APPENDICES

Abbreviations

Summary

Samenvatting

Acknowledgments

Biography

Biografie

List of publications

Abbreviations

ABCA1: ATP-binding cassette subfamily A member 1
ABCG5/8: ATP-binding cassette sub-fmaily G members 5/8
ALT: Alanine aminotransferase
ALP: alkaline phosphatase
APRI: AST / Platelets-Ratio-Index
ARFI: acoustic radiation force imaging
ASBT: apical sodium dependent bile acid transporter
ASBTi: apical sodium dependent bile acid transporter inhibitor
α-SMA: alpha-smooth muscle actin
AST: Aspartate aminotransferase
αMCA: α-muricholic acid
BA: bile acid
BAM: bile acid malabsorption
BBM: brush border membrane
BHT: butylated hydroxytoluene
BMI: body mass index
BW: bodyweight
CA: cholic acid
CDAA: choline-deficient L-amino acid-defined
CDCA: chenodeoxycholic acid
CF: cystic fibrosis
CFLD: cystic fibrosis-related liver disease
CFLI: cystic fibrosis-related liver involvement
CFTR: cystic fibrosis transmembrane conductance regulator
CFRD: cystic fibrosis-related diabetes
CMC: critical micellar concentration
COL1A1: collagen type 1 alpha 1
CSAA: choline-supplemented L-amino acid defined diet
CTGF: connective tissue growth factor
CVD: cardiovascular disease
CYP7A1: cholesterol 7α-hydroxylase
CYP27A1: sterol 27-hydroxylase
CYP7B1: oxysterol 7α-hydroxylase
CYP8B1: Cholesterol 12 α-hydroxylase
Cyp2c70: cytochrome P450, family 2, subfamily c, polypeptide 70
DCA: deoxycholic acid
DIOS: distal intestinal obstruction syndrome
EPI: exocrine pancreas insufficiency
ESPGHAN: European Society for Paediatric Gastroenterology Hepatology and Nutrition

FDA: Food and Drug Administration
FEV_1: forced expiratory volume in 1 second
Fgf15: fibroblast growth factor 15
FGF19: fibroblast growth factor 19
FGFR4: fibroblast growth factor receptor 4
FXR: farnesoid X receptor
GATA4: GATA binding factor 4
GC: gas chromatography
GCA: glycol-cholic acid
GGT: gamma-glutamyltransferase
GI: gastrointestinal
GLP-1: glucagon like peptide-1
GPBAR1: G-protein coupled bile acid receptor 1
GPCR19: G-protein coupled receptor 19
GSTα1: glutathione S-transferase A1
HDCA: hyodeoxycholic acid
HDL: high density lipoprotein
H&E: hematoxylin and eosin
HFD: high fat diet
HPS: hepatopulmonary syndrome
IBABP: intestinal bile acid-binding protein
iNOS: inducible nitric oxide synthase
ITT: insulin tolerance test
LCA: lithocholic acid
LC-MS: liquid chromatography-mass spectrometry
LDL: low density lipoprotein
LDLR1: lipoprotein receptor 1
LFD: low fat diet
LFTs: Liver function tests
LPL: lipoprotein lipase
LRH-1: liver receptor homologue-1
LXR: liver X receptor
MCA: muricholic acid
MCP1: monocyte chemoattractant protein 1
MDCA: murideoxycholic acid
NAFLD: non-alcoholic fatty liver disease
NAS: NAFLD activity score
NASH: non-alcoholic steatohepatitis
NPC1L1: Nieman-Pick C1-Like 1
NS: neutral sterols
OCA: obeticholic acid
OGTT: oral glucose tolerance test

PBC: primary biliary cholangitis
PC: phosphatidylcholine
PCSK9: proprotein convertase subtilsinkexin type 9
PEG: polyethylene glycol
PERT: pancreatic enzyme replacement therapy
pHFD: polyunsaturated fat high fat diet
PXR: pregnane X receptor
SFA: saturated fatty acid
SHP: small heterodimer partner
sHFD: saturated fat high fat diet
SIBO: small intestinal bacterial overgrowth
sLFD: saturated fat low fat diet
SREPB1c: sterol responsive element binding protein 1c
RER: respiratory exchange rate
T2DM: type 2 diabetes mellitus
Tβ-MCA: tauro-β-muricholic acid
TCA: tauro-cholic acid
TDCA: tauro-deoxycholic acid
TICE: transintestinal cholesterol excretion
TG: triglyceride
TGF-β: transforming growt factor beta
TGR5: takeda G protein-coupled receptor 5
tHFD: trans-fat high fat diet
TE: transient elastography
TIMP-1/2: tissue inhibitor of metalloproteinase-1/2
TNFα: tumor necrosis factor alpha
VDR: vitamin D receptor
VLDL: very low density lipoprotein
WT: wildtype
ZDF: Zucker Diabetic Fatty

A

Summary

Bile acids are produced by the liver to aid in the intestinal absorption of fat, cholesterol and fat-soluble vitamins. At the end of the small intestine bile acids are reabsorbed and resecreted by the liver into the bile. In recent years, several new functions of bile acids aside from their traditional role in fat absorption have been discovered, including effects on gut health, secretion of hormones and energy metabolism. These functions have consequences for various disease conditions but also highlight potential for development of new therapies.

