PUBLICATIONS

The PhD thesis is based on the following 3 scientific publications:

1. **Christensen L**, Roager HM, Astrup A & Hjorth MF.
   Microbial enterotypes in personalized nutrition and obesity management. American Journal of Clinical Nutrition. 2018 Oct 1;108(4):645-651. doi: 10.1093/ajcn/nqy175

2. **Christensen L,** Vuholm S, Roager HM, Nielsen DS, Krych L, Kristensen MB, Astrup A & Hjorth MF. *Prevotella* abundance predicts weight loss success in healthy, overweight adults consuming a whole-grain diet ad libitum: A post hoc analysis of a 6-wk randomized controlled trial. Journal of Nutrition. 2019 Dec 1;149(12):2174-2181. doi: 10.1093/jn/nxz198

3. **Christensen L,** Sørensen CV, Wøhlk FU, Kjølbæk L, Astrup A, Sanz Y, Hjorth MF & Benítez-Páez A. Microbial enterotypes beyond genus level: *Bacteroides* species as predictive biomarker for weight change upon controlled intervention with arabinoxylan oligosaccharides in overweight subjects. Gut Microbes. 2020 Nov 9;12(1):1847627. doi: 10.1080/19490976.2020.1847627

During the PhD period (2018-2021), I authored or co-authored 6 other publications:

- Brunse A, Martin L, Rasmussen TS, **Christensen L**, Cilieborg MS, Wiese M, Khakimov B, Pieper R, Nielsen DS, Sangild PT & Thymann T. Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. ISME J. 2019 Mar;13(3):720-733. doi: 10.1038/s41396-018-0301-z

- **Christensen L**, Thorning TK, Fabre O, Legrand R, Astrup A & Hjorth MF. Metabolic improvements during weight loss: The retrospective RNPC® cohort. Obesity Medicine. 2019 March. doi: 10.1016/j.obmed.2019.100085

- Hjorth MF, **Christensen L**, Kjølbæk L, Larsen LH, Roager HM, Kiilerich P, Kristiansen K & Astrup A. Pretreatment *Prevotella*-to-*Bacteroides* ratio and markers of glucose metabolism as prognostic markers for dietary weight loss maintenance. Eur J Clin Nutr. 2020 Feb;74(2):338-347. doi: 10.1038/s41430-019-0466-1

- Hjorth MF, **Christensen L**, Larsen TM, Roager HM, Krych L, Kot W, Nielsen DS, Ritz C & Astrup A. Pretreatment *Prevotella*-to-*Bacteroides* ratio and salivary amylase gene copy number as prognostic markers for dietary weight loss. Am J Clin Nutr. 2020 May 1;111(5):1079-1086. doi: 10.1093/ajcn/nqaa007

- Matusheski N, Caffrey A, **Christensen L**, Mezgec S, Surendran S, Hjorth MF, McNulty H, Pentieva K, Roager HM, Koroušić Seljak B, et al. Diets, nutrients, genes and the microbiome: Recent advances in personalised nutrition. Br J Nutr. 2021 pp. 1-24 24 s. doi: 10.1017/S0007114521000374

- Benítez-Páez A, Hess AL, Krautbauer S, Liebisch G, **Christensen L**, Hjorth MF, Larsen TM & Sanz Y. Sex, food, and the gut microbiota: disparate response to caloric restriction diet with fibre supplementation in women and men. Molecular Nutrition and Food Research. 2021 Feb 24;e2000996. doi: 10.1002/mnfr.202000996

SUMMARY

Weight loss diets have been tested for decades aiming at finding effective treatments to combat the continuing rise in obesity prevalence. However, studies consistently show that there is large variation in weight loss success among participants following such interventions. Several individual characteristics such as host genes and gut microbiota may help increase our understanding of why some subjects respond to a given weight loss diet and others do not. A compositional pattern of the microbiota, enterotypes, has during the last decade been associated with both dietary intake and metabolic health. Recently, enterotypes were also linked to weight loss success, as individuals with the *Prevotella* enterotype was shown to lose weight on a New Nordic diet, rich in fiber, while the group with a *Bacteroides* enterotype remained weight stable on the same diet.

Accordingly, the overall objective of this PhD thesis was to explore the potential of microbial enterotypes as biomarkers to individualize dietary treatment in obesity. More specifically, two independent studies examine whether enterotypes can predict weight loss among overweight individuals following consumption of high fiber diets compared to a control diet. The three objectives and consequent papers are described below:

1. To review the existing literature on enterotype stratification in dietary and medical interventions
2. To investigate weight loss success between enterotypes following whole grain consumption, and to explore if starch digestion in the upper gastrointestinal tract influences these potential enterotype-weight loss associations
3. To examine weight loss success between enterotypes following whole grain fiber (arabinoxylan-oligosaccharide) consumption and correlate relevant bacterial species with host metabolism

In **Paper 1**, we concluded that high-fiber diets seems to promote weight loss among *Prevotella* enterotype subjects, but not among *Bacteroides* enterotype subjects. In contrast, increasing bifidobacteria abundance in the gut for the *Bacteroides* enterotype appears to improve metabolic parameters, suggesting that this approach should be tested as an alternative weight loss strategy.

