Publications and Scientific Contributions

List of Included Papers

- Madsen MTB, Biltoft-Jensen AP, Trolle E, Lauritzen L, Michaelsen KF, Damsgaard CT. **WG intake, growth and metabolic markers in Danish infants and toddlers: a longitudinal study**. *Eur J Nutr*. 2022;61(7):3545-3557. doi:10.1007/s00394-022-02902-2
- Madsen MTB, Landberg R, Nielsen DS, Zhang Y, Anneberg OMR, Lauritzen L, Damsgaard CT. **Effects of Wholegrain Intake on Cardiometabolic Risk Markers, Gut Microbiota and Gut Health in Children: A Randomized Crossover Trial.** Submitted to American Journal of Clinical Nutrition.
- Madsen MTB, Christensen L, Zhang Y, Nielsen DS, Lauritzen L, Landberg R, Damsgaard CT. **Exploring the Impact of Gut Microbial Clusters on Early Cardiometabolic Risk Markers in a Randomized Wholegrain Intervention in Children**. In preparation.

Contributions to National and International Conferences

- The Danish Association for the Study of Obesity, annual meeting, November 2021, Nyborg. Poster presentation with the title: Wholegrain intake, body mass index and metabolic markers in Danish infants and toddlers – a longitudinal study.
- Danish Nutrition Society, annual meeting, October 2022, Copenhagen. Oral presentation with the title: High whole grain intake from rye and oat improves lipid metabolism and gut microbiota-derived metabolites among schoolchildren with overweight: a randomized controlled cross-over study.
- International Union of Nutritional Sciences - International Congress of Nutrition, December 2022, Tokyo. Oral presentation with the title: Wholegrain intake, growth and metabolic markers in Danish infants and toddlers – a longitudinal study. Received Young Investigator Excellent Abstract Award.
- NEXS Research day, April 2023. PhD blitz talk with the title: Wholegrain intake and cardiometabolic health in children.

Summary

Background: Wholegrain intake has been associated with reduced risk of cardiovascular disease and type 2 diabetes and linked to improved gut health in adults, however randomized studies involving mixed wholegrains have yielded inconsistent effects. In contrast, research has frequently shown beneficial cardiometabolic effects of wholegrain oats and wholegrain rye in adults, and this has also been suggested by an association study involving schoolchildren. However, only one randomized trial, using mixed wholegrain subtype, has been conducted in children and the underlying mechanisms are currently unresolved. Danish adults and children have high estimated wholegrain intakes compared to counterparts in other countries, but data on wholegrain intake among Danish infants and toddlers is lacking and the potential risk of high wholegrain intake on young children's growth is unclear. Furthermore, emerging evidence has demonstrated that the cardiometabolic effects of wholegrain interventions in adults may depend on the gut microbiota composition at baseline. At present, there is a lack of quantitative wholegrain recommendations for infants in Denmark, and the existing recommendation for the general population lacks support from studies involving children. Furthermore, it does not account for potential differences between wholegrain subtypes or individual responses to wholegrain intake.

Objective: The overall objective of the present thesis was to characterize wholegrain intake among young Danish children and elucidate the role of wholegrain intake, particularly from oats and rye, on children's growth, body composition, cardiometabolic markers, gut microbiota and gut wellbeing. Furthermore, it aims to elucidate potential underlying mechanisms behind the cardiometabolic effects of wholegrain consumption, with a particular focus on the gut microbiota and short-chain fatty acids (SCFA). Lastly, it sought to identify gut microbial profiles in children, which could potentially modulate the response to wholegrain intake.

Methods: This thesis included findings from an observational study of 439 young children (**Paper I**) and from a randomized crossover study of 55 schoolchildren (**Paper II** and **Paper III**). In **Paper I**, we used merged data from two previous cohorts of healthy Danish children at 9 and 36 months of age (corresponding to infants and toddlers), including 7-days dietary records, anthropometric measurements and blood samples. These data were used to characterize the intake of wholegrain and dietary fiber intake among Danish infants and toddlers and to study the

associations between wholegrain intake and blood lipids, glucose and insulin at the two time points. In **Paper II**, we investigated the cardiometabolic effects of wholegrain intake among healthy 8- to 13 years-old children with a high body mass index (BMI). We compared wholegrain oats and wholegrain rye treatment (WG) to refined grain treatment (RG), consumed *ad libitum* during 2x8 weeks in random order. Serum low-density lipoprotein cholesterol (LDL-C) and plasma insulin from fasting samples served as co-primary outcomes, and we evaluated compliance by self-reported study product intake and plasma alkylresorcinols (AR). Other outcomes included anthropometric measures, blood pressure (BP) and body composition by Dual-energy X-ray absorptiometry and additional fasting blood markers i.e. high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triacylglycerol (TG), inflammatory markers and SCFA. Furthermore, we included gut microbiota composition (16S rRNA) and SCFA from fecal samples and gut symptoms and stool characteristics from questionnaires. In **Paper III** we investigated whether Danish children could be clustered, based on their gut microbial composition. Furthermore, we explored whether the acquired gut microbial clusters determined the cardiometabolic response to wholegrain intake, by post-hoc analyses based on data from **Paper II**.

