. *Am J Pathol*. 2011;**178**(1):175–186.
2. Tan KP, Yang M, Ito S. Activation of nuclear factor (erythroid-2 like) factor 2 by toxic bile acids provokes adaptive defense responses to enhance cell survival at the emergence of oxidative stress. *Mol Pharmacol*. 2007;**72**(5):1380–1390.
3. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology*. 2017;**152**(7):1679–1694.e3.
4. Li T, Chiang JYL. Bile acid signaling in metabolic disease and drug therapy. *Pharmacol Rev*. 2014;**66**(4):948–983.
5. Prawitt J, Caron S, Staels B. Glucose-lowering effects of intestinal bile acid sequestration through enhancement of splanchnic glucose utilization. *Trends Endocrinol Metab*. 2014;**25**(5):235–244.
6. Trabelsi M-S, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, Perino A, Brighton CA, Sebti Y, Kluza J, Briand O, Dehondt H, Vallez E, Dorchies E, Baud G, Spinelli V, Hennuyer N, Caron S, Bantubungi K, Caiazzo R, Reimann F, Marchetti P, Lefebvre P, Bäckhed F, Gribble FM, Schoonjans K, Pattou F, Tailleux A, Staels B, Lestavel S. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun*. 2015;**6**:7629.
7. Brunt EM. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol*. 2010;**7**(4):195–203.
8. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol*. 2017;**14**(1):32–42.
9. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;**52**(5):1836–1846.
10. Verrijken A, Beckers S, Francque S, Hilden H, Caron S, Zegers D, Ruppert M, Hubens G, Van Marck E, Michielsen P, Staels B, Taskinen MR, Van Hul W, Van Gaal L. A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. *Obesity (Silver Spring)*. 2013;**21**(10):2138–2145.
11. Boursier J, Diehl AM. Nonalcoholic fatty liver disease and the gut microbiome. *Clin Liver Dis*. 2016;**20**(2):263–275.

12. Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. *Int J Obes (Lond)*. 2013;**37**(12):1553–1559.
13. Prinz P, Hofmann T, Ahnis A, Elbelt U, Goebel-Stengel M, Klapp BF, Rose M, Stengel A. Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients. *Front Neurosci*. 2015;**9**:199.
14. Sun W, Zhang D, Wang Z, Sun J, Xu B, Chen Y, Ding L, Huang X, Lv X, Lu J, Bi Y, Xu Q. Insulin resistance is associated with total bile acid level in type 2 diabetic and nondiabetic population: a cross-sectional study. *Medicine (Baltimore)*. 2016;**95**(10):e2778.
15. Haeusler RA, Astiarraga B, Camastra S, Accili D, Ferrannini E. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. *Diabetes*. 2013;**62**(12):4184–4191.
16. Bower G, Athanasiou T, Isla AM, Harling L, Li JV, Holmes E, Efthimiou E, Darzi A, Ashrafian H. Bariatric surgery and non-alcoholic fatty liver disease. *Eur J Gastroenterol Hepatol*. 2015; **27**(7):755–768.
17. Aranha MM, Cortez-Pinto H, Costa A, da Silva IBM, Camilo ME, de Moura MC, Rodrigues CMP. Bile acid levels are increased in the liver of patients with steatohepatitis. *Eur J Gastroenterol Hepatol*. 2008;**20**(6):519–525.
18. Lake AD, Novak P, Shipkova P, Aranibar N, Robertson D, Reily MD, Lu Z, Lehman-McKeeman LD, Cherrington NJ. Decreased hepatotoxic bile acid composition and altered synthesis in progressive human nonalcoholic fatty liver disease. *Toxicol Appl Pharmacol*. 2013;**268**(2):132–140.
19. Bechmann LP, Kocabayoglu P, Sowa J-P, Sydor S, Best J, Schlattjan M, Beilfuss A, Schmitt J, Hannivoort RA, Kilicarslan A, Rust C, Berr F, Tschopp O, Gerken G, Friedman SL, Geier A, Canbay A. Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. *Hepatology*. 2013;**57**(4):1394–1406.
20. Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC. Elevated hepatic fatty acid oxidation, high plasma fibroblast growth factor 21, and fasting bile acids in nonalcoholic steatohepatitis. *Eur J Gastroenterol Hepatol*. 2011;**23**(5):382–388.
21. Ferslew BC, Xie G, Johnston CK, Su M, Stewart PW, Jia W, Brouwer KLR, Barritt AS IV. Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. *Dig Dis Sci*. 2015; **60**(11):3318–3328.
22. Kalhan SC, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, Milburn M. Plasma metabolomic profile in non-alcoholic fatty liver disease. *Metabolism*. 2011;**60**(3):404–413.
23. Mouzaki M, Wang AY, Bandsma R, Comelli EM, Arendt BM, Zhang L, Fung S, Fischer SE, McGilvray IG, Allard JP. Bile acids and dysbiosis in non-alcoholic fatty liver disease. *PLoS One*. 2016; **11**(5):e0151829.
24. Francque S, Verrijken A, Caron S, Prawitt J, Paumelle R, Derudas B, Lefebvre P, Taskinen MR, Van Hul W, Mertens I, Hubens G, Van Marck E, Michielsen P, Van Gaal L, Staels B. PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *J Hepatol*. 2015;**63**(1):164–173.
25. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;**41**(6):1313–1321.
26. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Association for the Study of Liver Diseases; American College of Gastroenterology; American Gastroenterological Association. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol*. 2012;**107**(6):811–826.
27. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016;**64**(6):1388–1402.
28. Bedossa P, Poitou C, Veyrie N, Bouillot J-L, Basdevant A, Paradis V, Tordjman J, Clement K. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology*. 2012;**56**(5):1751–1759.
29. Pawlak M, Baugé E, Bourguet W, De Bosscher K, Lalloyer F, Tailleux A, Lebherz C, Lefebvre P, Staels B. The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice. *Hepatology*. 2014;**60**(5):1593–1606.
30. Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap—bile acids in metabolic control. *Nat Rev Endocrinol*. 2014;**10**(8):488–498.
31. Spinelli V, Lalloyer F, Baud G, Osto E, Kouach M, Daoudi M, Vallez E, Raverdy V, Goossens JF, Descat A, Doytcheva P, Hubert T, Lutz TA, Lestavel S, Staels B, Pattou F, Tailleux A. Influence of Roux-en-Y gastric bypass on plasma bile acid profiles: a comparative study between rats, pigs and humans. *Int J Obes (Lond)*. 2016; **40**(8):1260–1267.
32. Steiner C, von Eckardstein A, Rentsch KM. Quantification of the 15 major human bile acids and their precursor 7α-hydroxy-4-cholesten-3-one in serum by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;**878**(28):2870–2880.
33. Axelson M, Björkhem I, Reihnér E, Einarsson K. The plasma level of 7α-hydroxy-4-cholesten-3-one reflects the activity of hepatic cholesterol 7α-hydroxylase in man. *FEBS Lett*. 1991;**284**(2):216–218.
34. Schmucker DL, Ohta M, Kanai S, Sato Y, Kitani K. Hepatic injury induced by bile salts: correlation between biochemical and morphological events. *Hepatology*. 1990;**12**(5):1216–1221.
35. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature*. 2013; **499**(7456):97–101.
36. Perino A, Schoonjans K. TGR5 and immunometabolism: insights from physiology and pharmacology. *Trends Pharmacol Sci*. 2015; **36**(12):847–857.
37. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson JA, Gerard RD, Repa JJ, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab*. 2005;**2**(4):217–225.
38. Lien F, Berthier A, Bouchaert E, Gheeraert C, Alexandre J, Porez G, Prawitt J, Dehondt H, Ploton M, Colin S, Lucas A, Patrice A, Pattou F, Diemer H, Van Dorsselaer A, Rachez C, Kamilic J, Groen AK, Staels B, Lefebvre P. Metformin interferes with bile acid homeostasis through AMPK-FXR crosstalk. *J Clin Invest*. 2014;**124**(3): 1037–1051.
39. Sonne DP, van Nierop FS, Kulik W, Soeters MR, Vilsbøll T, Knop FK. Postprandial plasma concentrations of individual bile acids and FGF-19 in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2016;**101**(8):3002–3009.
40. Brufau G, Stellaard F, Prado K, Bloks VW, Jonkers E, Boverhof R, Kuipers F, Murphy EJ. Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. *Hepatology*. 2010;**52**(4): 1455–1464.
41. Min H-K, Kapoor A, Fuchs M, Mirshahi F, Zhou H, Maher J, Kellum J, Warnick R, Contos MJ, Sanyal AJ. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab*. 2012;**15**(5):665–674.

42. Legry V, Van Rooyen DM, Lambert B, Sempoux C, Poekes L, Español-Suñer R, Molendi-Coste O, Horsmans Y, Farrell GC, Leclercq IA. Endoplasmic reticulum stress does not contribute to steatohepatitis in obese and insulin-resistant high-fat-diet-fed *foz/foz* mice. *Clin Sci (Lond)*. 2014;**127**(7):507–518.
43. Steinacher D, Claudel T, Trauner M. Therapeutic mechanisms of bile acids and nor-ursodeoxycholic acid in non-alcoholic fatty liver disease. *Dig Dis*. 2017;**35**(3):282–287.
44. Roma MG, Toledo FD, Boaglio AC, Basiglio CL, Crocenzi FA, Sánchez Pozzi EJ. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications. *Clin Sci (Lond)*. 2011;**121**(12):523–544.
45. Le T-A, Chen J, Changchien C, Peterson MR, Kono Y, Patton H, Cohen BL, Brenner D, Sirlin C, Loomba R; San Diego Integrated NAFLD Research Consortium (SINC). Effect of colesevelam on liver fat quantified by magnetic resonance in nonalcoholic steatohepatitis: a randomized controlled trial. *Hepatology*. 2012;**56**(3):922–932.
46. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet*. 2015;**385**(9972):956–965.
47. Verbeke L, Mannaerts I, Schierwagen R, Govaere O, Klein S, Vander Elst I, Windmolders P, Farre R, Wenes M, Mazzone M, Nevens F, van Grunsven LA, Trebicka J, Laleman W. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. *Sci Rep*. 2016;**6**:33453.
48. Dubois-Chevalier J, Dubois V, Dehondt H, Mazrooei P, Mazuy C, Sérandour AA, Gheeraert C, Guillaume P, Baugé E, Derudas B, Hennuyer N, Paumelle R, Marot G, Carroll JS, Lupien M, Staels B, Lefebvre P, Eeckhoute J. The logic of transcriptional regulator recruitment architecture at cis-regulatory modules controlling liver functions. *Genome Res*. 2017;**27**(6):985–996.
49. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, Cai J, Qi Y, Fang ZZ, Takahashi S, Tanaka N, Desai D, Amin SG, Albert I, Patterson AD, Gonzalez FJ. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest*. 2015;**125**(1):386–402.
50. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, Brocker CN, Desai D, Amin SG, Bisson WH, Liu Y, Gavrilova O, Patterson AD, Gonzalez FJ. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat Commun*. 2015;**6**:10166.
51. Úbeda M, Lario M, Muñoz L, Borrero M-J, Rodríguez-Serrano M, Sánchez-Díaz A-M, del Campo R, Lledó L, Pastor Ó, García-Bermejo L, Díaz D, Álvarez-Mon M, Albillos A. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. *J Hepatol*. 2016;**64**(5):1049–1057.
52. Verbeke L, Farre R, Verbinnen B, Covens K, Vanuytsel T, Verhaegen J, Komuta M, Roskams T, Chatterjee S, Annaert P, Vander Elst I, Windmolders P, Trebicka J, Nevens F, Laleman W. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am J Pathol*. 2015;**185**(2):409–419.

PART II

BILE ACIDS IN BARIATRIC SURGERY: STUDY IN HUMANS AND IN PRECLINICAL MODELS OF MINIPIG (*Sus scrofa*).

The bariatric surgery is the most effective treatment for morbid obesity, since it reduces body weight and consequently, improves metabolism. Interestingly, many of the metabolic improvements following bariatric surgery occur before the body weight loss occurs, and the mechanism driving those improvements is unknown. Among the candidates proposed to participate to these changes are the bile acids, since their systemic concentrations increase and their proportions were reported to be modified early after the surgery in many clinical and pre-clinical studies (***See the Table in the Manuscript 1*, page 17**).

This chapter's introduction is a review summarizing the reported bile acid changes in humans undergoing bariatric surgery, the mechanisms underlying the bile acid changes and the potential signaling role of bile acids in the metabolic improvements after bariatric surgery (***Chávez-Talavera et al. Appetite 2019 submission in course. Manuscript 5 page 91***).

Since bile acids are one of the candidates proposed to mediate the early metabolic improvements following the Roux-en-Y gastric bypass (RYGB), we wondered what the mechanism underlying such bile acid increases is.

To address this question, we made a collaboration with the team of Pr. François Pattou (UMR 1190, EGID, University of Lille), that has validated a Göttingen-like minipig model of RYGB. We used this model since it presents the advantage to be an omnivorous human-sized mammal, allowing continuous sampling and simultaneous analysis of pre-hepatic portal and systemic venous blood. Dr. Grégory Baud, PhD student of François Pattou at the time of the study, performed the surgeries, and the bile acid dosages were performed by Emmanuelle Vallez, technician working in our team. I was trusted to interpret the bile acid data and search for the underlying hepatic mechanism, and write the manuscript under the direction of my PhD advisor and thanks to the intellectual training and contributions from Sophie Lestavel, Bart Staels and François Pattou. Since I was the responsible for this project I am co-first author of this manuscript.

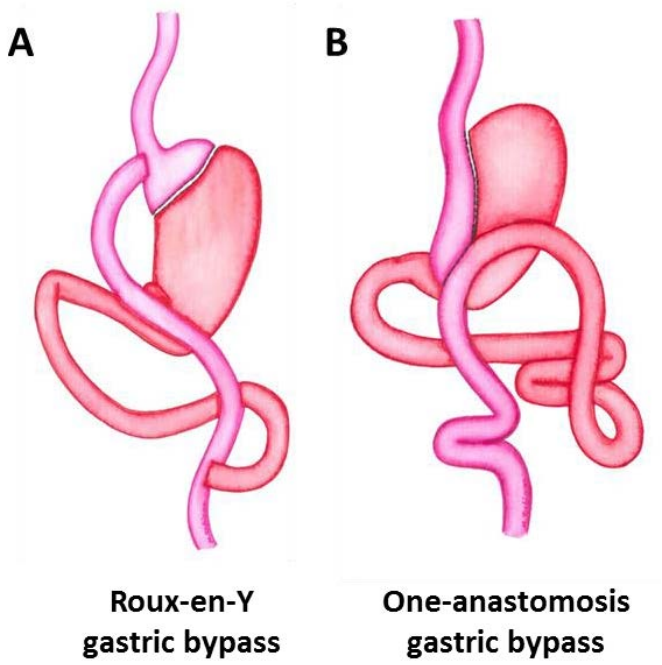


Figure 3. A. The Roux-en-Y gastric bypass (RYGB) is one of the most frequently performed bariatric procedures, consisting on the creation of a small gastric pouch from the cardiac portion of the stomach, the rest of the stomach being excluded. Then, the small intestine is segmented 50cm distal to the ligament of Treitz; the proximal segment constitutes the biliopancreatic limb. The distal segment is anastomosed to the small gastric pouch and constitutes the alimentary limb. The biliopancreatic limb is anastomosed with the alimentary limb to form the common limb, and the length of the latter is variable depending on the surgeon and healthcare facilities (Nguyen & Varela, 2017) **B. The One anastomosis gastric bypass (OAGB): Also known as minigastric bypass, encompasses a series of techniques characterized by the absence of the alimentary limb, the permanence of the excluded stomach (as in the RYGB) and the presence of a long biliopancreatic limb, it is therefore a less agresive malabsorptive procedure since the common limb is not so short (Mahawar *et al.*, 2016). *Adapted from Chávez-Talavera et al. Appetite 2019.***

Since our aim was to study the role of the liver in the RYGB-induced bile acid changes, we analyzed the portal venous blood and the systemic blood bile acids during a meal test before and after RYGB, and analyzed hepatic bile acid metabolism gene expression. We demonstrated that, postprandially, the portal bile acids are unaltered following bariatric surgery, whereas the systemic bile acid concentrations increase due to increased conjugated bile acid species. Since the organ between the portal and the systemic circulation is the liver, and since the liver is the one of the main organs where bile acid metabolism occurs, we analyzed in liver biopsies the expression of the bile acid metabolism genes. Our analysis revealed decreased expression of hepatic bile acid reuptake transporter genes, suggesting that selective hepatic reuptake occurs upon RYGB, and contributes to the plasma bile acid changes following bariatric surgery (***Chávez-Talavera & Baud et al. Int J Obes 2017. Manuscript 6, page 114***).

For the next section of this chapter, we focused on the intestinal bile acids, since they participate to the regulation of glucose homeostasis *via* their receptors in the intestine, and since the biliary limb has been hypothesized to mediate the metabolic improvements following bariatric surgery. We wondered whether the biliary limb mediates the metabolic changes of another bariatric surgery technique: the One-Anastomosis Gastric Bypass (OAGB) (Figure 3B), and whether intestinal bile acids participate to such metabolic improvements.

To answer this question, we continued the collaboration with François Pattou, and used the minipig preclinical model. Minipigs underwent OAGB or sham surgery with and without resection of the biliary limb. The minipigs were metabolically phenotyped 20 days after the surgery, including intestinal bile acid content all along the gastrointestinal tract and plasma bile acids. Dr. Camille Marciniak. PhD student under the supervision of François Pattou performed the surgeries and I made the intestinal bile acid analyses, dosed the plasma bile acids and C4 concentrations. I was responsible for the analysis of all the bile acid data and equally contributed to the interpretation and drafting of the manuscript with Camille Marciniak, for which I am co-first author of this article in preparation.

This work yielded novel results on the bile acid field. First, we show for the first time that the OAGB increases systemic bile acid concentrations, similarly as the RYGB. Next, we describe that glucose homeostasis improves simultaneously with a shift in the intestinal intra-luminal proportions of HDCA towards HCA upon OAGB. Finally, we studied the role of the biliary and the common limbs in the metabolic improvements and in the intestinal intra-luminal bile acid changes upon OAGB. Our data show that the resection of the biliary limb (or the corresponding intestinal segment in the sham model) reproduced the metabolic and bile acid changes as OAGB without resection, suggesting that the length of the common limb, but not that of the biliary limb, mediates such effects (***Chávez-Talavera & Marciniak et al. Article in preparation. Manuscript 7, page 120***).

The final part of this chapter is a study in humans performed in collaboration with the team of Elena Osto, from the Laboratory of Translational Nutrition Biology at the Univesity of Zurich, Zurich, Switzerland. For this section, the reader should know that bile acids circulate in the bloodstream bound to plasma components of the lipoprotein-depleted fraction, and the albumin exhibits the highest affinity to bind them (Ceryak, Bouscarel, & Fromm, 1993; Rudman & Kendall, 1957). However, bile acids are also transported by lipoproteins (Kramer, Buscher, Gerok, & Kurz, 1979; Steiner *et al.*, 2012). Indeed, total bile acids, transported by VLDL+LDL and HDL, represent 5-10% of the total plasma bile acids in physiological conditions (Buscher, Beger, Sauerbier, & Gerok, 1987; Hedenborg, Norman, & Ritzén, 1988; Steiner *et al.*, 2012). Additionally, the HDL are known to exert vaso-protective effects on the

endothelial cells by, among others, enhancing nitric oxide production or inhibition of apoptosis (Osto *et al.* 2015). These properties of HDL are known to be decreased in the context of obesity, and restored early upon RYGB. Since circulating bile acids also change early upon RYGB, we studied the bile acids bound to HDL in humans following RYGB and whether they are correlated with the improvement of the vaso-protective properties of HDL. Our data show that RYGB increases the bile acids bound to the HDL and that the increase in CA and CDCA is correlated with the restoration of HDL anti-apoptotic properties following surgery (***Chávez-Talavera & Jomard, et al. Article in preparation. Manuscript 8, page 148***).

MANUSCRIPT NUMBER 5

Chávez-Talavera *et al.* Bariatric surgery and bile acids in humans and pre-clinical models. Appetite 2019, article under submission.

Bariatric surgery and bile acids in humans and pre-clinical models.

Oscar Chávez-Talavera MD[1], Lorea Zubiaga MD-PhD[2], Camille Marciniak MD[2], Bart Staels PhD*[1], Anne Tailleux PhD*[1], Francois Pattou MD-PhD*[2]

[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011- EGID, F-59000 Lille, France.
[2]University Lille, Inserm, CHU Lille, U1190 Translational research for diabetes, 59000 Lille, France.

*Co-last authors

Corresponding author: Francois Pattou, MD.
e-mail: francois.pattou@univ-lille.fr
University Lille, Inserm, CHU Lille, U1190 Translational research for diabetes, 59000 Lille, France. European Genomic Institute for Diabetes, EGID, 59000 Lille, France.

Abstract (142/250)

Bariatric surgery is the most effective and long lasting treatment for morbid obesity, improving metabolism not only by inducing body weight loss, but also by early, weight-loss independent mechanisms. Bile acids are signaling molecules regulating metabolic homeostasis whose plasma concentrations increase upon many bariatric surgery techniques in humans. The aim of this review is to discuss the human studies reporting changes in fasting and post-prandial bile acids plasma concentrations upon the most common bariatric surgery techniques, and how human studies support the hypothesis that bile acids participate to the early effects of bariatric surgery. Additionally, we will present the currently available preclinical models of mouse, rat and pig in bariatric surgery, their pros and cons for the translational study of bile acid signaling and physiology, and the mechanistic experiments *in vivo* linking bile acid signaling with the metabolic effects of bariatric surgery.

Keywords

Bile acids, RYGB, VSG, LAGB, bariatric surgery, minipig

Introduction

The rising global prevalence of obesity and its complications represents a major public health problem worldwide, carrying severe metabolic comorbidities, decreased life quality and economic costs for society (Srivastava and Apovian 2018; Ng *et al.* 2014). Treatment of obesity consists on inducing weight loss, which can be achieved through lifestyle modifications, aiming to modify nutritional behavior and physical activity. Additionally, FDA-approved anti-obesity medications are available as adjunct therapy for patients failing to respond to lifestyle modifications alone. Most of these anti-obesity drugs induce only approximately 3–7% net weight loss (For review (Srivastava and Apovian 2018)). Although modest weight loss may provide clinical benefits, achieved weight loss by these methods is often not durable (Heymsfield and Wadden 2017).

Bariatric surgery is the most effective and sustained treatment for morbid obesity and its comorbidities (Nguyen and Varela 2017). There are many different techniques varying from purely restrictive to malabsortive, of which the Roux-en-Y gastric bypass (RYGB) and the sleeve gastrectomy (SG) are the most performed in the world. The techniques involving a bile diversion are characterized by the bypass of intestinal segments, whose different regions are well known to have a selective role in the absorption of specific nutrients. Bile diversion techniques are more effective in the long term for excess weight loss and reversal of comorbidities (Ramos-Molina *et al.* 2018). The biliopancreatic diversion - duodenal switch (BPD-DS) and the Roux-en-Y gastric bypass (RYGB) are the most commonly performed bile diversion techniques, due to their greater body-weight loss effects (Buchwald *et al.* 2009). However, the ideal length of the limbs to obtain best results and minimize adverse effects risk has not yet been established (Pinheiro *et al.* 2008). Other bariatric surgery techniques consist on surgically restricting the size of the stomach with or without resection of the excluded part, or on implementing exogenous tools that physically restrict the passage of the food to the gastric lumen. In this review, the main surgical techniques are gathered in 3 groups: bile diversion (RYGB, one-anastomosis gastric bypass (OAGB), BPD-DS), gastric resection (BPD-DS and SG) and gastric restriction (adjustable gastric band (AGB)) (Figure 1).

In addition to body weight loss, bariatric surgery also improves obesity-related co-morbidities such as type 2 diabetes (T2D) (D. E. Cummings and Rubino 2018), non-alcoholic steatohepatitis (Lassailly *et al.* 2015), high blood pressure (Owen, Yazdi, and Reisin 2017) and cardiovascular disease (Sjöström *et al.* 2012; Tailleux *et al.* 2015). Such improvements are often attributed to the body weight loss. However, some of these improvements appear before the patient loses weight (Carbajo *et al.* 2017; Sweeney and Morton 2014), and several potential mechanisms have been postulated: 1. Negative caloric balance (Isbell *et al.* 2010);

2. Food exclusion from the gastric antrum, duodenum and proximal jejunum, leading to decreased ghrelin secretion, resulting in improved insulin levels and glucose tolerance (Patel *et al.* 2014; Murata *et al.* 2002); 3. Increased rate of nutrient presentation the distal intestine, stimulating glucagon-like peptide 1 (GLP-1) and PYY release (Sethi and Parikh 2015); 4. Beneficial alterations in the gut microbiota (Liou *et al.* 2013); 5. Alterations in neural signaling (Patel *et al.* 2014); 6. Changes in glucose intestinal absorption (Baud *et al.* 2016); 7. Alterations in bile acid pool size, composition and signaling (Patel *et al.* 2014; Sethi and Parikh 2015). In this review we will present and discuss the reported changes in BA plasma concentrations and signaling upon bariatric surgery in humans and in the pre-clinical models of mice, rats and pigs in bariatric surgery, and how the currently available data support the hypothesis that BA signaling could be implicated in the metabolic improvements of bariatric surgery.

Bile acid metabolism

BAs are a family of molecules synthesized in hepatocytes from cholesterol by two pathways (Figure 2): the classical pathway produces the majority of BAs, it is initiated by the enzyme cytochrome P450 cholesterol 7α-hydroxylase (CYP7A1), and a biomarker of this pathway's activity rate is the plasma concentration of 7α-hydroxy-4-cholesten-3-one (C4). The remaining fraction of BAs is synthesized through the alternative, or acidic pathway, initiated by cytochrome P450 27α-hydroxylase CYP27A1. These pathways produce the primary BAs, *i.e.* BAs synthesized within the hepatocytes, that in humans and pigs can be subsequently hydroxylated by either the 12α-hydroxylase CYP8B1, producing the cholic acid (CA), the 6α-hydroxylase (CYP3A4) (Deo and Bandiera 2008) producing hyocholic acid, or none of the previous, producing chenodeoxycholic acid (CDCA) (of note, the major plasma BA families in humans are CA and CDCA). Exclusively in murine models, CDCA and UDCA can be 6β-hydroxylated by the 6β-hydroxylase CYP2C70 (Takahashi *et al.* 2016), producing the primary BAs α- and β-muricholic acid (MCA), respectively, whereas murine HCA is produced by bacterial transformation in the intestine. Noteworthy, the activity of the 12α-hydroxylase CYP8B1 is regulated by the insulin receptor-Akt pathway and the ratio of 12α-hydroxylated/non-12α-hydroxylated BAs is associated with changes in hepatocytes' insulin sensitivity (Haeusler *et al.* 2013).

BAs are subsequently conjugated to glycine or taurine and actively secreted by the bile salt export pump (BSEP) into the bile, which is stored and concentrated in the gallbladder during the inter-digestive period and released in the duodenum after meal ingestion to contribute to the emulsification and absorption of dietary fat. Along the gastrointestinal tract, the gut microbiota dehydroxylates and epimerizes the primary BAs to synthesize secondary BAs: CA into DCA, CDCA into LCA and UDCA, and HCA into HDCA in humans (Figure 2).

Additionally, in murine models β-MCA is transformed into ωMCA, and HCA is a secondary BA species (for review (Hofmann and Hagey 2008)). Free BAs are re-absorbed passively throughout the whole intestine, but the apical-sodium-dependent BA transporter (ASBT) expressed mainly in the distal ileum, takes up conjugated BA species. BAs are translocated to the basolateral pole of the enterocytes by the ileal bile acid binding protein (IABP) and secreted into the mesenteric venous blood *via* the organic solute transporter (OST) complex α/β, and then transported back to the liver in the portal blood. Approximately 5% of BAs resist intestinal reabsorption and are lost in feces and replaced by hepatic *de novo* synthesis. As BAs pass through the hepatic sinusoids, the hepatocytes reabsorb most conjugated BAs through their transporters: sodium/bile acid cotransporter (NTCP), organic-anion-transporting polypeptides (OATPs) and organic anion transporters (OATs), whereas free BAs can pass through the plasma membrane by passive diffusion. Although hepatic BA reuptake is highly efficient, a fraction of BAs spills into the systemic circulation reaching peripheral organs, where they act as signaling molecules by activating the nuclear farnesoid X receptor (FXR) and the Takeda G protein coupled receptor (TGR5), together regulating metabolic homeostasis, BA synthesis and meta-inflammation. The different BA species activate FXR and TGR5 with different potencies. Consequently, quantitative and qualitative variations of the BA pool composition could change the activity of these receptors and modulate metabolic pathways regulated by them (for review (Oscar Chávez-Talavera *et al.* 2017; Kuipers, Bloks, and Groen 2014)).

Importantly, BA variations in the metabolic syndrome and in the context of bariatric surgery have been studied in humans and in pre-clinical models of mouse, rats and pigs. It should be taken into account that the BA species proportions and types between these animal species present important variations, with diverse advantages and disadvantages (Spinelli *et al.* 2016). The rodent models present as advantage that their husbandry is cheaper and easier, and the availability of genetically-modified animals for mechanistic studies and the simplicity of the surgical procedures allowing larger sample sizes compared to larger mammals. However, muricholic species are the most abundant BAs in murine models, and increasing evidence suggests that they are important regulators of murine metabolic homeostasis *via* antagonistic effects of Tβ-MCA on FXR, rendering BA data on murine models not entirely translatable to humans. The development of CYP2C70 knock-out murine models would theoretically suppress 6β-hydroxylation and thus α- and β- MCA species synthesis, resulting in a “humanized BA pool” that could render BA results in these mice more clinically relevant. Another pre-clinical model for the study of BAs and bariatric surgery is the pig (*Sus scrofa*) strain Gottingen-like minipig (Baud *et al.* 2016), this strain is smaller than ordinary pigs, and presents as advantage a human size along with greater similarity to humans’ anatomy and nutrition, and the possibility of larger blood sampling volumes, allowing complex metabolic

tests with simultaneous sampling from catheters allowing access to different sites (e.g. portal or central venous catheters). Additionally, pigs don't synthesize the rodent α- and β- MCA species, rendering their BA pool more similar to humans. However, pigs' husbandry is expensive and requires larger space, surgical procedures are heavier and require special operating rooms, there are not currently available many pig knock-out models for mechanistic studies and pigs present a BA pool mainly represented by HCA and its derived secondary BA HDCA, which are minor BA species in humans, whose signaling on metabolism *via* FXR and TGR5 has not been studied and which are often neglected on plasma BA analysis. However, recent interest has emerged on 6α-hydroxylated BAs since they have been negatively associated with obesity and glucose homeostasis parameters in personal unpublished data and by other teams (Wewalka *et al.* 2014).

Patients undergoing bariatric surgery present co-morbidities associated with bile acid alterations.

Several studies have associated plasma BAs with obesity in humans, reporting vastly heterogeneous results (Prinz *et al.* 2015; Brufau *et al.* 2010; Friedrich, Marschall, and Lammert 2018; Glicksman *et al.* 2010; Haeusler *et al.* 2016; Cariou *et al.* 2011; Ahmad, Pfalzer, and Kaplan 2013; Steiner *et al.* 2011), likely due to differences in the characteristics of the studied populations and the high inter- and intra- individual variability of BAs (Steiner *et al.* 2011) and the fasting/fed metabolic status (Oscar Chávez-Talavera *et al.* 2019). Overall, obesity seems to be associated with increased fasting plasma BAs but with blunted meal-induced increases. Furthermore, morbidly obese patients undergoing bariatric surgery often present comorbidities that are associated with BA changes, such as insulin resistance (Haeusler *et al.* 2013; W. Sun *et al.* 2016; Ginos *et al.* 2018; Wewalka *et al.* 2014; Cariou *et al.* 2011; Kimberly *et al.* 2017) and type 2 diabetes (Brufau *et al.* 2010; Vincent *et al.* 2013; Jørgensen *et al.* 2015; Haeusler *et al.* 2013; Gerhard *et al.* 2013; Sonne *et al.* 2016; Suhre *et al.* 2010). Even though there are inconsistencies in the published data, BA metabolism alterations occur in obesity and its comorbidities in humans (for review (Oscar Chávez-Talavera *et al.* 2019)). Additionally, obese patients often present hypertriglyceridaemia treated with fibrates (PPARα agonists), which have been associated *in vitro*, *in vivo* and in humans with decreased BA synthesis *via* a PPARα-FXR crosstalk (Pineda Torra *et al.* 2003). Furthermore, approximately 25% of the patients undergoing bariatric surgery have had the gallbladder removed (Papasavas *et al.* 2006), and the impact of cholecystectomy in plasma BAs is unknown. Finally many obese patients undergoing bariatric surgery are treated with metformin, associated with altered gut microbiota (Forslund *et al.* 2015) that could impact intestinal BA transformation. A recent study suggested that the metabolic effects of metformin occur by decreasing *Bacteroides fragilis* abundance, leading to increased GUDCA

levels, which could inhibit FXR signaling (L. Sun *et al.* 2018). These results need to be further confirmed, but it cannot be excluded that metformin treatment on diabetic patients undergoing bariatric surgery could change BA pool composition and bias results of BAs and bariatric surgery.

Therefore, when studying BAs in the context of bariatric surgery, it is important to consider that the association of BA changes to metabolic improvements could be influenced by the metabolic improvements themselves or by the pharmacological treatments and not exclusively by the surgical intervention.

Plasma bile acid variations in the different bariatric surgery techniques

Bile diversion surgeries.

Bile diversion techniques surgically shift to the distal part of the intestine the anatomic place on which bile encounters chymus, and enhance the delivery of biliopancreatic secretion to the distal bowel. Clinical studies have demonstrated increased BAs upon bile diversion techniques (see below). The increase is reproducible in pre-clinical models, allowing to explore the underlying mechanisms. For instance, bile diversion alone reproduced the effects of RYGB on glucose metabolism in rats and mice (Goncalves *et al.* 2015; Flynn *et al.* 2015). Moreover, drainage of endogenous bile into the terminal ileum of rats enhanced satiety gut hormone response, reduced food intake, and lower body weight (Pournaras *et al.* 2012). Furthermore, a recent study reported that gallbladder bile diversion to the ileum reproduces the glycemic improvements of bariatric surgery in wild-type and TGR5 knock-out mice, but not in intestinal-specific FXR knock-out mice, or in presence of the GLP-1R antagonist exendin-9 or bile acid sequestration, pointing at the GLP-1-FXR intestinal axis as a crucial mediator of the metabolic improvements of bariatric surgery (Albaugh *et al.* 2019). The surgical modification is not entirely necessary, since the duodenal-jejunal bypass liner (DJBL), bypassing proximal intestine through a simple physical barrier between ingested food and the proximal intestine epithelium, increased fasting and postprandial free BAs and postprandial GLP-1 plasma levels after 6 months of DJBL placement, this suggested that the GLP-1 increase does not depend on the BA augmentations since only GLP-1 but not BA levels are increased 1 week after DJBL implantation (van Nierop *et al.* 2019).