The role of bile acid malabsorption in cystic fibrosis

Cystic fibrosis (CF) is a severe life shortening hereditary condition characterized by the absence or a defective cystic fibrosis transmembrane protein regulator (CFTR). In CF, CFTR dysfunction and the generation of thick viscous mucus causes dysfunctions in various organ systems including the lungs, intestine and liver. Novel therapeutic options directly targeting CFTR show promising results in improving life expectancy and quality of life in CF patients. However, most studies assess primarily pulmonary disease and insights in their effects on other complications which significantly contribute to morbidity and mortality such as the gastrointestinal system and the liver are lacking (liver disease is reviewed in **chapter 2**). While it is known that bile acid malabsorption is a consistent feature of the CF phenotype in both patients and animal models, the underlying cause and (clinical) consequences remain to be elucidated. In **chapter 3** we reviewed current literature on bile acid homeostasis and CF and speculated on its role in various CF related complications. We concluded that bile acid homeostasis is an attractive target for future CF research both for its potential to provide novel biomarkers and therapies.

While bile acid malabsorption in CF was first reported over three decades ago, clinical assessment is not done regularly. This is partly because current measurements are time-consuming and complicated. In **chapter 4** we studied whether we could assess bile acid malabsorption in CF indirectly by measurement of 7α-hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19 (FGF19), which are plasma surrogate markers for bile acid synthesis and absorption, respectively. We demonstrated that these markers are indeed associated with the bile acid malabsorption phenotype in the studied group of CF patients with a class III mutation and that they can be improved by CFTR potentiator treatment, illustrating their potential as biomarkers in clinical CF follow-up. Additionally, we

showed that improvements in FGF19 and C4 correlate poorly to other CF related outcomes, highlighting the role for organ specificity and modifying factors in CF.

Since targeted CFTR therapy is not effective in treatment of all CF patients and symptoms, there is still need for other therapeutic options targeting complications in different organ systems. In **chapter 5** we assessed the possibility to improve bile acid homeostasis using laxative treatment, which is not directly dependent on CFTR function. Previous studies have shown that treatment of CF mice with a commonly prescribed laxative, polyethylene glycol (PEG), could improve various gastrointestinal outcomes, such as inflammation and the microbiome. Therefore, we hypothesized that PEG also affects bile acid homeostasis in CF mice. We showed that PEG treatment could indeed decrease bile acid excretion and possibly restore intestinal FXR signaling in a CF mouse model. However, the magnitude of the phenotype and subsequent effect were dependent on the diet.

The role of bile acids and ASBT inhibition in intestinal dietary fatty acid and cholesterol (re)absorption

Lipid and bile acid homeostasis are tightly intertwined. Bile acids solubilize dietary lipids into micelles that facilitate the transport of these hydrophobic compounds over the unstirred water layer for their subsequent absorption into the enterocyte. While there is an abundance of data suggesting that a decrease or compositional change in the bile acid pool reduces cholesterol absorption, quantitative data of intestinal cholesterol fluxes are lacking. In **chapter 6**, we investigated intestinal cholesterol fluxes in two models of reduced intestinal cholesterol absorption by 1) directly inhibiting the main protein responsible for absorption, the Niemann-Pick C1-Like 1 (NPC1L1), using ezetimibe and 2) indirectly via reduction of the bile acid pool through genetic inactivation of the apical sodium dependent bile acid transporter (ASBT). We found that in both models fractional cholesterol absorption was nearly abrogated while fecal cholesterol excretion was increased to a similar degree. Combination of both models did not have an additive effect on cholesterol absorption or fecal excretion, indicating that (re)absorption of intestinal cholesterol was already completely abrogated in either of the two models separately. The calculated transintestinal cholesterol excretion (TICE) was similar in all three conditions. Therefore, we concluded that the majority of the fecal cholesterol in these models, originates from TICE that under physiological conditions is nearly fully reabsorbed.

Bile acid malabsorption induced by ASBT inhibition reduces the total bile acid pool size. We therefore hypothesized that a decrease in intestinal fat absorption

upon ASBT inhibition would be more pronounced for hydrophobic fatty acids, as these are more dependent on bile acids for their absorption. However, this has never been directly assessed. Hydrophobicity of fatty acids is determined by acyl chain length and saturation (number of double bonds). In **chapter 7** we measured intestinal fat absorption of different individual fatty acid species and demonstrate that upon ASBT inhibition total fatty acid absorption is reduced but this is most pronounced for long chain saturated and mono-unsaturated fatty acids, which are more hydrophobic.

The effects of ASBT inhibition on diet induced obesity, glucose homeostasis and non-alcoholic fatty liver disease (NAFLD)

Recently, bile acid homeostasis has emerged as an important regulator of glucose and lipid metabolism. ASBT inhibitor treatment has beneficial effects on both glucose and lipid metabolism in certain models of type 2 diabetes mellitus and non-alcoholic fatty liver disease (NAFLD). The mechanism underlying these benefits, however, is unclear. In **chapter 7** we showed that ASBT inhibition resulted in a more pronounced effect on lowering intestinal fat absorption of saturated compared to polyunsaturated fatty acids. Therefore, we further investigated the different effects of a lard based high fat diet (rich in long-chain saturated fatty acids) and a soybean oil based high fat diet (rich in polyunsaturated fatty acids) on weight gain, glucose homeostasis and hepatic steatosis in wildtype (WT) and ASBT knockout mice. We show that genetic inactivation of ASBT in mice protects them from diet induced obesity and disturbances in glucose metabolism only when feeding a lard based high-fat diet. In line with the observations using an ASBT inhibitor, the ratio of fecal excretion of saturated to polyunsaturated fatty acids was greater in ASBT knockout mice compared to WT mice. However, we were unable to firmly conclude that the difference in weight gain and glucose homeostasis between WT mice and ASBT knockout mice was due to differences in dietary fatty acid composition and subsequent absorption as the soybean oil based diet was overall less effective in inducing weight gain, even in WT mice. This indicates a generally different handling of dietary fatty acids in mice and stresses the importance of carefully choosing dietary fatty acid composition in murine models of diet induced obesity. Additionally, we demonstrated in **chapter 7** that ASBT inhibition is highly effective in preventing hepatic fat accumulation and these results were independent of the used diet.