Results: Slightly more than half of the infants and a lower proportion of toddlers adhered to the Danish daily wholegrain recommendation of 7.5g/MJ, while the majority of infants and toddlers followed the daily dietary fiber recommendation of 2 g/MJ (**Paper I**). Wholegrain intake in toddlerhood was inversely associated with plasma LDL-C ($P=0.05$), and directly associated with plasma glucose ($P<0.001$). Neither wholegrain intake nor dietary fiber intake demonstrated any significant association with age- and sex-adjusted height (HAZ) or BMI (BMIZ) in young children ($P\geq0.10$) (**Paper I**). In the randomized trial in schoolchildren, the wholegrain intakes were 108 ± 38 g/d and 3 ± 2 g/d in the WG and RG period, respectively and the reported wholegrain intake directly correlated with plasma AR ($P>0.001$) (**Paper II**). WG reduced serum LDL-C, TG and the TC:HDL-C ratio compared to RG (all: $P\leq0.035$; LDL-C: -0.13 (-0.22, -0.04) mmol/L, $P=0.008$), without any effect on plasma insulin. WG did not affect BMIZ and fat mass index (FMI), BP, plasma glucose or serum inflammatory markers compared to RG. WG modulated specific gut bacterial taxa, including increased relative abundance of *Faecalibacterium* ($P=0.031$) and SCFA, in particular plasma and fecal butyrate ($P\leq0.001$). Both butyrate and acetate in plasma and feces correlated with reduced LDL-C (**Paper II** and **Additional results from KORN**). Furthermore, WG reduced fatigue ($P=0.013$) and tended to

increase stool frequency compared to RG (**Paper II**). Two gut microbial clusters were identified in Danish schoolchildren at baseline: a *Firmicutes* and *Bacteroides* predominated cluster (cluster 1) and a *Bifidobacteria* and *Clostridium* predominated cluster (cluster 2), and the latter had slightly lower diversity (**Paper III**). These clusters modulated the effect of WG on FMI i.e. only children with cluster 1 had reduced FMI in response to WG compared to RG (**Paper III**).

Conclusion: Overall, this thesis demonstrated a favorable role of wholegrain consumption on children's health, in particular in relation to the lipid profile. This was partly indicated by an inverse association between wholegrain intake and plasma LDL-C in toddlers without any evidence of compromised growth in young children. Furthermore, intervention with wholegrain from oats and rye reduced several members of the lipid profile and in particular LDL-C. Results from this thesis do not support a role of wholegrain intake on BP or markers of glucose homeostasis and inflammation in children, and the direct association between wholegrain intake and glucose in toddlers may derive from non-fasting, and thus needs further investigation. We also found that wholegrain from oats and rye modulated the gut microbiota and increased SCFA production. Correlations indicated a potential link between increased SCFA and reduced LDL-C, suggesting that SCFA may drive parts of the observed beneficial effect on LDL-C. Furthermore, the identified baseline gut microbiota clusters in Danish schoolchildren determined the effect of wholegrain intake on FMI, suggesting a benefit for schoolchildren with a more diverse gut microbiota with butyrate-producing capacity. In summary, this thesis highlights the benefits of wholegrain intake, particularly from oats and rye, among children and particularly in relation to their blood lipids. It also indicates a benefit of wholegrain intake in relation to body composition in children with certain gut microbiota characteristics. Future wholegrain trials should be designed to elucidate the effects of wholegrain intake in young children and to explore other potential underlying mechanisms beyond SCFA in children.

Sammendrag (Danish summary)

Baggrund: Fuldkornsindtag er blevet associeret med en reduceret risiko for hjerte-kar-sygdomme og type 2-diabetes samt forbedret tarmsundhed hos voksne. Randomiserede studier med forskellige typer af fuldkorn har dog vist inkonsistente effekter på hjertemetaboliske risikomarkører. Derimod har studier hyppigt vist fordelagtige hjertemetabolske effekter af fuldkorn fra havre og rug hos voksne, og dette er også blevet antydet i et associationsstudie hos skolebørn. Imidlertid er der kun blevet udført et randomiseret studie med forskellige typer af fuldkorn hos børn, og de underliggende mekanismer er i øjeblikket uafklarede. Voksne og børn i Danmark har et højt estimeret indtag af fuldkorn sammenlignet med folk i andre lande, men data på fuldkornsindtag blandt danske småbørn mangler, og den potentielle risiko ved et højt fuldkornsindtag på småbørns vækst er uvis. Desuden har nyere forskning vist, at de hjertemetabolske virkninger af fuldkornsindtag hos voksne muligvis er afhængig af individets bakteriesammensætning i tarmen. På nuværende tidspunkt mangler der en kvantitativ anbefaling af fuldkornsindtag til børn under to år i Danmark, og den eksisterende anbefaling for den generelle befolkning mangler evidens fra randomiserede studier hos børn. Desuden tager anbefalingen ikke hensyn til potentielle forskelle mellem typer af fuldkorn eller individuelle respons på fuldkornsindtag.