Roux-en-Y gastric bypass (RYGB)

Fasting plasma BAs increase upon RYGB in humans, but the reported BA species are heterogeneous between studies probably due to variations in the surgical technique, time upon surgery, nutritional state or patients BMI, IR and T2D status. Some of the studies have reported early increases in fasting plasma BAs (Jansen *et al.* 2011; Pournaras *et al.* 2012;

Nakatani *et al.* 2009; Spinelli *et al.* 2016; Jørgensen *et al.* 2015; Albaugh *et al.* 2015; Ferrannini *et al.* 2015; Fiamoncini *et al.* 2018), whereas other studies suggest that the increase is evident at one year upon RYGB (Simonen *et al.* 2012; Werling *et al.* 2013; Steinert *et al.* 2013; Dutia *et al.* 2015; Patti *et al.* 2009; Fiamoncini *et al.* 2018) or upon 20% loss of body weight (Kohli *et al.* 2013). Only few studies have reported no changes in fasting plasma BAs (Steinert *et al.* 2013; Dutia *et al.* 2015; Sachdev *et al.* 2016). Similarly, post-prandial plasma BAs have been reported to increase both, early upon surgery (Ahmad, Pfalzer, and Kaplan 2013) and in the long term (Werling *et al.* 2013; Ahmad, Pfalzer, and Kaplan 2013; Kohli *et al.* 2013; Dutia *et al.* 2015; Sachdev *et al.* 2016; De Giorgi *et al.* 2015; Steinert *et al.* 2013; Jørgensen *et al.* 2015), along with qualitative increase in conjugated BA species (Sachdev *et al.* 2016; Werling *et al.* 2013). The BA changes following RYGB were not associated with the BMI decrease (Dirksen *et al.* 2013), nor with the insulin sensitivity. In line, the RYGB improves insulin resistance and increases the 12α-hydroxylated/non12α-hydroxylated BA ratio (Dutia *et al.* 2015), in contradiction with the results in patients without surgery, showing a positive correlation of insulin resistance with the 12α-hydroxylated/non12α-hydroxylated BA ratio (Haeusler *et al.* 2013). Conversely, plasma BA increase is greater in T2D patients presenting remission upon RYGB than in those patients whose T2D did not remit (Gerhard *et al.* 2013), and women with diabetes remission upon RYGB presented decreased fecal BAs compared to non-remitters (de Siqueira Cardinelli *et al.* 2019), suggesting that BAs could be implicated in RYGB-mediated T2D improvements. Intestinal FXR and TGR5 signaling could be implicated to these metabolic improvements, since BA levels positively correlate with plasma GLP-1 concentrations (Patti *et al.* 2009; Steinert *et al.* 2013) -regulated by FXR and TGR5 in the intestine-, and FGF19 levels -an intestinal FXR activation marker- increase upon RYGB (Gerhard *et al.* 2013; Jansen *et al.* 2011).

Plasma BA concentrations also increase upon RYGB in murine and pig pre-clinical models (Spinelli *et al.* 2016; Bhutta *et al.* 2015; O. Chávez-Talavera *et al.* 2017). Mechanistic studies in murine models have suggested that it could be due to increased hepatic *de novo* BA synthesis, increased intestinal BA reuptake along with gut microbiota modifications or increased portal blood waste potentially associated with lower hepatic BA recapture from portal blood (Goncalves *et al.* 2015; Bhutta *et al.* 2015; Liou *et al.* 2013; Ryan *et al.* 2014; Spinelli *et al.* 2016). Adittionally, the expression of hepatic bile acid recapture transporters decreased upon RYGB, along with increased BAs in minipigs (O. Chávez-Talavera *et al.* 2017) and in rats (Bhutta *et al.* 2015). Recently, the BA composition all along the gastro-intestinal tract was described in rats upon RYGB reporting no changes upon RYGB and VSG, notwithstanding alterations in the gut microbiota, suggesting that intestinal selective reuptake might not be involved in the systemic BA changes (Duboc *et al.* 2018).

Bilio-pancreatic diversion - duodenal switch (BPD-DS)

BPD-DS is highly similar to RYGB, but the length of the limbs is markedly different. BPD-DS is highly malabsorptive since most of the intestine is bypassed. To daate, this technique is less used, since it has higher risk of side effects and higher mortality rates (Schauer *et al.* 2015). Similarly as in RYGB, fasting plasma BA concentrations after BPD-DS occur (Ramos-Molina *et al.* 2018; Ferrannini *et al.* 2015) and also when compared with BMI-matched non-surgical subjects (Ferrannini *et al.* 2015). The underlying mechanisms may be similar to the RYGB, since the techniques have many similarities.

One anastomosis gastric bypass (OAGB)

The OAGB is a new technique with rising interest since it is a more rapid and safe procedure with effective results. However, the long-term effects are still unclear and the impact of this surgery on plasma BAs in humans is still unknown. Personal unpublished data demonstrate that OAGB increases plasma BAs similarly as RYGB in the preclinical model of minipig (data not shown).

Gastric resection surgery:

Vertical sleeve gastrectomy (VSG)

It is unclear whether VSG alters plasma BAs due to the contradictory reported results. Increased plasma BAs have been reported in the fasting state early (Nakatani *et al.* 2009; Jahansouz *et al.* 2016) or 1 year after VSG (Steinert *et al.* 2013), and also postprandially (Khan *et al.* 2016) in humans. Contrarily, different teams showed unaltered total fasting (Haluzíková *et al.* 2013; Escalona *et al.* 2016; Belgaumkar *et al.* 2016) and post-prandial (Steinert *et al.* 2013) BAs upon VSG or not quantitative but just qualitative changes (Belgaumkar *et al.* 2016). However, similarly as in RYGB, BA and GLP-1 levels positively correlate after VSG in humans (Steinert *et al.* 2013).

Notwithstanding the inconclusive data in humans, pre-clinical models of VSG support a mechanistic role of BAs in VSG effects. Since VSG increased plasma BAs in rats (B. P. Cummings *et al.* 2012) and in mice (Ding *et al.* 2016; Haluzíková *et al.* 2013; Myronovych *et al.* 2014), correlating with GLP-1 levels (Ding *et al.* 2016), FGF19 (Haluzíková *et al.* 2013) and with body weight loss (Myronovych *et al.* 2014). Additionally, studies in FXR- (Ryan *et al.* 2014) and TGR5-deficient (McGavigan *et al.* 2015; Ding *et al.* 2016) mice suggest that BA receptors are necessary for the metabolic improvements of VSG.

Gastric restraint surgery

Laparoscopic adjustable gastric banding

This restrictive procedure doesn't seem to induce long-lasting changes in fasting (Pournaras *et al.* 2012) and postprandial (Kohli *et al.* 2013) BAs in humans, although one study suggested that it decreased fasting BAs (Kohli *et al.* 2013). However, it cannot be excluded that transitory changes could occur, since two studies show that plasma BA increased 1 month (Nakatani *et al.* 2009) and 3 months (Thöni *et al.* 2017) after LAGB, with BA levels returning to baseline 1 year after (Thöni *et al.* 2017).

Conclusion

In humans, BAs increase upon bariatric surgery techniques involving enhancement and divertion of biliopancreatic secretions to the distal segments of the intestine (RYGB, BPD-DS, SG) but not techniques involving gastric restriction alone (AG). Besides the quantitative increase, there are also qualitative changes. The human studies present vast heterogeneity, probably due to differences in the studied postoperative time points, surgical techniques, nutritional state of the patients, metabolic co-morbidities and treatments. Mechanistic studies in humans are hardly achievable, but BAs in bariatric surgery can be studied in pre-clinical models, the choice of the experimental animal species should be based on the needs of the experimental protocol and resemblance to humans for translational research. The causes of BA changes and the role of BAs in the multiple metabolic improvements of bariatric surgery in humans remain unknown. Although mice models suggest that BAs participate to bariatric surgery improvements, to date, there is no scientific clinical evidence proving that BA changes are responsible for the metabolic effects of bariatric surgery.

Acknowledgments:

We thank Maite Zubiaga for her assistance in the original artwork on bariatric procedures. This work was supported by Agence Nationale pour la Recherche (ANR-10-LABX-46 and ANR-10-INBS-08; ProFI project, "Infrastructures Nationales en Biologie et Santé"; "Investissements d'Avenir" call) and by Société Francophone du diabète (SFD). OCT received a PhD fellowship from the French Ministry of Research. BS is a recipient of an Advanced ERC Grant (694717).

References

Ahmad, N. N., A. Pfalzer, and L. M. Kaplan. 2013. “Roux-En-Y Gastric Bypass Normalizes the Blunted Postprandial Bile Acid Excursion Associated with Obesity.” *International Journal of Obesity* 37 (12): 1553–59. https://doi.org/10.1038/ijo.2013.38.

Albaugh, Vance L., Babak Banan, Joseph Antoun, Yanhua Xiong, Yan Guo, Jie Ping, Muhammed Alikhan, Blake Austin Clements, Naji N. Abumrad, and Charles Robb Flynn. 2019. “Role of Bile Acids and GLP-1 in Mediating the Metabolic Improvements of Bariatric Surgery.” *Gastroenterology* 156 (4): 1041-1051.e4. https://doi.org/10.1053/j.gastro.2018.11.017.

Albaugh, Vance L., Charles Robb Flynn, Steven Cai, Yi Xiao, Robyn A. Tamboli, and Naji N. Abumrad. 2015. “Early Increases in Bile Acids Post Roux-En-Y Gastric Bypass Are Driven by Insulin-Sensitizing, Secondary Bile Acids.” *The Journal of Clinical Endocrinology and Metabolism* 100 (9): E1225-1233. https://doi.org/10.1210/jc.2015-2467.

Baud, Gregory, Mehdi Daoudi, Thomas Hubert, Violeta Raverdy, Marie Pigeyre, Erik Hervieux, Magalie Devienne, *et al.* 2016. “Bile Diversion in Roux-En-Y Gastric Bypass Modulates Sodium-Dependent Glucose Intestinal Uptake.” *Cell Metabolism* 23 (3): 547–53. https://doi.org/10.1016/j.cmet.2016.01.018.

Belgaumkar, Ajay P., Royce P. Vincent, Kirstin A. Carswell, Robin D. Hughes, Jamie Alaghband-Zadeh, Ragai R. Mitry, Carel W. le Roux, and Ameet G. Patel. 2016. “Changes in Bile Acid Profile After Laparoscopic Sleeve Gastrectomy Are Associated with Improvements in Metabolic Profile and Fatty Liver Disease.” *Obesity Surgery* 26 (6): 1195–1202. https://doi.org/10.1007/s11695-015-1878-1.

Bhutta, Hina Y., Neetu Rajpal, Wendy White, Johannes M. Freudenberg, Yaping Liu, James Way, Deepak Rajpal, *et al.* 2015. “Effect of Roux-En-Y Gastric Bypass Surgery on Bile Acid Metabolism in Normal and Obese Diabetic Rats.” *PloS One* 10 (3): e0122273. https://doi.org/10.1371/journal.pone.0122273.

Brufau, Gemma, Frans Stellaard, Kris Prado, Vincent W. Bloks, Elles Jonkers, Renze Boverhof, Folkert Kuipers, and Elizabeth J. Murphy. 2010. “Improved Glycemic Control with Colesevelam Treatment in Patients with Type 2 Diabetes Is Not Directly Associated with Changes in Bile Acid Metabolism.” *Hepatology (Baltimore, Md.)* 52 (4): 1455–64. https://doi.org/10.1002/hep.23831.

Buchwald, Henry, Rhonda Estok, Kyle Fahrbach, Deirdre Banel, Michael D. Jensen, Walter J. Pories, John P. Bantle, and Isabella Sledge. 2009. “Weight and Type 2 Diabetes after Bariatric Surgery: Systematic Review and Meta-Analysis.” *The American Journal of Medicine* 122 (3): 248-256.e5. https://doi.org/10.1016/j.amjmed.2008.09.041.

Carbajo, Miguel A., Enrique Luque-de-León, José M. Jiménez, Javier Ortiz-de-Solórzano, Manuel Pérez-Miranda, and María J. Castro-Alija. 2017. “Laparoscopic One-Anastomosis Gastric Bypass: Technique, Results, and Long-Term Follow-Up in 1200 Patients.” *Obesity Surgery* 27 (5): 1153–67. https://doi.org/10.1007/s11695-016-2428-1.

Cariou, Bertrand, Maud Chetiveaux, Yassine Zaïr, Etienne Pouteau, Emmanuel Disse, Béatrice Guyomarc’h-Delasalle, Martine Laville, and Michel Krempf. 2011. “Fasting Plasma Chenodeoxycholic Acid and Cholic Acid Concentrations Are Inversely Correlated with Insulin Sensitivity in Adults.” *Nutrition & Metabolism* 8 (1): 48. https://doi.org/10.1186/1743-7075-8-48.

Chávez-Talavera, O., G. Baud, V. Spinelli, M. Daoudi, M. Kouach, J.-F. Goossens, E. Vallez, *et al.* 2017. “Roux-En-Y Gastric Bypass Increases Systemic but Not Portal Bile Acid Concentrations by Decreasing Hepatic Bile Acid Uptake in Minipigs.” *International Journal of Obesity (2005)* 41 (4): 664–68. https://doi.org/10.1038/ijo.2017.7.

Chávez-Talavera, Oscar, Joel Haas, Guillaume Grzych, Anne Tailleux, and Bart Staels. 2019. “Bile Acid Alterations in Nonalcoholic Fatty Liver Disease, Obesity, Insulin Resistance and Type 2 Diabetes: What Do the Human Studies Tell?” *Current Opinion in Lipidology*, March. https://doi.org/10.1097/MOL.0000000000000597.

Chávez-Talavera, Oscar, Anne Tailleux, Philippe Lefebvre, and Bart Staels. 2017. “Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia and NAFLD.” *Gastroenterology*, February. https://doi.org/10.1053/j.gastro.2017.01.055.

Cummings, Bethany P., Ahmed Bettaieb, James L. Graham, Kimber L. Stanhope, Mark Kowala, Fawaz G. Haj, Michael L. Chouinard, and Peter J. Havel. 2012. “Vertical Sleeve Gastrectomy Improves Glucose and Lipid Metabolism and Delays Diabetes Onset in UCD-T2DM Rats.” *Endocrinology* 153 (8): 3620–32. https://doi.org/10.1210/en.2012-1131.

Cummings, David E., and Francesco Rubino. 2018. “Metabolic Surgery for the Treatment of Type 2 Diabetes in Obese Individuals.” *Diabetologia* 61 (2): 257–64. https://doi.org/10.1007/s00125-017-4513-y.

De Giorgi, Sara, Vanessa Campos, Leonie Egli, Ulrike Toepel, Guillaume Carrel, Bertrand Cariou, Dominique Rainteau, Philippe Schneiter, Luc Tappy, and Vittorio Giusti. 2015. “Long-Term Effects of Roux-En-Y Gastric Bypass on Postprandial Plasma Lipid and Bile Acids Kinetics in Female Non Diabetic Subjects: A Cross-Sectional Pilot Study.” *Clinical Nutrition (Edinburgh, Scotland)* 34 (5): 911–17. https://doi.org/10.1016/j.clnu.2014.09.018.

Deo, Anand K., and Stelvio M. Bandiera. 2008. “Identification of Human Hepatic Cytochrome P450 Enzymes Involved in the Biotransformation of Cholic and Chenodeoxycholic Acid.” *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 36 (10): 1983–91. https://doi.org/10.1124/dmd.108.022194.

Ding, Lili, Kyle M. Sousa, Lihua Jin, Bingning Dong, Byung-Wook Kim, Ricardo Ramirez, Zhenzhou Xiao, *et al.* 2016. “Vertical Sleeve Gastrectomy Activates GPBAR-1/TGR5 to Sustain Weight Loss, Improve Fatty Liver, and Remit Insulin Resistance in Mice.” *Hepatology* 64 (3): 760–73. https://doi.org/10.1002/hep.28689.

Dirksen, C., N. B. Jørgensen, K. N. Bojsen-Møller, U. Kielgast, S. H. Jacobsen, T. R. Clausen, D. Worm, *et al.* 2013. “Gut Hormones, Early Dumping and Resting Energy Expenditure in Patients with Good and Poor Weight Loss Response after Roux-En-Y Gastric Bypass.” *International Journal of Obesity (2005)* 37 (11): 1452–59. https://doi.org/10.1038/ijo.2013.15.

Duboc, Henri, Caroline Chong Nguyen, Jean-Baptiste Cavin, Lara Ribeiro-Parenti, Anne-Charlotte Jarry, Dominique Rainteau, Lydie Humbert, *et al.* 2018. “Roux-En-Y Gastric-Bypass and Sleeve Gastrectomy Induces Specific Shifts of the Gut Microbiota without Altering the Metabolism of Bile Acids in the Intestinal Lumen.” *International Journal of Obesity (2005)*, January. https://doi.org/10.1038/s41366-018-0015-3.

Dutia, R., M. Embrey, C. S. O’Brien, S. O’Brien, R. A. Haeusler, K. K. Agénor, P. Homel, *et al.* 2015. “Temporal Changes in Bile Acid Levels and 12α-Hydroxylation after Roux-En-Y Gastric Bypass Surgery in Type 2 Diabetes.” *International Journal of Obesity (2005)* 39 (5): 806–13. https://doi.org/10.1038/ijo.2015.1.

Escalona, Alex, Rodrigo Muñoz, Veronica Irribarra, Sandra Solari, Fidel Allende, and Juan Francisco Miquel. 2016. “Bile Acids Synthesis Decreases after Laparoscopic Sleeve Gastrectomy.” *Surgery for Obesity and Related Diseases: Official Journal of the American Society for Bariatric Surgery* 12 (4): 763–69. https://doi.org/10.1016/j.soard.2015.11.015.

Ferrannini, Ele, Stefania Camastra, Brenno Astiarraga, Monica Nannipieri, Jose Castro-Perez, Dan Xie, Liangsu Wang, Manu Chakravarthy, and Rebecca A. Haeusler. 2015. “Increased Bile Acid Synthesis and Deconjugation After Biliopancreatic Diversion.” *Diabetes* 64 (10): 3377–85. https://doi.org/10.2337/db15-0214.

Fiamoncini, Jarlei, Carina Fernandes Barbosa, José Rubens Arnoni Junior, José Celestino Araújo Junior, Cinthia Taglieri, Tiago Szego, Barbara Gelhaus, Heraldo Possolo de Souza, Hannelore Daniel, and Thais Martins de Lima. 2018. “Roux-En-Y Gastric Bypass Surgery Induces Distinct but Frequently Transient Effects on Acylcarnitine, Bile Acid and Phospholipid Levels.” *Metabolites* 8 (4). https://doi.org/10.3390/metabo8040083.

Flynn, Charles Robb, Vance L. Albaugh, Steven Cai, Joyce Cheung-Flynn, Phillip E. Williams, Robert M. Brucker, Seth R. Bordenstein, Yan Guo, David H. Wasserman, and Naji N. Abumrad. 2015. “Bile Diversion to the Distal Small Intestine Has Comparable Metabolic Benefits to Bariatric Surgery.” *Nature Communications* 6: 7715. https://doi.org/10.1038/ncomms8715.

Forslund, Kristoffer, Falk Hildebrand, Trine Nielsen, Gwen Falony, Emmanuelle Le Chatelier, Shinichi Sunagawa, Edi Prifti, *et al.* 2015. “Disentangling Type 2 Diabetes and Metformin Treatment Signatures in the Human Gut Microbiota.” *Nature* 528 (7581): 262–66. https://doi.org/10.1038/nature15766.

Friedrich, Dana, Hanns-Ulrich Marschall, and Frank Lammert. 2018. “Response of Fibroblast Growth Factor 19 and Bile Acid Synthesis after a Body Weight-Adjusted Oral Fat Tolerance Test in Overweight and Obese NAFLD Patients: A Non-Randomized Controlled Pilot Trial.” *BMC Gastroenterology* 18 (1): 76. https://doi.org/10.1186/s12876-018-0805-z.

Gerhard, Glenn S., Amanda M. Styer, G. Craig Wood, Stephen L. Roesch, Anthony T. Petrick, Jon Gabrielsen, William E. Strodel, Christopher D. Still, and George Argyropoulos. 2013. “A Role for Fibroblast Growth Factor 19 and Bile Acids in Diabetes Remission after Roux-En-Y Gastric Bypass.” *Diabetes Care* 36 (7): 1859–64. https://doi.org/10.2337/dc12-2255.

Ginos, Bigina N. R., Sandi L. Navarro, Yvonne Schwarz, Haiwei Gu, Dongfang Wang, Timothy W. Randolph, Ali Shojaie, *et al.* 2018. “Circulating Bile Acids in Healthy Adults Respond Differently to a Dietary Pattern Characterized by Whole Grains, Legumes and Fruits and Vegetables Compared to a Diet High in Refined Grains and Added Sugars: A Randomized, Controlled, Crossover Feeding Study.” *Metabolism: Clinical and Experimental* 83: 197–204. https://doi.org/10.1016/j.metabol.2018.02.006.

Glicksman, C., D. J. Pournaras, M. Wright, R. Roberts, D. Mahon, R. Welbourn, R. Sherwood, J. Alaghband-Zadeh, and C. W. le Roux. 2010. “Postprandial Plasma Bile Acid Responses in Normal Weight and Obese Subjects.” *Annals of Clinical Biochemistry* 47 (Pt 5): 482–84. https://doi.org/10.1258/acb.2010.010040.

Goncalves, Daisy, Aude Barataud, Filipe De Vadder, Jennifer Vinera, Carine Zitoun, Adeline Duchampt, and Gilles Mithieux. 2015. “Bile Routing Modification Reproduces Key Features of Gastric Bypass in Rat.” *Annals of Surgery* 262 (6): 1006–15. https://doi.org/10.1097/SLA.0000000000001121.

Haeusler, Rebecca A., Brenno Astiarraga, Stefania Camastra, Domenico Accili, and Ele Ferrannini. 2013. “Human Insulin Resistance Is Associated with Increased Plasma

Levels of 12α-Hydroxylated Bile Acids." *Diabetes* 62 (12): 4184–91. https://doi.org/10.2337/db13-0639.

Haeusler, Rebecca A., Stefania Camastra, Monica Nannipieri, Brenno Astiarraga, Jose Castro-Perez, Dan Xie, Liangsu Wang, Manu Chakravarthy, and Ele Ferrannini. 2016. "Increased Bile Acid Synthesis and Impaired Bile Acid Transport in Human Obesity." *The Journal of Clinical Endocrinology and Metabolism* 101 (5): 1935–44. https://doi.org/10.1210/jc.2015-2583.

Haluzíková, D., Z. Lacinová, P. Kaválková, J. Drápalová, J. Křížová, M. Bártlová, M. Mráz, *et al.* 2013. "Laparoscopic Sleeve Gastrectomy Differentially Affects Serum Concentrations of FGF-19 and FGF-21 in Morbidly Obese Subjects." *Obesity (Silver Spring, Md.)* 21 (7): 1335–42. https://doi.org/10.1002/oby.20208.

Heymsfield, Steven B., and Thomas A. Wadden. 2017. "Mechanisms, Pathophysiology, and Management of Obesity." *The New England Journal of Medicine* 376 (3): 254–66. https://doi.org/10.1056/NEJMra1514009.

Hofmann, A. F., and L. R. Hagey. 2008. "Bile Acids: Chemistry, Pathochemistry, Biology, Pathobiology, and Therapeutics." *Cellular and Molecular Life Sciences: CMLS* 65 (16): 2461–83. https://doi.org/10.1007/s00018-008-7568-6.

Isbell, James M., Robyn A. Tamboli, Erik N. Hansen, Jabbar Saliba, Julia P. Dunn, Sharon E. Phillips, Pamela A. Marks-Shulman, and Naji N. Abumrad. 2010. "The Importance of Caloric Restriction in the Early Improvements in Insulin Sensitivity after Roux-En-Y Gastric Bypass Surgery." *Diabetes Care* 33 (7): 1438–42. https://doi.org/10.2337/dc09-2107.

Jahansouz, Cyrus, Hongliang Xu, Ann V. Hertzel, Federico J. Serrot, Nicholas Kvalheim, Abigail Cole, Anasooya Abraham, *et al.* 2016. "Bile Acids Increase Independently From Hypocaloric Restriction After Bariatric Surgery." *Annals of Surgery* 264 (6): 1022–28. https://doi.org/10.1097/SLA.0000000000001552.

Jansen, Peter L. M., Jochem van Werven, Edo Aarts, Frits Berends, Ignace Janssen, Jaap Stoker, and Frank G. Schaap. 2011. "Alterations of Hormonally Active Fibroblast Growth Factors after Roux-En-Y Gastric Bypass Surgery." *Digestive Diseases (Basel, Switzerland)* 29 (1): 48–51. https://doi.org/10.1159/000324128.

Jørgensen, Nils B., Carsten Dirksen, Kirstine N. Bojsen-Møller, Viggo B. Kristiansen, Birgitte S. Wulff, Dominique Rainteau, Lydie Humbert, *et al.* 2015. "Improvements in Glucose Metabolism Early after Gastric Bypass Surgery Are Not Explained by Increases in Total Bile Acids and Fibroblast Growth Factor 19 Concentrations." *The Journal of Clinical Endocrinology and Metabolism* 100 (3): E396-406. https://doi.org/10.1210/jc.2014-1658.

Khan, Farooq H., Lindsey Shaw, Wujuan Zhang, Rosa Maria Salazar Gonzalez, Sarah Mowery, Melissa Oehrle, Xueheng Zhao, *et al.* 2016. “Fibroblast Growth Factor 21 Correlates with Weight Loss after Vertical Sleeve Gastrectomy in Adolescents.” *Obesity (Silver Spring, Md.)* 24 (11): 2377–83. https://doi.org/10.1002/oby.21658.

Kimberly, W. Taylor, John F. O’Sullivan, Anjali K. Nath, Michelle Keyes, Xu Shi, Martin G. Larson, Qiong Yang, *et al.* 2017. “Metabolite Profiling Identifies Anandamide as a Biomarker of Nonalcoholic Steatohepatitis.” *JCI Insight* 2 (9). https://doi.org/10.1172/jci.insight.92989.

Kohli, Rohit, David Bradley, Kenneth D. Setchell, J. Christopher Eagon, Nada Abumrad, and Samuel Klein. 2013. “Weight Loss Induced by Roux-En-Y Gastric Bypass but Not Laparoscopic Adjustable Gastric Banding Increases Circulating Bile Acids.” *The Journal of Clinical Endocrinology and Metabolism* 98 (4): E708-712. https://doi.org/10.1210/jc.2012-3736.

Kuipers, Folkert, Vincent W. Bloks, and Albert K. Groen. 2014. “Beyond Intestinal Soap--Bile Acids in Metabolic Control.” *Nature Reviews. Endocrinology* 10 (8): 488–98. https://doi.org/10.1038/nrendo.2014.60.

Lassailly, Guillaume, Robert Caiazzo, David Buob, Marie Pigeyre, Hélène Verkindt, Julien Labreuche, Violeta Raverdy, *et al.* 2015. “Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese Patients.” *Gastroenterology* 149 (2): 379–88; quiz e15-16. https://doi.org/10.1053/j.gastro.2015.04.014.

Liou, Alice P., Melissa Paziuk, Jesus-Mario Luevano, Sriram Machineni, Peter J. Turnbaugh, and Lee M. Kaplan. 2013. “Conserved Shifts in the Gut Microbiota Due to Gastric Bypass Reduce Host Weight and Adiposity.” *Science Translational Medicine* 5 (178): 178ra41. https://doi.org/10.1126/scitranslmed.3005687.

McGavigan, Anne K, Darline Garibay, Zachariah M Henseler, Jack Chen, Ahmed Bettaieb, Fawaz G Haj, Ruth E Ley, Michael L Chouinard, and Bethany P Cummings. 2015. “TGR5 Contributes to Glucoregulatory Improvements after Vertical Sleeve Gastrectomy in Mice.” *Gut* [Epub ahead of print] (October): gutjnl-2015-309871. https://doi.org/10.1136/gutjnl-2015-309871.

Murata, Masahiro, Yasuhiko Okimura, Keiji Iida, Michihiro Matsumoto, Hideaki Sowa, Hidesuke Kaji, Masayasu Kojima, Kenji Kangawa, and Kazuo Chihara. 2002. “Ghrelin Modulates the Downstream Molecules of Insulin Signaling in Hepatoma Cells.” *The Journal of Biological Chemistry* 277 (7): 5667–74. https://doi.org/10.1074/jbc.M103898200.

Myronovych, Andriy, Michelle Kirby, Karen K. Ryan, Wujuan Zhang, Pinky Jha, Kenneth Dr Setchell, Phillip J. Dexheimer, Bruce Aronow, Randy J. Seeley, and Rohit Kohli. 2014. “Vertical Sleeve Gastrectomy Reduces Hepatic Steatosis While Increasing

Serum Bile Acids in a Weight-Loss-Independent Manner." *Obesity (Silver Spring, Md.)* 22 (2): 390–400. https://doi.org/10.1002/oby.20548.

Nakatani, Hiroshi, Kazunori Kasama, Takashi Oshiro, Mitsuhiro Watanabe, Hiroshi Hirose, and Hiroshi Itoh. 2009. "Serum Bile Acid along with Plasma Incretins and Serum High-Molecular Weight Adiponectin Levels Are Increased after Bariatric Surgery." *Metabolism: Clinical and Experimental* 58 (10): 1400–1407. https://doi.org/10.1016/j.metabol.2009.05.006.

Ng, Marie, Tom Fleming, Margaret Robinson, Blake Thomson, Nicholas Graetz, Christopher Margono, Erin C Mullany, *et al.* 2014. "Global, Regional and National Prevalence of Overweight and Obesity in Children and Adults 1980-2013: A Systematic Analysis." *Lancet (London, England)* 384 (9945): 766–81. https://doi.org/10.1016/S0140-6736(14)60460-8.

Nguyen, Ninh T., and J. Esteban Varela. 2017. "Bariatric Surgery for Obesity and Metabolic Disorders: State of the Art." *Nature Reviews. Gastroenterology & Hepatology* 14 (3): 160–69. https://doi.org/10.1038/nrgastro.2016.170.

Nierop, Frederik Samuel van, Charlotte de Jonge, Wim Kulik, Nicole Bouvy, Frank G. Schaap, Steven W. Olde Damink, Sander Rensen, Johannes A. Romijn, Jan Willem M. Greve, and Maarten R. Soeters. 2019. "Duodenal-Jejunal Lining Increases Postprandial Unconjugated Bile Acid Responses and Disrupts the Bile Acid-FXR-FGF19 Axis in Humans." *Metabolism: Clinical and Experimental* 93 (April): 25–32. https://doi.org/10.1016/j.metabol.2018.12.009.

Owen, Jonathan G., Farshid Yazdi, and Efrain Reisin. 2017. "Bariatric Surgery and Hypertension." *American Journal of Hypertension* 31 (1): 11–17. https://doi.org/10.1093/ajh/hpx112.

Papasavas, Pavlos K., Daniel J. Gagné, Federico A. Ceppa, and Philip F. Caushaj. 2006. "Routine Gallbladder Screening Not Necessary in Patients Undergoing Laparoscopic Roux-En-Y Gastric Bypass." *Surgery for Obesity and Related Diseases: Official Journal of the American Society for Bariatric Surgery* 2 (1): 41–46; discussion 46-47. https://doi.org/10.1016/j.soard.2005.10.006.

Patel, Rajesh T., Alpana P. Shukla, Soo Min Ahn, Marlus Moreira, and Francesco Rubino. 2014. "Surgical Control of Obesity and Diabetes: The Role of Intestinal vs. Gastric Mechanisms in the Regulation of Body Weight and Glucose Homeostasis." *Obesity (Silver Spring, Md.)* 22 (1): 159–69. https://doi.org/10.1002/oby.20441.

Patti, Mary-Elizabeth, Sander M. Houten, Antonio C. Bianco, Raquel Bernier, P. Reed Larsen, Jens J. Holst, Michael K. Badman, *et al.* 2009. "Serum Bile Acids Are Higher in Humans with Prior Gastric Bypass: Potential Contribution to Improved Glucose and

Lipid Metabolism." *Obesity (Silver Spring, Md.)* 17 (9): 1671–77. https://doi.org/10.1038/oby.2009.102.

Pineda Torra, Inés, Thierry Claudel, Caroline Duval, Vladimir Kosykh, Jean-Charles Fruchart, and Bart Staels. 2003. "Bile Acids Induce the Expression of the Human Peroxisome Proliferator-Activated Receptor Alpha Gene via Activation of the Farnesoid X Receptor." *Molecular Endocrinology* 17 (2): 259–72. https://doi.org/10.1210/me.2002-0120.

Pinheiro, Jose S., Carlos A. Schiavon, Paulo B. Pereira, Jose L. Correa, Patricia Noujaim, and Ricardo Cohen. 2008. "Long-Long Limb Roux-En-Y Gastric Bypass Is More Efficacious in Treatment of Type 2 Diabetes and Lipid Disorders in Super-Obese Patients." *Surgery for Obesity and Related Diseases: Official Journal of the American Society for Bariatric Surgery* 4 (4): 521–25; discussion 526-527. https://doi.org/10.1016/j.soard.2007.12.016.

Pournaras, Dimitri J., Clare Glicksman, Royce P. Vincent, Shophia Kuganolipava, Jamie Alaghband-Zadeh, David Mahon, Jan H. R. Bekker, *et al.* 2012. "The Role of Bile after Roux-En-Y Gastric Bypass in Promoting Weight Loss and Improving Glycaemic Control." *Endocrinology* 153 (8): 3613–19. https://doi.org/10.1210/en.2011-2145.

Prinz, Philip, Tobias Hofmann, Anne Ahnis, Ulf Elbelt, Miriam Goebel-Stengel, Burghard F. Klapp, Matthias Rose, and Andreas Stengel. 2015. "Plasma Bile Acids Show a Positive Correlation with Body Mass Index and Are Negatively Associated with Cognitive Restraint of Eating in Obese Patients." *Frontiers in Neuroscience* 9: 199. https://doi.org/10.3389/fnins.2015.00199.

Ramos-Molina, Bruno, Daniel Castellano-Castillo, Juan Alcaide-Torres, Óscar Pastor, Resi de Luna Díaz, Jordi Salas-Salvadó, Javier López-Moreno, *et al.* 2018. "Differential Effects of Restrictive and Malabsorptive Bariatric Surgery Procedures on the Serum Lipidome in Obese Subjects." *Journal of Clinical Lipidology* 12 (6): 1502–12. https://doi.org/10.1016/j.jacl.2018.07.006.

Ryan, Karen K., Valentina Tremaroli, Christoffer Clemmensen, Petia Kovatcheva-Datchary, Andriy Myronovych, Rebekah Karns, Hilary E. Wilson-Pérez, *et al.* 2014. "FXR Is a Molecular Target for the Effects of Vertical Sleeve Gastrectomy." *Nature* 509 (7499): 183–88. https://doi.org/10.1038/nature13135.

Sachdev, Saachi, Qi Wang, Charles Billington, John Connett, Leaque Ahmed, William Inabnet, Streamson Chua, Sayeed Ikramuddin, and Judith Korner. 2016. "FGF 19 and Bile Acids Increase Following Roux-En-Y Gastric Bypass but Not After Medical Management in Patients with Type 2 Diabetes." *Obesity Surgery* 26 (5): 957–65. https://doi.org/10.1007/s11695-015-1834-0.

Schauer, Daniel P., David E. Arterburn, Edward H. Livingston, Karen J. Coleman, Steve Sidney, David Fisher, Patrick O'Connor, David Fischer, and Mark H. Eckman. 2015. "Impact of Bariatric Surgery on Life Expectancy in Severely Obese Patients with Diabetes: A Decision Analysis." *Annals of Surgery* 261 (5): 914–19. https://doi.org/10.1097/SLA.0000000000000907.