While the effects of ASBT inhibition on hepatic steatosis are robust, it is not known whether ASBT inhibition also prevents progression from mere hepatic

steatosis to more clinically relevant inflammation and fibrosis. In **chapter 8** we showed that in a dietary choline deficiency mouse model of NAFLD that fibrosis was not prevented. In addition, ASBT inhibition did not reduce steatosis or fat absorption. The reason for the absence of these previously consistent effects of ASBT inhibition in the context of choline deficiency was not found. Combined consideration of the results from **chapter 7,** that showed diet independent effects of ASBT inhibition on fecal fat excretion and hepatic steatosis similarly, and **chapter 8**, that showed absence of both effects, suggests that there is a possible link between the decreased fat absorption and anti-steatotic effects of ASBT inhibition. While these results add to our knowledge on the role of ASBT inhibition in NAFLD development, caution should be taken in drawing far reaching conclusions as 1) mice have a bile acid profile distinct from humans and 2) steatosis induced by dietary choline deficiency is highly different from human NAFLD pathophysiology. Future research on ASBT inhibition using different NAFLD models is warranted.

In conclusion, the studies in this thesis highlight a prominent and complex role for bile acid homeostasis in regulating metabolism. This is relevant for multisystem diseases that affect bile acid homeostasis, such as cystic fibrosis, where bile acid malabsorption potentially affects a multitude of complications. Interestingly, modulating bile acid homeostasis (i.e. by ASBT inhibition) might also be exploited for prevention or novel treatment options in chronic metabolic disorders such as NAFLD or type 2 diabetes mellitus.

Samenvatting

Galzouten worden geproduceerd door de lever en uitgescheiden in de proximale dunne darm om de opname van vet, cholesterol en vet-oplosbare vitamines te bevorderen. Aan het einde van de dunne darm worden galzouten opgenomen en opnieuw uitgescheiden door de lever in de gal. In de afgelopen jaren zijn er verschillende nieuwe functies van galzouten ontdekt waaronder effecten op de darmgezondheid, de uitscheiding van hormonen en het energiemetabolisme. Deze functies hebben gevolgen voor aandoeningen waar het galzoutmetabolisme is aangedaan maar bieden ook de mogelijkheid tot ontwikkeling van nieuwe therapieën.

De rol van galzoutmalabsorptie in cystische fibrose

Cystische fibrose (CF) is een ernstige levensbekortende genetische aandoening gekarakteriseerd door de afwezigheid of non functionaliteit van het cystic fibrosis transmembrane protein regulator (CFTR) eiwit. In CF, dysfunctie van CFTR en de vorming van dik visceus slijm resulteren in stoornissen van verschillende orgaansystemen zoals de longen, darmen en lever. Nieuwe geneesmiddelen die direct op het CFTR eiwit werken laten veelbelovende resultaten zien in het verbeteren de kwaliteit van leven in CF patiënten. De meeste studies met deze geneesmiddelen richten zich echter primair op CF gerelateerde longproblemen en er is derhalve een gebrek aan inzicht op de effecten op andere complicaties, bijvoorbeeld het gastro-intestinale systeem en de lever (besproken in **hoofdstuk 2**), die significant bijdragen aan morbiditeit en mortaliteit. Galzoutmalabsorptie is een consistente observatie van het CF fenotype, zowel in patiënten als diermodellen. De onderliggende oorzaak en (klinische) consequenties van deze observatie zijn onduidelijk. In **hoofdstuk 3** bespreken we de huidige literatuur over galzouthomeostase en CF en speculeren over de mogelijke rol die galzoutmalabsorptie speelt in verschillende CF gerelateerde complicaties. We concluderen dat galzouthomeostase een interessant onderwerp is voor toekomstige CF studies gericht op de ontwikkeling van nieuwe biomarkers en therapieën.

Galzoutmalabsorptie in CF is meer dan dertig jaar geleden voor het eerst gerapporteerd in de wetenschappelijke literatuur, echter wordt het in de klinische praktijk zelden beoordeeld. Enerzijds komt dit doordat de klinische consequenties niet geheel duidelijk zijn en anderzijds doordat de huidige meetmethoden complex en moeilijk uit te voeren zijn. In **hoofdstuk 4** hebben we onderzocht of we

galzoutmalabsorptie in CF kunnen meten met behulp van 7α-hydroxy-4-cholesten-3-one (C4) en fibroblast growth factor 19 (FGF19), plasma surrogaat markers voor respectievelijk galzoutsynthese en absorptie. We tonen aan dat CF patiënten met een klasse III mutatie inderdaad een verstoring hebben van deze markers in een patroon dat overeenkomt met het galzoutmalabsorptie fenotype. Tevens laten we zien dat deze markers (gedeeltelijk) normaliseren na behandeling met het medicijn ivacaftor, een CFTR modulator. De verbeteringen in FGF19 en C4 correleren slecht met andere CF gerelateerde uitkomsten, zoals longfunctie en body-mass index, wat de orgaan specificiteit en de aanwezigheid van modificerende factoren in CF benadrukt. Deze resultaten illustreren de potentiële waarde voor deze biomarkers in CF onderzoek en klinische follow-up.