Formål: Det overordnede formål med denne afhandling var at karakterisere fuldkornsindtaget blandt danske småbørn og klarlægge rollen af fuldkornsindtag, især fra havre og rug, for børns vækst, kropssammensætning, hjertemetaboliske markører samt tarmens mikrobiota og velbefindende. Derudover havde den til formål at klarlægge potentielle underliggende mekanismer bag de hjertemetabolske virkninger af fuldkornsindtag, med særlig fokus på tarmens mikrobiota og kortkædede fedtsyrer (SCFA). Endelig søgte den at identificere profiler i tarmens mikrobiota, som potentielt kunne have betydning for individets respons på fuldkornsindtag.

Metoder: Denne afhandling benyttede data fra et prospektivt studie af 439 småbørn (**Artikel I**) og fra et randomiseret overkrydsningsstudie blandt 55 skolebørn (**Artikel II** og **Artikel III**). I **Artikel I** anvendte vi sammenflettede data fra to tidligere kohorter af sunde danske småbørn fra 9 og 36 måneder, herunder 7-dages kostregistreringer, antropometriske målinger og blodprøver. Disse data blev brugt til at karakterisere indtaget af fuldkorn og kostfiber blandt danske småbørn ved 9 og 36 måneder og til at undersøge sammenhængen mellem indtag af fuldkorn og

blodlipider, glukose og insulin på de to tidspunkter. I **Artikel II** undersøgte vi de hjertemetabolske effekter af fuldkornsindtag hos sunde 8- til 13-årige børn med et højt body mass index (BMI). Vi sammenlignede effekten af fuldkorn fra havre og rug (WG) med raffinerede korn (RG), som blev indtaget *ad libitum* i 2x8 uger i tilfældig rækkefølge. Serum lavdensitetslipoproteinkolesterol (LDL-C) og plasma insulin udgjorde de primære udfald, og vi vurderede compliance ved selvrapporteret indtag af forsøgsprodukterne og plasma alkylresorcinoler (AR). Øvrige udfald omfattede antropometriske mål, blodtryk (BP) og kropssammensætning ved DEXA-skanning samt andre markører i blod såsom højdensitetslipoproteinkolesterol (HDL-C), total kolesterol (TC), triacylglycerol (TG), inflammationsmarkører og SCFA. Desuden inkluderede vi tarmens mikrobiota sammensætning (16S rRNA) og SCFA fra afføringsprøver samt tarmsymptomer og afføringskarakteristika fra spørgeskemaer. I **Artikel III** undersøgte vi, om danske børn kunne profileres baseret på deres sammensætning af tarmens mikrobiota. Derudover undersøgte vi om de erhvervede tarm mikrobiota profiler bestemte det hjertemetaboliske respons på fuldkornsindtag, ved post-hoc analyser baseret på data fra **Artikel II**.

Resultater: Lidt mere end halvdelen af de 9 måneder gamle børn og en lavere andel af de 36 måneder gamle børn overholdt den danske daglige fuldkornsanbefaling på 7,5 g/MJ, mens flertallet af småbørnene fulgte den daglige anbefaling for kostfiber på 2 g/MJ (**Artikel I**). Indtaget af fuldkorn ved 36 måneder var omvendt associeret med plasma LDL-C (P=0,05) og direkte associeret med plasma glukose (P<0,001). Hverken indtag af fuldkorn eller kostfiber viste nogen signifikant sammenhæng med alders- og kønsjusteret højde (HAZ) eller BMI (BMIZ) hos småbørn (P≥0,10) (**Artikel I**). I det randomiserede studie blandt skolebørn var indtaget af fuldkorn 108 ± 38 g/dag i WG perioden og 3 ± 2 g/dag i RG perioden, og det rapporterede fuldkornsindtag var direkte korreleret med plasma AR (P>0,001) (**Artikel II**). WG reducerede serum LDL-C, TG og TC:HDL-C ratioen i forhold til RG (alle: P≤0,035; LDL-C: -0,13 (-0,22, -0,04) mmol/L, P=0,008), uden nogen effekt på plasma insulin. WG påvirkede ikke BMIZ og fedtmasseindeks (FMI), blodtryk, plasma glukose eller serum inflammationsmarkører sammenlignet med RG. WG havde en effekt på specifikke tarmbakterier, herunder øget relativ forekomst af *Faecalibacterium* (P=0,031) og øgede SCFA, især butyrat i plasma og afføring (P≤0,001). Både butyrat og acetat i plasma og afføring korrelerede med sænket LDL-C (**Artikel II** og yderligere resultater fra KORN). Derudover reducerede WG træthed (P=0,013) og havde en tendens til at øge afføringsfrekvensen sammenlignet med RG (**Artikel II**). To tarmmikrobiota