Sethi, Monica, and Manish Parikh. 2015. "Bariatric Surgery versus Intensive Medical Weight Management for Type 2 Diabetes." *Advances in Surgery* 49: 157–71. https://doi.org/10.1016/j.yasu.2015.04.004.

Simonen, M., N. Dali-Youcef, D. Kaminska, S. Venesmaa, P. Käkelä, M. Pääkkönen, M. Hallikainen, *et al.* 2012. "Conjugated Bile Acids Associate with Altered Rates of Glucose and Lipid Oxidation after Roux-En-Y Gastric Bypass." *Obesity Surgery* 22 (9): 1473–80. https://doi.org/10.1007/s11695-012-0673-5.

Siqueira Cardinelli, Camila de, Raquel Susana Torrinhas, Priscila Sala, Marcos Albieri Pudenzi, Célio Fernando F Angolini, Mariane Marques da Silva, Natasha Mendonça Machado, Graziela Ravacci, Marcos N. Eberlin, and Dan L. Waitzberg. 2019. "Fecal Bile Acid Profile after Roux-En-Y Gastric Bypass and Its Association with the Remission of Type 2 Diabetes in Obese Women: A Preliminary Study." *Clinical Nutrition (Edinburgh, Scotland)*, January. https://doi.org/10.1016/j.clnu.2018.12.028.

Sjöström, Lars, Markku Peltonen, Peter Jacobson, C. David Sjöström, Kristjan Karason, Hans Wedel, Sofie Ahlin, *et al.* 2012. "Bariatric Surgery and Long-Term Cardiovascular Events." *JAMA* 307 (1): 56–65. https://doi.org/10.1001/jama.2011.1914.

Sonne, David P., F. Samuel van Nierop, Willem Kulik, Maarten R. Soeters, Tina Vilsbøll, and Filip K. Knop. 2016. "Postprandial Plasma Concentrations of Individual Bile Acids and FGF-19 in Patients With Type 2 Diabetes." *The Journal of Clinical Endocrinology and Metabolism* 101 (8): 3002–9. https://doi.org/10.1210/jc.2016-1607.

Spinelli, V., F. Lalloyer, G. Baud, E. Osto, M. Kouach, M. Daoudi, E. Vallez, *et al.* 2016. "Influence of Roux-En-Y Gastric Bypass on Plasma Bile Acid Profiles: A Comparative Study between Rats, Pigs and Humans." *International Journal of Obesity (2005)* 40 (8): 1260–67. https://doi.org/10.1038/ijo.2016.46.

Srivastava, Gitanjali, and Caroline M. Apovian. 2018. "Current Pharmacotherapy for Obesity." *Nature Reviews. Endocrinology* 14 (1): 12–24. https://doi.org/10.1038/nrendo.2017.122.

Steiner, Carine, Alaa Othman, Christoph H. Saely, Philipp Rein, Heinz Drexel, Arnold von Eckardstein, and Katharina M. Rentsch. 2011. "Bile Acid Metabolites in Serum: Intraindividual Variation and Associations with Coronary Heart Disease, Metabolic

Syndrome and Diabetes Mellitus." *PloS One* 6 (11): e25006. https://doi.org/10.1371/journal.pone.0025006.

Steinert, Robert E., Ralph Peterli, Sylvia Keller, Anne C. Meyer-Gerspach, Jürgen Drewe, Thomas Peters, and Christoph Beglinger. 2013. "Bile Acids and Gut Peptide Secretion after Bariatric Surgery: A 1-Year Prospective Randomized Pilot Trial." *Obesity* 21 (12): E660–68. https://doi.org/10.1002/oby.20522.

Suhre, Karsten, Christa Meisinger, Angela Döring, Elisabeth Altmaier, Petra Belcredi, Christian Gieger, David Chang, *et al.* 2010. "Metabolic Footprint of Diabetes: A Multiplatform Metabolomics Study in an Epidemiological Setting." *PloS One* 5 (11): e13953. https://doi.org/10.1371/journal.pone.0013953.

Sun, Lulu, Cen Xie, Guang Wang, Yue Wu, Qing Wu, Xuemei Wang, Jia Liu, *et al.* 2018. "Gut Microbiota and Intestinal FXR Mediate the Clinical Benefits of Metformin." *Nature Medicine* 24 (12): 1919–29. https://doi.org/10.1038/s41591-018-0222-4.

Sun, Wanwan, Di Zhang, Zhengyi Wang, Jichao Sun, Baihui Xu, Ying Chen, Lin Ding, *et al.* 2016. "Insulin Resistance Is Associated With Total Bile Acid Level in Type 2 Diabetic and Nondiabetic Population: A Cross-Sectional Study." *Medicine* 95 (10): e2778. https://doi.org/10.1097/MD.0000000000002778.

Sweeney, Timothy E., and John M. Morton. 2014. "Metabolic Surgery: Action via Hormonal Milieu Changes, Changes in Bile Acids or Gut Microbiota? A Summary of the Literature." *Best Practice & Research. Clinical Gastroenterology* 28 (4): 727–40. https://doi.org/10.1016/j.bpg.2014.07.016.

Tailleux, Anne, Konstantinos Rouskas, François Pattou, and Bart Staels. 2015. "Bariatric Surgery, Lipoprotein Metabolism and Cardiovascular Risk." *Current Opinion in Lipidology* 26 (4): 317–24. https://doi.org/10.1097/MOL.0000000000000197.

Takahashi, Shogo, Tatsuki Fukami, Yusuke Masuo, Chad N. Brocker, Cen Xie, Kristopher W. Krausz, C. Roland Wolf, Colin J. Henderson, and Frank J. Gonzalez. 2016. "Cyp2c70 Is Responsible for the Species Difference in Bile Acid Metabolism between Mice and Humans." *Journal of Lipid Research* 57 (12): 2130–37. https://doi.org/10.1194/jlr.M071183.

Thöni, Veronika, Alexandra Pfister, Andreas Melmer, Barbara Enrich, Karin Salzmann, Susanne Kaser, Claudia Lamina, *et al.* 2017. "Dynamics of Bile Acid Profiles, GLP-1, and FGF19 After Laparoscopic Gastric Banding." *The Journal of Clinical Endocrinology and Metabolism* 102 (8): 2974–84. https://doi.org/10.1210/jc.2017-00235.

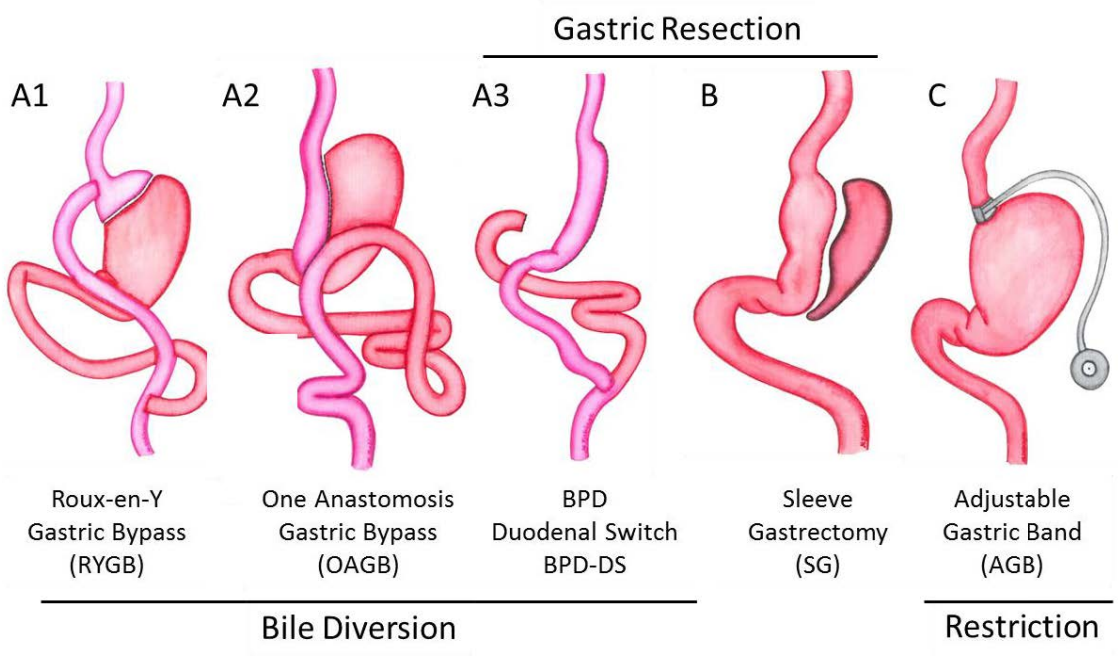
Vincent, Royce P., Sohail Omar, Samer Ghozlan, David R. Taylor, Gemma Cross, Roy A. Sherwood, Lars Fandriks, *et al.* 2013. "Higher Circulating Bile Acid Concentrations in

Obese Patients with Type 2 Diabetes." *Annals of Clinical Biochemistry* 50 (Pt 4): 360–64. https://doi.org/10.1177/0004563212473450.

Werling, Malin, Royce P. Vincent, Gemma F. Cross, Hanns-Ulrich Marschall, Lars Fändriks, Hans Lönroth, David R. Taylor, Jamshid Alaghband-Zadeh, Torsten Olbers, and Carel W. Le Roux. 2013. "Enhanced Fasting and Post-Prandial Plasma Bile Acid Responses after Roux-En-Y Gastric Bypass Surgery." *Scandinavian Journal of Gastroenterology* 48 (11): 1257–64. https://doi.org/10.3109/00365521.2013.833647.

Wewalka, Marlene, Mary-Elizabeth Patti, Corinne Barbato, Sander M. Houten, and Allison B. Goldfine. 2014. "Fasting Serum Taurine-Conjugated Bile Acids Are Elevated in Type 2 Diabetes and Do Not Change with Intensification of Insulin." *The Journal of Clinical Endocrinology and Metabolism* 99 (4): 1442–51. https://doi.org/10.1210/jc.2013-3367.

Figure 1



Title: Most commonly used bariatric surgery procedures

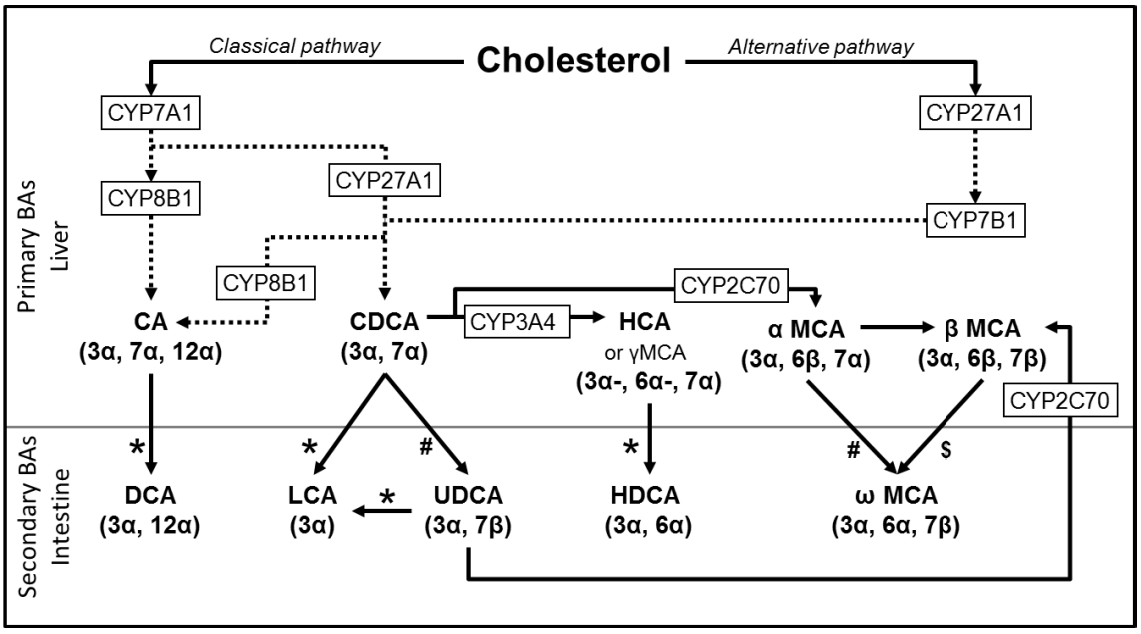
Legend: Technically, these bariatric procedures can be classified into three main groups: bile diversion surgeries, gastric resection surgeries and gastric restriction surgery.

Bile diversion surgeries: The Roux-en-Y gastric bypass (RYGB) (A1): is one of the most frequently performed bariatric procedures, consisting on the creation of a small gastric pouch from the cardiac portion of the stomach, the rest of the stomach being excluded. Then, the small intestine is segmented 50cm distal to the ligament of Treitz; the proximal segment constitutes the biliopancreatic limb. The distal segment is anastomosed to the small gastric pouch and constitutes the alimentary limb. The biliopancreatic limb is anastomosed with the alimentary limb to form the common limb, and the length of the latter is variable depending on the surgeon and healthcare facilities (Nguyen and Varela 2017). The biliopancreatic diversion (BPD) (A2): consists on a first phase sleeve gastrectomy followed by an intestinal second phase, consisting of gut section around 250cm distal from the ligament of Treitz. The proximal end constitutes the biliopancretic limb and the distal end forms the alimentary limb, which is climbed up and anastomosed to the duodenum. These procedures form a very short common limb, approximately 50cm from the ileocecal valve (Marceau *et al.*, 1998). The One anastomosis gastric bypass (OAGB) (A3): Also known as minigastric bypass, encompasses a series of techniques characterized by the absence of the alimentary limb, the permanence of the excluded stomach (as in the RYGB) and the presence of a long biliopancreatic limb (as in the BPD), it is therefore a less agresive malabsorptive procedure since, unlike BPD, the common limb is not so short (Mahawar *et al.*, 2016).

B. Gastric resection surgery: The Sleeve Gastrectomy (SG): This is the currently most performed technique in the world, consisting on a longitudinal resection of the stomach which starts from the antrum, at 5-6cm from the pylorus and finishing at the gastroesophageal junction. The gastric tube is measured with a bougie size 36 or 40-Fr, to get a final stomach size of approximately 150cc. In this technique the gastrointestinal circuit is not modified. (Rosenthal *et al.*,2012).

C. Gastric restraint surgery: The Adjustable Gastric Band (AGB): The AGB does not modify the anatomy of the gastrointestinal tract. It consists on the placement of an adjustable silicone band around the upper portion of the stomach, accessing through the flaccid pars to the posterior wall of the stomach, creating thus a small gastric pouch above the gastric band (Beitner *et al.*, 2016).

Figure 2



Title: Main steps of hepatic and intestinal bile acid synthesis.

Legend: Main enzymes and steps involved in the hepatic and intestinal synthesis of bile acids. For details, see in the text. : Intestinal microbiota 7-dehydroxylation (*), 7-epimerization (#), 6-epimerization ($).

MANUSCRIPT NUMBER 6

Chávez-Talavera, Baud & Spinelli *et al.* Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. Int J Obes (Lond), 2017. Apr 41(4):664-668.

International Journal of Obesity (2017) **41,** 664–668

www.nature.com/ijo

SHORT COMMUNICATION

Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs

O Chávez-Talavera[1,2,3,4,5,9], G Baud[3,6,7,9], V Spinelli[1,2,3,4,9], M Daoudi[3,6,7], M Kouach[8], J-F Goossens[8], E Vallez[1,2,3,4], R Caiazzo[3,6,7], M Ghunaim[3,6,7], T Hubert[3,6,7], S Lestavel[1,2,3,4], A Tailleux[1,2,3,4,10], B Staels[1,2,3,4,10] and F Pattou[3,6,7,10]

Roux-en-Y gastric bypass (RYGB) surgery is widely used in the management of morbid obesity. RYGB improves metabolism independently of weight loss by still unknown mechanisms. Bile acids (BAs) are good candidates to explain this benefit, since they regulate metabolic homeostasis and their systemic concentrations increase upon RYGB. Here we analyzed the mechanisms underlying the increase in systemic BA concentrations after RYGB and the role of the liver therein. To this aim, we used the Göttingen-like minipig, a human-size mammalian model, which allows continuous sampling and simultaneous analysis of pre-hepatic portal and systemic venous blood. BA concentrations and pool composition were measured in portal blood, containing intestinal reabsorbed BAs and compared to systemic blood during a standardized meal test before and after RYGB. Systemic total BA concentrations increased after RYGB, due to an increase in conjugated BAs. Interestingly, the ratio of portal:systemic conjugated BAs decreased after RYGB, indicating a role for the liver in systemic BA concentrations changes. In line, hepatic expression of BA transporter genes decreased after RYGB. Our results show that the increase in systemic BAs after surgery is due to decreased selective hepatic recapture. Thus, alterations in hepatic function contribute to the increase in systemic BAs after RYGB.

International Journal of Obesity (2017) **41,** 664–668; doi:10.1038/ijo.2017.7

INTRODUCTION

Roux-en-Y gastric bypass (RYGB) is one of the most widely used bariatric surgery techniques to treat morbid obesity. In addition to weight loss, RYGB reduces cardiovascular risk by improving cardiovascular risk factors such as dyslipidemia, non-alcoholic fatty liver disease and type 2 diabetes.[1,2] Interestingly, some of these improvements occur, at least in part, in a weight loss-independent manner.[3] The mechanisms underlying the metabolic changes after RYGB are complex and not yet fully understood. Among the proposed hypotheses to explain the metabolic changes are increased production of gastrointestinal hormones (glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP)),[4–6] changes in the gut microbiota[7,8] and qualitative as well as quantitative changes in bile acid (BA) pool composition, metabolism and signaling.[9,10] Indeed, systemic BA concentrations consistently increase after RYGB in clinical studies and animal models.[11,12] In humans several studies consistently reported an increase in systemic BAs in the fasting state one year after RYGB,[13–15] as well as in the post-prandial state.[16,17] However, the kinetics of systemic BA increase after surgery is still a matter of debate: while some studies did not find changes in fasting BAs one or 3 months after RYGB,[18,19] others reported increased fasting BAs already within a few days,[20] 1[(refs 20–23)] and 3 months[23,24] after RYGB. Furthermore, one study reported an increase in conjugated BAs in the post-prandial state 4 weeks after RYGB, as also observed in our minipig model.[20]

BAs are synthesized and conjugated in the liver, secreted into the duodenum after meal ingestion to facilitate intestinal lipid absorption, reabsorbed in the ileum and finally, transported back to the liver by the portal vein. Most BAs are recaptured by the hepatocytes *via* transporters, such as the sodium/bile acid cotransporter (NTCP), the organic anion transporter (OAT), and the organic anion-transporting polypeptide (OATP). However, a fraction of BAs escapes hepatic uptake, flows into the systemic circulation and reaches peripheral organs.[25] BAs are signaling molecules which modulate the activity of the nuclear farnesoid X receptor (FXR) and the plasma membrane receptor TGR5, both involved in metabolic homeostasis. Thus, quantitative and qualitative changes in BA concentrations may change the activity of these receptors and potentially regulate metabolic pathways.[26] As such, studies in mice have recently pointed to the BA/FXR signaling pathway to participate in the beneficial metabolic effects of bariatric surgery.[27]

To study the mechanisms underlying the increase in peripheral BA concentrations after RYGB, BA concentrations and pool composition were analyzed in pre-hepatic and systemic circulation during a meal test, when BAs undergo entero-hepatic circulation. Experiments were performed in Göttingen-like minipigs, which present similar hormonal responses[28] and increased BA plasma concentrations as humans after RYGB.[29] Interestingly, this experimental model allows continuous sampling of pre-hepatic portal and systemic venous blood (VB). In this study, we demonstrate that the increase in BA systemic concentration after

[1]Université de Lille, U1011 - EGID, Lille, France; [2]Inserm, U1011, Lille, France; [3]CHU Lille, Lille, France; [4]Institut Pasteur de Lille, Lille, France; [5]PECEM, Faculty of Medicine, UNAM, Mexico City, Mexico; [6]Université de Lille, U1190-EGID, Lille, France; [7]Inserm, U1190, Lille, France and [8]Centre Universitaire de Mesures et d'Analyses, Université de Lille, Lille France. Correspondence: Professor B Staels, Inserm UMR1011, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP245, Lille 59019 France.
E-mail: Bart.Staels@pasteur-lille.fr
[9]Co-first authors.
[10]Co-last authors.
Received 8 July 2016; revised 14 December 2016; accepted 17 December 2016; accepted article preview online 17 January 2017; advance online publication, 7 February 2017

RYGB could be explained, at least in part, by a reduction in BA hepatic recapture.

MATERIALS AND METHODS

In vivo experiments

Minipig animal model and surgical procedures. RYGB was performed on adult (16–24-month old) Göttingen-like, non-obese, non-diabetic male minipigs (*Sus scrofa*), as previously described.[28] Briefly, animals were fasted overnight and RYGB was performed under general anaesthesia by midline laparotomy. For the experiment, animals were pre-medicated with an intramuscular injection of ketamine (Ketamine1000, Virbac, Carros, France, 10 mg/kg of body weight) and xylazine (Sédaxylan, CEVA Santé Animale, Libourne France, 2.5 mg/Kg of body weight). A 4% concentration of isoflurane (Aerrane, Baxter, France) was used during the surgery. A small gastric pouch (30–50 ml) was constructed and the intestine was divided at seventy centimeters from the duodeno-jejunal junction, using a linear staplers device. The jejunal end of the Roux-en-Y limb was brought up and anastomosed to the gastric pouch using a linear stapler (Ethicon, Issy-les-Moulineaux, France). After surgery, minipigs were closely monitored and treated with transdermal fentanyl (Recuvyra 2,6 mg/Kg, Eli Lilly, Neuilly-sur-Seine, France). Water intake started on the first postoperative day, and *ad libitum* oral feeding was allowed 72 h after surgery. The marker of metabolic efficiency of surgery was assessed by measuring the expected increase in post-prandial GLP-1 plasma concentration, as assessed by Verhaeghe *et al.*[28] The surgical interventions were performed in the University and Hospital Department for Experimental Research of the Lille 2 University, France, in accordance with French regulations for animal experimentation (approval code: CEEA75 152012).

Meal tolerance tests. Single-lumen radiopaque silicone catheters (Hickman; Bard, Trappes, France) were placed in the portal vein and in the right atrium of minipigs and then exteriorized on the neck of the animals to allow repeated blood sampling. After an overnight fasting, each animal was submitted to a 10-min meal test using a nasogastric tube before surgery and within the first month after surgery. The meal consisted on 200 ml of Ensure Plus (Abott France, Rungis, France) and a 20 g solid energy bar Ovomaltine (Ovomaltine France, Cergy-Pontoise, France). It contained overall 387 kcal, 13 g of fat, 15 g of protein, 22 g of simple carbohydrates (including sucrose and approximately 10 g of glucose and 32 g starch). The systemic and portal venous blood samples were obtained at 0, 15, 30, 60, 90 and 120 min from each catheter.

Portal and systemic blood sampling. Venous blood samples were collected during the meal tolerance test before and after the surgery in K_2EDTA plastic blood collection tubes (BD Vacutainer, New Jersey, US) from the central venous and portal catheters ($n = 4$). After centrifugation (5000 r.p.m. for 10 min at 4 °C), plasma was immediately separated and stored at –80 °C until analyzed.

Liver biopsy sampling. The right-lobe liver biopsies were taken by laparotomy under general anaesthesia from 16 h-fasted minipigs during RYGB ($n = 7$) and 4.5 ± 1.5 weeks after the surgery, at the time of sacrifice ($n = 8$). All tissues were snap frozen in liquid nitrogen and stored at − 80 °C until RNA extractions.

Bile acid analysis

Bile acid plasma concentrations were determined under blinded conditions by phase liquid chromatography associated to tandem mass spectrometry (LC-MS/MS) in Multiple Reaction Monitoring mode, after extraction by protein precipitation as previously published,[29,30] the use of internal deutered standards allowed the BA quantification. The limit of BA detection by LC-MS/MS is: 1.5 nM for GCA, GCDCA, GDCA, GUDCA, GLCA, TCDCA, TDCA, TUDCA, TLCA; 3 nM for GHCA, TCA THCA; and 15 nM for CA, CDCA, DCA, UDCA, LCA, HCA.

RNA extraction and quantification by qPCR

Total RNA was extracted from liver biopsies using Extract-All Reagent (Eurobio, Courteboeuf, France) according to the manufacturer's protocol under blinded conditions. After DNAse treatment (Fermentas, St Rémy Les Chevreuse, France), total RNA (0.5–1 mg) was reverse transcribed using High Capacity Multiscribe Reverse Transcriptase (Applied Biosystems, St Aubin, France) according to the manufacturer's protocol. qPCR with cDNA from reverse transcription was performed using the Master MIX SYBR Green Brillant Fast III (Agilent, Santa Clara, CA, USA) on a MX4000 apparatus (Stratagene, USA) using the following primers: *NTCP* forward: 5′-GATGGGACCCTGAAGGACAAGG-3′, reverse: 5′-CCACG TTGAGGACAGAGAGCAC-3′; *OATP* forward: 5′-TCCGATCCTTGGCT TTTCACTGG-3′, reverse: 5′-AAGCACCAACCCAACGAGAGTC-3′; *OAT* forward: 5′-GCTACTTGATACGGGACTGGCG-3′, reverse: 5′-TACCTAT GGGCGTCCTCCACAC-3′. The results are presented using the DDCt method and normalized to a reference gene (cyclophilin). Controls were set at 1 and all conditions were expressed comparatively to control.

Statistical analysis

Data were analyzed using the GraphPad-Prism V 5.0 (GraphPad Software, La Jolla, CA, USA) statistics software and presented as means ± s.e.m. Sample size was determined based on our experience from previous studies, as in Verhaeghe *et al.*[28] BA data were analyzed by two-way repeated measures analysis of variance followed by Sidak *post hoc* test. Gene expression data were compared using unpaired Student's *t*-test. Differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Post-prandial BAs were measured in conscious animals before and after RYGB, in both pre-hepatic portal VB, which contains the BAs reabsorbed from the intestine, and in systemic VB, *via* catheters placed in the portal vein and right atrium, respectively. Similar as in humans,[10,18,31] fasting and post-prandial total BA concentrations increased in systemic VB after RYGB (Figure 1a). This increase was due to a significant elevation in conjugated BAs (Figure 1b), whereas the change in free BAs was not significant due to larger inter-individual variations (Figure 1c), as recently reported in humans. Since different BA species activate FXR and TGR5 with different potencies, the RYGB-induced modifications in BA pool size and composition could modulate the metabolic processes regulated by these receptors and participate in the metabolic changes upon RYGB. Interestingly, post-prandial total BA concentrations in portal VB decreased after surgery (Figure 1d) due to a significant reduction in conjugated BAs (Figure 1e), whereas free BAs in portal VB did not change after RYGB (Figure 1f). The changes in portal VB suggest that the intestine is also involved in the RYGB-induced BA concentration changes, possibly *via* alterations in BA deconjugation by the Bile Salt Hydrolase (BSH) of the microbiota or in intestinal BA recapture. Qualitative analysis revealed a non-significant tendency to increase in the secondary: primary BA ratio in portal VB (data not shown), whereas portal free BAs (products of BSH) were not influenced by RYGB. The latter observation is suggestive of alterations in intestinal BA absorption whereas alterations in the gut microbiota appear to be less involved.

Calculation of the portal/systemic BA ratio before and after RYGB evidenced a role for the liver in the changes in systemic BA concentrations. RYGB surgery significantly decreased the fasting and post-prandial portal/systemic ratio of total BAs (Figure 1g).

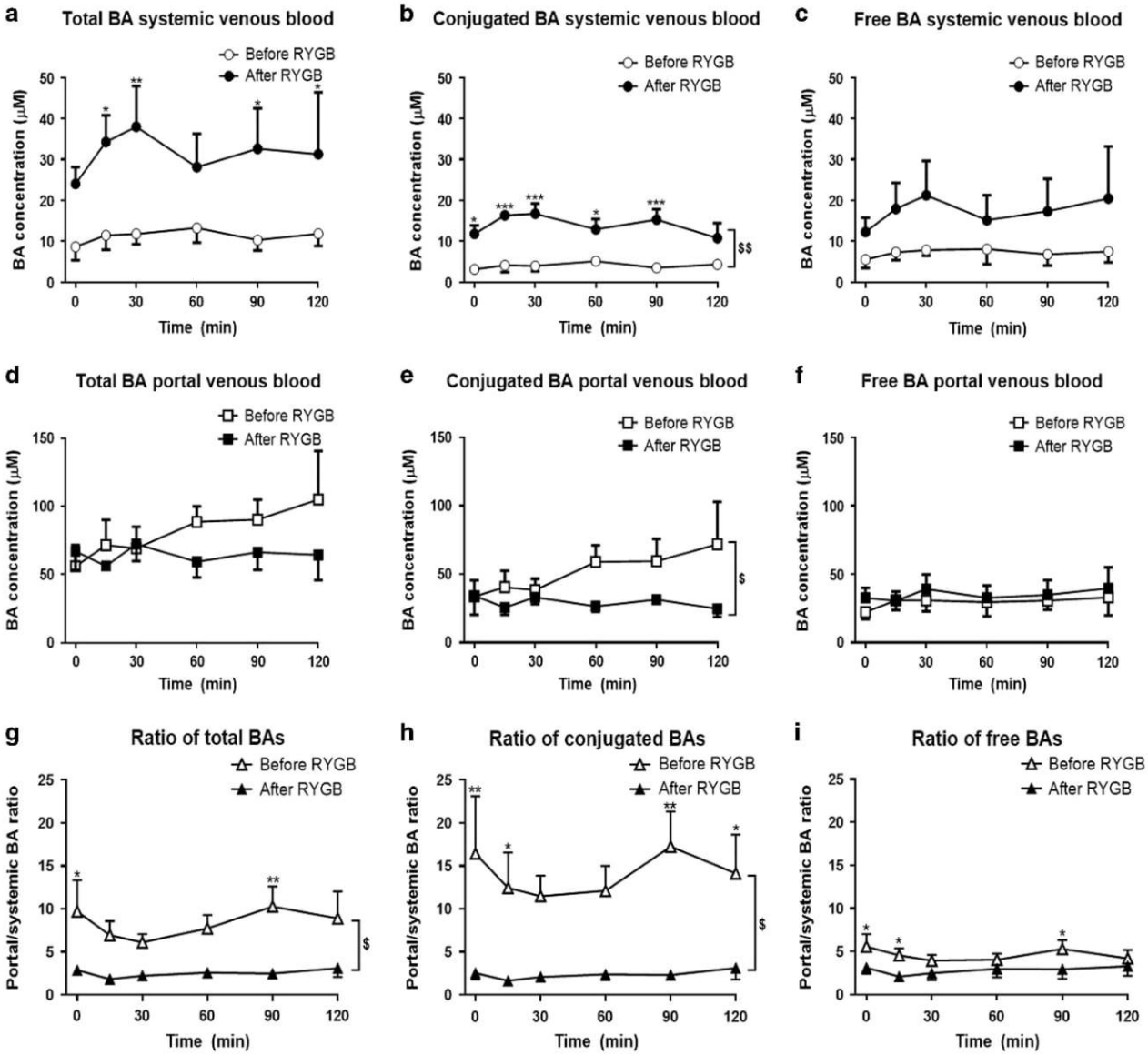


Figure 1. RYGB differently affects free and conjugated BA concentrations in systemic and portal venous blood. Total (**a**, **d**), conjugated (**b**, **e**) and free (**c**, **f**) bile acid concentrations during a meal test in systemic (**a**–**c**) and portal (**d**–**f**) venous blood, in minipigs ($n = 4$) before and after Roux-en-Y gastric bypass. Portal/systemic total (**g**), conjugated (**h**) and free (**i**) BA ratio before and after RYGB. Values are expressed as means ± s.e.m. Repeated measures two-way analysis of variance, $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ and Sidak *post hoc* test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

This decrease was almost exclusively due to conjugated BAs (Figure 1h) and, to a lesser extent, to free BAs (Figure 1i). In line, the area under the curve (AUC) of conjugated BA concentrations during the meal test decreased after RYGB in portal VB (Figure 2b), which may point to an effect of RYGB on transporter-mediated conjugated BA reuptake in the intestine. Interestingly, the AUCs of systemic BA concentrations increased (Figures 2a–c), again mainly due to a significant increase in the AUC of conjugated BAs, notwithstanding their decrease in portal VB (Figure 2b). These observations strongly suggest that quantitative and qualitative alterations in hepatic BA recapture occur after RYGB. To test this hypothesis, the expression of BA recapture transporter genes, such as *NTCP, OAT* and *OATP* was assessed in liver biopsies taken before and after RYGB. The expression of all three genes was reduced after RYGB, with a strongest effect on *NTCP* expression, which mainly transports conjugated BAs (Figure 2d).

We speculate that the more pronounced increase in systemic conjugated BA species is related to the fact that their reuptake depends entirely on active transport mechanisms (NTCP, OATP, OAT), whereas free BAs can still passively diffuse across the sinusoidal membrane of hepatocytes, resulting in a less pronounced increase in systemic free BAs. The surgical modification of the gastrointestinal tract could increase portal blood flow, and consequently alter systemic BA concentrations. However, this seems to be less plausible in our minipig model, since we observed important qualitative differences after RYGB, whereas a hemodynamic effect would similarly increase all BA species.

The present study presents some limitations: the mechanisms by which RYGB modifies hepatic BA metabolism remain unknown and further studies considering the inter-organ BA signaling with particular focus on the intestine are required to determine the underlying mechanisms. In line, analysis of the expression of BA

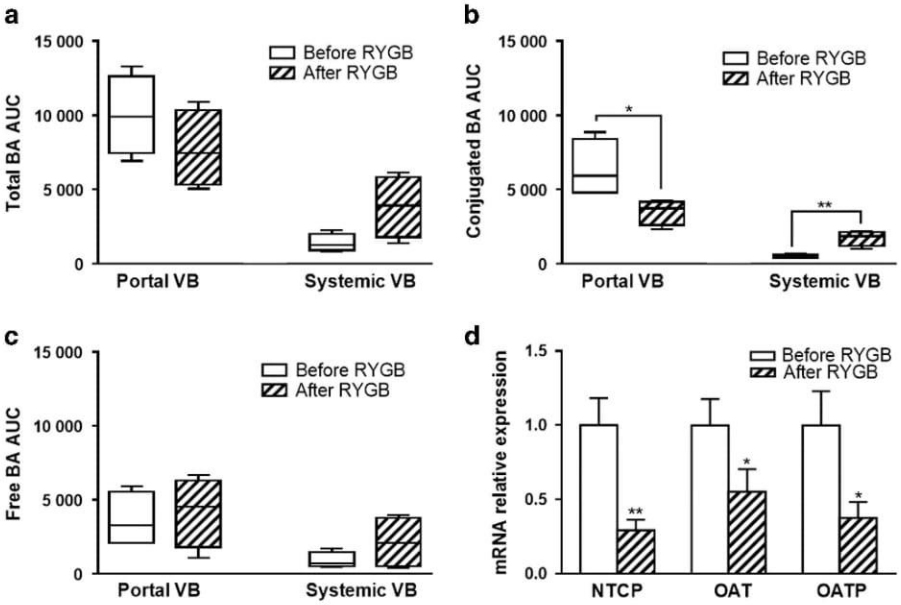


Figure 2. RYGB decreases conjugated BA uptake by the liver. AUC calculated from concentration curves of total (**a**), conjugated (**b**) and free bile acids (**c**) during a meal test in systemic venous blood and portal venous blood in minipigs ($n=4$) before and after the acronym:RYGB. Expression of genes (**d**) involved in BA recapture (*NTCP, OAT* and *OATP*) in pig liver biopsies before ($n=7$) and after ($n=8$) RYGB. Results are normalized to cyclophilin expression. Values are expressed as means ± s.e.m. Student's *t*-test, $^{*}P<0.05$, $^{**}P<0.01$.

transporters in gut biopsies is necessary. Finally, even though indirect arguments indicate a minor role for changes in microbiota, a detailed gut flora analysis would be necessary for conclusive evidence.