Gerichte CFTR modulerende therapie laat veelbelovende resultaten zien maar is nog niet effectief in de behandeling van alle CF patiënten en symptomen. Er is derhalve nog steeds noodzaak tot onderzoek naar andere therapeutische mogelijkheden om CF gerelateerde complicaties te behandelen of te voorkomen. In **hoofdstuk 5** hebben we gekeken of het mogelijk was om de galzouthomeostase in CF te verbeteren met behulp van behandeling met een laxeermiddel. Eerdere studies hebben laten zien dat behandeling van CF muizen met een veel voorgeschreven laxeermiddel, polyethylene glycol (PEG), een gunstig effect heeft op verschillende darmparameters zoals ontsteking en de microbiota. In **hoofdstuk 5** laten we zien in dat behandeling met PEG de galzoutexcretie verlaagd in CF muizen en dat daarmee gepaard een verhoging optreed van intestinale FXR activatie. De omvang van zowel de galzoutmalabsorptie als het effect van PEG behandeling was echter sterk afhankelijk van het type dieet dat werd gebruikt.

De rol van galzouten en ASBT in de intestinale absorptie van vetzuren en cholesterol

Vet- en galzoutmetabolisme zijn nauw met elkaar verbonden. Galzouten verhogen de oplosbaarheid van hydrofobe verbindingen, zoals cholesterol en vetzuren, door vorming van micellen in de darm. Deze micellen faciliteren transport van vetten over de waterlaag van de darm om daarna geabsorbeerd te kunnen worden door de enterocyt. Er is een verscheidenheid aan data die suggereren dat een verlaging of verandering in de samenstelling van de galzoutpool, de cholesterol absorptie verlaagt. Er is echter geen precieze kwantificatie van de intestinale cholesterol fluxen bij dergelijke veranderingen. In **hoofdstuk 6** hebben we onderzocht hoe de intestinale cholesterol fluxen beïnvloed worden in twee

modellen van verlaagde cholesterol absorptie namelijk door 1) directe remming van het belangrijkste cholesterol absorptie eiwit, Niemann-Pick C1-Like 1 (NPC1L1), middels ezetimibe, en 2) indirecte remming door inactivatie van de apical sodium dependent bile acid transporter (ASBT) en daaropvolgende verlaging van de galzoutpool. We laten zien dat in beide modellen de fractionele cholesterol absorptie zeer sterk verlaagd wordt. Tegelijkertijd was de fecale uitscheiding van cholesterol in beide modellen in gelijke mate verhoogd wat resulteerde in een vergelijkbare berekende flux van transintestinale cholesterol excretie (TICE). Combinatie van beide modellen had geen additief effect op zowel cholesterol absorptie als berekende TICE. Hieruit concludeerden we dat het merendeel van het fecaal uitgescheiden cholesterol in deze modellen TICE representeert dat onder fysiologische omstandigheden bijna volledig geresorbeerd wordt.

Galzoutmalabsorptie veroorzaakt door remming van ASBT vermindert de grootte van de galzoutpool. Aangezien hydrofobe vetzuren meer afhankelijk zijn van galzouten voor absorptie, is een vermindering in intestinale vetabsorptie ten gevolge van ASBT remming waarschijnlijk sterker voor hydrofobe vetzuren. Echter is deze relatie nooit direct onderzocht. Hydrofobiciteit van vetzuren wordt bepaald door de lengte van de acylketen en de saturatie (aantal dubbele bindingen). In **hoofdstuk 7** meten we intestinale vetabsorptie van verschillende individuele vetzuren. We laten zien dat het geven van een ASBT remmer de totale vetzuurabsorptie remt maar dat dit het meest uitgesproken is voor de hydrofobe lange keten verzadigde en mono-onverzadigde vetzuren, die meer afhankelijk zijn van galzouten voor absorptie.

De effecten van ASBT remming op dieet geïnduceerde gewichtstoename, glucosehomeostase en non-alcoholic fatty liver disease (NAFLD)

Recent onderzoek laat zien dat galzouthomeostase een belangrijke regulator is van zowel glucose- als vetmetabolisme. Behandeling met een ASBT inhibitor verbetert de glucose- en vetstofwisseling in type 2 diabetes mellitus en non-alcoholic fatty liver disease (NAFLD) in verschillende (pre)klinische studies. Het mechanisme dat ten grondslag ligt aan deze effecten is echter onduidelijk. Vanwege de eerder aangetoonde effecten van ASBT remming op intestinale vetabsorptie, hebben we in **hoofdstuk 7** verder onderzocht wat het effect is van twee hoog-vet diëten met verschillende compositie op de ontwikkeling van overgewicht en daaraan gerelateerde metabole dysfunctie. In **hoofdstuk 7** laten we zien dat genetische inactivatie van ASBT in muizen bescherming biedt tegen