profiler blev identificeret hos danske skolebørn: en profil domineret af *Firmicutes* og *Bacteroides* (profil 1) og en profil domineret af *Bifidobacterium* og *Clostridium* (profil 2), hvor sidstnævnte havde lidt lavere diversitet (**Artikel III**). Disse profiler påvirkede effekten af WG på FMI; kun børn med profil 1 fik reduceret deres FMI som respons på WG i forhold til RG (**Artikel III**).

Konklusion: Denne afhandling påviste den gavnlige rolle af fuldkornsindtag i forhold til børns sundhed, især i relation til lipidprofilen. Dette blev delvist indikeret af en omvendt association mellem fuldkornsindtag og plasma LDL-C hos småbørn uden tegn på hæmmet vækst. Intervention med fuldkorn fra havre og rug sænkede flere blodlipider, og især LDL-C. Resultaterne fra denne afhandling understøtter ikke en rolle for fuldkorn i forhold til blodtryk eller markører for glukosehomeostase og inflammation hos børn, og den direkte sammenhæng mellem fuldkornsindtag og glukose hos småbørn kan skyldes at de ikke var fastende, så dette kræver yderligere undersøgelse. Vi fandt også, at fuldkorn fra havre og rug påvirkede tarmens mikrobiota og øgede produktionen af SCFA. Korrelationer indikerede en potentiel forbindelse mellem øget SCFA og reduceret LDL-C, hvilket tyder på, at SCFA kan bidrage delvist til den observerede gavnlige effekt på LDL-C. Desuden bestemte de identificerede baseline tarmmikrobiota profiler hos danske skolebørn effekten af fuldkornindtag på FMI, og antyder fordele for skolebørn med en mere divers tarmmikrobiota med butyrat-producerende kapacitet. Overordnet set fremhæver denne afhandling fordelene ved at spise fuldkorn, især fra havre og rug, blandt børn, og især med fokus på deres blodlipider. Den indikerer også fordele ved fuldkornsindtag i forhold til kropsammensætning hos børn med visse karakteristika i tarmmikrobiotaen. Fremtidige fuldkornsstudier bør designes til at belyse effekterne af fuldkornindtag hos småbørn og undersøge andre potentielle underliggende mekanismer ud over SCFA hos børn.

Abbreviations

16S rRNA: 16S Ribosomal RNA
AACC: American Association for Clinical Chemistry
AMPK: Adenosine Monophosphate-Activated Protein Kinase
AR: Alkylresorcinols
BMI: Body Mass Index
BMIZ: BMI Z-score
BP: Blood Pressure
CCK: Cholecystokinin
CRP: C-Reactive Protein
CVD: Cardiovascular Disease
DBP: Diastolic Blood Pressure
DXA: Dual-Energy X-ray Absorptiometry
EFSA: European Food Safety Authority
FFA: Free Fatty Acids
FGID: Functional Gastrointestinal Disorders
FM %: Fat Mass Percent
FMI: Fat Mass Index
FOS: Fructooligosaccharides
GLP-1: Glucagon-Like Peptide 1
GPR41: G-protein-coupled receptor 41
GPR43: G-protein-coupled receptor 43
HAZ: Height-for-Age Z-score
HDAC: Histone Deacetylase
HDL-C: High-Density Lipoprotein Cholesterol
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance
IBS: Irritable Bowel Syndrome
IL-6: Interleukin-6
ITF: Inulin-type Fructans
LDL-C: Low-Density Lipoprotein Cholesterol
NEXS: Department of Nutrition, Exercise, and Sports
NNR2023: Nordic nutrition recommendations from 2023
PYY: Peptide YY
RG: refined grain treatment (in the KORN study)
SBP: Systolic Blood Pressure
SCFA: Short-Chain Fatty Acids
T2D: Type 2 Diabetes
TC: Total Cholesterol
TC:HDL-C: Total Cholesterol:High-Density Lipoprotein Cholesterol ratio
TG: Triacylglycerol
TNF-α: Tumor Necrosis Factor-Alpha
VLDL: Very-Low-Density Lipoprotein
WC: Waist Circumference
WG: Wholegrain oats and wholegrain rye treatment (in the KORN study)
WHO: World Health Organization

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