Our study compares for the first time BA pool changes between pre-hepatic portal and systemic venous blood, before and after RYGB, in a human-size mammalian model presenting better anatomic similarities to humans than rodent models, and which reproduces the metabolic improvements after RYGB. Altogether, our results suggest that alterations in hepatic function contribute to the increase in systemic BAs after RYGB, and that decreased hepatic BA recapture may be a mechanism involved in these modifications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Audray Quenon, Amandine Descat, Bruno Derudas, Arnold Dive, Michel Pottier and Mathieu Fourdrinier for excellent technical assistance. VS was supported by a grant from the Fondation pour la Recherche Médicale (FRM) Grant FDT20140930804. This work was supported by Grants from Région Nord-Pas de Calais, FEDER, INSERM, ANR (FXREn), Société Francophone du Diabète (SFD), Institut Universitaire de France and European Genomic Institute for Diabetes (EGID, ANR-10-LABX-46), and European Commission.

REFERENCES

1 Eliasson B, Liakopoulos V, Franzén S, Näslund I, Svensson A-M, Ottosson J *et al.* Cardiovascular disease and mortality in patients with type 2 diabetes after bariatric surgery in Sweden: a nationwide, matched, observational cohort study. *Lancet Diabetes Endocrinol* 2015; **3**: 847–854.
2 Tailleux A, Rouskas K, Pattou F, Staels B. Bariatric surgery, lipoprotein metabolism and cardiovascular risk. *Curr Opin Lipidol* 2015; **22**: 317–324.
3 Caiazzo R, Lassailly G, Leteurtre E, Baud G, Verkindt H, Raverdy V *et al.* Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study. *Ann Surg* 2014; **260**: 893–898; discussion 898–899.
4 Osto E, Doytcheva P, Corteville C, Bueter M, Dörig C, Stivala S *et al.* Rapid and body weight-independent improvement of endothelial and high-density lipoprotein function after Roux-en-Y gastric bypass: role of glucagon-like peptide-1. *Circulation* 2015; **131**: 871–881.
5 Dirksen C, Bojsen-Møller KN, Jørgensen NB, Jacobsen SH, Kristiansen VB, Naver LS *et al.* Exaggerated release and preserved insulinotropic action of glucagon-like peptide-1 underlie insulin hypersecretion in glucose-tolerant individuals after Roux-en-Y gastric bypass. *Diabetologia* 2013; **56**: 2679–2687.
6 Laferrère B, Heshka S, Wang K, Khan Y, McGinty J, Teixeira J *et al.* Incretin levels and effect are markedly enhanced 1 month after Roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care* 2007; **30**: 1709–1716.
7 Sweeney TE, Morton JM. The human gut microbiome: a review of the effect of obesity and surgically induced weight loss. *JAMA Surg* 2013; **148**: 563–569.
8 Osto M, Abegg K, Bueter M, le Roux CW, Cani PD, Lutz TA. Roux-en-Y gastric bypass surgery in rats alters gut microbiota profile along the intestine. *Physiol Behav* 2013; **119**: 92–96.
9 Sweeney TE, Morton JM. Metabolic surgery: action via hormonal milieu changes, changes in bile acids or gut microbiota? A summary of the literature. *Best Pract Res Clin Gastroenterol* 2014; **28**: 727–740.
10 Penney NC, Kinross JM, Newton RC, Purkayastha S. The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: A systematic review. *Int J Obes* 20052015; **17**: 1565–1574.
11 Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T *et al.* Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab* 2015; **22**: 228–238.
12 Bhutta HY, Rajpal N, White W, Freudenberg JM, Liu Y, Way J *et al.* Effect of Roux-en-Y gastric bypass surgery on bile acid metabolism in normal and obese diabetic rats. *PloS One* 2015; **10**: e0122273.
13 Gerhard GS, Styer AM, Wood GC, Roesch SL, Petrick AT, Gabrielsen J *et al.* A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. *Diabetes Care* 2013; **36**: 1859–1864.
14 Patti M-E, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ *et al.* Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obes Silver Spring Md* 2009; **17**: 1671–1677.
15 Simonen M, Dali-Youcef N, Kaminska D, Venesmaa S, Käkelä P, Pääkkönen M *et al.* Conjugated bile acids associate with altered rates of glucose and lipid oxidation after Roux-en-Y gastric bypass. *Obes Surg* 2012; **22**: 1473–1480.
16 Werling M, Vincent RP, Cross GF, Marschall H-U, Fändriks L, Lönroth H *et al.* Enhanced fasting and post-prandial plasma bile acid responses after Roux-en-Y gastric bypass surgery. *Scand J Gastroenterol* 2013; **48**: 1257–1264.
17 Sachdev S, Wang Q, Billington C, Connett J, Ahmed L, Inabnet W *et al.* FGF 19 and bile acids increase following Roux-en-Y Gastric bypass but not after medical management in patients with type 2 diabetes. *Obes Surg* 2016; **26**: 957–965.

18 Dutia R, Embrey M, O'Brien S, Haeusler RA, Agénor KK, Homel P *et al.* Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. *Int J Obes 2005* 2015; **39**: 806–813.
19 Steinert RE, Peterli R, Keller S, Meyer-Gerspach AC, Drewe J, Peters T *et al.* Bile acids and gut peptide secretion after bariatric surgery: A 1-year prospective randomized pilot trial. *Obesity* 2013; **21**: E660–E668.
20 Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. *Int J Obes* 2013; **37**: 1553–1559.
21 Pournaras DJ, Glicksman C, Vincent RP, Kuganolipava S, Alaghband-Zadeh J, Mahon D *et al.* The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. *Endocrinology* 2012; **153**: 3613–3619.
22 Albaugh VL, Flynn CR, Cai S, Xiao Y, Tamboli RA, Abumrad NN. Early Increases in Bile Acids Post Roux-en-Y Gastric Bypass Are Driven by Insulin-Sensitizing, Secondary Bile Acids. *J Clin Endocrinol Metab* 2015; **100**: E1225–E1233.
23 Nakatani H, Kasama K, Oshiro T, Watanabe M, Hirose H, Itoh H. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. *Metabolism* 2009; **58**: 1400–1407.
24 Jansen PLM, van Werven J, Aarts E, Berends F, Janssen I, Stoker J *et al.* Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery. *Dig Dis Basel Switz* 2011; **29**: 48–51.
25 Mazuy C, Helleboid A, Staels B, Lefebvre P. Nuclear bile acid signaling through the farnesoid X receptor. *Cell Mol Life Sci* 2014; **72**: 1631–1650.
26 Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. *Physiol Rev* 2009; **89**: 147–191.
27 Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R *et al.* FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014; **509**: 183–188.
28 Verhaeghe R, Zerrweck C, Hubert T, Tréchot B, Gmyr V, D'Herbomez M *et al.* Gastric bypass increases postprandial insulin and GLP-1 in nonobese minipigs. *Eur Surg Res* 2014; **52**: 41–49.
29 Spinelli V, Lalloyer F, Baud G, Osto E, Kouach M, Daoudi M *et al.* Influence of Roux-en-Y gastric bypass on plasma bile acid profiles: a comparative study between rats, pigs and humans. *Int J Obes* 2016; **40**: 1260–1267.
30 Broeders EPM, Nascimento EBM, Havekes B, Brans B, Roumans KHM, Tailleux A *et al.* The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. *Cell Metab* 2015; **28**: 418–426.
31 Ferrannini E, Camastra S, Astiarraga B, Nannipieri M, Castro-Perez J, Xie D *et al.* Increased bile acid synthesis and deconjugation after biliopancreatic diversion. *Diabetes* 2015; **26**: 3377–3385.

MANUSCRIPT NUMBER 7

Chávez-Talavera & Marciniak *et al.* The length of the biliary limb drives the weight loss effect of One Anastomosis Gastric Bypass independently of glucose and bile acid changes in minipigs. Article in preparation.

Title: The length of the biliary limb drives the weight loss effect of One Anastomosis Gastric Bypass independently of glucose and bile acid changes in minipigs.

Short title: Role of the common limb in gastric bypass

C. Marciniak*[1], O. Chávez-Talavera*[2], G. Baud[1], A. Quenon[1], A. Descat[3], E Vallez[2], JF Gossens[3], M Kouach[3], V. Vangelder[1], L. Zubiaga[1], M. Daoudi[1],B. Derudas[2], P. Pigny[4], A. Klein[5], V. Gmyr, V. Raverdy[1], T. Hubert[1], R. Caiazzo[1], S. Lestavel[2], B. Laferrère[6], B. Staels[$2], A. Tailleux[$2], F. Pattou[$1]

[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1190 - EGID, F-59000 Lille, France
[2]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011 - EGID, F-59000 Lille, France
[3]Mass Spectrometry Department, PSM-GRITA, Pharmacy Faculty, F-59000 Lille, France.
[4]University Lille, Inserm, CHU Lille, U1172 Jean-Pierre Aubert Research Center, 59000 Lille, France
[5]CHU Lille, Metabolism and Glycosylation Diseases, Biology Pathology Center, 59000 Lille, France
[6]Columbia University College of Physicians and Surgeons, Division of Endocrinology, New York, NY 10032, United States of America.

* Co-first authors
[$] Co-last authors

Grant support: This work was supported by European Genomic Institute for Diabetes (ANR-10-LABX-46), and Fondation Francophone de Recherche sur le Diabète (FFRD-2015-1). OCT received a PhD fellowship from the French Ministry of Research. BS is a recipient of an Advanced ERC Grant (694717).

Corresponding author: François Pattou, correspondence information: General Endocrine Surgery, Lille University Hospital. Rue M Polonovski, 59000 Lille, France. e-mail: francois.pattou@univ-lille.fr

Disclosures: CM, OCT, GB, AQ, AD, EV, JFG, MK, VV, LZ, MD, BD, PP, AK, VG, VR, TH, RC, SL, BS, AT and FP declare no conflict of interest.

Author Contributions:

Study concept and design: CM, GB, VG, VR, TH, RC, FP. Surgical procedures: CM, GB, AQ, VV. Acquisition of data: CM, OCT, GB, AQ, AD, EV, VV, LZ, MD, BD, PP, AK. Analysis and interpretation of data: CM, OCT, GB, VV, LZ, VG, RC, SL, AT, BS, FP. Drafting of the manuscript: CM, OCT, AT, BS, FP. Critical revision of the manuscript for important intellectual content: BL. Statistical analysis: CM, OCT. Obtained funding, administrative, technical or material support: VR, TH, BS, FP. Study supervision: AT, BS, FP.

Abstract

Background & Aims: The alimentary limb has been proposed to be the main driver of the weight-loss independent metabolic improvements in the bariatric surgery procedure. However, the One Anastomosis Gastric Bypass (OAGB) procedure, consisting of one long biliary limb and a short common limb, induces stronger metabolic beneficial effects compared to RYGB in humans, despite the lack of an alimentary limb. The aim of this study was to assess the role of the biliary and common limbs in the weight-loss and metabolic effects upon OAGB.

Methods: OAGB and sham surgery, both with or without resection of the biliary limb were performed in Gottingen-like minipigs. 15 days after surgery, weight loss and metabolic changes were assessed.

Results: OAGB decreased body weight, improved glucose homeostasis, increased postprandial GLP-1 and fasting plasma BAs, and qualitatively changed intestinal BAs. Resection of the biliary limb prevented the body weight loss effects of OAGB and attenuated the postprandial GLP-1 increase. Glucose homeostasis along with changes in plasma and intestinal BAs occurred upon OAGB regardless of the biliary limb length. Resection alone reproduced the glucose homeostasis effects and the changes in intestinal BAs.

Conclusions: A long biliary limb is necessary for weight loss in OAGB, whereas the metabolic and BA changes are mediated by the length of the common limb.

Keywords: bariatric surgery, biliary limb, common limb, mini-bypass, bile acids

INTRODUCTION

The rapidly rising global prevalence of obesity and its complications represent a major public health problem, decreasing quality of life and carrying substantial economic costs for society (Srivastava and Apovian 2018; Ng *et al.* 2014). Bariatric surgery induces substantial and sustained body weight loss, and is considered the most effective treatment for morbid obesity, since in addition to body mass loss, it also reverses obesity's co-morbidities, such as type 2 diabetes and non-alcoholic fatty liver disease (Lassailly *et al.* 2015; Srivastava and Apovian 2018). Several bariatric surgery techniques have been developed over time, but the most studied and, until recently, most performed, is the Roux-en-Y gastric bypass (RYGB) (Buchwald, Buchwald, and McGlennon 2014).

Many metabolic improvements upon RYGB are due to weight loss, but certain changes occur before the body weight loss (Carbajo *et al.* 2017; Sweeney and Morton 2014). The mechanisms are not entirely known, but it has been proposed that, in RYGB, the exclusion of a portion of the stomach and proximal part of the intestine to form a small gastric pouch (**Supplemental Figure 1A**) leads to reduced meal volumes and early satiety (Elder and Wolfe 2007), provoking caloric restriction (Mattison *et al.* 2017). Moreover, the duodenal diversion, present in RYGB, results in altered duodenal sensing of the nutrients (Breen *et al.* 2012), and bypassing the proximal part of the intestine causes accelerated nutrient transit, leading to enhanced postprandial release of incretins (*e.g.* glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP)) and satiety peptides (*e.g.* peptide YY (PYY), oxyntomodulin (OXY)) (Laferrère *et al.* 2007; Oliván *et al.* 2009; Laferrère *et al.* 2010). Furthermore, bile diversion, also found in RYGB, decreases glucose absorption in the common limb (Baud *et al.* 2016). Other suggested mechanisms include hypertrophy of the alimentary limb with increased splanchnic glucose utilization and changes in glucose transporter expression (Cavin *et al.* 2016; Saeidi *et al.* 2013), beneficial modifications of the gut microbiota (Anhê *et al.* 2017) and changes in bile acid (BA) pool size and composition (Dutia *et al.* 2015; Spinelli *et al.* 2016; O. Chávez-Talavera *et al.* 2017), which modulate energy metabolism through activation of the farnesoid X receptor (FXR) and the Takeda G protein coupled receptor 5 (TGR5).

Experimental data support a key role for the alimentary limb as mediator of the improvement of glucose homeostasis upon RYGB (Saeidi *et al.* 2013; Baud *et al.* 2016). However, results from a recently developped surgical technique, the One Anastomosis Gastric Bypass (OAGB) (Rutledge 2001), challenges these data. OAGB results in weight loss and beneficial metabolic effects, which are similar or even supperior to those elicited by RYGB (Carbajo *et al.* 2017; Wang *et al.* 2017). Similarly as RYGB, OAGB (**Supplemental Figure 1A**) excludes a portion of the stomach and the proximal intestine, but it differs from RYGB by the types of

limbs: while RYGB creates an alimentary limb, a short biliary limb and a common limb, OAGB does not have an alimentary limb and is composed solely by a long-loop biliary limb and a common limb **(Supplementary Figure 1B).** The mechanism of action of OAGB is not entirely clear, since OAGB lacks an alimentary limb, thought to be responsible for the RYGB-induced metabolic effects.

The superior metabolic effects of OAGB in comparison with RYGB point at a potential mechanistic role of the biliary limb (Miyachi *et al.* 2016), and therefore certain surgeons adjust the length of the biliary limb according to the BMI of their patients in OAGB (Charalampos *et al.* 2019) and RYGB (Mahawar *et al.* 2016). However, modulation of the length of the biliary limb consequently affects the length of the common limb, where biliopancreatic secretions and alimentary nutrients meet, and where absorption takes place. OAGB could also alter BAs, since unlike in the normal intestine, where BAs transit along with the chyme, the biliopancreatic secretions transit alone in OAGB. It is thus conceivable that similarly as in RYGB, the anatomic variations of OAGB could induce changes in intestinal or circulating BAs and contribute to the early metabolic improvements following bariatric surgery by modulating the activity of the BA receptors FXR and TGR5. The aim of this study is to investigate the role of the biliary/common limbs in the effects of OAGB on body weight and metabolic effects. For that, we analyzed glucose homeostasis and BA composition in plasma and within the intestine of minipigs submitted to sham surgery or OAGB, with and without resection of an intestinal segment. We hypothesized that: OAGB impacts glucose homeostasis and BAs and that this also occurs in absence of the biliary limb.

MATERIALS AND METHODS

Animal facilities

All animal procedures were performed in the University and Hospital Department for Experimental Research of the University of Lille, France, in accordance with French regulations for animal experimentation [approval code:CEEA 152012]. A total of 24 healthy adult female minipigs Göttingen-like weighing 53.9±1.7kg (Pannier's breeding) were used. All pigs were kept in individual pens. All pens were equiped with a feeding through and a drinking nipple. Pigs were fed a standard diet (Uneal cooperative, Aire sur la Lys, France) at 1.5% of their body weight per day, mixed with water (1 volume for 1 volume) for a pureed consistency, in two meals during the morning.

Animals were fasted overnight and the surgical procedures were performed under general anaesthesia by midline laparotomy. For the experiment, animals were pre-medicated with an intramuscular injection of ketamine (Ketamine1000 , Virbac, Carros, France, 10 mg/kg of

body weight) and xylazine (Sédaxylan , CEVA Santé Animale, Libourne France, 2.5 mg/kg of body weight). A 2% concentration of isoflurane (Aerrane®, Baxter, France) was used during the surgery. A single-lumen radiopaque silicone catheter (Hickman; Bard, Trappes, France) was initially placed in a jugular vein and exteriorized on the neck of the animal for repeated non-invasive blood samples.

Surgical Procedures

Animals were randomly assigned to either one of the four procedures (n=6-7 per group): sham, OAGB, sham with intestinal resection (Sham-res) or OAGB with resection of the biliaray limb (OAGB-res) **(Figure 1A-D)**.

OAGB and OAGB-res: after dissection of the oesogastric junction, a 100mL gastric pouch was constructed using linear staplers (Proximate®, green cartridges, Ethicon, Issy-les-Moulineaux, France). As minipig intestine is approximately twice the size of that of humans, performed a distal OAGB to amplify the observed effects. The distal portion of the ileum (200 cm before the ileo-caecal junction) was taken up and anastomosed to the gastric pouch (Proximate®, blue cartridges, Ethicon, Issy-les-Moulineaux, France) to form an omega loop. In the OAGB-res procedure, the proximal portion of the jejunum (50 cm from the duodenojejunal junction) was taken up and anastomosed to the gastric pouch. The intestine between the gastrojejunal anastomosis and the distal portion of the ileum (200 cm before the ileocaecal junction) was then resected and an entero-enteral anastomosis was performed.

Sham and Sham-res: The Sham procedure consisted in oesogastric junction dissection, gastrotomy, enterotomy, and repair. The Sham-res procedure consisted in resection of the intestine, starting 50 cm from the duodeno-jejunal junction and down to 200 cm before the ileo-caecal junction and then entero-enteral anastomosis.

Post-operative care

After surgery, minipigs were closely monitored and treated with fentanyl transdermal (Recuvyra® 2.6 mg/Kg, Eli Lilly, Neuilly-sur-Seine, France). Antibioprophylaxy by lincomycine and spectinomycine (LINCO-SPECTIN®, 0.1mL/kg, ZOETIS, Malakoff, France) was administered for five days. Water intake started on the first postoperative day, and similar oral feeding was allowed 72 hours after surgery. At the end of the study, minipigs were euthanized by injection of Embutramide, Mebezonium and Tetracaine (T61®, 0.3 mL/kg, INTERVET, Beaucouzé cedex, France).

Mixed-meal test

15 days after the surgery, after overnight fasting, mixed meal was adminisitered *via* a previously placed nasogastric tube over 10 minutes. The 200 ml liquid mixed meal test

(MMT) (Fortimel Energy, Nutricia Nutrition Clinique, Saint-Ouen, France) included a 20 g crushed solid energy bar of Ovomaltine (Ovomaltine, Wander SA, Neuenegg, Suisse) and was composed of 14 g fat, 14 g protein, 50 gr of carbohydrate (23 g simple sugars, and 27 g starch) for a total of 386 kcal, and included 30 g of D-Xylose (Sigma-Aldrich, Saint Louis, Etats-Unis). Venous blood samples were collected from a central venous catheter before and at 15, 30, 60, 90, 120 and 180 minutes after completion of the meal. Blood samples were immediately kept on ice until centrifugation at 5,000 rpm for 10 minutes. Plasma or serum aliquots were stored at -80°C until analysis.

Intestinal liquid sampling

During and 20 days after surgery, under general anesthesia, a 50 cm-long intestinal segment was clamped and 40 mL of distilled water were injected and then re-aspired from the intestinal lumen (20 cm-long segment and 20 mL for the short biliary limb). Intestinal liquid samples were aliquoted and immediately frozen at -80 °C until BA analysis.

Liver and intestine biopsy sampling

Right-lobe liver biopsies were taken by laparotomy under general anaesthesia from fasted minipigs before surgery and 20 days after the surgery. Intestine biopsies were taken by laparotomy under general anaesthesia from fasted minipigs at 20 days after surgery. All tissues were immediately snap frozen in liquid nitrogen and stored at -80°C until RNA extractions.

Biochemical analysis

Blood glucose was measured in duplicate immediatly after sampling using the amperometric glucoseoxydase method (glucose meter, FreeStyle Optium®, Abbott, Rungis, France). Plasma D-Xylose concentration was measured by a colorimetric micromethod with phloroglucinol as previously described (Eberts *et al.* 1979). Insulin was measured by ELISA (Access ultrasensitive Insulin, Beckman Coulter, Brea, USA), and total GLP-1 by RIA (GLP-1T-36HK, Millipore-IDS, France). Plasma concentrations of BAs and 7alpha-hydroxy-4-cholesten-3-one (C4) were determined after extraction by protein precipitation as previously published (Legry *et al.* 2017). Intestinal intra-luminal BA profiles were performed similarly as in plasma. Feces and caecal content of BA profiles were quantified after extraction on samples lyophilized at -80ºC to avoid bacterial BA transformation as previously described (Humbert *et al.* 2012). The 21 BA species were quantified by phase liquid chromatography associated to tandem mass spectrometry (LC-MS/MS) in the different biological samples. Due to the non quantitative intraluminal liquid sampling, the intra-luminal BA species concentrations are expressed as percentage or ratios of the total BAs.

Statistical analyses

Results were expressed as mean ± SEM. Continuous variables were analyzed by the unpaired or paired one-way ANOVA or Kruskal Wallis according to the data distribution. Variables measured during the mixed-meal test were compared to baseline values, and the areas under curves (AUC) were calculated using the trapezoidal method. One-way repeated measures ANOVA and *post hoc* Tukey's multiple comparisons test were used to compare differences of AUC. A p value <0.05 was considered as significant. All statistical analyses were made using Prism 7 for Mac OS X (GraphPad La Jolla, CA, USA).

RESULTS

OAGB induces body weight loss only in presence of a long biliary limb

Unlike the Sham surgery, OAGB progressively and strongly reduces body weight during the 20-day follow-up after surgery **(Figure 2).** Shortening of the biliary limb (BL) in OAGB (OAGB-res) reduced but not abolished the body weight loss compared to OAGB. The intestinal resection alone also slightly reduced body weight in Sham-res. These results suggest that a long biliary limb contributes to the weight loss effect of OAGB.

The improvements of glucose homeostasis are not driven by the length of the biliary limb but rather by a short common limb upon OAGB.

Compared with Sham, OAGB improves oral glucose tolerance **(Figure 3A, 3B)**, decreases intestinal D-xylose absorption **(Figure 3C, 3D)** and increases fasting (**Supplemental figure 2**) and postprandial GLP-1 **(Figure 3E, 3F)**, without changing insulin concentration **(Figure 3G, 3H)** during a meal test.

Compared with the Sham group, both groups with intestinal resection (Sham-res and OAGB-res) improved oral glucose tolerance **(Figure 3A, 3B),** decreased D-Xylose absorption **(Figure 3C, 3D),** increased fasting GLP-1 plasma concentration -the difference is not statistically significant for OAGB-res (**Supplemental Figure 2**)- and increased postprandial GLP-1 (**Figure 3E, 3F**) during the meal test. Similarly as in OAGB, there are no changes in the insulinemia in both groups with BL resection. Taken together, these data suggest that glucose homeostasis improvements occur in presence of a short common limb regardless of the resection of the BL or the OAGB.

Intestinal intra-luminal BAs are not modified by biliary limb length but by the length of the common limb.

In order to determine whether metabolic improvements are associated with BA changes, we measured BA profiles in different segments along the gastro-intestinal tract (GIT) from bile to feces in the Sham (**Supplemental Figure 4A**) and in OAGB (**Supplemental Figure 4B)** groups. As expected, in the Sham group, the free/conjugated BA ratio progressively increases from bile to feces (**Supplemental Figure 3C)**. Compared to Sham (**Supplemental Figure 3A, 3C**), the OAGB group (**Supplemental Figure 3B, 3D**) displays strongly and significantly increased primary/secondary BA ratios and free/conjugated BA ratios in all the segments of the GIT due to a shift from HDCA (a major secondary BA) to HCA (a major primary BA) in bile (**Supplemental Figure 4A**), in all intestinal compartments (**Supplemental Figure 4B-F**) and in feces (**Supplemental Figure 4G**), whereas minor BA species in minipigs (CA, CDCA, DCA, LCA and UDCA) are poorly affected by OAGB (**Supplemental Figure 4A-5G)**.

Interestingly, both BL resection groups (Sham-res and OAGB-res) also display a higher primary/secondary BA ratio (**Figure 3A)** due to a shift from HDCA to HCA both in bile (**Figure 3A, 3C)** and cecum **(Figure 3A, 3D)** which are representative of the whole GIT similarly as upon OAGB.

Interestingly, fasting plasma BAs do not reflect intestinal intra-luminal BAs as shown in (**Supplemental Figure 5**). OAGB increased total BA concentrations (**Supplemental Figure 5A**) without changing neither the Free/Conjugated (**Supplemental Figure 5B**) nor the 1ary/2ary BA retios (**Supplemental Figure 5C**). The shift from HDCA to HCA observed in the GIT was not found in plasma (**Supplemental Figure 5E, 5D**).

DISCUSSION

To elucidate the role of the biliary and common limbs on body weight loss and metabolic changes upon bariatric surgery, we performed OAGB, presenting a long biliary limb and no alimentary limb **(Figure 1B, 1D**). We studied these model with and without resection of the biliary limb, and compared it with sham surgery with and without resection of the intestinal segment that constitutes the biliary limb in OAGB, in a validated minipig model of bariatric surgery (Baud *et al.* 2016).

First we showed that the OAGB induces body weight loss compared to Sham. The body weight lowering effects of OAGB are attenuated in the models with resection (OAGB-res vs

Sham-res), suggesting that the resected intestinal segment, that constitutes the long biliary llimb of OAGB, plays a crucial mechanistic role for the body weight lowering effects of bariatric surgery. Interestingly, oral glucose tolerance, D-xylose absorption and GLP-1 secretion improved similarly in OAGB, Sham-res and OAGB-res compared to Sham, suggesting that the biliary limb is not necessary for the glucose homeostasis improvements but it's necessary for the body weight effects of OAGB.

The mechanism remains unknown. However, since OAGB but not RYGB induces nutrient malabsortpion (Robert *et al.* 2019), and since the main difference between OAGB and RYGB is the long biliary limb, we hypothesized that shortening the biliary limb could attenuate the malabsortion induced by the OAGB and therefore reduce the body-weight loss effect. The common limb -where most of nutrient absorption occurs- was similar in OAGB, Sham-res and OAGB-res, but the weight loss was different, supporting the hypothesis that the biliary limb length could modulate the body weight loss effect.

The long biliary limb probably uses nutrients absorbed from the bloodstream to sustain itself and not only those coming from the instinal lumen, as described for the alimentary limb in RYGB (Saedi *et al.* 2014). Another possibility is that, when resected, the amount of energy necessary to sustain that intestinal segment would no longer be consumed, decreasing the intestinal energy expenditure, and attenuate the body weight loss. In line with this hypothesis, resection of the biliary limb in a rat model of duodenal-jejunal bypass prevented the weight loss effect induced by the surgery (Miyachi *et al.* 2016).

The glucose homeostasis changes were similar in OAGB, OAGB-res, and Sham-res, where the biliary limb was either long (OAGB), short (OAGB-res) or absent (Sham-res), suggesting that the glucose metabolism improvements are not mediated by the biliary limb length. In contrast, the common limb was equally short in the three procedures, suggesting that it could be involved in glucose homeostasis ameliorations, but that it might not be crucial for the differential effects on GLP-1 and body weight loss. The improved metabolism in the sham-res group is in line with recently published data in rats (Prada-Oliveira *et al.* 2019).

Since BAs change in other bariatric surgery techniques, modulate metabolic homeostasis and are current candidates to mediate weight-loss independent improvements of bariatric surgery, we analyzed plasma and intestinal BAs upon OAGB with and without resection of the biliary limb. We report for the first time increased fasting plasma BAs upon OAGB, similarly as found upon other bariatric surgery techniques (Oscar Chávez-Talavera *et al.* 2017). Fasting plasma BAs increased regardless of the biliary limb length, but the concentrations of the groups with intestinal resection seem to be lower and the mechanism remains unclear. Interestingly, intestinal resection in the Sham-res group alone was not

enough to increase BA concentrations, whereas it improved glucose homeostasis, and partially improved body weight, suggesting that circulating fasting plasma BAs might not be major drivers of the glucose homeostasis or body weight improvements upon the surgery, but this does not exclude that BAs in other compartiments could be implicated (*e.g.* in the intestinal lumen). In RYGB, the fasting plasma BAs increase is mainly due to conjugated BA species in minipigs (O. Chávez-Talavera *et al.* 2017). In contrast, in OAGB (and also in OAGB-res) the free/conjugated BA ratio is unaltered, and this qualitative discrepancy between surgical techniques suggests that the mechanism driving the BA increase is different. Additionally, a previous study reported that changes in 12α-OH/non-12α-OH BA ratio occur upon RYGB in humans (Dutia *et al.* 2015), but our data show that OAGB doesn't alter this parameter in minipigs. Finally, preclinical data in rodents suggested that the BA receptors FXR and TGR5 (McGavigan *et al.* 2015) could mediate the metabolic improvements, since bile diversion to the ileum reproduced the metabolic effects of bariatric surgery in TGR5 knockout mice but not in intestine-specific FXR knockout mice. The authors showed that the GLP-1 - FXR axis (Trabelsi *et al.* 2015) could be implicated in such improvements (Albaugh *et al.* 2019), suggesting that BA signaling is a topic that has not been fully elucidated, probably participating to the bariatric surgery metabolic effects, and that should be further studied in different pre-clinical models to confirm whether the reported results are present also in humans.

Conclusion

In minipigs, a long biliary limb is necessary for weight loss after OAGB, but not for the metabolic effects, which seem to be mediated by the length of the common limb. Similarly as other bile-diverting procedures, OAGB increases systemic fasting plasma BAs. A short common limb was enough to induce metabolic changes regardless of the OAGB. Suggesting that it is a crucial player in the metabolic improvements following bariatric surgery, warranting further study.

REFERENCES

Albaugh, Vance L., Babak Banan, Joseph Antoun, Yanhua Xiong, Yan Guo, Jie Ping, Muhammed Alikhan, Blake Austin Clements, Naji N. Abumrad, and Charles Robb Flynn. 2019. “Role of Bile Acids and GLP-1 in Mediating the Metabolic Improvements of Bariatric Surgery.” Gastroenterology 156 (4): 1041-1051.e4. https://doi.org/10.1053/j.gastro.2018.11.017.

Anhê, Fernando F., Thibault V. Varin, Jonathan D. Schertzer, and André Marette. 2017. “The Gut Microbiota as a Mediator of Metabolic Benefits after Bariatric Surgery.” Canadian Journal of Diabetes 41 (4): 439–47. https://doi.org/10.1016/j.jcjd.2017.02.002.

Baud, Gregory, Mehdi Daoudi, Thomas Hubert, Violeta Raverdy, Marie Pigeyre, Erik Hervieux, Magalie Devienne, *et al.* 2016. “Bile Diversion in Roux-En-Y Gastric Bypass Modulates Sodium-Dependent Glucose Intestinal Uptake.” Cell Metabolism 23 (3): 547–53. https://doi.org/10.1016/j.cmet.2016.01.018.

Breen, Danna M., Brittany A. Rasmussen, Andrea Kokorovic, Rennian Wang, Grace W. C. Cheung, and Tony K. T. Lam. 2012. “Jejunal Nutrient Sensing Is Required for Duodenal-Jejunal Bypass Surgery to Rapidly Lower Glucose Concentrations in Uncontrolled Diabetes.” Nature Medicine 18 (6): 950–55. https://doi.org/10.1038/nm.2745.

Buchwald, H., J. N. Buchwald, and T. W. McGlennon. 2014. “Systematic Review and Meta-Analysis of Medium-Term Outcomes after Banded Roux-En-Y Gastric Bypass.” Obesity Surgery 24 (9): 1536–51. https://doi.org/10.1007/s11695-014-1311-1.

Carbajo, Miguel A., Enrique Luque-de-León, José M. Jiménez, Javier Ortiz-de-Solórzano, Manuel Pérez-Miranda, and María J. Castro-Alija. 2017. “Laparoscopic One-Anastomosis Gastric Bypass: Technique, Results, and Long-Term Follow-Up in 1200 Patients.” Obesity Surgery 27 (5): 1153–67. https://doi.org/10.1007/s11695-016-2428-1.

Cavin, Jean-Baptiste, Anne Couvelard, Rachida Lebtahi, Robert Ducroc, Konstantinos Arapis, Eglantine Voitellier, Françoise Cluzeaud, *et al.* 2016. “Differences in Alimentary Glucose Absorption and Intestinal Disposal of Blood Glucose After Roux-En-Y Gastric Bypass vs Sleeve Gastrectomy.” Gastroenterology 150 (2): 454-464.e9. https://doi.org/10.1053/j.gastro.2015.10.009.

Charalampos, Theodoropoulos, Natoudi Maria, Vrakopoulou Gavriella Zoi Vrakopoulou, Triantafyllou Tania, Dimitrios Raptis, Zografos George, Leandros Emmanouil, and Albanopoulos Konstantinos. 2019. “Tailored One Anastomosis Gastric Bypass: 3-Year Outcomes of 94 Patients.” Obesity Surgery 29 (2): 542–51. https://doi.org/10.1007/s11695-018-3572-6.

Chávez-Talavera, O., G. Baud, V. Spinelli, M. Daoudi, M. Kouach, J.-F. Goossens, E. Vallez, *et al.* 2017. “Roux-En-Y Gastric Bypass Increases Systemic but Not Portal Bile Acid Concentrations by Decreasing Hepatic Bile Acid Uptake in Minipigs.” International Journal of Obesity (2005) 41 (4): 664–68. https://doi.org/10.1038/ijo.2017.7.