obesitas en gerelateerde problemen in glucosemetabolisme ten gevolge van een hoog-vet dieet gebaseerd op dierlijk (varkens-) vet, hoog in verzadigde vetten. Deze veranderingen werden niet gezien op een hoog-vet dieet gebaseerd op sojaboonolie, hoger in meervoudig onverzadigde vetten. In overeenstemming met de eerdere observaties van een ASBT remmer op vetabsorptie, was de proportionele fecale uitscheiding van verzadigde ten opzichte van meervoudig onverzadigde vetzuren groter in ASBT knockout muizen in vergelijking met wildtype (WT) muizen. De reden dat er geen verschil in gewichtstoename was tussen ASBT knockout en WT muizen op het sojaboonolie dieet, zou derhalve het resultaat kunnen zijn van betere opname van meervoudig onverzadigde vetten in ASBT knockout muizen. Echter, in vergelijking met het op varkensvet gebaseerde hoog-vet dieet, zorgde het op sojaboonolie gebaseerde dieet ook in de WT controle muizen voor minder gewichtstoename. Dit wijst op een algemene verschillende verwerking van dieetvetzuren in muizen en onderstreept het belang van het kiezen van de juiste compositie van een hoog-vet dieet voor metabole studies.

In **hoofdstuk 7** tonen we tevens aan dat remming van ASBT zeer effectief is in het voorkomen van steatose in de lever en dat dit effect onafhankelijk is van de samenstelling van het gebruikte dieet. Hoewel de effecten van ASBT inhibitie op leversteatose robuust waren, was het onduidelijk of het ook de progressie van steatose naar de ernstigere NAFLD verschijnselen, ontsteking en fibrose, remt. Derhalve hebben wij in **hoofdstuk 8** de effecten van ASBT remming op NAFLD onderzocht in een choline deficiënt muismodel dat, in tegenstelling tot de meeste dieet geïnduceerde NAFLD muismodellen, wél inflammatie en fibrose ontwikkelt. Behandeling met een ASBT remmer in een choline deficiënt muismodel kon de ontwikkeling van fibrose niet voorkomen. Tevens werden de eerder consistente effecten op steatose evenals de vermindering van vetabsorptie niet geobserveerd. De onderliggende oorzaak voor het uitblijven van deze eerder consistente effecten van ASBT remming in de context van een choline deficiënt dieet werd niet gevonden. Als zowel de data van hoofdstuk 7, waar robuuste dieet onafhankelijke effecten werden gezien op vetabsorptie én steatose met ASBT inhibitie, en hoofdstuk 8, waar beide genoemde effecten afwezig waren, samen worden overwogen, kan men denken aan een mogelijke link tussen de verminderde vetabsorptie en de anti-steatose effecten van ASBT remming. Meer experimenten zijn noodzakelijk om dit verband aan te tonen dan wel uit te sluiten.

De resultaten van deze studies zijn van belang voor onze kennis over ASBT remming en de effecten op NAFLD. Echter is het belangrijk geen verregaande

conclusies te trekken omdat 1) het galzoutprofiel van muizen zeer verschillend is van mensen en 2) het choline deficiëntie model ver af staat van de NAFLD pathofysiologie in patiënten. Studies in andere modellen van NAFLD zijn noodzakelijk om de resultaten te verifiëren en de mogelijke implicaties voor patiënten te duiden.

De studies in dit proefschrift benadrukken de prominente doch complexe rol van galzouthomeostase in de regulatie van metabolisme. Deze rol is relevant voor multisysteemaandoeningen die de galzouthomeostase aandoen, zoals CF, waar galzoutmalabsorptie mogelijk bijdraagt aan verschillende complicaties. Tevens is moduleren van de galzouthomeostase (bijvoorbeeld door ASBT remming) een interessant nieuw aangrijpingspunt voor de preventie of behandeling van chronische metabole ziekten zoals NAFLD of type 2 diabetes mellitus.

Acknowledgements

This is arguably the easiest and simultaneously the hardest part of my thesis to write. It is obvious that this booklet would not be in front of you without the help and support of many great people. However, to include everyone and put the appropriate gratitude into words is difficult, especially for me, but here is a shot at it. Let me start by expressing my thanks to you, as in everyone who takes the time to pick up this piece of work, look at it, opened it up, browse through it and read some parts of it (even if it is only the acknowledgements). The knowledge that someone would be interested enough to pick up something that I wrote, means a lot to me. As for my gratitude towards the contribution of specific people to my (personal) life and work during my PhD time I could most likely write a separate book but I'll do my best to keep it brief and hope I'm not forgetting anyone.

First-off, I like to express major gratitude towards my supervisors **Hans Jonker** and **Henkjan Verkade**. I admire both of you, not only as scientists/researchers (your knowledge and expertise are outstanding!) but also as people. Either of you left necessary room for (discussing) personal aspects of life besides the science and professional parts which to me was really important to keep me on track. Arguably, there is more to life than science ;). The most important point I need to thank you both equally for, is the fact that you always kept believing in me and you were there to support me every step of the way. Even towards the end when I was bombarding you with manuscripts and questions to finish my thesis before new year's eve. Fun Christmas days!

Hans, thank you for your open personality and approachability. You always made time when I stepped into your office for questions or needed your opinion on something. Thank you for giving off a calm and controlled vibe in our meetings, keeping me focused while my mind was all over the place. Thanks for giving me the opportunity to go to different conferences, supporting me in my decision to go to the US and offering me a post-doc position on your new project. I also want to thank you and Henkjan both for that very short, spur of the moment, trip that we took to discuss the CF pig model in Germany. Even though it ended up amounting to nothing (so far!), the conversations and jokes in the endless car ride were worth the trip in itself.