In **Paper 2**, we found that overweight adults with high *Prevotella* abundances lost more weight than individuals with low abundances of *Prevotella*, when consuming whole grains *ad libitum*. Moreover, the digestive capacity for starch degradation (amylase gene, AMY1) was found to mediate the weight loss predictability of *Prevotella*, as it was only among the participants with a low AMY1 copy number

that *Prevotella* was linked to weight loss, and thus, not among the participants with a high AMY1 copy number.

In **Paper 3**, a *Bacteroides* species was found to be a better predictor of body weight regulation than the proxy of enterotypes, *Prevotella*-to-*Bacteroides* ratio, following consumption of whole grain fibers (arabinoxylan-oligosaccharides). Also, this data revealed a potentially new subtype of the *Bacteroides* enterotype with increased abundance of *B. cellulosilyticus,* which is linked to weight gain, increased cholesterol synthesis, and is inversely correlated with specific arabinoxylan-degrading clades of *Prevotella (copri)*.

In summary, the work presented in this PhD supports a link between microbial enterotypes and body weight, when a diet rich in whole grain fibers is consumed. However, the results also underline that future studies should explore enterotypes beyond genus level and include relevant host genes (i.e. AMY1) in combination with microbial metabolites (i.e. SCFA) in comprehensive multi omics analyses to fully entangle the interactions between enterotype-related species, dietary fiber, and host metabolism.

SUMMARY IN DANISH

I årtier er vægttabsdiæter blevet undersøgt med det formål at finde effektive behandlinger til at bekæmpe den fortsatte stigning i fedme. Studierne viser imidlertid relativt konsekvent at der er stor variation i vægttabssucces blandt deltagerne efter sådanne interventioner. Flere individuelle karakteristika såsom genetik og tarmbakterier kan måske hjælpe med at øge vores forståelse af hvorfor nogle personer responderer på en given vægttabsdiæt, mens andre ikke gør. Et mønster i tarmbakteriesammensætning, "enterotyper", er i løbet af det sidste årti blevet forbundet med både kostindtag og metabolisk sundhed. For nyligt blev enterotyper også linket til vægttabssucces, da man viste at individer med *Prevotella* enterotype tabte sig effektivt på en Ny Nordisk kost, rig på kostfibre, mens individer med en *Bacteroides* enterotype forblev vægtstabile.

I forlængelse heraf, var det overordnede mål med denne PhD-afhandling at undersøge potentialet for enterotyper som biomarkører til at individualisere diætbehandling mod fedme. Mere specifikt i to uafhængige undersøgelser, at vurdere om enterotyper kunne forudsige vægttab blandt overvægtige individer efter indtag af fuldkornskostfibre sammenlignet med en kontrol diæt. De tre formål og resulterende artikler er beskrevet nedenfor:

1. At gennemgå den eksisterende litteratur om enterotype stratificering i kost og medicinske studier

2. At undersøge vægttabssucces mellem enterotyper efter fuldkornsindtag. Derudover at undersøge om fordøjelse af stivelse i den øvre del af mave-tarmkanalen påvirker potentielle enterotype-vægttabs associationer

3. At undersøge vægttabssucces mellem enterotyper efter indtag af specifikke fuldkornsfibre (arabinoxylan-oligosakkarider) og korrelere relevante bakteriearter med værtsmetabolisme

I **artikel 1** konkluderede vi, at fiberrige diæter ser ud til at fremme vægttab blandt forsøgspersoner med *Prevotella* enterotypen, men ikke blandt *Bacteroides*-enterotype forsøgspersoner. I modsætning hertil tyder studier på at en stigning i forekomst af bifidobakterier kan forbedre metaboliske parametre for *Bacteroides*-enterotypen, hvilket antyder at denne tilgang kunne undersøges som en alternativ vægttabsstrategi.

I **artikel 2** fandt vi at overvægtige voksne med høj forekomst af *Prevotella* tabte sig mere end overvægtige voksne med lav forekomst af *Prevotella*, når de spiste fuldkornsrig kost *ad libitum*. Desuden viste data at fordøjelseskapaciteten for stivelsesnedbrydning (spyt amylase gen, AMY1) medierede *Prevotella*'s evne til at forudsige vægttab, da det kun var blandt deltagerne med lavt antal

AMY1 kopier at *Prevotella* var linket til vægttab, og dermed ikke blandt deltagerne med et højt AMY1 kopi nummer.

I **artikel 3** fandt vi at en *Bacteroides* art var en bedre "biomarkør" for kropsvægtregulering end enterotyper efter indtag af fuldkornsfibre (arabinoxylan-oligosakkarider). Disse data afslørede også en potentielt ny undergruppe af *Bacteroides* enterotypen med øget forekomst af *B. cellulosilyticus*, som var korreleret med vægtøgning, øget kolesterolsyntese, og var omvendt korreleret med specifikke arabinoxylan-nedbrydende grupper af *Prevotella (copri)*.

Sammenfattende understøtter arbejdet i denne PhD afhandling en sammenhæng mellem enterotyper og vægtændring, når personer med overvægt spiser en diæt rig på fuldkornfibre. Resultaterne understreger imidlertid også, at fremtidige undersøgelser bør studere enterotyper på et dybt taksonomisk niveau og omfatte relevante værtsgener (AMY1) i kombination med mikrobielle metabolitter (fx kortkædede fedtsyrer) i omfattende multi-omics analyser for fuldt ud at forstå interaktionerne mellem enterotype-relaterede arter, kostfibre og værtsmetabolisme.

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