Chávez-Talavera, Oscar, Anne Tailleux, Philippe Lefebvre, and Bart Staels. 2017. “Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia and NAFLD.” Gastroenterology, February. https://doi.org/10.1053/j.gastro.2017.01.055.

Duboc, Henri, Caroline Chong Nguyen, Jean-Baptiste Cavin, Lara Ribeiro-Parenti, Anne-Charlotte Jarry, Dominique Rainteau, Lydie Humbert, *et al.* 2018. “Roux-En-Y Gastric-Bypass and Sleeve Gastrectomy Induces Specific Shifts of the Gut Microbiota without Altering the Metabolism of Bile Acids in the Intestinal Lumen.” International Journal of Obesity (2005), January. https://doi.org/10.1038/s41366-018-0015-3.

Dutia, R., M. Embrey, C. S. O’Brien, S. O’Brien, R. A. Haeusler, K. K. Agénor, P. Homel, *et al.* 2015. “Temporal Changes in Bile Acid Levels and 12α-Hydroxylation after Roux-En-Y Gastric Bypass Surgery in Type 2 Diabetes.” International Journal of Obesity (2005) 39 (5): 806–13. https://doi.org/10.1038/ijo.2015.1.

Eberts, T. J., R. H. Sample, M. R. Glick, and G. H. Ellis. 1979. “A Simplified, Colorimetric Micromethod for Xylose in Serum or Urine, with Phloroglucinol.” Clinical Chemistry 25 (8): 1440–43.

Elder, Katherine A., and Bruce M. Wolfe. 2007. “Bariatric Surgery: A Review of Procedures and Outcomes.” Gastroenterology 132 (6): 2253–71. https://doi.org/10.1053/j.gastro.2007.03.057.

Goncalves, Daisy, Aude Barataud, Filipe De Vadder, Jennifer Vinera, Carine Zitoun, Adeline Duchampt, and Gilles Mithieux. 2015. “Bile Routing Modification Reproduces Key Features of Gastric Bypass in Rat.” Annals of Surgery 262 (6): 1006–15. https://doi.org/10.1097/SLA.0000000000001121.

Humbert, Lydie, Marie Anne Maubert, Claude Wolf, Henri Duboc, Myriam Mahé, Dominique Farabos, Philippe Seksik, *et al.* 2012. “Bile Acid Profiling in Human Biological Samples: Comparison of Extraction Procedures and Application to Normal and Cholestatic Patients.” Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences 899 (June): 135–45. https://doi.org/10.1016/j.jchromb.2012.05.015.

Laferrère, Blandine, Stanley Heshka, Krystle Wang, Yasmin Khan, James McGinty, Julio Teixeira, Allison B. Hart, and Blanca Olivan. 2007. “Incretin Levels and Effect Are Markedly Enhanced 1 Month after Roux-En-Y Gastric Bypass Surgery in Obese Patients with Type 2 Diabetes.” Diabetes Care 30 (7): 1709–16. https://doi.org/10.2337/dc06-1549.

Laferrère, Blandine, Nicholas Swerdlow, Baani Bawa, Sara Arias, Mousumi Bose, Blanca Oliván, Julio Teixeira, James McGinty, and Kristina I. Rother. 2010. “Rise of Oxyntomodulin in Response to Oral Glucose after Gastric Bypass Surgery in Patients with Type 2 Diabetes.” The Journal of Clinical Endocrinology and Metabolism 95 (8): 4072–76. https://doi.org/10.1210/jc.2009-2767.

Lassailly, Guillaume, Robert Caiazzo, David Buob, Marie Pigeyre, Hélène Verkindt, Julien Labreuche, Violeta Raverdy, *et al.* 2015. “Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese Patients.” Gastroenterology 149 (2): 379–88; quiz e15-16. https://doi.org/10.1053/j.gastro.2015.04.014.

Legry, Vanessa, Sven Francque, Joel T. Haas, An Verrijken, Sandrine Caron, Oscar Chávez-Talavera, Emmanuelle Vallez, *et al.* 2017. “Bile Acid Alterations Are Associated with Insulin Resistance, but Not with NASH in Obese Subjects.” The Journal of Clinical Endocrinology and Metabolism, August. https://doi.org/10.1210/jc.2017-01397.

Mahawar, Kamal K., Parveen Kumar, Chetan Parmar, Yitka Graham, William R. J. Carr, Neil Jennings, Norbert Schroeder, Shlok Balupuri, and Peter K. Small. 2016. “Small Bowel Limb Lengths and Roux-En-Y Gastric Bypass: A Systematic Review.” Obesity Surgery 26 (3): 660–71. https://doi.org/10.1007/s11695-016-2050-2.

Mattison, Julie A., Ricki J. Colman, T. Mark Beasley, David B. Allison, Joseph W. Kemnitz, George S. Roth, Donald K. Ingram, Richard Weindruch, Rafael de Cabo, and Rozalyn M. Anderson. 2017. “Caloric Restriction Improves Health and Survival of Rhesus Monkeys.” Nature Communications 8: 14063. https://doi.org/10.1038/ncomms14063.

McGavigan, Anne K, Darline Garibay, Zachariah M Henseler, Jack Chen, Ahmed Bettaieb, Fawaz G Haj, Ruth E Ley, Michael L Chouinard, and Bethany P Cummings. 2015. “TGR5 Contributes to Glucoregulatory Improvements after Vertical Sleeve Gastrectomy in Mice.” Gut [Epub ahead of print] (October): gutjnl-2015-309871. https://doi.org/10.1136/gutjnl-2015-309871.

Miyachi, Tomohiro, Munenori Nagao, Chikashi Shibata, Yoshiro Kitahara, Naoki Tanaka, Kazuhiro Watanabe, Takahiro Tsuchiya, Fuyuhiko Motoi, Takeshi Naitoh, and Michiaki Unno. 2016. “Biliopancreatic Limb Plays an Important Role in Metabolic Improvement after Duodenal-Jejunal Bypass in a Rat Model of Diabetes.” Surgery 159 (5): 1360–71. https://doi.org/10.1016/j.surg.2015.11.027.

Ng, Marie, Tom Fleming, Margaret Robinson, Blake Thomson, Nicholas Graetz, Christopher Margono, Erin C Mullany, *et al.* 2014. “Global, Regional and National Prevalence of Overweight and Obesity in Children and Adults 1980-2013: A Systematic Analysis.” Lancet (London, England) 384 (9945): 766–81. https://doi.org/10.1016/S0140-6736(14)60460-8.

Oliván, Blanca, Julio Teixeira, Mousumi Bose, Baani Bawa, Tangel Chang, Heather Summe, Hongchan Lee, and Blandine Laferrère. 2009. “Effect of Weight Loss by Diet or Gastric Bypass Surgery on Peptide YY3-36 Levels.” Annals of Surgery 249 (6): 948–53. https://doi.org/10.1097/SLA.0b013e3181a6cdb0.

Patrício, Bárbara G., Tiago Morais, Marta Guimarães, Simon Veedfald, Bolette Hartmann, Linda Hilsted, Jens J. Holst, Mário Nora, and Mariana P. Monteiro. 2019. “Gut Hormone Release after Gastric Bypass Depends on the Length of the Biliopancreatic Limb.” International Journal of Obesity (2005) 43 (5): 1009–18. https://doi.org/10.1038/s41366-018-0117-y.

Prada-Oliveira, J. Arturo, Alonso Camacho-Ramirez, Jesús Salas-Alvarez, Francisco Javier Campos-Martinez, Alfonso M. Lechuga-Sancho, David Almorza-Gomar, Manuel Blandino-Rosano, and Gonzalo M. Perez-Arana. 2019. “GLP-1 Mediated Improvement of the Glucose Tolerance in the T2DM GK Rat Model after Massive Jejunal Resection.” Annals of Anatomy = Anatomischer Anzeiger: Official Organ of the Anatomische Gesellschaft 223 (February): 1–7. https://doi.org/10.1016/j.aanat.2019.01.007.

Robert, Maud, Philippe Espalieu, Elise Pelascini, Robert Caiazzo, Adrien Sterkers, Lita Khamphommala, Tigran Poghosyan, *et al.* 2019. “Efficacy and Safety of One

Anastomosis Gastric Bypass versus Roux-En-Y Gastric Bypass for Obesity (YOMEGA): A Multicentre, Randomised, Open-Label, Non-Inferiority Trial." Lancet (London, England) 393 (10178): 1299–1309. https://doi.org/10.1016/S0140-6736(19)30475-1.

Rutledge, R. 2001. "The Mini-Gastric Bypass: Experience with the First 1,274 Cases." Obesity Surgery 11 (3): 276–80. https://doi.org/10.1381/096089201321336584.

Saeidi, Nima, Luca Meoli, Eirini Nestoridi, Nitin K. Gupta, Stephanie Kvas, John Kucharczyk, Ali A. Bonab, Alan J. Fischman, Martin L. Yarmush, and Nicholas Stylopoulos. 2013. "Reprogramming of Intestinal Glucose Metabolism and Glycemic Control in Rats after Gastric Bypass." Science (New York, N.Y.) 341 (6144): 406–10. https://doi.org/10.1126/science.1235103.

Spinelli, V., F. Lalloyer, G. Baud, E. Osto, M. Kouach, M. Daoudi, E. Vallez, *et al.* 2016. "Influence of Roux-En-Y Gastric Bypass on Plasma Bile Acid Profiles: A Comparative Study between Rats, Pigs and Humans." International Journal of Obesity (2005) 40 (8): 1260–67. https://doi.org/10.1038/ijo.2016.46.

Srivastava, Gitanjali, and Caroline M. Apovian. 2018. "Current Pharmacotherapy for Obesity." Nature Reviews. Endocrinology 14 (1): 12–24. https://doi.org/10.1038/nrendo.2017.122.

Sweeney, Timothy E., and John M. Morton. 2014. "Metabolic Surgery: Action via Hormonal Milieu Changes, Changes in Bile Acids or Gut Microbiota? A Summary of the Literature." Best Practice & Research. Clinical Gastroenterology 28 (4): 727–40. https://doi.org/10.1016/j.bpg.2014.07.016.

Thazhath, Sony S., Tongzhi Wu, Richard L. Young, Michael Horowitz, and Christopher K. Rayner. 2014. "Glucose Absorption in Small Intestinal Diseases." Expert Review of Gastroenterology & Hepatology 8 (3): 301–12. https://doi.org/10.1586/17474124.2014.887439.

Trabelsi, Mohamed-Sami, Mehdi Daoudi, Janne Prawitt, Sarah Ducastel, Véronique Touche, Sama I. Sayin, Alessia Perino, *et al.* 2015. "Farnesoid X Receptor Inhibits Glucagon-like Peptide-1 Production by Enteroendocrine L Cells." Nature Communications 6 (July): 7629. https://doi.org/10.1038/ncomms8629.

Wang, Fu-Gang, Zhao-Peng Yu, Wen-Mao Yan, Ming Yan, and Mao-Min Song. 2017. "Comparison of Safety and Effectiveness between Laparoscopic Mini-Gastric Bypass

and Laparoscopic Sleeve Gastrectomy: A Meta-Analysis and Systematic Review." Medicine 96 (50): e8924. https://doi.org/10.1097/MD.0000000000008924.

Wewalka, Marlene, Mary-Elizabeth Patti, Corinne Barbato, Sander M. Houten, and Allison B. Goldfine. 2014. "Fasting Serum Taurine-Conjugated Bile Acids Are Elevated in Type 2 Diabetes and Do Not Change with Intensification of Insulin." The Journal of Clinical Endocrinology and Metabolism 99 (4): 1442–51. https://doi.org/10.1210/jc.2013-3367.

Wichmann, Anita, Ava Allahyar, Thomas U. Greiner, Hubert Plovier, Gunnel Östergren Lundén, Thomas Larsson, Daniel J. Drucker, Nathalie M. Delzenne, Patrice D. Cani, and Fredrik Bäckhed. 2013. "Microbial Modulation of Energy Availability in the Colon Regulates Intestinal Transit." Cell Host & Microbe 14 (5): 582–90. https://doi.org/10.1016/j.chom.2013.09.012.

Yousseif, Ahmed, Julian Emmanuel, Efthimia Karra, Queensta Millet, Mohamed Elkalaawy, Andrew D. Jenkinson, Majid Hashemi, *et al.* 2014. "Differential Effects of Laparoscopic Sleeve Gastrectomy and Laparoscopic Gastric Bypass on Appetite, Circulating Acyl-Ghrelin, Peptide YY3-36 and Active GLP-1 Levels in Non-Diabetic Humans." Obesity Surgery 24 (2): 241–52. https://doi.org/10.1007/s11695-013-1066-0.

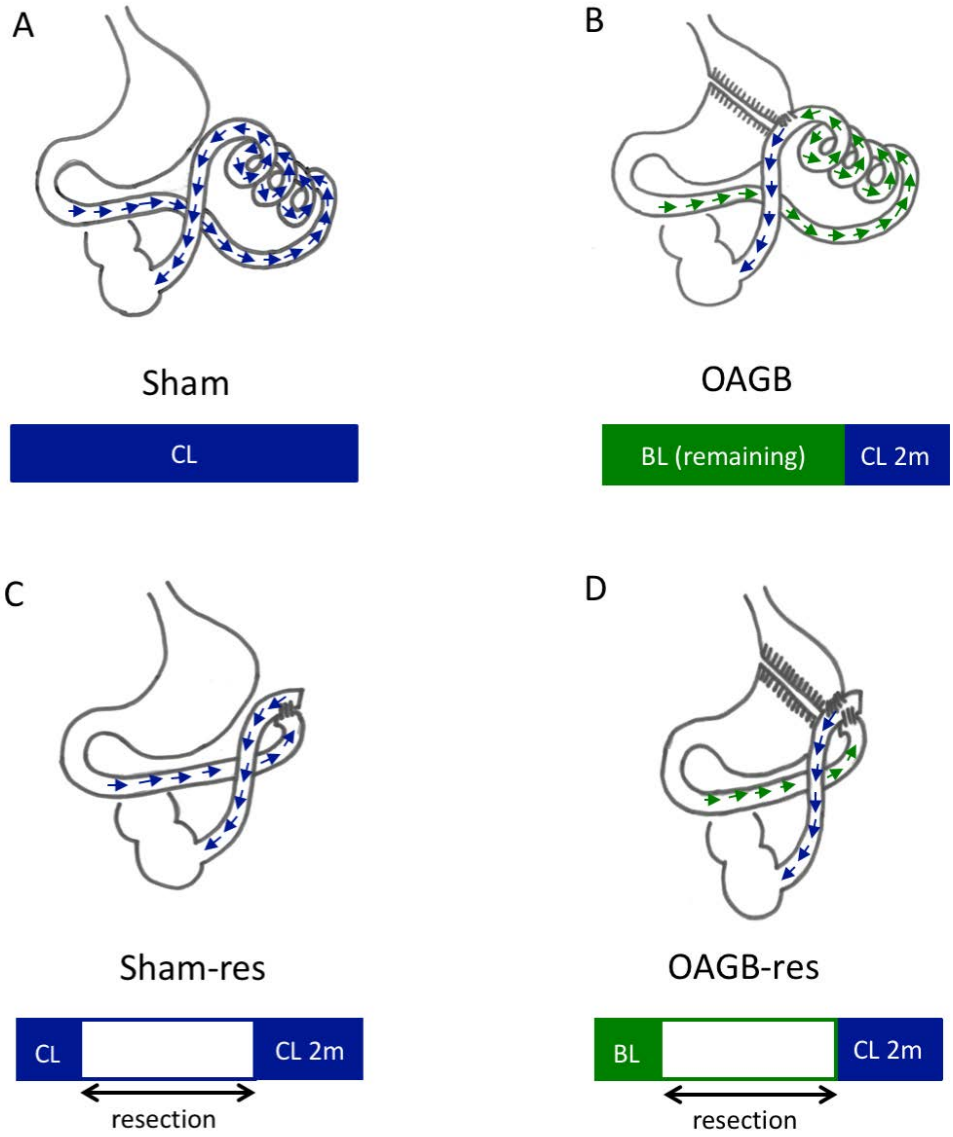


Figure 1.

Title: Graphic representations of the surgical procedures.

Legend: (A) Sham, (B) OAGB, (C) Sham-res,(D) OAGB-res. BL : Biliary limb in green, CL: Common limb in blue, resected biliary limb: white with green lining. Green arrow: transit in the biliary limb (BL), blue arrow: transit in the common limb (CL).

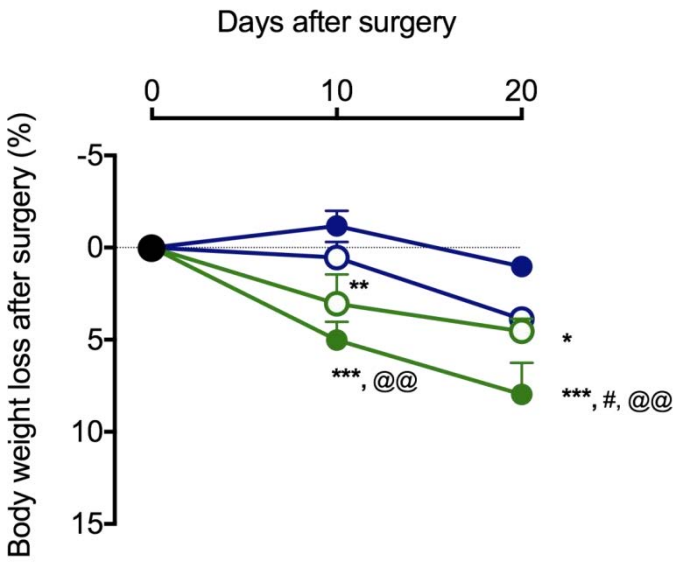


Figure 2.

Title: Body weight loss after the surgical procedures

Legend: Time-course of post-operative body weight expressed as percentage of preoperative body weight. Blue dot: Sham; open blue circle: Sham-res; Green dot: OAGB; open green circle: OAGB-res. Data are expressed as mean+/-SEM. N=6-7/group. Two-way ANOVA, *p<0.05 *vs* Sham, ** p<0.01 *vs* Sham, ***p<0.001 *vs* Sham, @@ p<0.01 *vs* Sham-res, # p<0.05 *vs* OAGB-res.

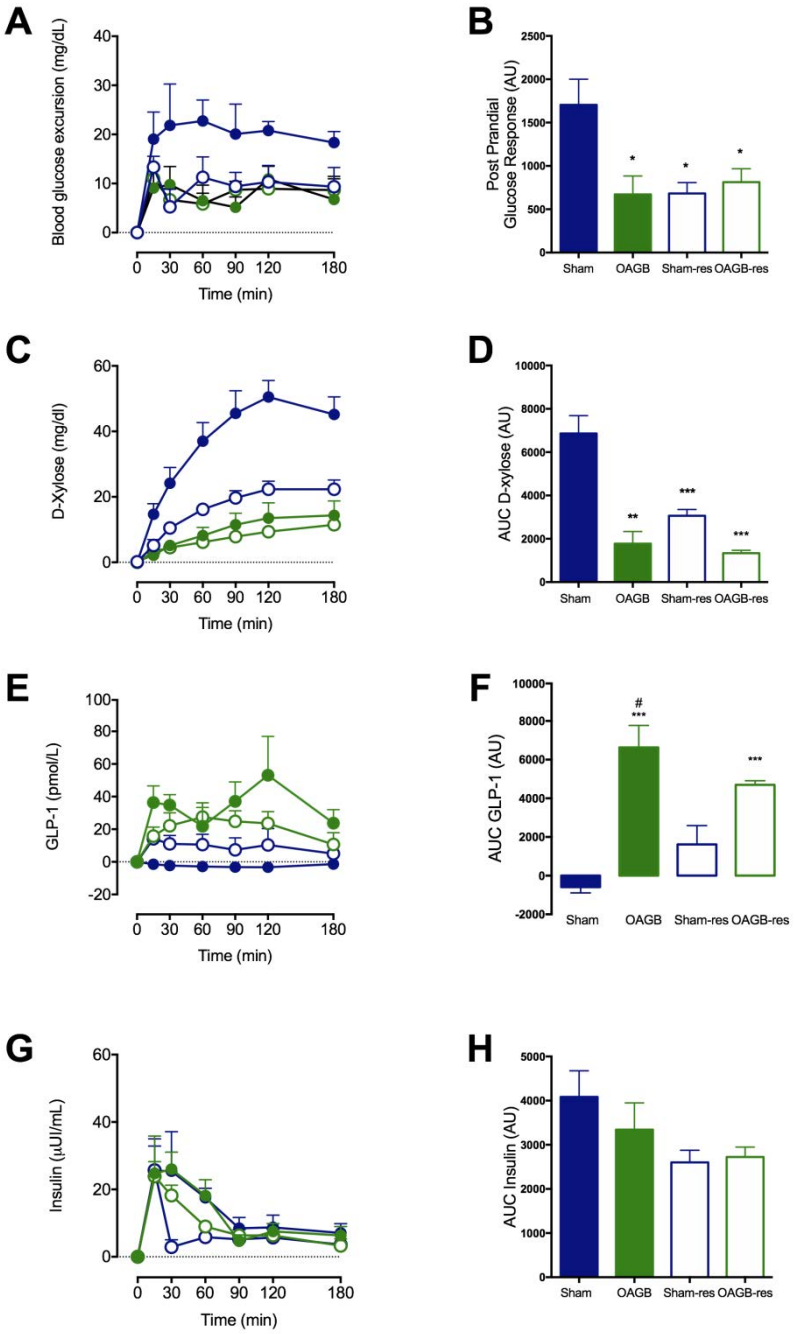


Figure 3.

Title: Metabolic effects of the surgery procedures during the mixed-meal test

Legend: (A) blood glucose excursion, (B) post-prandial glucose response, (C) D-Xylose aborption, (D) D-Xylose excursion curve, (E) plasma GLP-1 concentration, (F) GLP-1 excursion curve, (G) insulinemia, (H) insulinemia excursion curve. Blue dot: Sham; halved blue dot: Sham-res; Green dot: OAGB; halved green dot: OAGB-res. Data are expressed as mean+/-SEM. N=6-8/group. One-way ANOVA, *p<0.05 *vs* Sham, ** p<0.01 *vs* Sham, ***p<0.001 *vs* Sham, # p<0.05 *vs* OAGB-res.

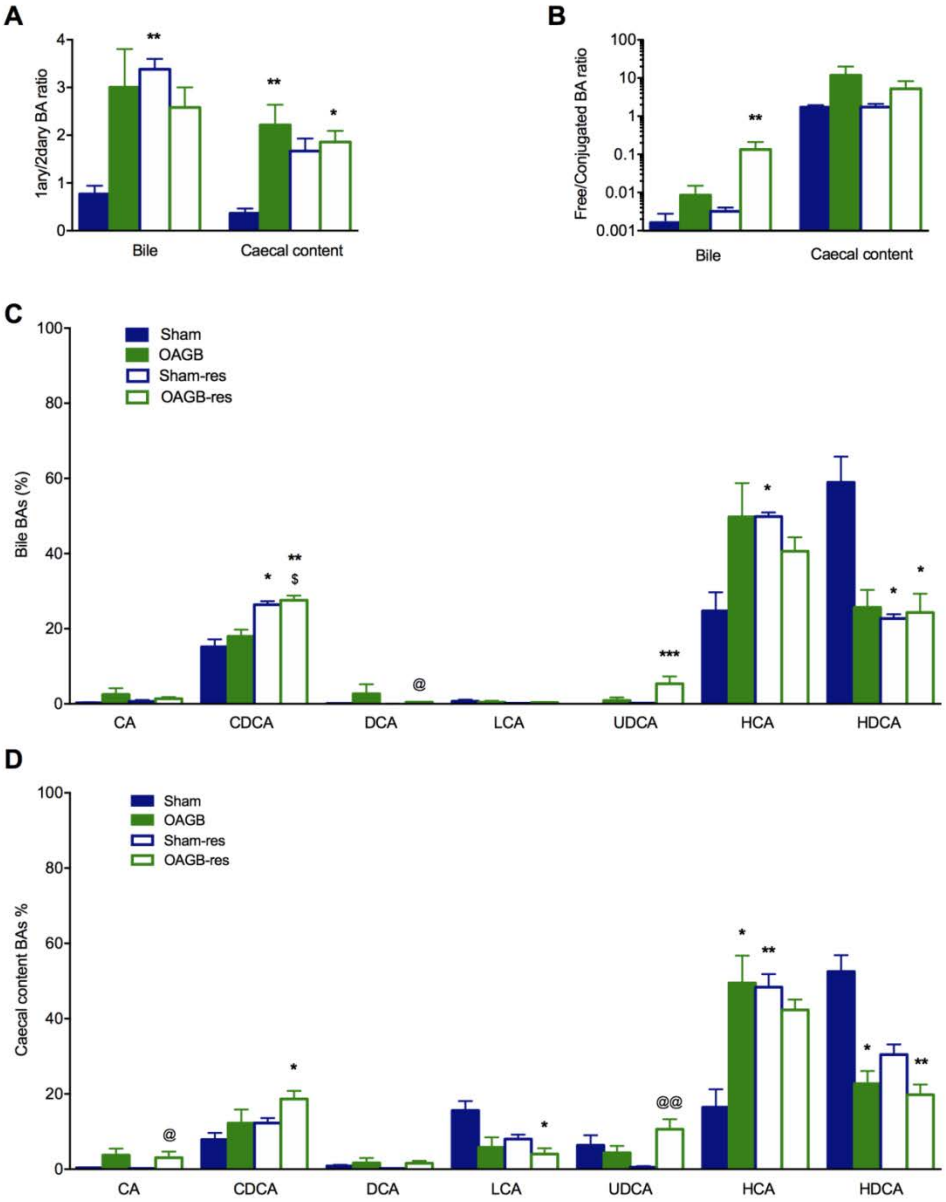
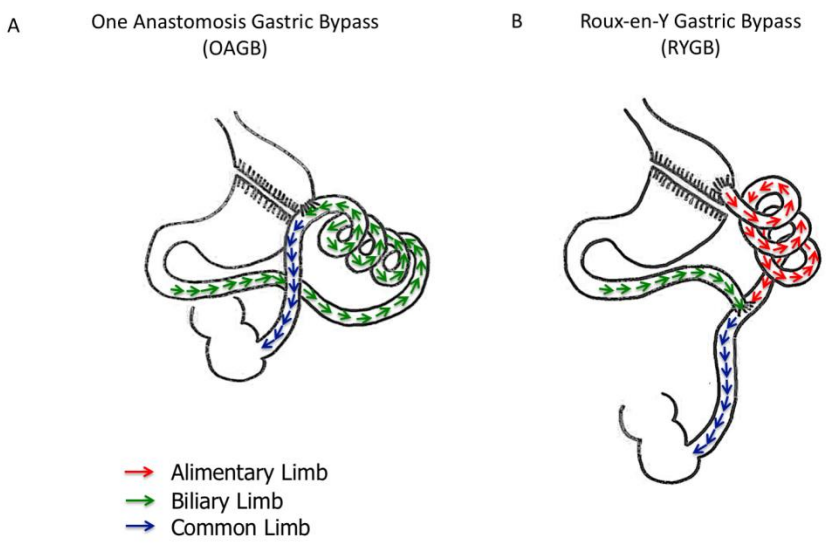


Figure 4

Title: BA profiles in bile and caecal content after the surgical procedures

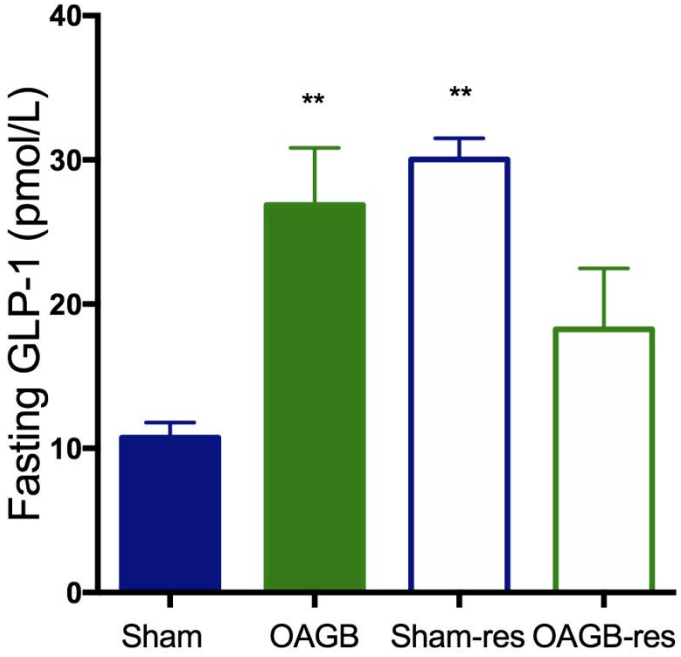
Legend: (A) primary/secondary BA ratio in bile and caecal content, (B) Free/conjugated BA ratio in bile and caecal content, (C) total BA species in bile expressed as percentage of the total BAs, (D) total BA species in caecal content expressed as percentage of the total BAs. Blue: Sham; green: OAGB; blue with white strips: Sham-res; green with white stripes: OAGB-res**.** Data are expressed as mean+/-SEM. N=5-6/group. A-D: Kruskal Wallis test, *p<0.05 *vs* Sham, ** p<0.01 *vs* Sham, @ p<0.05 *vs* Sham-res, @@ p<0.01 *vs* Sham-res, $ p<0.05 *vs* OAGB.



Supplemental Figure 1.

Title: Comparision of surgical procedures

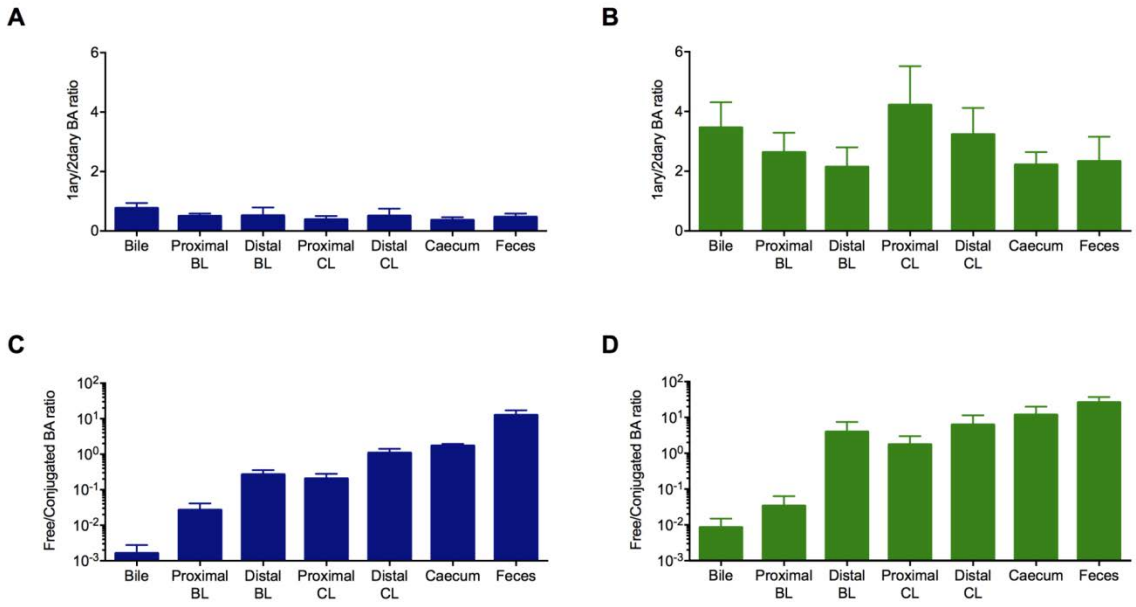
Legend: (A) OAGB, (B) RYGB.



Supplemental Figure 2

Title: Fasting plasma GLP-1 concentrations after the surgical procedures

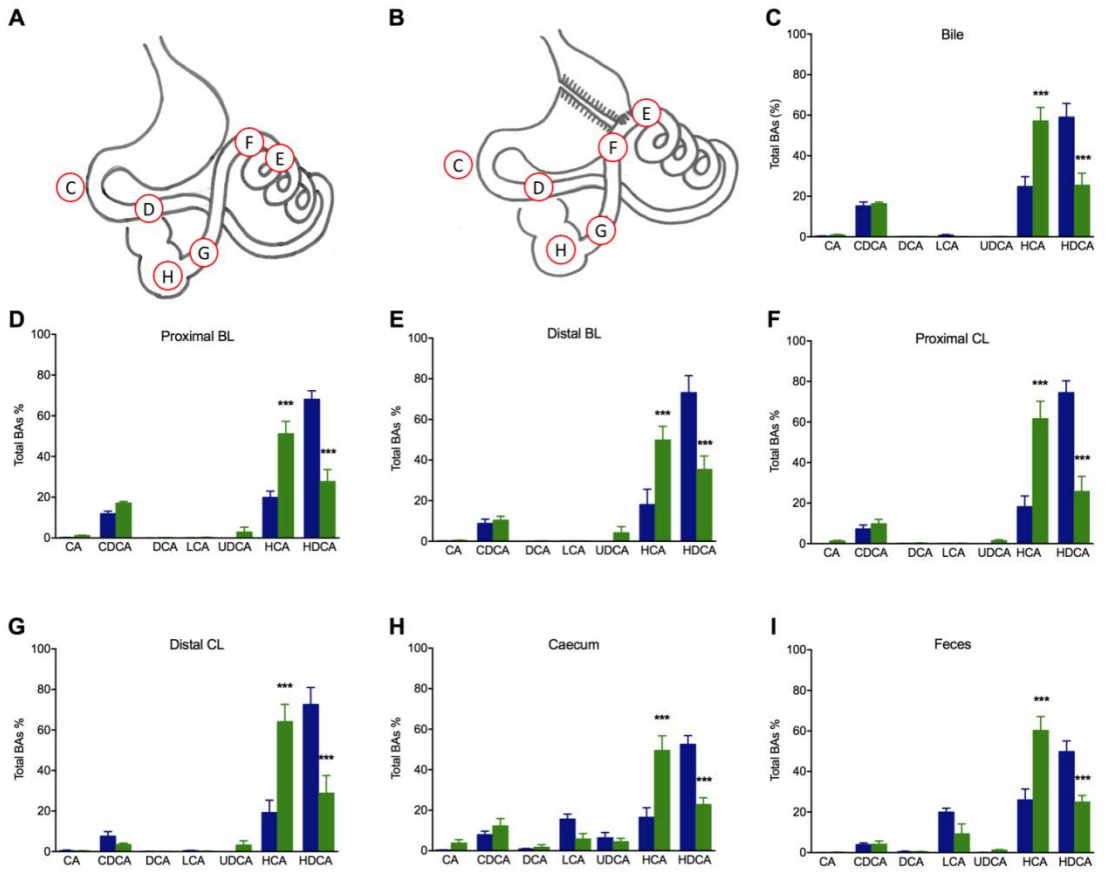
Legend: Fasting plasmatic GLP-1 15 days after surgery. Blue : Sham; green: OAGB; white with blue lining: Sham-res; white with green lining: OAGB-res. Data are expressed as mean+/-SEM. N=4-6/group. One way ANOVA, ** $p<0.01$ *vs* Sham.