Henkjan, I owe you a lot of gratitude to be where I am right now. I was hesitant, very hesitant, to take on the path to the PhD. Many sleepless nights preceded my decision to opt for the MD/PhD trajectory. In the conversations with you leading up to this decision, you never tried to convince me of what was the right decision, or

that there even was a right decision. You told me your story and helped me order my thought processes in a way that I don't think I could have done by myself. These conversations were not about the science or the project but about life, experience and emotion. Dubiety has always been a cornerstone of me as a person. Therefore (unsurprisingly), doubts were there everywhere along the way. However, when in real dire need, you were there to talk about them in every manner that was necessary, even if that meant sacrificing your own time on very busy clinic weeks. I am beyond grateful for that. Also, thanks for taking me seriously and getting me antibiotics (when my GP wouldn't) while we were in Germany and my leg was swelling like a ripe tomato (ha!).

I would also like to thank **Frank Bodewes,** co-author and co-promotor. While this thesis ended up with less actual clinical studies than I would have wanted due to a variety of reasons (most likely the main being, 2 years is not a very long time on the research clock), I would really like to thank you for your input, especially on the clinical related factors of the CF work. With your help I managed to write and get my first big grant, the Christina Onderzoek Subsidie (COS) 2017! I want to especially thank you for your immense help on the ivacaftor paper which, if not for you, it would for sure be still floating somewhere in the realm of 'sort of finished but unpublished manuscripts'.

I would like to thank **the reading committee**, prof. dr. R.P.J. Oude Elferink, prof. dr. S.C.D. van IJzendoorn and prof. dr. J.A. Kuivenhoven for taking the time to assess my thesis and deeming it sufficient to be accepted and ready for public defense. Thanks to the other **PIs of the department**, giving valuable input in meetings. Especially, dr. Janine Kruit (big thanks also for the opportunities to practice my teaching!), prof. Folkert Kuipers and prof. Bert Groen, your input at (BaCh/transport) meetings was of invaluable importance to my scientific development and thesis chapters.

A big thank you to my **paranymphs**.
Anna, you started out as a student of Marcela, became my student and you are now a PhD candidate yourself. I have seen you develop in this time in the lab, both as a scientist and as a person. Besides being my colleague and collaborator, you also grew to be a valuable friend. You have an amazingly talented and intelligent mind. I am grateful for the talks we had where both laughs and frustrations were shared. Even though we had our differences, your presence was vital along the

way. Without your help and collaboration, I would definitely not be where I am right now. This should be readily apparent by looking at the authors on the chapters. It is an honor to have you as my paranymph!
Bendiks, a buddy in medicine, science and party. Thank you for your caring and uplifting personality. You were always there to help me out when I needed it, even if that meant sitting down and doing nothing but writing down glucose numbers for a few hours. Thank you so much for being my paranymph!

I would like to express major gratitude to **everyone who helped me in my time in Atlanta**. First and foremost I want to thank prof. **Paul Dawson** for his expert guidance and mentorship. You have taught me so much in the relatively short period I was in the United States. Your incredible knowledge about everything bile acid related is astounding. Taking me to the MDI laboratory in Maine for the 'Frontiers of Hepatobiliary and Gastrointestinal Physiology' and meeting/receiving lessons (even on 'how to eat lobster'!) from some of the (other!) titans in the field like Jim Boyer, was an unforgettable experience.
I would also like to greatly thank dr. **Anuradha Rao**. Without your help and close collaboration, Anu, I would not have amounted to anything in my time in Atlanta. You are an absolutely amazing researcher. I am very glad to have witnessed and learned from your work in the lab that you carried out with skilled and meticulous precision.
Both of you, Paul and Anu, have an extremely dedicated work ethic while at the same time being very welcoming and open towards me. You wanted me to enjoy my stay, both at work and in my spare time. You showed me Atlanta and made me feel cared for. I hope you are taking enough time for yourselves, go for runs and enjoy the occasional good craft brew!
A massive thanks as well to the other people in the lab in Atlanta, prof. Saul Karpen, Jianing (I spelt it right this time?!), Kim and Ashley, for helping me out and being great colleagues.
Aside from the people that guided me professionally (AND also personally!), I need to thank some people that made my time in the US generally even more valuable. Specifically I would like to name: Tanner (I'm getting back into the gym!), Bejan (hope you will ultra-run to the Netherlands someday), Niora, Elizabeth, Kimberley, Dylan, Jessie, Katie and Anne (If you have Ghost?...). Raegan, you taught me so many things in such a short time and while you definitely did not make my time easier, you did make it more meaningful. Rachel, thank you for the endless talks

about life and music and taking me on the road in California while I was going through a rough patch mentally and physically.