Supplemental Figure 3

Title: 1ary/2dary and Free/Conjugated BA ratios along the GI tract upon Sham (blue) or OAGB (green) surgery

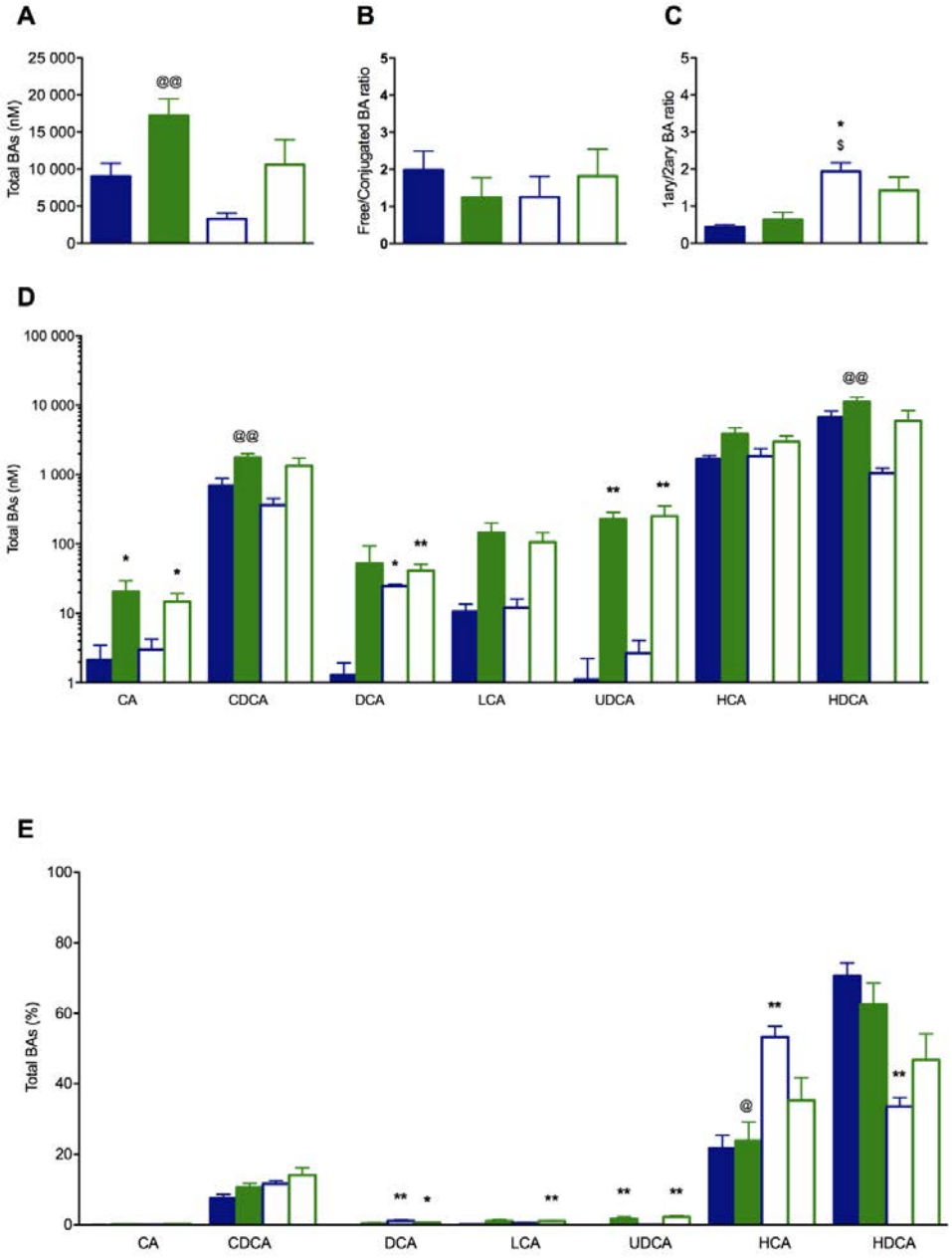
Legend: BL, Biliary limb; CL, common limb. Data are expressed as mean+/-SEM. N=6-8/group.



Supplemental Figure 4

Title: BA species concentrations along the gastro-intestinal tract from bile to feces

Legend: Intestinal intra-luminal sampling location in (A) Sham and (B) OAGB. BA species concentrations in (C) bile, (D) proximal BL, (E) distal BL, (F) proximal CL, (G) distal CL, (H) caecum, (I) feces. Data are expressed as mean+/-SEM. N=4-6/group. One way ANOVA, *** $p<0.001$ *vs* Sham.



Supplemental Figure 5

Title: Plasma BA concentrations after the surgical procedures

Legend: (A) Total BAs, (B) Free/conjugated BA ratio, (C) primary/secondary BA ratio, (D) total BAs species, expressed as concentrations, (E) total BAs species, expressed as pecentages. Blue: Sham; green: OAGB; blue with white strips: Sham-res; green with white stripes: OAGB-res. Data are expressed as mean+/-SEM. N=5-7/group. A-E: Kruskal-Wallis *p<0.05 *vs* Sham, ** p<0.01 *vs* Sham, @ p<0.05 *vs* Sham-res, @@ p<0.01 *vs* Sham-res, $ p<0.05 *vs* OAGB.

MANUSCRIPT NUMBER 8

The Functional Relevance of BAs in the Improvement of HDL-mediated Endothelial Protection After Bariatric Surgery.

Oscar Chávez-Talavera MD*[1], Anne Jomard MSc*[2,3], Petia Doytcheva PhD[4], Amandine Descat MSc[5], Mostafa Kouach PhD[5], Jean-Francois Goossens PhD[5], Marco Büter MD-PhD[6], Amy Taheri MSc[3,4], Christian Wolfrum PhD[2], Arnold von Eckardstein MD[3], Thomas A. Lutz DVM, PhD[4], Frank Ruschitzka[7], Olivier Briand[1], Sophie Lestavel[1], Anne Tailleux PhD[#1], Bart Staels PhD[#1], Elena Osto MD-PhD[#2,3,7]

[1]Université de Lille, Inserm, CHU, Institut Pasteur de Lille, U1011-EGID, F-59000 Lille, France.
[2]Laboratory of Translational Nutrition Biology, IFNH, ETH Zurich, Zurich, Switzerland.
[3]Institute of Clinical Chemistry, University Hospital of Zurich, Zurich, Switzerland.
[4]Institute of Veterinary Physiology, University of Zurich and Zurich Center for Integrative Human Physiology (ZIHP), Zurich, Switzerland.
[5]Plateau de Spectrométrie de Masse, PSM-GRITA EA 7365, Faculté de Pharmacie, F-59000 Lille, France.
[6]Department of Surgery &Transplantation, University Hospital of Zurich, Zurich, Switzerland.
[7]Heart Center Zurich, University Hospital of Zurich, Zurich, Switzerland.

* Co-first authors
[#] Co-last authors

ABSTRACT

Aim (background & objectives): Roux-en-Y gastric bypass (RYGB) reduces cardiovascular mortality and improves high density lipoprotein (HDL)-mediated vaso-protection. Bile acids (BAs) are signaling molecules increasingly recognized as regulators of cardio-metabolic homeostasis. BAs circulate in blood bound mainly to albumin and HDL. The signaling role of HDL-bound BAs (HDL-BAs) is unknown. We hypothesize that HDL could facilitate BA delivery to endothelial cells to regulate vaso-protective properties. Circulating BAs systematically increase upon RYGB, and are candidates to contribute to the early, weight-loss independent metabolic improvements after surgery. We aimed to study whether RYGB changes the composition of HDL-BAs and their contribution to the RYGB-mediated improvement of HDL vaso-protective properties.

Method: HDL were isolated from 25 morbidly obese patients before and 1 year after RYGB. The HDL-BA composition was determined by LC/MS-MS and the HDL vaso-protective properties were evaluated *ex-vivo* in human aortic endothelial cells (HAEC). The size of HDL particles were determined by NMR spectroscopy in plasma.

Results: Interestingly, the increase in plasma total BA concentrations observed 1 year after RYGB also translated in HDL-BAs. Obesity-induced HDL dysfunction (HDL-mediated endothelial NO production, anti-apoptotic effects, cholesterol efflux capacity, VCAM-1 expression and PON-1 activity) were reversed post-operatively. RYGB changed HDL-bound BAs, agonists of the nuclear farnesoid X receptor (FXR) (cholic acid (CA) and chenodeoxy-CA (CDCA)), or TGR5 (deoxy-CA (DCA)). The specific enrichment of HDL-CA correlated with improved HDL's endothelial anti-apoptotic capacity ($p=0.006$), and there was a strong trend for HDL-CDCA ($p=0.07$). This suggests that an interaction between endothelial cells and BAs may underlie the improved HDL functionality after RYGB. Aditionally, RYGB increased HDL size, that significantly correlated with total HDL-CDCA ($p<0.018$).

Conclusions: RYGB improves the vaso-protective function of HDL and increases HDL-BAs. The results suggest that HDL-BAs could contribute to the improved endothelial-protective effects of HDL *via* endothelial activation of FXR and TGR5.

INTRODUCTION

Obesity is a global health concern, associated to several co-morbidities, including cardio-vascular (CV) disease and Type 2 Diabetes Mellitus (T2DM) (Flegal *et al.* 2013). An early hallmark of CV disease is dysfunction of endothelial cells (EC), widely considered the gatekeepers of vascular function (Anderson 1999). Roux-en-Y Gastric Bypass (RYGB) is currently the gold-standard for obesity treatment, not only able to reduce bodyweight, but also to improve co-morbidities, such as T2DM and to reduce CV morbidity (Romeo *et al.* 2012; Sjöström *et al.* 2012) and mortality (Sjöström *et al.* 2007; Lent *et al.* 2017) compared to non-surgical body weight loss strategies. We showed that endothelial dysfunction and endothelial protective properties of high-density lipoprotein (HDL) were improved early after RYGB in a body-weight independent manner in part *via* Glucagon-like peptide-1 (GLP-1) mediated pathways (Osto *et al.* 2015). HDL are complex particles that carry a variety of different lipids, enzymes and proteins, which all have a potential signalling role. In recent years, research has been focusing on characterizing HDL structure and molecular composition to better understand what the determinants of HDL's vaso-protective function are. It has been established that pathological states can alter HDL structure and composition, causing it to become a pro-inflammatory, pro-apoptotic particle, and thus contributing to dysfunction at the level of the EC (Lüscher *et al.* 2014; Besler *et al.* 2011). We and others have shown that metabolic intervention, such as diet (Tabet *et al.* 2016), exercise (Adams *et al.* 2013) and bariatric surgery (Osto *et al.* 2015), can restore HDL function. HDL particles can be divided into several subclasses based on shape and size (Kontush *et al.* 2015), with each subclass having a particular functional profile. Moreover, several studies have identified specific HDL components that can further influence functionality, including sphingosine-1-phosphate content (Sattler *et al.* 2015), phospholipid content (Yancey *et al.* 2000), and apolipoprotein subtype (Wolfrum, Poy, and Stoffel 2005; Ruiz, Okada, and Dahlbäck 2017). However, the specific components of HDL owing to restored functionality post-RYGB have not been fully identified (Osto *et al.* 2015). Among different candidates, bile acids (BAs) could be implicated in the functional improvements of HDL after RYGB.

Total plasma BAs are significantly increased in the systemic circulation post-RYGB (Spinelli *et al.* 2016; Chávez-Talavera *et al.* 2017), and their functional relevance after bariatric surgery is under intense investigation. Moreover, BA circulate bound to carrier proteins, with up to 10% being bound to lipoproteins (HDL, LDL, VLDL), with a preferential association to the HDL fraction (Steiner *et al.* 2012). HDL particles are known to interact with EC, and functionally induce nitric oxide (NO) production or inhibit apoptosis, among other protective properties (Besler *et al.* 2011). It is therefore conceivable that BA bound to HDL may have an additional or a synergistic signalling role.

BAs are signalling molecules, regulating their own synthesis from cholesterol in the liver, glucose and lipid metabolism. Among other functions, BAs have already been characterized to have beneficial effects in the vascular system (Porez *et al.* 2012; Chávez-Talavera *et al.* 2017). Farnesoid-X-receptor (FXR) activation, a bile acid nuclear receptor, has been shown to improve EC function through increased transcription of endothelial nitric oxide synthase (eNOS) (J. Li *et al.* 2008). Further, Takeda G-protein coupled receptor 5 (TGR5) activation, has been shown to favour vasorelaxation by inhibiting the vasoconstrictor endothelin-1 (Renga *et al.* 2010), and through increase in H_2S-producing enzyme cystathionine γ-lyase (Renga *et al.* 2015). The aim of this study is to investigate whether HDL-BAs change upon RYGB and whether such changes contribute to the improvement of HDL endothelial properties in humans.

METHODS

Study population

Studies were performed according to the principles of the Declaration of Helsinki. The Local Research and Ethics committee approved the study (KEK-ZH 2013 0260), and all patients gave written consent. The surgery group consisted of 25 patients undergoing primary laparoscopic proximal RYGB surgery. The RYGB procedure was performed as described in Weber *et al.* 2004. All patients underwent a clinical, biochemical and pre-operative evaluation, and all patients adhered to the study protocol and completed follow-up. Patients with unstable medical conditions such as recent coronary syndromes (within six months), congestive heart failure, systemic infection, acute illness, malignancy, or pregnancy, substance abuse, more than three alcoholic drinks per day, or psychiatric illness were excluded.

Blood sampling

Blood samples were obtained from fasting patients before RYGB and 1-year post RYGB surgery. Serum was collected in non-additive vacutainers, and plasma was collected in BD P800 vacutainers (containing DPPIV and protease inhibitors) that were kept at +4°C before and after blood collection.

Biochemical analysis

Plasma lipid profiles (total cholesterol, HDL-cholesterol and triglycerides) and fasting glycemia were measured in-house at the Institute for Clinical Chemistry, University Hospital Zürich on a Roche cobas® Integra 8000 device. LDL-cholesterol was estimated using the

Friedewald formula. Insulin and GLP-1 measurement were performed using a customized human duplex insulin/active GLP-1 Meso Scale Discovery 96-well plates following the provided protocol (Meso Scale Discovery, Gaithersburg. MD, USA).

HDL isolation

HDL was isolated from fresh, fasting plasma by density gradient ultracentrifugation (HDL density 1.063 to 1.21 g/cm^3), as previsouly described (Riwanto *et al.* 2013 ; Besler *et al.* 2011). Potassium bromide (Merck KGaA, Darmstadt, Germany) was used to adjust the density. Purity of HDL was assessed by SDS-PAGE and subsequent Coomassie Blue staining of the gel.

Cell Culture

Human aortic endothelial cells (HAEC) were obtained from (Lonza, Basel, Switzerland) and cultured in endothelial cell basal medium-2 (Lonza) supplemented with endothelial growth medium–SingleQuots as indicated by the manufacturer (37°C, 95% O_2, 5% CO_2). HAECs were grown to sub-confluency and rendered quiescent before experiments by incubation in medium containing 0.5% fetal bovine serum. For cholesterol efflux experiments, the murine macrophage cell line J774 was cultured on 75-cm^2 flasks, in 10% FBS, 4.5g/l glucose RPMI medium 1640 (GIBCO, Life Technologies, Grand Island, NY, USA).

Endothelial-protective properties of HDL

Endothelial-protective properties of HDL were performed as described in (Osto *et al.* 2015). Briefly, HDL-stimulated (50µg/mL) NO production from HAECs was tested *in-vitro* with the 4,5-diaminofluorescein (DAF-2, Sigma 205391-02) fluorescent probe. HDL (100µg/mL) anti-apoptotic properties were assessed in HAECs using a commercial kit (Roche Biochemical Cell Death Determination ELISA^{PLUS}). Arylesterase activity of HDL-associated paraoxonase-1 (PON-1) was measured in whole serum by ultraviolet spectrophotometry. Endothelial protein expression of vascular cell adhesion molecule-1 (VCAM-1), anti-human VCAM-1 antibody BBA19 R&D System, was determined in tumor necrosis factor- (TNF-) (100pg/mL) stimulated HAECs treated with isolated HDL (100µg/mL) using in-cell Western blot. NADPH oxidase activity in HAECs treated with isolated HDL (50µg/mL) was measured using a commercially available kit (Abcam, ab65349). Cholesterol efflux capacity of HDL from J774 macrophage-like cells was measured by determining ^{3}H cholesterol extracellular to intracellular ratio after stimulation with 2.8% apolipoprotein B depleted serum.

NMR analysis

Nuclear magnetic resonance spectroscopy (NMR) was used to determine lipoprotein subclass and abundance using a commercially available service (*AXINON® lipoFIT®-S100*) in 1 mL of human serum.

BA determination in plasma and in HDL

BAs were extracted from plasma or HDL after protein precipitation with iced methanol. Twenty-one BA species were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described previously (Spinelli *et al.* 2016), using 5 deutered BAs (D4-CA, D4-GCA, D4-TCA, D4-CDCA, D4-GCDCA) as internal standard for quantification by high-performance liquid chromatography (UFLC-XR device; Shimadzu, Kyoto, Japan) coupled to tandem mass spectrometry (MS/MS) (QTRAP 5500 hybrid system, equipped with a Turbo VTM ion source; Sciex, Foster City, CA, USA). Plasma BA concentrations were expressed in concentrations (nM), whereas HDL-BAs were transformed to ng/L by multiplying the concentration times the molecular weight, and then normalized to the amount of proteins in the HDL solution from which BAs were extracted. The HDL-BAs are therefore expressed as BA(ng)/HDLprot(g).

Statistical analysis.

Data distribution was evaluated with D'Agostino & Pearson omnibus normality tests. Quantitative variables are presented as Median [inter quartile range (IQR)] due to the data non normal distribution, with appropriate non-parametric paired comparison tests (Wilcoxon rank-sum test). The Spearman's R correlation coefficients were calculated to evaluate the association between two quantitative variables. A p value <0.05 was considered statistically significant. The analyses were performed using R software version 3.5.1 (The R Foundation for Statistical Computing, R Core Team, Vienna, 2018), with the RStudio interface or with Prism 6 for Windows (GraphPad La Jolla, CA, USA).

RESULTS

RYGB decreased BMI, improved glucose homeostasis, lipid profiles and vaso-protective properties of isolated HDL 1 year after surgery.

All patients were morbidly obese as indication for the bariatric surgery. As expected, RYGB significantly improved the BMI, glucose metabolism (fasting GLP-1, FPG, insulinaemia and HOMA-2R), lipid profiles (total cholesterol, LDL-C, HDL-C, plasma triglycerides (TG)) of the patients, and vaso-protective properties of the isolated HDL, as shown in **Table 1**.

RYGB increases total plasma BAs and HDL-BAs

As expected, total plasma BAs concentrations increase upon RYGB (**Figure 1a**). Interestingly Total HDL-BAs also increased (**Figure 1b**). The RYGB-induced change in plasma Total BAs positively correlated with the changes in Total HDL-BAs (**Figure 1c**). This correlation is due to Total Free (**Figure 1d**), Total Glyco (**Figure 1e**) and Total Tauro (**Figure 1f**) BA species, suggesting that RYGB changes similarly BAs in plasma and those bound to HDL.

The increased content of CA in HDL is negatively correlated with apoptosis of HDL-treated HAEC cells.

In order to analyze whether the changes in HDL-BAs and the improvement of the HDL vaso-protective properties induced by RYGB could be associated, correlations between HDL-BAs and the different endothelial properties of HDL were analyzed. The improvement in the HDL anti-apoptotic properties has a significant, negative correlation with the RYGB-induced increase in Total HDL-Free BAs and Total HDL-CA (**Table 2, Supplemental Figure 1**). Whereas a similar marginal correlation was found with Total HDL-CDCA. None of the other RYGB-improvements in endothelial vaso-protective properties of HDL were correlated with the RYGB-induced increase in HDL-BA species.

RYGB increases HDL size, and the increase is correlated with the RYGB-induced increase in CDCA but not with the vaso-protective properties of HDL.

RYGB significantly increased the size of HDL (**Figure 2**), this increase was correlated with the increase in the HDL content of conjugated but not free CDCA (**Table 3).** Neither the other HDL-BAs nor the improvement in vaso-protective properties of HDL were correlated with the change in size of HDL.

DISCUSSION

We have shown that RYGB simultaneously improves vaso-protective properties of HDL (Osto *et al.* 2015), decreases cardiovascular mortality (Sjöström *et al.* 2007; Lent *et al.* 2017) and qualitatively / quantitatively changes plasma BAs. We studied the relationship between these factors, and show for the first time that HDL-BAs increase upon RYGB, and that HDL-BAs reflect circulating BAs in the lipoprotein-depleted fraction. The mechanisms of HDL-BA enrichment is still unknown, and how this enrichment may contribute to function.

Interestingly, the specific increase in HDL-CA is correlated with the improvement of the anti-apoptotic properties of HDL after RYGB, suggesting that HDL-CA could be involved in one of these improvements. It should be taken into account that patients undergoing RYGB have both altered BAs concentrations (Cariou *et al.* 2011; Prinz *et al.* 2015), and that these BA alterations could contribute to the loss of endothelial vaso-protection of HDL in the context of obesity.

Interestingly, we have shown that HDL size increases upon RYGB, and that this increase is correlated with the increased content of conjugated CDCA in HDL, suggesting that it could be responsible for the increased size upon RYGB. However, the increased size of HDL did not correlate with their vaso-protective properties *in vitro*.

The mechanism by which HDL-bound BAs could signal on the endothelial cell has never been explored. BAs circulate in the bloodstream bound to plasma components of the lipoprotein-depleted fraction, and the albumin exhibits the highest affinity to bind them (Rudman and Kendall 1957; Ceryak, Bouscarel, and Fromm 1993). However, BAs are also transported by lipoproteins (KRAMER *et al.* 1979; Steiner *et al.* 2012). Indeed, total BAs, transported by VLDL+LDL and HDL, represent 5-10% of the total plasma BA pool in physiological conditions (Buscher *et al.* 1987; Steiner *et al.* 2012; Hedenborg, Norman, and Ritzén 1988). Whether the BAs bound to HDL have a signaling activity on the endothelium and how they gain access to their receptors in the endothelial cells is still an open question.

To exert their signaling activity, BAs would require binding to their receptors. In the case of TGR5, BA binding could occur only by simple proximity to the cell membrane. Contrarily, in the case of the nuclear receptor FXR, BA internalization is required. Free BAs passively diffuse inside the cell but it is unknown how conjugated and more hydrophilic BAs are delivered to FXR in tissues that don't express the known BA transporters for internalization.

Additionally, the endothelial cells present within the entero-hepatic cycle are exposed to micromolar BA concentrations, allowing them to activate FXR and TGR5. However, nanomolar BA concentrations in the systemic circulation, to which peripheral endothelial cells are exposed, are below the EC_{50} of FXR and TGR5 (T. Li and Chiang 2014), and their binding to the receptor probably needs to be facilitated. In contrast to the albumin, the lipoproteins are internalized by most tissues, therefore lipoproteins could contribute to carry and deliver BA directly to their target extrahepatic cells and tissues and participate to the activation of FXR and TGR5.

Given that TGR5 activation does not require the internalization of its ligands, we speculate that HDL binding to their receptors could approach BAs to TGR5 facilitating its activation.

Another possibility is that BAs could dissociate from HDL once the HDL is docked on the cell membrane and then be either passively transported across the plasma membrane (for lipophilic free BAs) or be transferred to their transporters.

We also speculate that HDL could serve as a shuttle for BA entrance, either *via* holoparticle endocytosis of HDL or by facilitating BA transport across the membrane by unknown transporters (e.g. SR-B1, which could participate to the BA uptake as it promotes selective cholesteryl ester uptake from HDL). Moreover, similar to cholesterol, BAs could also be effluxed from the cells by the HDL. Overall, we speculate that the HDL could be an important mediator for transport and potentially reverse transport of BAs, and as such, participate in BA signaling.

CONCLUSIONS

The RYGB-induced increased content of CA in HDL is correlated with the improvement of HDL anti-apoptotic properties upon RYGB, suggesting that BAs bound to the lipoproteins could have a signaling role and participate in HDL functionality in endothelial cells. Our new results warrant further study exploring the signaling role of BAs bound to HDL in peripheral tissues and especially in the endothelium.

REFERENCES

Adams, Volker, Christian Besler, Tina Fischer, Meliana Riwanto, Friederike Noack, Robert Höllriegel, Andreas Oberbach, *et al.* 2013. “Exercise Training in Patients with Chronic Heart Failure Promotes Restoration of High-Density Lipoprotein Functional Properties.” *Circulation Research* 113 (12): 1345–55. https://doi.org/10.1161/CIRCRESAHA.113.301684.

Anderson, T. J. 1999. “Assessment and Treatment of Endothelial Dysfunction in Humans.” *Journal of the American College of Cardiology* 34 (3): 631–38.

Besler, Christian, Kathrin Heinrich, Lucia Rohrer, Carola Doerries, Meliana Riwanto, Diana M. Shih, Angeliki Chroni, *et al.* 2011. “Mechanisms Underlying Adverse Effects of HDL on ENOS-Activating Pathways in Patients with Coronary Artery Disease.” *The Journal of Clinical Investigation* 121 (7): 2693–2708. https://doi.org/10.1172/JCI42946.

Buscher, Hans-Peter, Mathias Beger, Hella Sauerbier, and Wolfgang Gerok. 1987. “Bile Salt Shift from Albumin to High-Density Lipoprotein in Cholestasis.” *Hepatology* 7 (5): 900–905.

Cariou, Bertrand, Maud Chetiveaux, Yassine Zaïr, Etienne Pouteau, Emmanuel Disse, Béatrice Guyomarc'h-Delasalle, Martine Laville, and Michel Krempf. 2011. "Fasting Plasma Chenodeoxycholic Acid and Cholic Acid Concentrations Are Inversely Correlated with Insulin Sensitivity in Adults." *Nutrition & Metabolism* 8 (1): 48. https://doi.org/10.1186/1743-7075-8-48.

Ceryak, Susan, Bernard Bouscarel, and Hans Fromm. 1993. "Comparative Binding of Bile Acids to Serum Lipoproteins and Albumin." *Journal of Lipid Research* 34 (10): 1661–1674.

Chávez-Talavera, Oscar, Anne Tailleux, Philippe Lefebvre, and Bart Staels. 2017. "Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia and NAFLD." *Gastroenterology*, February. https://doi.org/10.1053/j.gastro.2017.01.055.

Flegal, Katherine M., Brian K. Kit, Heather Orpana, and Barry I. Graubard. 2013. "Association of All-Cause Mortality with Overweight and Obesity Using Standard Body Mass Index Categories: A Systematic Review and Meta-Analysis." *JAMA* 309 (1): 71–82. https://doi.org/10.1001/jama.2012.113905.

Hedenborg, G., A. Norman, and A. Ritzén. 1988. "Lipoprotein-Bound Bile Acids in Serum from Healthy Men, Postprandially and during Fasting." *Scandinavian Journal of Clinical and Laboratory Investigation* 48 (3): 241–45.

Kontush, Anatol, Mats Lindahl, Marie Lhomme, Laura Calabresi, M. John Chapman, and W. Sean Davidson. 2015. "Structure of HDL: Particle Subclasses and Molecular Components." *Handbook of Experimental Pharmacology* 224: 3–51. https://doi.org/10.1007/978-3-319-09665-0_1.

Kramer, Werner, Hans-Peter Buscher, Wolfgang Gerok, and Gerhart Kurz. 1979. "Bile Salt Binding to Serum Components." *European Journal of Biochemistry* 102 (1): 1–9.

Lent, Michelle R., Peter N. Benotti, Tooraj Mirshahi, Glenn S. Gerhard, William E. Strodel, Anthony T. Petrick, Jon D. Gabrielsen, *et al.* 2017. "All-Cause and Specific-Cause Mortality Risk After Roux-En-Y Gastric Bypass in Patients With and Without Diabetes." *Diabetes Care* 40 (10): 1379–85. https://doi.org/10.2337/dc17-0519.

Li, Jiang, Annette Wilson, Ramalinga Kuruba, Qiuhong Zhang, Xiang Gao, Fengtian He, Li-Ming Zhang, Bruce R. Pitt, Wen Xie, and Song Li. 2008. "FXR-Mediated Regulation of ENOS Expression in Vascular Endothelial Cells." *Cardiovascular Research* 77 (1): 169–77. https://doi.org/10.1093/cvr/cvm016.

Li, Tiangang, and John Y. L. Chiang. 2014. "Bile Acid Signaling in Metabolic Disease and Drug Therapy." *Pharmacological Reviews* 66 (4): 948–83. https://doi.org/10.1124/pr.113.008201.

Lüscher, Thomas F., Ulf Landmesser, Arnold von Eckardstein, and Alan M. Fogelman. 2014. "High-Density Lipoprotein: Vascular Protective Effects, Dysfunction, and Potential as Therapeutic Target." *Circulation Research* 114 (1): 171–82. https://doi.org/10.1161/CIRCRESAHA.114.300935.

Osto, Elena, Petia Doytcheva, Caroline Corteville, Marco Bueter, Claudia Dörig, Simona Stivala, Helena Buhmann, *et al.* 2015. "Rapid and Body Weight-Independent Improvement of Endothelial and High-Density Lipoprotein Function after Roux-En-Y Gastric Bypass: Role of Glucagon-like Peptide-1." *Circulation* 131 (10): 871–81. https://doi.org/10.1161/CIRCULATIONAHA.114.011791.

Porez, Geoffrey, Janne Prawitt, Barbara Gross, and Bart Staels. 2012. "Bile Acid Receptors as Targets for the Treatment of Dyslipidemia and Cardiovascular Disease." *Journal of Lipid Research* 53 (9): 1723–37. https://doi.org/10.1194/jlr.R024794.

Prinz, Philip, Tobias Hofmann, Anne Ahnis, Ulf Elbelt, Miriam Goebel-Stengel, Burghard F. Klapp, Matthias Rose, and Andreas Stengel. 2015. "Plasma Bile Acids Show a Positive Correlation with Body Mass Index and Are Negatively Associated with Cognitive Restraint of Eating in Obese Patients." *Frontiers in Neuroscience* 9: 199. https://doi.org/10.3389/fnins.2015.00199.

Renga, Barbara, Mariarosaria Bucci, Sabrina Cipriani, Adriana Carino, Maria Chiara Monti, Angela Zampella, Antonella Gargiulo, Roberta d'Emmanuele di Villa Bianca, Eleonora Distrutti, and Stefano Fiorucci. 2015. "Cystathionine γ-Lyase, a H2S-Generating Enzyme, Is a GPBAR1-Regulated Gene and Contributes to Vasodilation Caused by Secondary Bile Acids." *American Journal of Physiology. Heart and Circulatory Physiology* 309 (1): H114-126. https://doi.org/10.1152/ajpheart.00087.2015.

Renga, Barbara, Andrea Mencarelli, Piero Vavassori, Vincenzo Brancaleone, and Stefano Fiorucci. 2010. "The Bile Acid Sensor FXR Regulates Insulin Transcription and Secretion." *Biochimica Et Biophysica Acta* 1802 (3): 363–72. https://doi.org/10.1016/j.bbadis.2010.01.002.

Romeo, Stefano, Cristina Maglio, Maria Antonella Burza, Carlo Pirazzi, Kajsa Sjöholm, Peter Jacobson, Per-Arne Svensson, Markku Peltonen, Lars Sjöström, and Lena M. S. Carlsson. 2012. "Cardiovascular Events after Bariatric Surgery in Obese Subjects with Type 2 Diabetes." *Diabetes Care* 35 (12): 2613–17. https://doi.org/10.2337/dc12-0193.

Rudman, Daniel, and Forrest E. Kendall. 1957. "Bile Acid Content of Human Serum. II. The Binding of Cholanic Acids by Human Plasma Proteins1." *Journal of Clinical Investigation* 36 (4): 538–42. https://doi.org/10.1172/JCI103451.

Ruiz, Mario, Hiromi Okada, and Björn Dahlbäck. 2017. "HDL-Associated ApoM Is Anti-Apoptotic by Delivering Sphingosine 1-Phosphate to S1P1 & S1P3 Receptors on

Vascular Endothelium." *Lipids in Health and Disease* 16 (1): 36. https://doi.org/10.1186/s12944-017-0429-2.

Sattler, Katherine, Markus Gräler, Petra Keul, Sarah Weske, Christina-Maria Reimann, Helena Jindrová, Petra Kleinbongard, *et al.* 2015. "Defects of High-Density Lipoproteins in Coronary Artery Disease Caused by Low Sphingosine-1-Phosphate Content: Correction by Sphingosine-1-Phosphate-Loading." *Journal of the American College of Cardiology* 66 (13): 1470–85. https://doi.org/10.1016/j.jacc.2015.07.057.

Sjöström, Lars, Kristina Narbro, C. David Sjöström, Kristjan Karason, Bo Larsson, Hans Wedel, Ted Lystig, *et al.* 2007. "Effects of Bariatric Surgery on Mortality in Swedish Obese Subjects." *The New England Journal of Medicine* 357 (8): 741–52. https://doi.org/10.1056/NEJMoa066254.

Sjöström, Lars, Markku Peltonen, Peter Jacobson, C. David Sjöström, Kristjan Karason, Hans Wedel, Sofie Ahlin, *et al.* 2012. "Bariatric Surgery and Long-Term Cardiovascular Events." *JAMA* 307 (1): 56–65. https://doi.org/10.1001/jama.2011.1914.

Spinelli, V., F. Lalloyer, G. Baud, E. Osto, M. Kouach, M. Daoudi, E. Vallez, *et al.* 2016. "Influence of Roux-En-Y Gastric Bypass on Plasma Bile Acid Profiles: A Comparative Study between Rats, Pigs and Humans." *International Journal of Obesity (2005)* 40 (8): 1260–67. https://doi.org/10.1038/ijo.2016.46.

Steiner, Carine, Adriaan G. Holleboom, Ratna Karuna, Mohammad M. Motazacker, Jan Albert Kuivenhoven, Ruth Frikke-Schmidt, Anne Tybjaerg-Hansen, Lucia Rohrer, Katharina M. Rentsch, and Arnold von Eckardstein. 2012. "Lipoprotein Distribution and Serum Concentrations of 7α-Hydroxy-4-Cholesten-3-One and Bile Acids: Effects of Monogenic Disturbances in High-Density Lipoprotein Metabolism." *Clinical Science* 122 (8): 385–400. https://doi.org/10.1042/CS20110482.

Tabet, Fatiha, Luisa F. Cuesta Torres, Kwok Leung Ong, Sudichhya Shrestha, Sébastien A. Choteau, Philip J. Barter, Peter Clifton, and Kerry-Anne Rye. 2016. "High-Density Lipoprotein-Associated MiR-223 Is Altered after Diet-Induced Weight Loss in Overweight and Obese Males." *PloS One* 11 (3): e0151061. https://doi.org/10.1371/journal.pone.0151061.

Wolfrum, Christian, Matthew N. Poy, and Markus Stoffel. 2005. "Apolipoprotein M Is Required for Prebeta-HDL Formation and Cholesterol Efflux to HDL and Protects against Atherosclerosis." *Nature Medicine* 11 (4): 418–22. https://doi.org/10.1038/nm1211.