A major thanks to the **Jonker group** and **all other colleagues at the UMCG**.
Marcela, thanks for showing me the ropes in the lab, supervising me and believing in me. I valued our talks, even when it was just sheer complaining about stuff not working out or shit data or life struggles. It was an honor to be your paranymph! Irene, thank you for the awesome time in Copenhagen and sharing the love for craft brews at WarPigs brewpub (:, Vera, thanks for all the good lunch talks about music and nutrition! Dicky, first as a technician, later as a PhD colleague and officemate, I must have asked you a thousand questions and you were always willing to help, cheers! Maaike B., I did not know making stinky chow cookies with ezetimibe could be so bonding. Good luck with them nasty TICE protocols! Marleen D., thanks for the good times in the lab (where you ALWAYS were.. or the CDP of course) and for working up all those samples from the experiment I finished literally the day before flying to the US. Chapter 7 would otherwise not have existed without you.
All other PhD and Post-Doc colleagues including: Tim (smart, skilled and (seemingly?!) always happy, thanks for your help!), Jan Freark (your knowledge is outstanding and thanks for all the practical tips in the lab!) , Onne (thanks for the good conversations while being 'in the hood' extracting those sterols), Mirjam, Archie, Marleen S., Fabio, Rima, Weilin, Karin, Ana, Mathilda, Lars, Jiufang, Christy, Andrea, Angela, Martijn, Sarah (thanks for helping me on the microbiota stuff even though it did not make it to the book in the end o:), Antoine, Joanne, Sandra (thanks for the period of nice and well needed coffee break talks!).
Big high five to my officemates for creating a good atmosphere for the heavy writing hours: Yana (you were an admirable example, always hardworking and seeing you after office hours), Ali (thanks for the good talks and helping each other out near the end of both our PhDs!), Daniel (thank you for always being open for a chat about virtually anything, very highly needed from time to time), Guido (a refreshing good taste in music and coffee, the important stuff!) and Natalia (you brought the necessary smiles and happiness to the office!).
Without the expertise and knowledge of the technicians I would probably have made it nowhere. A big thank you to: Renze, thanks for measuring all those big series of GC samples for me time after time. Niels, thank you for always being so friendly, open and helpful in the lab. I would usually bother you first with lab related questions because I thought (still do) you knew everything in the lab and you were

always willing to help. A true tower of strength! Rick, thank you for all your help with the bile cannulations. The days were usually intense but I remember them also as fun times with many good talks about music. Theo, thank you for all the time you invested in the in depth TICE calculations and explanations. Vincent, your critical advice on statistics was of great help!
Thanks also to all the other technicians for helping me out with questions or assays or just created a nice atmosphere in the lab including: Henk (always noting my choice of shirts!), Aycha (always great to see you around the lab for fun chats and thanks for organizing fun beer evenings!), Angelika (all that genotyping...), Theo B. (all those bloodspots...), Martijn (all those plasma and bile samples...), Trijnie, Ingrid, Tjasso, Manon, Jaenette & Dianne.

'Musica Salvum Me Facit'

Without **music** I don't know where I would be. I could write pages of odes to all the bands and artists that I listened to, went to shows of and helped me carry on during my PhD period but I will spare you the time. So here is a thank you to handful of important ones: Nick Cave, The Rattlesnakes (Frank, Dean, Gareth), Death Alley (Douwe, Oeds, Dennis, Uno), Une Misère (Jón, Finnbogi, too bad you guys couldn't play at my party :'( ), Code Orange, Iron Maiden, Zola Jesus, Deafheaven, Converge and many many many many more…
I would also like to give a shout out to the podcasts that kept my ears and brain busy while doing repetitive lab work. They kept me critical and taught me about skills and factors to improve a various aspects of my daily of life. I listened to a big variety of podcasts but I want to especially thank Danny Lennon, Tim Ferriss, Peter Attia and Sam Harris.

Lastly I want to thank the most important people in my personal life.
I have to mention some **my amazing friends** who kept me going and are always there to remind of enjoyments in life. Cindy, thank you for somehow always knowing how to level with me in talking about struggles in life. I will never forget that damn long Skype conversation in the US, discussing more important stuff in ~1.5 hours then I do with most people in a lifetime. Kyra, reconnecting after all those years was of vital importance to keep me going. Introducing me to new friends and experiences was highly necessary at the time. Thanks for being you! To both of you Cindy & Kyra, my best friends, while I would rather see you much more often, I feel the connection. Even when we don't see each other, I know you are there when I need it. Nothing will tear us up again, certainly not for 5 years!

Thanks to the core Groningen crew, Esther, Helen, Karin, Marije, Michelle & Maurits, for all the amazing evenings together! Thanks to the 'vatteranen', Jackie, Naomi, Bas, Femke, Jaccomijn, Joost (big additional thanks for making this book look rad!), Ruud, Sabine, Simone, Stefan, for all the talks, the parties, the affection and the hugs. Much love as well to all my concert buddies, including but definitely not limited to: Ashley (I cannot tell you how massive of a support you've been over the years, we both went through some rough patches at similar times, thanks for being there!), Jeroen, Dirk, Tim, Koen, Cynthia, Maud, Mathijs, Gerald, Richard, Luna, Leroy, Peter, Jamie, Jurgen, Tessa, Kor, Annegreet & Nynke. Beers were drunk, asses wobbled and heads banged! Cheers (:
Also, a shout out to all the people I met traveling, both when on holiday and for conferences! You are way too many to start naming but don't think I forgot about you or those great times o: !

Mom, **Nicoline**, thank you for always being concerned about my wellbeing above anything else. You always tried to poke through my wordless worries and were available for talks, whether it was about my scientific or personal struggles.
Dad, **Peter**, thank you for always being at the ready for small stupid things such as coming over to pick up my bike and get it fixed. These things saved me so much time and worries.
To both of you, mom and dad, I know I have definitely not always been easy to talk to (especially throughout my PhD period) but be cognizant of the fact that I always felt your love and readiness to be there for me. Thanks again for all of that and keeping faith in my abilities (which I lost 99% of the time)!
Lennart, blood brother, there is no one in this life I have more admiration for than you. Your staunch determination and dedication to your sports, science and everything else that you do in life is a major example. I might have gotten my PhD before you but I'm sure your thesis will blow mine out of the water when the time comes. I will always look up to you and am beyond proud to call you my brother and friend.

To all,
From the biliary bile acid secretions in my liver, to the deep dark microbial depths of my intestine, packed on the FGF19 rafts speeding in my venous portal blood flow, through my body, to my heart,

Thank you! 

Biography

Ivo Pieter van de Peppel was born on May 24th, 1991 in Brandwijk (gemeente Graafstroom), The Netherlands. He grew up in Rhenen and in 2006 moved to Assen where he finished pre-university education (VWO-gymnasium) at the CS Vincent van Gogh College. In 2009 he started studying medicine and obtained his degree in 2016. Most of his clinical rotations were done at the University Medical Center Groningen and Bethesda hospital in Hoogeveen, with a 3 month internship at the St. Kizito hospital in Mikumi, Tanzania. His studies were concluded at the department of internal medicine at the Wilhelmina hospital in Assen. Concurrently he studied Philosophy of Specific Discipline for which he received his bachelor degree in 2014. During his medicine bachelor he conducted two month-long research internships at the BRAINlab (University of Copenhagen, Prof. R. Kupers) and the department of nephrology (University of Groningen, Dr. M.A.J. Seelen). He conducted his master research internship working on cystic fibrosis and bile acid metabolism under supervision of Dr. M. Doktorova-Demmin, Prof. J.W. Jonker and Prof. H.J. Verkade. This lead to a successful application for an MD-PhD trajectory of 2 years which was extended for a 6 month period to do a project in Atlanta working on non-alcoholic fatty liver disease and ASBT inhibition with Dr. A. Rao supervised by Prof. P.A. Dawson and Prof. S.J. Karpen.

Biografie

Ivo Pieter van de Peppel was geboren op 24 mei 1991 te Brandwijk (gemeente Graafstroom). Hij groeide op in Rhenen en verhuisde in 2006 naar Assen waar hij zijn VWO-gymnasium diploma behaalde op het CS Vincent van Gogh college. In 2009 startte hij met de studie geneeskunde en verkreeg zijn doktersdiploma (Msc, Master of Science) in 2016. Hij liep het merendeel van zijn coschappen in het Universitair Medisch Centrum Groningen en Bethesda ziekenhuis in Hoogeveen met een extra stage van 3 maanden in het St. Kizito ziekenhuis in Mikumi, Tanzania. Hij rondde zijn studies af met een semiartsstage op de afdeling interne geneeskunde bij het Wilhelmina ziekenhuis te Assen. Naast geneeskunde studeerde hij ook Wijsbegeerte van een Bepaald Wetenschapsgebied waarvoor hij in 2014 zijn bachelorsdiploma ontving. Tijdens zijn geneeskundebachelor deed hij twee onderzoeksstages van een maand bij het BRAINlab (Universiteit van Kopenhagen, Prof. R. Kupers) en bij de afdeling nefrologie (Rijksuniversiteit Groningen, Dr. M.A.J. Seelen). Voor zijn master onderzoeksstage deed hij onderzoek naar cystische fibrose en galzoutmetabolisme onder supervisie van Dr. M. Doktorova-Demmin, Prof. J.W. Jonker en Prof. H.J. Verkade. Dit leidde tot een succesvolle MD-PhD aanvraag voor een 2 jarig promotie traject. Dit werd verlengd voor een project van 6 maanden bij de Emory University in Atlanta om te werken aan non-alcoholic fatty liver disease en ASBT remming samen met Dr. A. Rao onder supervisie van Prof. P.A. Dawson en Prof. S.J. Karpen.

List of publications

Van de Peppel IP, Bertolini A, Jonker JW, Bodewes FAJA, Verkade HJ. Diagnosis, follow-up and treatment of cystic fibrosis-related liver disease. *Current opinion in pulmonary medicine* 2017; 23(6): 562-569

Van de Peppel IP, Doktorova M, Berkers G, De Jonge HR, Houwen RHJ, Verkade HJ, Jonker JW, Bodewes FAJA. Ivacaftor restores FGF19 regulated bile acid homeostasis in cystic fibrosis patients with an S1251N or a G551D gating mutation. *Journal of Cystic Fibrosis* 2019; 18(2): 286-293

Van de Peppel IP, Bodewes FAJA, Verkade HJ, Jonker JW. Bile acid homeostasis in gastrointestinal and metabolic complications of cystic fibrosis. *Journal of Cystic Fibrosis* 2018 Sep; doi:10.1016/j.jcf.2018.08.009. [Epub ahead of print]

Bertolini A, Van de Peppel IP, Doktorova-Demmin M, Bodewes FAJA, De Jonge H, Bijvelds M, Verkade HJ, Jonker JW. Defective FXR-FGF15 signaling and bile acid homeostasis in cystic fibrosis mice can be restored by the laxative polyethylene glycol. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 2019;316(3):G404-G411

Van de Peppel IP, Bertolini A, Van Dijk TH, Groen AK, Jonker JW, Verkade HJ. Efficient reabsorption of transintestinally excreted cholesterol is a strong determinant for cholesterol disposal in mice. *Under review, Journal of Lipid Research*.