Yancey, P. G., M. de la Llera-Moya, S. Swarnakar, P. Monzo, S. M. Klein, M. A. Connelly, W. J. Johnson, D. L. Williams, and G. H. Rothblat. 2000. "High Density Lipoprotein Phospholipid Composition Is a Major Determinant of the Bi-Directional Flux and Net

Movement of Cellular Free Cholesterol Mediated by Scavenger Receptor BI." *The Journal of Biological Chemistry* 275 (47): 36596–604. https://doi.org/10.1074/jbc.M006924200.

n=25	Before		After		p value
Age (years)	45.1 [45.6 - 51.2]				
Sex (women/men)	16/9				
BMI	44.2 [40.2 - 48.2]	25/25	30.6 [25.9 - 34.4]	23/25	< 0,0001
Glucose metabolism					
GLP-1 (pg/mL)	0.6 [0.2 - 0.8]	25/25	2.2 [1.2 - 6.8]	25/25	< 0,0001
Insulin (U/mL)	16.1 [8.8 - 24.8]	25/25	214.7	25/25	< 0,0001
HOMA2-IR	2.2 [1.2 - 3.3]	25/25	3.9 [2.5 - 5.4]	23/25	0.0011
FPG (mg/dL)	96.9 [92.4 - 110.9]	25/25	85.9 [74.7 - 93.7]	24/25	0.0002
Lipid profiles					
Total cholesterol (mmol/L)	4.7 [4.2 - 5.5]	25/25	4.2 [3.7 - 4.5]	21/25	0.0002
LDL-C (mmol/L)	2.9 [2.6 - 3.6]	25/25	2.0 [1.7 - 2.5]	19/25	< 0,0001
HDL-C (mmol/L)	0.1 [0.9 - 1.2]	25/25	1.4 [1.1 - 1.8]	22/25	< 0,0001
TG (mmol/L)	1.5 [1.1 - 2.2]	25/25	0.8 [0.7 - 1.1]	20/25	0.0023
Vaso-protective properties of HDL					
VCAM-1 expression	75.4 [58.8 - 83.1]	25/25	53.0 [47.6 - 59.6]	25/25	0.0008
NO production	24.3 [-10.3 - 66.4]	25/25	89.72	25/25	< 0,0001
Cholesterol efflux	100.0 [90.0 - 108.1]	25/25	112.2 [97.5 - 134.1]	25/25	0.0023
Apoptosis	58.2 [34.6 - 73.3]	25/25	41.8 [36.7 - 44.9]	25/25	0.0136
PON activity	249.5 [161.2 - 625.2]	25/25	7014.0 [5438.0 - 8166.0]	25/25	< 0,0001

Table 1. Clinico-biological characteristics of the patients before and 1 year after RYGBP.

RYGB decreased BMI, improved glucose homeostasis, lipid profiles and vaso-protective properties of isolated HDL 1 year after surgery.

VCAM-1 expression: inhibition from TNF-α stimulated cells. NO: DAF-2 Fluorescence. Cholesterol Efflux: Cholesterol Efflux from J774 macrophages. Apoptosis: cytoplasmic DNA-histone complexes. PON: paraoxonase activity.

Data are presented as medians [IQR]. Wilcoxon tests.

Δ (After-Before)	VCAM1		NO expression		Cholesterol efflux		Apoptosis		PON activity	
						R				
Total HDL-BAs	0.11	0.61	0.07	0.75	0.30	0.14	-0.36	0.07	-0.16	0.43
Total HDL-Free BAs	-0.04	0.83	0.23	0.25	0.06	0.76	**-0.52***	0.01	-0.25	0.22
Total HDL-Glyco BAs	0.18	0.38	-0.07	0.75	0.32	0.11	-0.15	0.47	-0.01	0.95
Total HDL-Tauro BAs	0.13	0.52	-0.02	0.94	0.33	0.10	-0.04	0.84	0.00	1.00
Total HDL-1ary BAs	0.01	0.95	0.03	0.90	0.23	0.27	-0.37	0.06	-0.14	0.51
Total HDL-2dary BAs	0.17	0.42	0.14	0.50	0.24	0.24	-0.24	0.23	-0.21	0.29
Total HDL-CA	-0.05	0.81	0.09	0.65	0.23	0.26	**-0.52****	0.01	0.11	0.58
Total HDL-CDCA	0.02	0.94	0.00	1.00	0.22	0.28	-0.35	0.08	-0.14	0.50
Total HDL-DCA	0.15	0.48	0.10	0.64	0.22	0.28	-0.20	0.33	-0.20	0.34
Total HDL-LCA	-0.19	0.35	0.21	0.31	0.02	0.92	-0.09	0.64	-0.38	0.06
Total HDL-UDCA	-0.17	0.42	0.08	0.69	-0.08	0.69	-0.35	0.08	-0.35	0.08
Total HDL-HCA	-0.23	0.26	-0.11	0.59	0.13	0.53	-0.35	0.08	-0.01	0.98
Total HDL-HDCA	0.01	0.98	-0.26	0.20	0.03	0.87	0.00	0.99	0.13	0.54
Total HDL-BAs	0.11	0.61	0.07	0.75	0.30	0.14	-0.36	0.07	-0.16	0.43

Table 2. Correlation of the increase in HDL-bound BAs and the improvement of endothelial protective properties of HDL.

Legend: Total HDL-Free BAs negatively correlate with apoptosis due to a significant correlation of Total HDL-CA and a marginally significant correlation of total HDL-CDCA (See Supplemental Figure 1). *R*: Spearman's correlation coefficients.

Δ (After-Before)	HDL size	
	R	p
VCAM1 expression	-0.038	0.869
NO expression	-0.175	0.449
Cholesterol efflux	0.422	0.057
Apoptosis	-0.124	0.591
PON activity	0.044	0.849
Total HDL-BAs	0.534	**0.013**
Total HDL-CA	0.279	0.221
Total HDL-CDCA	0.510	**0.018**
Total HDL-DCA	0.290	0.202
Total HDL-LCA	-0.050	0.828
Total HDL-UDCA	0.067	0.773
Total HDL-HCA	0.318	0.160
Total HDL-HDCA	0.333	0.140

Table 3. Correlation of RYGB-induced changes in HDL-BAs and size of HDL.

Changes in HDL size is correlated with Total-HDL-CDCA but not with HDL vaso-protective properties.

Legend: *R*: Spearman's correlation coefficients.

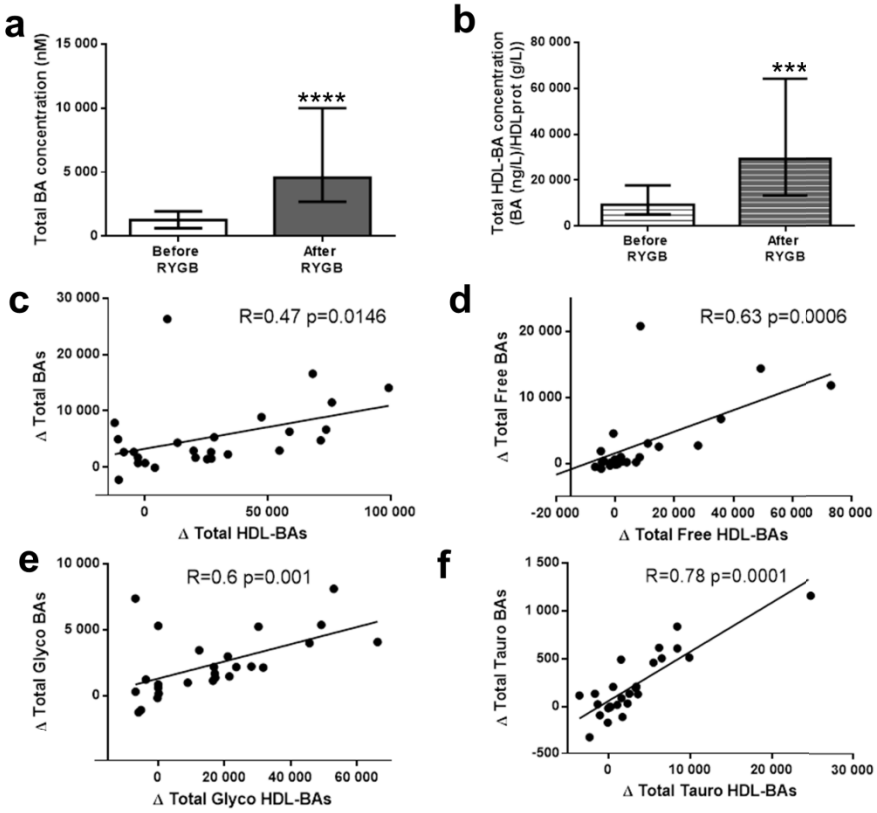


Figure 1. Effect of RYGB on plasma and HDL-BAs.

Legend: RYGB increased plasma BAs **(a)** and HDL-BAs **(b)**. The increase (concentrations after minus concentrations before, noted Δ) was similar in plasma and in HDL for Total BAs **(c)**, Total Free-BAs **(d)**, Total Glyco-BAs **(e)**, and Total Tauro-BAs **(f)**.

Data are presented as medians ± IQR or Δ (after - before). Wilcoxon test: ***p<0.001, ****p<0.0001. R: Spearman's correlation coefficients.

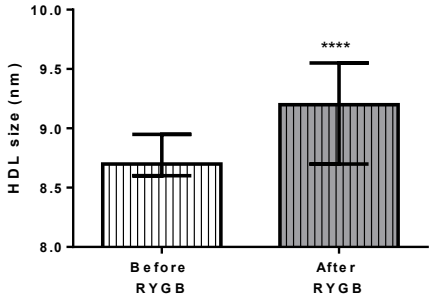
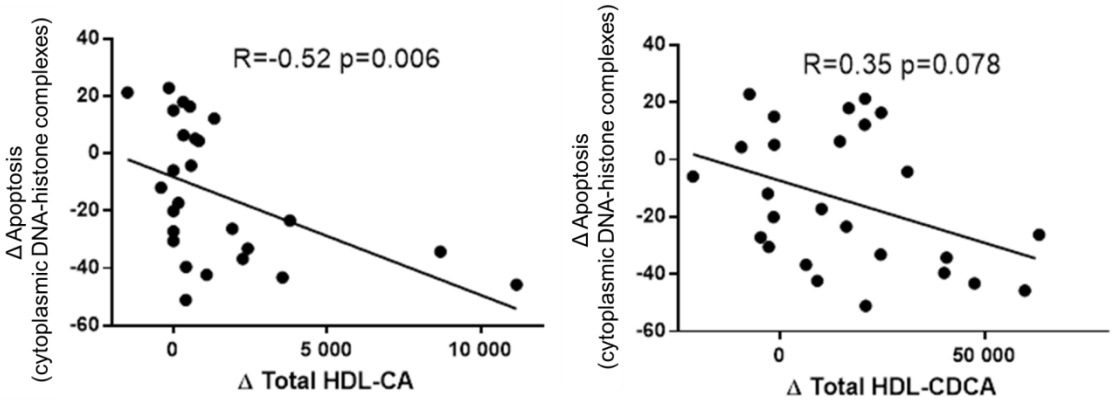


Figure 2. HDL size of patients before and after RYGB.

Legend: HDL size increased after surgery.

Data are presented as medians ± IQR, Wilcoxon test: ****p<0.0001.



Supplemental Figure 1. Dot plots of the significant correlations in the Table 2. Spearman's correlation coefficients.

GENERAL CONCLUSION

The discovery of the BA receptors FXR in 1999 (Makishima *et al.*, 1999) and TGR5 in 2003 (Kawamata *et al.*, 2003) opened a new area of interest for human physiology, revealing a novel role for BAs as actors of a complex and intricate inter-organ dialogue between the liver, the intestine, its microbiota, and the other peripheral tissues to modulate the metabolic homeostasis, in addition to their first discovered role in lipid solubilization. The role of BAs in cardiovascular diseases is complex and not yet entirely known.

One of the major challenges for researchers in the BA field is the inter-species variability on BA pool composition of the pre-clinical models used for the study of metabolic diseases, which in addition display different proportions of BA families in their BA pool, could also present structural and functional differences of their BA receptors, making pre-clinical data hardly translatable to humans. Particularly, murine models are the most used pre-clinical animal model in medical research, however, they are not entirely appropriate for the study of BA physiology due to the recently described expression of the 6β-hydroxylase CYP2C70 (Takahashi *et al.*, 2016), responsible for the synthesis of α, β and ωMCA, major BA species in murine models but absent in humans, which, for some of them, elicit completely different effects on BA receptors (*e.g.* tauro-conjugated βMCA is a FXR antagonist). The development and metabolic phenotyping of rodent models invalidated for the expression of CYP2C70 will possibly allow in the future performing studies in mice with humanized BA pool, yielding results closer to the human physiology, although probably not entirely mimicking human BA signaling in mice. Similarly, the pre-clinical model of minipig used during my PhD, lacks of murine CYP2C70, but presents as major BA species the 6α-hydroxylated BAs HCA and HDCA, whose proportion in humans systemic circulation is inferior to 1%, and whose signaling role is still unknown, notwithstanding that this model allowed us to study portal blood, intestinal content and liver biopsies that would have been otherwise impossible to access in human patients. In conclusion, pre-clinical models are imperfect for BA study but indispensable for mechanistic research and potentially adaptable to resemble humans.

To date, the main BA receptors (FXR and TGR5) are known to be highly expressed in different organs where their specific role remains not totally elucidated. It cannot be excluded that other molecules different than BAs could also activate them. Similarly, given the wide diversity of BA species in nature, it is conceivable that other BA receptors could exist in humans, and that BA species present in other animal species could keep vestigial signaling roles for humans, similarly as we expect that a humanized BA pool will evoke in mice human physiology.

The wide structural diversity of the different BA species present in humans, their respective specific proportions and their different affinities to modulate their receptors activities is intriguing. Intuitively, one could think that humans do not necessitate so many different BA species for the sole solubilization of dietary fat; the evolutionary conservation of BA synthesis pathways in humans, carrying a cost to synthesize the enzymatic machinery responsible for the BA diversity, as well as the energetic costs that the different BA species synthesis *per se* represent, suggests that all of these BA species have/might have had a particular role in humans either by activating their receptors, by their physicochemical properties and/or by their recently described inter-communication with the gut microbiota.

Another unanswered question is whether and how BAs activate their receptors in many peripheral tissues, where their circulating concentrations are below the EC50 of their receptors. During my thesis we hypothesized that the lipoproteins -and particularly the HDL- could act as shuttles delivering BAs to the different peripheral tissues, and we speculated that it could occur by (I) internalization of the BAs at the same time than the HDL, (II) internalization of HDL components (as BAs), but not the entire HDL or (III) approaching BAs to the membrane receptor TGR5 without HDL internalization, with the help of HDL enchorage to the membrane *via* HDL-Rc such as SRB-1. Our results (manuscript 8) showing the interesting association of the increase in CA content of HDL with improved endothelial apoptosis *ex vivo* upon RYGB constitutes the proof of concept that BAs associated to HDL may modulate a physiopathological function. This association paves the way to new research aiming to determine the utility of HDL as vehicles directed to evoke beneficial effects of different BA species in the context of cardiovascular diseases in diverse cell types. For example, it is known that HDL interact with macrophages and foam cells for cholesterol efflux, it is conceivable that BAs bound to HDL could thusly interact with such cells (known to express FXR/TGR5) and modulate their function.

The main contribution of this thesis in the BA field is the proof that BAs are not good biomarkers for predicting type 2 diabetes in patients with prediabetes. Although this hypothesis has been long and widely stated due to the association of BA alterations with metabolic diseases, it had never been tested until now. The lack of association of the BAs with the risk of progression of glucose-homeostasis disorders suggests that BA changes in these diseases might be one of the metabolic consequences but not necessarily driving forces for the pathogenesis of these disorders. The mechanisms driving such changes could be promiscuous signaling pathways of metabolic diseases interfering with BA metabolic routes, whose enzymes participate to other biological functions in humans. However, this thesis fails to prove whether BAs are drivers or markers in the metabolic diseases. Some mechanistic studies link BA metabolism with glucose homeostasis. Haeusler *et al.* (Haeusler,

Pratt-Hyatt, Welch, Klaassen, & Accili, 2012), reported that the hepatic insulin receptor modulates the 12α-hydroxylase CYP8B1 *via* FoxO1, therefore modifying the 12α-hydroxylated/non-12α-hydroxylated BA ratio. However, our experimental conditions yielded results failing to reproduce the association of these BAs with insulin resistance in humans, mice and pigs. Moreover, the work by Hoogerland *et al.* (Hoogerland *et al.* Hepatology 2019), reports that bile acid synthesis in mice is controlled by the glucose-6-phosphate, the primary metabolite of glucose, by increasing the expression of CYP8A1 *via* carbohydrate response element-binding protein (ChREBP). Additionally, a recent study suggested that infusion of bile acids in the jugular vein leads to increased systemic bile acids and suppresses hepatic insulin sensitivity in lean mice, and this effect is lost when bile acids are infused in the portal vein, probably due to hepatic first-pass effect (Syring, K. E., *et al.* 2019). Further studies should be aimed to study the mechanism causing the BA alterations reported in human glucose homeostasis disorders in order to reproduce them and determine whether they are "eggs or hens" in the natural history of metabolic diseases.

In the field of NAFLD, we can conclude that BAs are unlikely suitable as biomarkers for the diagnosis or leading to the decision of biopsy taking in NAFLD patients, probably due to the large inter-individual variations, feeding state of the patietnts and circadian fluctuations in circulaging BAs. The lack of association of NAFLD lesions with BA changes is astonishing, since BA metabolism is zonated and since NASH lesions alter the normal zonation of the liver. Even though BAs were not associated with NASH *per se*, BA signaling is an attractive therapeutic target for NASH treatment, but whether BAs are a driving force in the pathogenesis and progression of NAFLD or just innocuous bystanders remains undetermined.

In the particular context of bariatric surgery, we described that selective changes in hepatic BA recapture is a mechanism that contributes to explain the increased and altered BA concentrations following RYGB in minipigs. Interestingly, such results have been reproduced in rats (Bhutta *et al.*, 2015) and in the context of bile diversion alone (Goncalves *et al.*, 2015). Surprisingly, we failed to reproduce such changes in the ABOS cohort, suggesting that caution should be taken when translating pre-clinical data to humans. A recent study showed that NTCP deficiency (NTCP being one of the main BA transporters for hepatic reuptake) in mice protects from obesity and hepatosteatosis (Donkers *et al.*, 2019), both of which are improved early by the RYGB. The mechanism underlying the gut-liver communication that alters hepatic BA reuptake remains an open question. Furthermore, the role of the intestine in the RYGB-induced BA modifications remains unknown, and further studies should focus on intestinal BA recapture and the role of the surgically modified intestinal limbs in the BA changes. The altered 1ary/2dary intra-luminal intestinal BA ratio described in my thesis

suggest that modifications in the gut microbiota occur upon bariatric surgery, which could contribute to the metabolic improvements by altering BA signaling in the entero-hepatic circulation or even in the circulating BA modifications upon surgery.

The regulation of metabolism by BAs is very complex and its study requires a multidisciplinary approach, particularly from the microbiota experts. The recently described mutual regulation of BAs and the gut microbiota, and the dysbiosis present in metabolic diseases suggests that the BA alterations could change human metabolism in ways that are not described yet *via* microbial metabolites (*e.g.* gut microbiota-derived short chain fatty acids are known to modulate metabolism in humans), and contribute to the pathophysiology of metabolic disorders or improvements following bariatric surgery (also known to modify the gut microbiota). It remains undetermined whether modulation of the gut microbiota could elicit changes in the BA pool in humans that could have therapeutic effects for the treatment of metabolic diseases or even in cholestasis disorders. Since BAs exert antimicrobial properties, BAs could contribute to the metabolic regulation by changing the gut microbiota.

Noteworthy, despite BAs are probably unsuitable biomarkers of metabolic diseases, and despite their role in the pathophysiology of metabolic diseases is still poorly evidenced, BA receptors and BA metabolic pathways undoubtedly remain good targets for the treatment of glucose and cholesterol homeostasis disorders, as well as NASH. Indeed, pharmacological modulation of FXR and TGR5, as well as the targeting of downstream BA signaling (ASBT, FGF19, CYP8B1, NTCP) will allow the development of better treatments for metabolic disorders.

BIBLIOGRAPHY

Bhutta, H. Y., Rajpal, N., White, W., Freudenberg, J. M., Liu, Y., Way, J., … Chen, L. (2015). Effect of Roux-en-Y gastric bypass surgery on bile acid metabolism in normal and obese diabetic rats. *PloS One*, *10*(3), e0122273. https://doi.org/10.1371/journal.pone.0122273

Buscher, H.-P., Beger, M., Sauerbier, H., & Gerok, W. (1987). Bile salt shift from albumin to high-density lipoprotein in cholestasis. *Hepatology*, *7*(5), 900–905.

Ceryak, S., Bouscarel, B., & Fromm, H. (1993). Comparative binding of bile acids to serum lipoproteins and albumin. *Journal of Lipid Research*, *34*(10), 1661–1674.

Chávez-Talavera, O., Tailleux, A., Lefebvre, P., & Staels, B. (2017). Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia and NAFLD. *Gastroenterology*. https://doi.org/10.1053/j.gastro.2017.01.055

Donkers, J. M., Kooijman, S., Slijepcevic, D., Kunst, R. F., Roscam Abbing, R. L., Haazen, L. C., … Van de Graaf, S. F. (2019). NTCP deficiency in mice protects against obesity and hepatosteatosis. *JCI Insight*, *5*. https://doi.org/10.1172/jci.insight.127197

Goncalves, D., Barataud, A., De Vadder, F., Vinera, J., Zitoun, C., Duchampt, A., & Mithieux, G. (2015). Bile Routing Modification Reproduces Key Features of Gastric Bypass in Rat. *Annals of Surgery*, *262*(6), 1006–1015. https://doi.org/10.1097/SLA.0000000000001121

Haeusler, R. A., Pratt-Hyatt, M., Welch, C. L., Klaassen, C. D., & Accili, D. (2012). Impaired generation of 12-hydroxylated bile acids links hepatic insulin signaling with dyslipidemia. *Cell Metabolism*, *15*(1), 65–74. https://doi.org/10.1016/j.cmet.2011.11.010

Hedenborg, G., Norman, A., & Ritzén, A. (1988). Lipoprotein-bound bile acids in serum from healthy men, postprandially and during fasting. *Scandinavian Journal of Clinical and Laboratory Investigation*, *48*(3), 241–245.

Hoogerland, JA*. et al.* (2019). Glucose-6-Phosphate Regulates Hepatic Bile Acid Synthesis in Mice. Hepatology. doi: 10.1002/hep.30778. [Epub ahead of print]

Kawamata, Y., Fujii, R., Hosoya, M., Harada, M., Yoshida, H., Miwa, M., … Fujino, M. (2003). A G protein-coupled receptor responsive to bile acids. *The Journal of Biological Chemistry*, *278*(11), 9435–9440. https://doi.org/10.1074/jbc.M209706200

Kramer, W., Buscher, H.-P., Gerok, W., & Kurz, G. (1979). Bile salt binding to serum components. *European Journal of Biochemistry*, *102*(1), 1–9.

Kuipers, F., Bloks, V. W., & Groen, A. K. (2014). Beyond intestinal soap--bile acids in metabolic control. *Nature Reviews. Endocrinology*, *10*(8), 488–498. https://doi.org/10.1038/nrendo.2014.60

Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., & Staels, B. (2009). Role of bile acids and bile acid receptors in metabolic regulation. *Physiological Reviews*, *89*(1), 147–191. https://doi.org/10.1152/physrev.00010.2008

Makishima, M., Okamoto, A. Y., Repa, J. J., Tu, H., Learned, R. M., Luk, A., … Shan, B. (1999). Identification of a nuclear receptor for bile acids. *Science (New York, N.Y.)*, *284*(5418), 1362–1365. https://doi.org/10.1126/science.284.5418.1362

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., … Gakidou, E. (2014). Global, regional and national prevalence of overweight and obesity in children and adults 1980-2013: A systematic analysis. *Lancet (London, England)*, *384*(9945), 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

Nguyen, N. T., & Varela, J. E. (2017). Bariatric surgery for obesity and metabolic disorders: State of the art. *Nature Reviews. Gastroenterology & Hepatology*, *14*(3), 160–169. https://doi.org/10.1038/nrgastro.2016.170

Osto, E., Doytcheva, P., Corteville, C., Bueter, M., Dörig, C., Stivala, S., … Lüscher, T. F. (2015). Rapid and body weight-independent improvement of endothelial and high-density lipoprotein function after Roux-en-Y gastric bypass: Role of glucagon-like peptide-1. *Circulation*, *131*(10), 871–881. https://doi.org/10.1161/CIRCULATIONAHA.114.011791

Rudman, D., & Kendall, F. E. (1957). Bile Acid Content of Human Serum. II. The Binding of Cholanic Acids by Human Plasma Proteins1. *Journal of Clinical Investigation*, *36*(4), 538–542. https://doi.org/10.1172/JCI103451

Schaap, F. G., Trauner, M., & Jansen, P. L. M. (2014). Bile acid receptors as targets for drug development. *Nature Reviews. Gastroenterology & Hepatology*, *11*(1), 55–67. https://doi.org/10.1038/nrgastro.2013.151

Srivastava, G., & Apovian, C. M. (2018). Current pharmacotherapy for obesity. *Nature Reviews. Endocrinology*, *14*(1), 12–24. https://doi.org/10.1038/nrendo.2017.122

Steiner, C., Holleboom, A. G., Karuna, R., Motazacker, M. M., Kuivenhoven, J. A., Frikke-Schmidt, R., … von Eckardstein, A. (2012). Lipoprotein distribution and serum concentrations of 7α-hydroxy-4-cholesten-3-one and bile acids: Effects of monogenic disturbances in high-density lipoprotein metabolism. *Clinical Science*, *122*(8), 385–400. https://doi.org/10.1042/CS20110482

Syring, K. E. … McGuinness O. P. (2019). Systemic bile acids induce insulin resistance in a TGR5-independent manner. *Am J Physiol Endocrinol Metab, 316*; E782-E796

Takahashi, S., Fukami, T., Masuo, Y., Brocker, C. N., Xie, C., Krausz, K. W., … Gonzalez, F. J. (2016). Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. *Journal of Lipid Research*, *57*(12), 2130–2137. https://doi.org/10.1194/jlr.M071183

APPENDICES

MANUSCRIPT NUMBER 9

Chávez-Talavera & Spinelli *et al.* Metabolic effects of bile acid sequestration: impact on cardiovascular risk factors. Current Opinion in Endocrinology, Diabetes and Obesity, 2016. Apr 23(2):138-144

Metabolic effects of bile acid sequestration: impact on cardiovascular risk factors

Valeria Spinelli[a,b,c,d,*], *Oscar Chávez-Talavera*[a,b,c,d,*], *Anne Tailleux*[a,b,c,d], *and Bart Staels*[a,b,c,d]

Purpose of review
This article discusses the impact of bile acid sequestrants (BAS) on cardiovascular risk factors (CVRFs), on the basis of recent (pre)clinical studies assessing the metabolic impact of modulation of enterohepatic bile acid signaling via the bile acid receptors farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5).

Recent findings
BAS decrease low-density lipoprotein-cholesterol by stimulating *de novo* hepatic bile acid synthesis and lowering intestinal lipid absorption, and improve glucose homeostasis in type 2 diabetes mellitus, at least in part by increasing GLP-1 production, via intestinal TGR5- and FXR-dependent mechanisms. Intestinal and peripheral FXR and TGR5 modulation also affects peripheral tissues, which can contribute to the reduction of CVRFs.

Summary
Bile acids are regulators of metabolism acting in an integrated interorgan manner via FXR and TGR5. Modulation of the bile acid pool size and composition, and selective interference with their receptors could, therefore, be a therapeutic approach to decrease CVRFs. Even though clinical cardiovascular outcome studies using BAS are still lacking, the existing data point to BAS as an efficacious pharmacological approach to reduce CVRFs.

Keywords
bile acid sequestrants, bile acids, cardiovascular risk, FGF15/19, farnesoid X receptor, GLP-1, TGR5

INTRODUCTION

Cardiovascular disease (CVD) is the first cause of death worldwide (WHO 2015). CVD is precipitated by cardiovascular risk factors (CVRFs), such as dyslipidemia [high low-density lipoprotein-cholesterol (LDL-C), low high-density lipoprotein-cholesterol (HDL-C), high triglycerides], type 2 diabetes mellitus (T2DM), and nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH). Statins are the first-line treatment for dyslipidemia [1], but they do not completely eliminate cardiovascular risk, increase the risk of T2DM and are not tolerated by all patients [2]. Among the current nonstatin lipid-lowering therapies are the bile acid sequestrants (BAS), which are positively charged resins that bind and sequester bile acids in the intestine to form an insoluble complex, hence disrupting the enterohepatic cycle by interfering with intestinal bile acid reabsorption. BAS exert beneficial effects on LDL-C by enhancing hepatic cholesterol turnover for *de-novo* synthesis of bile acids, to compensate for BAS-induced fecal bile acid loss. BAS were initially used to manage hypercholesterolemia, but were recently found to improve glucose homeostasis in T2DM patients [3,4]. This beneficial effect of BAS on glucose homeostasis led to the repositioning of colesevelam and colestimide for the treatment of diabetes (in the USA and Japan, respectively).

Bile acids also act as signaling molecules regulating metabolism by activating at least two receptors, farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5), which are

[a]Univ Lille, UMR1011, EGID, [b]Inserm, UMR1011, [c]CHU Lille and [d]Institut Pasteur de Lille, U1011, Lille, France

Correspondence to Professor Bart Staels, Institut Pasteur de Lille, 1 Rue du Professeur Calmette, BP245, 59019 Lille, France.
Tel: +33 320877388; fax: +33 320877360;
e-mail: Bart.Staels@pasteur-lille.fr

*Valeria Spinelli and Oscar Chávez-Talavera have contributed equally to the writing of this article.

Curr Opin Endocrinol Diabetes Obes 2016, 23:138–144
DOI:10.1097/MED.0000000000000235

KEY POINTS

- BAS exert beneficial effects on CVRFs by decreasing LDL-C, total cholesterol, and improving glycemic control in T2D.
- The beneficial effects on T2DM are at least in part due to increased BAS-induced GLP-1 production by enteroendocrine intestinal cells, occurring through combined FXR repression in the ileum and TGR5 activation in the colon.
- BAS could also alter metabolism by deactivating FXR in the liver, which may enhance glucose utilization, lipogenesis, and steatosis.
- FXR and/or TGR5 activity in the intestine impacts on bile acid pool composition, resulting in metabolic effects not only in enterohepatic tissues, but also in peripheral organs, especially adipose tissue, thus impacting whole body metabolism.
- BAS decrease cardiovascular events in hypercholesterolemic patients, whereas their effects on morbi-mortality in type 2 diabetic patients remain to be established.

expressed in metabolic tissues, where they control energy homeostasis. As the different bile acid species activate these receptors with different potencies, changes in their pool size and composition can impact on metabolic regulation. Hence, variations of the bile acid pool following the use of BAS [5] can also contribute to the beneficial effects of BAS. In this review, we discuss recent findings on bile acids acting as signaling molecules in an integrated inter-organ metabolic system via their receptors, with a particular focus on enterohepatic organs. We propose the hypothesis that BAS impact on CVRFs, and potentially on CVD, by modulating the bile acid pool size and composition.

BILE ACIDS ARE SIGNALING MOLECULES REGULATING ENERGY HOMEOSTASIS

Bile acids are synthesized in the hepatocytes from cholesterol by the action of at least 17 enzymes. The end products are primary bile acids, which are conjugated with taurine (mainly in rodents) or glycine (mainly in humans) generating tauro-conjugated or glyco-conjugated bile acids, respectively. Bile acids are stored in the gallbladder in the fasting state and secreted in the duodenum after a meal. In the intestine, the primary bile acids are metabolized to secondary bile acids by the action of gut bacterial enzymes. After facilitating intestinal lipid absorption, about 95% of intestinal bile acids are absorbed and 5% are excreted in the feces and replaced by *de novo* synthesis. Absorbed bile acids are transported back to the liver through the portal vein, where the majority is recaptured by the hepatocytes. However, detectable amounts of bile acids escape the hepatic reuptake and reach the peripheral organs. Hence, a steep bile acid concentration gradient exists between portal and peripheral blood under normal physiological conditions. The cycling of bile acids from the liver to the intestine is known as the enterohepatic circulation (Fig. 1a).

Bile acids modulate metabolism by activating several receptors, including the nuclear receptor FXR and the membrane G-protein coupled receptor TGR5 (GPBAR1). FXR, which is widely expressed (liver, intestine, pancreas, adipose tissue, kidney, brain and immune cells), is a transcription factor regulating genes controlling lipid, glucose, bile acid metabolism, and inflammation [6]. TGR5 is expressed in adipose tissue, muscle, intestine, pancreas, gallbladder, lung, immune cells, brain, spinal cords, and the enteric nervous system (in mice). TGR5 regulates energy expenditure and body weight and exerts anti-inflammatory actions [7]. Not all bile acids activate FXR and TGR5 with the same potency. Thus, quantitative and/or qualitative changes of the bile acid pool can modify metabolism and inflammatory control by differential FXR and TGR5 activation. As animal models are required for genetic studies, it is important to note that specific differences in the composition of the bile acid pool exist among species. Indeed, since certain bile acid species exclusively present in rodents have been recently identified as FXR antagonists [8], extrapolation of these findings in animals to humans is difficult.

QUANTITATIVE AND QUALITATIVE CHANGES IN THE BILE ACID POOL: IMPACT ON CARDIOVASCULAR RISK FACTORS

Several CVRFs are modified in association with alterations in the bile acid pool (Fig. 1b). Obese individuals display increased plasma bile acid concentrations, which positively correlate with the BMI [9]. Insulin resistance is associated with alterations in the 12α-hydroxylated/non-12α-hydroxylated bile acid ratio, because of a lack of insulin repression of Cyp8b1 induction by Foxo1 [10]. Type 2 diabetic patients display increased synthesis of the primary bile acid cholic acid, and increased input rate and pool size of the secondary bile acid deoxycholic acid [5]. Patients with NASH display deregulated hepatic bile acid metabolism, with decreased hepatic cholic acid and increased hepatic tauro-conjugated bile acid levels [11].

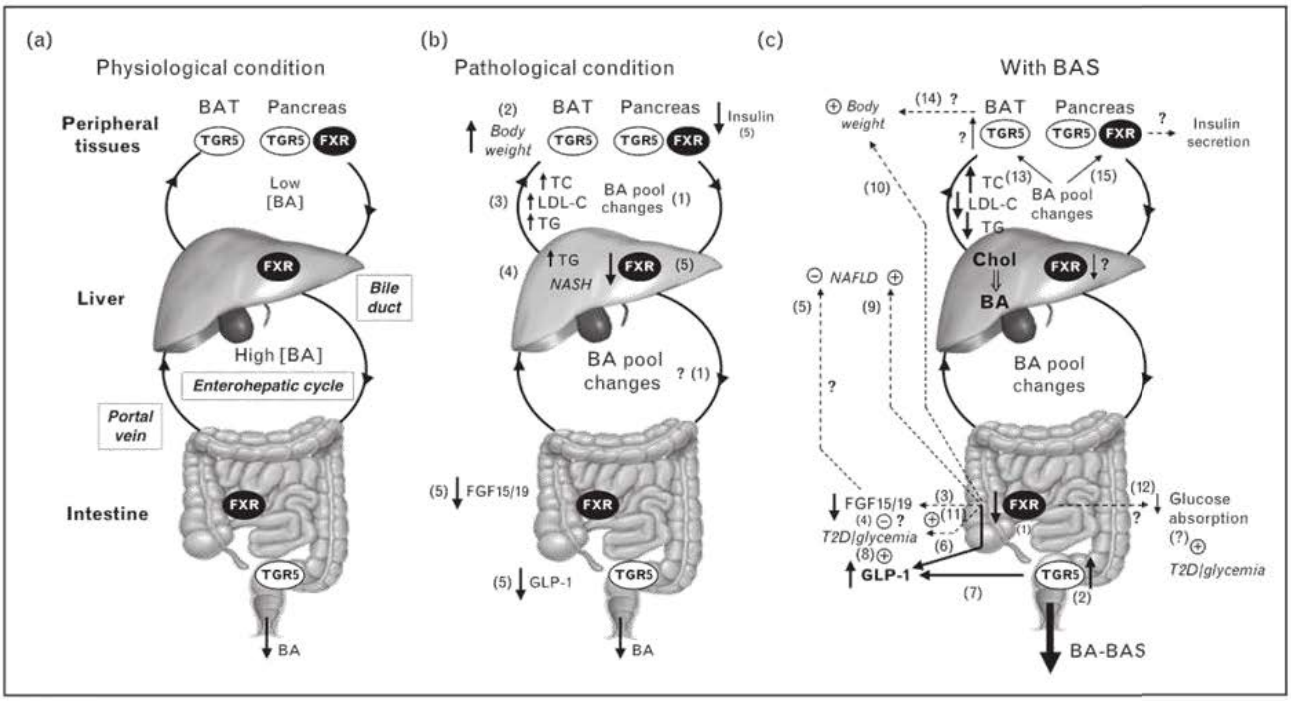


FIGURE 1. (a) Enterohepatic circulation of bile acids. Bile acids are synthesized and conjugated in the liver, stored in the gallbladder, and secreted in the duodenum via the bile duct after meal ingestion. They are modified by the intestinal gut flora, reabsorbed in the intestine and transported back to the liver by the portal vein. A small fraction of bile acids escapes and reaches peripheral organs. Bile acid concentrations within the enterohepatic cycle are in the order of mmol/l in bile and μmol/l in portal vein, whereas only in the low μmol/l in systemic blood. In physiological conditions, bile acids control the energy balance via FXR and TGR5 in enterohepatic and peripheral organs. (b) Alterations of bile acid metabolism and metabolic parameters in the pathological condition of the metabolic syndrome. Metabolic alterations are associated with quantitative/qualitative perturbations of the bile acid pool (1), obesity (2), dyslipidemia (high total cholesterol, low-density lipoprotein-cholesterol and triglyceride plasma concentrations) (3), NAFLD/NASH (increased hepatic triglycerides and NASH) (4), diabetes (insulin resistance and decreased insulinemia, decreased GLP-1 and fibroblast growth factor (FGF)19, and decreased hepatic FXR expression in mice) (5). Arrows: alterations characterizing metabolic pathological conditions. (c) Effect of BAS on CVRFs via action on FXR and TGR5 in enterohepatic tissues. Bile acid sequestration inactivates FXR (1) and may activate TGR5 (2) in the intestine. FXR inactivation decreases the production of FGF15/19 (3), which may limit potential positive metabolic effects of FGF19 on type 2 diabetes (4) and NAFLD (5). Both FXR inactivation (6) and TGR5 activation (7) increase GLP-1 production, improving glucose control (8). Intestinal FXR inactivation protects from NAFLD (9), body weight gain (10), glucose intolerance (11) and may retard intestinal glucose absorption (12). BAS treatment changes systemic bile acid pool composition and hence likely FXR and TGR5 activity. This may impact on brown adipose tissue (BAT) energy expenditure via TGR5 activation (13) improving body weight control (14), and insulin secretion from the pancreas (15) in addition to the stimulation of insulin secretion because of the increased GLP-1. Solid arrows: BAS known effects. Dotted arrows: putative effects of intestinal action of BAS on CVRFs, based on animals studies. Thin arrows: putative consequences of BAS treatment on peripheral tissues. Question marks: hypothetical action that would need further studies. BAS, bile acid sequestrants; CVRFs, cardiovascular risk factors; FXR, farnesoid X receptor; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

Systemic bile acid concentrations increase after Roux-en-Y Gastric Bypass (RYGB) surgery in preclinical models [12] and humans [13▪,14▪]. Changes in bile acid signaling may thus potentially contribute to the body weight-independent metabolic improvements seen after surgery (ex. remission from T2D and NASH) [14▪]. RYGB surgery has cardiovascular protective effects in rats [15▪] and humans [16], and reduces cardiovascular and overall mortality in humans [17]. Interestingly, bile diversion alone reproduces the metabolic effects of RYGB on glucose metabolism in rats and mice [18▪,19▪], and has beneficial effects on insulin sensitivity and cholesterol levels in T2DM patients [20▪] pointing to bile and/or bile acid as modulators of glycemic control after RYGB.

ACTION MECHANISMS OF BILE ACIDS ON CARDIOVASCULAR RISK FACTORS

Bile acids, glucose metabolism, and GLP-1

Bile acids regulate glucose homeostasis via FXR in the liver, which controls glycolysis and neoglucogenesis during nutritional transition periods [6]. Perturbations in hepatic bile acid metabolism also alter the secretion of insulin in response to glucose by pancreatic islets [21] revealing that bile acids connect also the liver and pancreas. Indeed, changes in bile acid pool composition result in different activation patterns of FXR and TGR5 in the pancreas, both regulators of insulin secretion [22,23].

Bile acids regulate intestinal glucagon like peptide-1 (GLP-1) production and secretion by enteroendocrine L cells. TGR5 activation in the colon induces proglucagon gene expression [4] and GLP-1 secretion [24]. Recent data indicate that this induction of GLP-1 secretion is because of basolateral rather than apical activation of the receptor, hence requiring bile acid absorption via the intestinal bile acid transporter [25]. These findings are at odds with a previous study showing that bile acid sequestrants, by forming bile acids–BAS complexes, induce GLP-1 secretion by binding to and activating apical TGR5 in enteroendocrine L cells of the colon [26]. However, FXR activation has been shown to repress proglucagon gene expression and GLP-1 secretion in the ileum [27▪▪]. Hence, chronic BAS treatment increases proglucagon gene expression by deactivating intestinal FXR, because of decreased intracellular levels of absorbed bile acids. In line, treatment of ob/ob mice with colesevelam improves glucose metabolism in an FXR-dependent manner [27▪▪]. As GLP-1 regulates glucose homeostasis, islet hormone secretion, appetite and body weight, gut motility and immune function [28], bile acids and their sequestration may influence T2DM, at least in part, via GLP-1. Bile acids can also modulate glucose homeostasis (and subsequently T2DM) via the action of intestinal FXR on the kinetics of glucose absorption, which is delayed in FXR-deficient mice [4], and hepatic FXR on glucose handling by the liver [4].

Bile acids, intestinal farnesoid X receptor signaling, and the gut microbiota

Treatment with antibiotics or tempol increases the levels of conjugated tauro-β-muricholic acid (TβMCA), an FXR antagonist, in the intestine by decreasing *Lactobacillus*, a genus which produces a bile acid deconjugation enzyme [29]. Inhibition of intestinal FXR activity by TβMCA in tempol-treated wild-type mice is associated with lower high-fat diet (HFD)-induced obesity and insulin resistance [29]. Moreover, intestinal FXR inhibition also inhibits intestinal ceramide biosynthesis, decreasing the levels of ceramides reaching the liver through the portal vein, subsequently inhibiting sterol regulatory element-binding protein-1c, lipogenesis, and steatosis, a process reversed by ceramide administration [30▪▪]. In line with these studies inducing microbiota changes, intestine-specific FXR deficiency also leads to lower body weight gain and insulin-resistance [29], as well as to decreased intestinal ceramide synthesis and hepatic steatosis [30▪▪]. Thus, inhibiting intestinal FXR either by microbiota-mediated changes of bile acid profiles and the subsequent TβMCA increase, or by genetic intestinal-specific FXR disruption, protects against NAFLD, obesity and glucose-intolerance and thus improves some CVRFs.

Moreover, recent data show that HFD-induced body weight gain, liver steatosis, and adipose tissue inflammation are promoted by the gut microbiota in a FXR-dependent manner [31▪▪]. These effects of the HFD are related to alterations in intestinal flora composition in FXR whole body knock-out mice. Hence, in one hand the microbiota regulate FXR activation, whereas, on the other hand, FXR modulates the microbial composition, impacting on obesity and CVRFs [31▪▪]. The use of antibiotics to modulate the microbiota and metabolic phenotype has also been tested in humans [32]. A single blind randomized controlled trial in 20 obese male subjects with metabolic syndrome treated with vancomycin for 7 days showed that antibiotic treatment decreased gram-positive bacteria (mainly *Firmicutes*) and increased gram-negative bacteria (mainly *Proteobacteria*). This change in microbiota composition translated into changes in bile acid pool composition, with an increase in primary bile acids. Interestingly, the microbiota alterations induced by vancomycin decreased peripheral insulin sensitivity, indicating a link between gut microbiota and metabolic effects in humans [32]. These observations point to important relationships between bile acids, the microbiota, FXR and CVRFs.

Bile acids, adipose tissue, and energy expenditure

By contrast, intestine-specific activation of FXR with Fexaramine increased energy expenditure and improved metabolism in obese mice through the induction of the expression of genes regulating mitochondrial biogenesis, fatty acid oxidation, thermogenesis, browning of white adipose tissue, and overall protecting against diet-induced weight gain [33▪]. These effects were ascribed to changes in

plasma bile acid pool composition, secondary to FXR-induced fibroblast growth factor (FGF)-15 expression in the intestinal epithelium, a FXR target gene, and key mediator of the intestinal control of hepatic bile acid synthesis. This increase of FGF15 in mice induces a shift to a bile acid pool enriched in TGR5 agonists, resulting in enhanced energy expenditure via TGR5-dependent brown adipose tissue (BAT) activation [33▪]. Interestingly, a recent study in healthy humans showed that oral administration of chenodeoxycholic acid (CDCA) for 2 days increases BAT activity and energy expenditure likely via TGR5 activation in brown adipocytes [34▪▪].

Bile acids, farnesoid X receptor, and FGF15/19 signaling

Enterocytic FXR-induced expression of FGF15/19 represses hepatic bile acid synthesis through the cell surface receptor complex FGFR4/βKlotho. FGF19 levels are reduced in patients with NAFLD [35]. High plasma levels of FGF19 are associated with T2DM remission following RYGB [36], suggesting FGF19 as a candidate participating in the body weight loss-independent metabolic improvements following RYGB surgery. Hence, FXR activation by promoting FGF15/19 secretion may result in additional metabolic effects. Treatment of mice with FGF19, the human ortholog of mouse FGF15, reduces plasma glucose by regulating postprandial glucose homeostasis, suppressing hepatic gluconeogenesis and inducing protein and glycogen synthesis, without stimulating lipogenesis [37]. Moreover, FGF19 directly acts on the brain by beneficially impacting on systemic glucose control in rodents [38]. FGF19 also reduces liver fat content, total cholesterol, and triglycerides concentrations and protects from NAFLD/NASH in rodents [39,40]. Bile acids and FGF19 repress the expression of apolipoprotein(a) [apo(a)] , a highly atherogenic lipoprotein [41], as well as hepatic expression of paraoxonase 1 [42], an antiatherogenic component of HDL. If these results are confirmed in humans, the bile acid-induced FXR-dependent FGF15/19 pathway could also modulate CVRFs (T2DM, obesity, NASH, and inflammation) impacting on these metabolic functions. However, the homology between FGF15 and FGF19 being relatively low, translational studies in humans and primates are necessarily required. Moreover, studies are necessary to determine whether the results obtained in rodent models with FGF19 are specific to the FGFR4/βKlotho pathway. Furthermore, in humans, FGF19 levels are positively correlated with tumor size of hepatocellular carcinomas [43], and short interfering RNA knock-down of FGF19 or FGFR4 inhibits proliferation of human hepatocellular carcinoma cell lines [44]. These observations question the suitability of targeting FGF19 for the treatment of cardiometabolic diseases. In humans, FGF19 levels rather decrease upon BAS treatment [5,45], the consequences of which are currently unclear (both on potential positive metabolic, as on negative promitogenic effects).

Bile acids, farnesoid X receptor, and lipoprotein metabolism

Bile acids affect CVRFs by modulating hepatic FXR activity, thereby regulating lipid metabolism. FXR regulates plasma lipid and lipoprotein metabolism by decreasing the expression of apo CIII and apo(a), and increasing apoCII [6] and apoF, an inhibitor of Cholesteryl Ester Transfer Protein [46]. Moreover, FXR also influences lipoprotein remodeling by controlling the expression of the Phospholipid Transfer Protein, Hepatic Lipase and Cholesteryl Ester Transfer Protein. FXR diminishes hepatic lipid content by increasing peroxisome proliferator-activated receptor α and repressing SREBP-1c gene expression, thus inhibiting lipogenesis and consequently lowering triglyceride plasma concentrations [6]. In line, a phase III clinical study has shown that administration of obeticholic acid, a FXR agonist, improves NASH [47▪▪]. By contrast, BAS increase plasma triglyceride levels [48] and worsen steatosis. These effects could be related to decreased FGF19 levels, and/or the deactivation of hepatic FXR. In fact, BAS administration decreases systemic plasma CDCA (the most potent bile acid FXR activator), whereas cholic acid and deoxycholic acid (less potent FXR activators) increase [5]. Lower FXR activation could in turn lead to lower lipogenesis repression.

Bile acids protect from atherosclerosis via TGR5, whose activation decreases proinflammatory cytokine production and induces antiatherosclerotic effects [49]. In line, dual activation of FXR and TGR5 with Intercept Pharmaceuticals-767 has protective effects from atherosclerosis in mice [50]. The effects of FXR on murine atherosclerosis are less clear, with different responses depending on the model [51]. The effects of BAS on CVRFs via action on FXR and TGR5 in enterohepatic tissues are represented in Fig. 1c.

CLINICAL EVIDENCE OF BILE ACID SEQUESTRANTS ON CARDIOVASCULAR RISK FACTORS AND CARDIOVASCULAR DISEASE

BAS improve the lipid profile in hypercholesterolemic patients, in mono or combination therapy, by decreasing total cholesterol, LDL-C and apoB. The

effect of BAS on plasma HDL-C is less consistent, with some, but not all studies reporting an increase. Moreover, BAS may increase plasma triglycerides, which, considering the simultaneous decrease of apoB-containing lipoprotein cholesterol concentrations, unlikely increase cardiovascular risk. Nevertheless, it may limit their use in hypertriglyceridemic patients at risk for pancreatitis [48]. In patients with NASH, colesevelam administration induces a small but measurable increase in liver fat [52]. There is only one multicenter randomized double blind clinical trial testing the efficacy of a BAS (i.e., cholestyramine) on CVD. The Lipid Research Clinics Coronary Primary Prevention Trial showed a significant reduction of CHD death and nonfatal myocardial infarction, without change in deaths from all causes [48]. In T2DM patients, BAS decrease HbA1c and fasting glycaemia, which are CVRFs [3,48], but studies testing the effects of BAS on cardiovascular morbidity and mortality in T2DM patients, on top of current standard of care treatment, are still lacking. A prospective, longitudinal, double-blind clinical trial is required to assess the impact of BAS on CVD.

CONCLUSION

BAS reduce CVRFs by regulating lipid and glucose metabolism as well as inflammation control. Analysis of their systemic effects indicates that inactivating FXR in the intestine and activating FXR in the liver could represent a good strategy to modulate CVRFs. Mechanistic studies of the effects of bile acids on enterohepatic organs and their crosstalk with peripheral tissues identify BAS as a useful pharmacological option to reduce cardiovascular risk through decreased plasma LDL-C concentrations and improved glucose metabolism. As BAS do not directly act on the liver, but could interfere with the action of hepatic FXR on lipid metabolism via the changes in bile acid pool composition (enhancing hepatic steatosis), it would be of interest to assess the effects of coadministration of BAS with a hepatic FXR agonist on metabolic control.

Acknowledgements

B.S. is a member of the Institut Universitaire de France.

Financial support and sponsorship

V.S. was supported by a grant from the Fondation pour la Recherche Médicale (FRM) Grant FDT20140930804. This work was supported by Grants from Région Nord-Pas de Calais, FEDER, ANR (FXREn), the Société Francophone du Diabète (SFD), the EFSD/Novo Nordisk Programme, the European Genomic Institute for Diabetes (EGID, ANR-10-LABX-46), and European Commission.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Greenland P, Lauer MS. Cholesterol lowering in 2015: still answering questions about how and in whom. JAMA 2015; 314:127–128.
2. Aiman U, Najmi A, Khan RA. Statin induced diabetes and its clinical implications. J Pharmacol Pharmacother 2014; 5:181–185.
3. Hansen M, Sonne DP, Knop FK. Bile acid sequestrants: glucose-lowering mechanisms and efficacy in type 2 diabetes. Curr Diab Rep 2014; 14:482–491.
4. Prawitt J, Caron S, Staels B. Glucose-lowering effects of intestinal bile acid sequestration through enhancement of splanchnic glucose utilization. Trends Endocrinol Metab 2014; 25:235–244.
5. Brufau G, Stellaard F, Prado K, *et al.* Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. Hepatol 2010; 52:1455–1464.
6. Mazuy C, Helleboid A, Staels B, Lefebvre P. Nuclear bile acid signaling through the farnesoid X receptor. Cell Mol Life Sci 2014; 72:1631–1650.
7. Duboc H, Taché Y, Hofmann AF. The bile acid TGR5 membrane receptor: from basic research to clinical application. Dig Liver Dis 2014; 46:302–312.
8. Sayin SI, Wahlström A, Felin J, *et al.* Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013; 17:225–235.
9. Prinz P, Hofmann T, Ahnis A, *et al.* Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients. Neuroendocr Sci 2015; 9:199–299.
10. Haeusler RA, Astiarraga B, Camastra S, *et al.* Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. Diabetes 2013; 62:4184–4191.
11. Lake AD, Novak P, Shipkova P, *et al.* Decreased hepatotoxic bile acid composition and altered synthesis in progressive human nonalcoholic fatty liver disease. Toxicol Appl Pharmacol 2013; 268:132–140.
12. Bhutta HY, Rajpal N, White W, *et al.* Effect of roux-en-Y gastric bypass surgery on bile acid metabolism in normal and obese diabetic rats. PloS One 2015; 10:e0122273.
13. ■ Dutia R, Embrey M, O'Brien S, *et al.* Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. Int J Obes 2015; 39:806–813.

The clinical study in obese T2D patients shows the temporal changes of bile acid concentrations and pool composition after RYGB in the fasted and postprandial states, with a particular focus on the ratio 12αhydroxylated/non-12αhydroxylated bile acids. This study shows the crucial importance of the time after surgery, with changes in bile acids occurring 2 years but not 1 month after surgery.

14. ■ Penney NC, Kinross JM, Newton RC, Purkayastha S. The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: a systematic review. Int J Obes 2015; 39:1565–1574.

Using a systematic search on literature databases, this review analyses the role of bile acids in humans and animals in bariatric surgery-induced metabolic effects. This review also discusses the possible involvement of bile acids in the decrease in cancers after surgery in relation to potential carcinogenic properties of high bile acid levels.

15. ■ Osto E, Doytcheva P, Corteville C, *et al.* Rapid and body weight-independent improvement of endothelial and high-density lipoprotein function after roux-en-Y gastric bypass: role of glucagon-like peptide-1. Circulation 2015; 131:871–881.

The mechanism of action of RYGB on cardioprotection is studied in rats and humans, showing beneficial effects of surgery on endothelial function and protective properties of HDL via GLP-1 by mechanisms independent of the GLP-1 receptor pathway. This study links lipoproteins, incretins and bile acids in the RYGB-induced improvement of cardiovascular risk.

16. Tailleux A, Rouskas K, Pattou F, Staels B. Bariatric surgery, lipoprotein metabolism and cardiovascular risk. Curr Opin Lipidol 2015; 26:317–324.

17. Eliasson B, Liakopoulos V, Franzén S, Näslund I, *et al.* Cardiovascular disease and mortality in patients with type 2 diabetes after bariatric surgery in Sweden: a nationwide, matched, observational cohort study. Lancet Diabetes Endocrinol 2015; 3:847–854.
18. ▪ Goncalves D, Barataud A, De Vadder F, *et al.* Bile routing modification reproduces key features of gastric bypass in rat. Ann Surg 2015; 262:1006–1015.

The study, performed in rats, provides the proof of concept that RYGB-induced bile acid changes are involved in the metabolic improvements occurring after surgery.

19. ▪ Flynn CR, Albaugh VL, Cai S, *et al.* Bile diversion to the distal small intestine has comparable metabolic benefits to bariatric surgery. Nat Commun 2015; 6:7715–7729.

The study, performed in mice, provides the proof of concept that RYGB-induced bile acid changes are involved in the metabolic improvements occurring after surgery.

20. ▪ Ferrannini E, Camastra S, Astiarraga B, *et al.* Increased bile acid synthesis and deconjugation after biliopancreatic diversion. Diabetes 2015; 64:3377–3385.

The clinical study compares the effects of biliopancreatic diversion (BPD) and RYGB on bile acid synthesis and pool composition of circulating bile acids. Despite both interventions increase plasma bile acids, BPD vs. RYGB increases vs. decreases bile acid synthesis markers, preferentially modulates unconjugated vs. conjugated bile acids, and induces sustained vs. normalized changes after 1 year, suggesting the existence of different mechanisms upon BPD vs. RYGB-induced changes in bile acid pool composition.

21. Seyer P, Vallois D, Poitry-Yamate C, *et al.* Hepatic glucose sensing is required to preserve β cell glucose competence. J Clin Invest 2013; 123:1662–1676.
22. Düfer M, Hörth K, Krippeit-Drews P, Drews G. The significance of the nuclear farnesoid X receptor (FXR) in β cell function. Islets 2012; 4:333–338.
23. Kumar DP, Rajagopal S, Mahavadi S, *et al.* Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β cells. Biochem. Biophys Res Commun 2012; 427:600–605.
24. Bala V, Rajagopal S, Kumar DP, *et al.* Release of GLP-1 and PYY in response to the activation of G protein-coupled bile acid receptor TGR5 is mediated by Epac/PLC-ε pathway and modulated by endogenous H2S. Front Physiol 2014; 5:420–431.
25. Brighton CA, Rievaj J, Kuhre RE, *et al.* Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. Endocrinology 2015; 156:3961–3970.
26. Potthoff MJ, Potts A, He T, *et al.* Colesevelam suppresses hepatic glycogenolysis by TGR5-mediated induction of GLP-1 action in DIO mice. Am J Physiol Gastrointest Liver Physiol 2013; 304:G371–380.
27. ▪▪ Trabelsi MS, Daoudi M, Prawitt J, *et al.* Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. Nat Commun 2015; 6:7629–7642.

The study identifies a role of FXR in L-cell regulation of proglucagon gene expression and GLP-1 secretion via mechanisms involving the carbohydrate-responsive element-binding protein pathway. This complements the previously reported role of TGR5, the membrane bile acid receptor, in the control of incretin synthesis in the intestine.

28. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 2013; 17:819–837.
29. Li F, Jiang C, Krausz KW, *et al.* Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun 2013; 4:2384–2394.
30. ▪▪ Jiang C, Xie C, Li F, *et al.* Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest 2015; 125:386–402.

Altering gut microbiota with either tempol or antibiotics in mice modulates FXR activity in the intestine, and subsequently liver lipid metabolism through interference with intestinal ceramide synthesis and hepatic lipogenesis. In this context, inhibition of FXR in the intestine has beneficial hepatic effects.

31. ▪▪ Parseus A, Sommer N, Sommer F, *et al.* Microbiota-induces obesity requires farnesoid X receptor. Gut 2016. (in press).

Using germ-free/conventional, FXR-KO/FXR-WT mice and microbiota transfer, this study shows that FXR is necessary for maintaining gut microbiota composition, which promotes obesity and associated disorders, and that this phenotype is transferable between mice.

32. Vrieze A, Out C, Fuentes S, *et al.* Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol 2014; 60:824–831.
33. ▪ Fang S, Suh JM, Reilly SM, *et al.* Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med 2015; 21:159–165.

Intestine-specific pharmacological FXR activation confers beneficial metabolic effects in the liver (glucose production) and adipose tissue browning. The authors propose that activation of FXR in the intestine has beneficial metabolic effects.

34. ▪▪ Broeders EPM, Nascimento EBM, Havekes B, *et al.* The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. Cell Metab 2015; 22:418–426.

TGR5 activation by CDCA treatment rapidly increases brown adipose tissue activity and promotes energy expenditure in healthy humans via mitochondrial uncoupling in adipocytes. This clinical study provides a proof of concept that energy expenditure can be stimulated by bile acids in humans, as previously shown in the mouse.

35. Reue K, Lee JM, Vergnes L. Regulation of bile acid homeostasis by the intestinal Diet1-FGF15/19 axis. Curr Opin Lipidol 2014; 25:140–147.
36. Gerhard GS, Styer AM, Wood GC, *et al.* A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. Diabetes Care 2013; 36:1859–1864.
37. Kir S, Beddow SA, Samuel VT, *et al.* FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 2011; 331:1621–1624.
38. Morton GJ, Matsen ME, Bracy DP, *et al.* FGF19 action in the brain induces insulin-independent glucose lowering. J Clin Invest 2013; 123:4799–4808.
39. Liu WY, Xie D-M, Zhu GQ, *et al.* Targeting fibroblast growth factor 19 in liver disease: a potential biomarker and therapeutic target. Expert Opin Ther Targets 2015; 19:675–685.
40. Rysz J, Gluba-Brzózka A, Mikhailidis DP, Banach M. Fibroblast growth factor 19-targeted therapies for the treatment of metabolic disease. Expert Opin Investig Drugs 2015; 24:603–610.
41. Chennamsetty I, Claudel T, Kostner KM, *et al.* FGF19 signaling cascade suppresses APOA gene expression. Arterioscler. Thromb Vasc Biol 2012; 32:1220–1227.
42. Gutierrez A, Ratliff EP, Andres AM, *et al.* Bile acids decrease hepatic paraoxonase 1 expression and plasma high-density lipoprotein levels via FXR-mediated signaling of FGFR4. Arterioscler Thromb Vasc Biol 2006; 26:301–306.
43. Hyeon J, Ahn S, Lee JJ, *et al.* Expression of fibroblast growth factor 19 is associated with recurrence and poor prognosis of hepatocellular carcinoma. Dig Dis Sci 2013; 58:1916–1922.
44. Miura S, Mitsuhashi N, Shimizu H, *et al.* Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. BMC Cancer 2012; 12:56–71.
45. Beysen C, Murphy EJ, Deines K, *et al.* Effect of bile acid sequestrants on glucose metabolism, hepatic de novo lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: a randomised controlled study. Diabetologia 2012; 55:432–442.
46. Li L, Liu H, Peng J, *et al.* Farnesoid X receptor up-regulates expression of lipid transfer inhibitor protein in liver cells and mice. Biochem Biophys Res Commun 2013; 441:880–885.
47. ▪▪ Neuschwander-Tetri BA, Loomba R, Sanyal AJ, *et al.* Farnesoid X nuclear receptor ligand obeticholic acid for noncirrhotic, nonalcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet Lond Engl 2015; 385:956–965.

Obeticholic acid treatment of NASH patients for 72 weeks significantly decreases the NAFLD Activity Score assessed by histological analysis of liver biopsies, without inducing the resolution of NASH. However, increased total cholesterol, LDL-cholesterol and insulin resistance, which are CVRFs, were observed also.

48. Out C, Groen AK, Brufau G. Bile acid sequestrants: more than simple resins. Curr Opin Lipidol 2012; 23:43–55.
49. Pols TWH, Nomura M, Harach T, *et al.* TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab 2011; 14:747–757.
50. Miyazaki-Anzai S, Masuda M, Levi M, *et al.* Dual activation of the bile acid nuclear receptor FXR and G-protein-coupled receptor TGR5 protects mice against atherosclerosis. PloS One 2014; 9:e108270.
51. Porez G, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. J Lipid Res 2012; 53:1723–1737.
52. Le TA, Chen J, Changchien C, *et al.* Effect of colesevelam on liver fat quantified by magnetic resonance in nonalcoholic steatohepatitis: a randomized controlled trial. Hepatol Baltim Md 2012; 56:922–932.

LIST OF PUBLISHED ARTICLES AT THE DATE OF THE THESIS DEFENSE

- Bile acid alterations in nonalcoholic fatty liver disease, obesity, insulin resistance and type 2 diabetes: what do the human studies tell? **Chávez-Talavera O***, Haas J*, Grzych G, Tailleux A, Staels B. Curr Opin Lipidol. 2019 Jun;30(3):244-254. *co-first authors

- Bile acid alterations are associated with insulin resistance, but not with NASH in obese subjects. Legry V, Francque S, Haas J, Verrijken A, Caron S, **Chávez-Talavera O**, Vallez E, Vonghia L, Dirinck E, Verhaegen A, Kouach M, Lestavel S, Lefebvre P, Van Gaal L, Tailleux A, Paumelle R, Staels B. J Clin Endocrinol Metab, 2017. Oct 1;102(10):3783-3794.

- Bile Acid Control of Metabolism and Inflammation in Obesity, Type 2 Diabetes, Dyslipidemia, and Nonalcoholic Fatty Liver Disease. **Chávez-Talavera O***, Tailleux A*, Lefebvre P, Staels B. Gastroenterology, 2017. May 152(7):1679-1694.e3. *co-first authors

- Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. **Chávez-Talavera O***, Baud G*, Spinelli V*, Daoudi M, Mostafa K, Goossens MK, Vallez E, Caiazzo R, Ghunaim, Hubert T, Lestavel S, Tailleux A, Staels B, Pattou F. Int J Obes (Lond), 2017. Apr 41(4):664-668. *co-first authors

- Metabolic effects of bile acid sequestration: impact on cardiovascular risk factors. **Chávez-Talavera, O*,** Spinelli V*, Tailleux A, Staels B. Current Opinion in Endocrinology, Diabetes and Obesity, 2016. Apr 23(2):138-144 *co-first authors

CONGRESS ATTENDANCE AND PRESENTATIONS DURING PHD

International congresses

- Leduc Consortium Meeting. Rome, Italy, August 31st - September 2nd 2016. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. *Oral presentation*.
- 4th symposium EGID: liver, diabetes and cardiovascular risks. Lille, France. November 29th- 30th 2016. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. *Poster presentation. Best poster award*.
- 7th Düsseldorf-Maastricht-Lille Symposium on Translational Diabetes Research. German Diabetes Center, Dusseldorf, Germany. May 29th – 30th , 2017. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. *Oral presentation*.
- 3d EGID thematic school. Bruges, Belgium. September 20th – 22th 2017. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. *Oral presentation*.
- 26th meeting of the European Group for the study of Insulin Resistance (EGIR). Lille, France. June 7th and 8th 2018.
- Falk Foundation. XXV International Bile Acid Meeting: Bile Acids in Health and Disease 2018 July 6 – 7, Dublin, Ireland. Modification of the intestinal intra-luminal bile acid pool composition upon bariatric surgery in a pre-clinical minipig model. Poster presentation; Bile acid alterations are associated with insulin resistance but not nash in obese patients. Poster presentation; Topical intestinal TGR5 agonists promote Glucagon Like Peptide-1 secretion and improve glucose tolerance. *Poster presentation*.
- 9th Düsseldorf-Maastricht-Lille Symposium on Translational Diabetes Research. European Genomic Institute for Diabetes, Lille, France. July 3d – 4th , 2019. Plasma bile acids do not predict the conversion from prediabetes to type 2 diabetes: results from the IT-DIAB cohort study. *Oral presentation*.

National congresses

- XVI André Verbert Meeting, EDBSL, Université de Lille. Lille, France. September 2016.
- Congrés de la Société Francophone de Diabète. Lille, France. March 28th – 31st 2017.
- André Verbert Meeting, EDBS, Université de Lille. Lille, France. September 14th 2017.
- 1ère Journée de la Recherche. Faculté de Pharmarcie. Université de Lille. November 10th 2017. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. *Poster presentation. Second prize of poster awards.*
- 2eme Journée de la Recherche. Faculté Pharmacie. Université de Lille. July 11th 2018. Metabolic effects of chronic intestinal-specific TGR5 activation. *Poster presentation*.
- 15th annual Meeting of the French Atherosclerosis Society (NSFA). Biarritz, France. 19 - 21 June 2019. The functional relevance of bile acids in the improvement of HDL-mediated endothelial protection after bariatric surgery. *Poster and oral presentation.*

Résumé

En plus de leur rôle dans la solubilisation des lipides alimentaires, les acides biliaires sont des molécules de signalisation régulant leur propre métabolisme, l'homéostasie du glucose et des lipides, la dépense énergétique, la fonction cardiovasculaire et l'inflammation, en modulant le Farnesoid X Receptor (FXR) et le Takeda G protein coupled Receptor 5 (TGR5). En effet, des modifications dans les concentrations des acides biliaires sont associées aux maladies métaboliques et ce sont des candidats pour participer à la pathophysiologie de ces maladies ou prédire leur progression.
Dans la première partie de cette thèse nous avons étudié les modifications des acides biliaires dans le contexte de l'obésité, l'insulinorésistance, le diabète de type 2 et la stéatohépatite non alcoolique. Nous avons montré que les acides biliaires sont corrélés avec l'homéostasie du glucose chez l'Homme, mais qu'ils ne sont pas des prédicteurs de la bascule du prédiabète en diabète de type 2 dans un étude de cohorte.
La deuxième partie de cette thèse est dédiée à l'étude des acides biliaires dans la chirurgie bariatrique. Nos résultats ont montré que la chirurgie bariatrique réduit la recapture hépatique des acides biliaires, provoquant leur augmentation dans la circulation systémique, et que ce n'est pas l'anse biliaire mais l'anse commune qui est responsable des modifications métaboliques après la chirurgie bariatrique chez le minipig. Ensuite, nous avons montré chez l'Homme que les acides biliaires liés aux lipoprotéines de haute densité (HDL) augmentent après la chirurgie bariatrique, et que cette augmentation est corrélée avec la restauration de leurs fonctions vaso-protectrices.

Cette thèse a été préparée à l'UMR1011 Inserm, Institut Pasteur de Lille, Université de Lille - EGID. 1 rue du professeur Calmette. 59000 Lille, France.

Abstract

In addition to their role in the solubilization of dietary lipids, bile acids are signaling molecules regulating their own metabolism, glucose and lipid homeostasis, energy expenditure, cardiovascular function and inflammation *via* the activation of the Farnesoid X Receptor (FXR) and the Takeda G protein coupled Receptor 5 (TGR5). Indeed, changes in bile acid concentrations are associated with metabolic diseases and therefore they are candidates to participate in the pathophysiology of these diseases or predict their progression.
In the first part of this thesis, we studied bile acid changes in the context of obesity, insulin resistance, type 2 diabetes and non-alcoholic steatohepatitis. We demonstrated that bile acids are correlated with glucose homeostasis in humans, but that they are not predictors for the progression from prediabetes to type 2 diabetes in a longitudinal cohort study.
In the second part of this thesis, we studied the bile acids in the context of bariatric surgery. Our results showed that bariatric surgery reduces the hepatic recapture of certain bile acids, causing them to increase in the systemic circulation. Additionally, we showed that it is not the bile limb but the common limb the one responsible for metabolic changes after bariatric surgery in the minipig. Finally, we showed in humans that bile acids linked to high-density lipoproteins (HDL) increase after bariatric surgery, and that this increase is correlated with the restoration of their vasoprotective functions.

This thesis was prepared at the UMR1011 Inserm, Institut Pasteur de Lille, University Lille - EGID. 1 rue du professeur Calmette. 59000 Lille, France.