

Diet and gut microbiota in cardiometabolic health:  
Studies from the Danish Cohort Diet, Cancer and Health – Next Generations

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**ABSTRACT**

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Cardiometabolic diseases (CMD) are the leading cause of death globally. Diet is a key preventive factor of CMD and a determinant of gut microbiota. Gut microbiota, diet and their interactions have been associated with CMD. In observational studies, diet is measured by self-reported instruments, that need to be validated before use. Gut bacteria vary over time within an individual, making it challenging to study their relationship with health outcomes. Moreover, different dietary patterns may be associated differently with gut microbiota, but few studies exist.

The overall aim of this thesis work was to investigate the role of diet, gut microbiota and their interplay in cardiometabolic health. The MAX sub-cohort from the Diet, Cancer and Health – Next generations (DCH-NG) cohort was established to: validate the DCH-NG food frequency questionnaire (FFQ), validate a dietary quality score (DQS) and associate it with CMD risk factors, investigate gut microbiota temporal variability and associate these with dietary patterns and investigate the direct and indirect effects of a healthy Nordic and Mediterranean diet on CMD risk factors, mediated by gut microbiota.

The FFQ provided satisfactory ranking of individuals according to energy and nutrient intakes. The DQS was useful to rank individuals into groups of having unhealthy, average and healthy dietary habits. Healthy dietary habits were associated with lower levels of several CMD risk factors. Among bacterial genera, 39% had moderate to good reproducibility (ICC>0.5). Gut microbial subgroups (*Bacteroides*, *Prevotella* 9 and *Ruminococcaceae*) were identified and adherence to plant-based dietary patterns differed between subgroups. Healthy Nordic and Mediterranean diets were associated with lower levels of adiposity, but no indirect effect mediated by gut microbiota (*Prevotella*-to-*Bacteroides* ratio) was found. However, healthy Nordic and Mediterranean diets were associated with lower levels of lipidemia and hs-CRP, mediated by adiposity.

In conclusion, the DCH-NG FFQ can be used to rank individuals according to dietary intake in epidemiological studies and the DQS is a good indicator of overall diet quality. Different dietary patterns associated differently with gut microbial subgroups and specific genera. There was an effect of diet on CMD risk factors, though this effect was not mediated by the gut microbiota.

*Keywords:* food frequency questionnaire, 24-hour dietary recall, validity, reproducibility, dietary patterns, nutrients, gut microbiota, temporal variation, risk factors, cardiometabolic diseases, cohort study, epidemiology

## RESUMÈ

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Kardiometaboliske sygdomme (CMD) er den mest dominerende dødsårsag på globalt plan. Kosten spiller en afgørende rolle i forbindelse med forebyggelse af CMD og har også en stor indflydelse på tarmmikrobiotaen. Undersøgelser peger på, at der er en sammenhæng mellem tarmmikrobiotaen, kosten samt deres interaktion i forhold til risiko for CMD. I observationelle studier anvendes selvrapporteringsværktøjer, som fx spørgeskemaer, til måling af kostindtag. Disse bør valideres før brug. Tarmbakterier varierer over tid hos individer, hvilket er en udfordring i studier, der undersøger tarmbakterierne i relation til kost og sygdom. Derudover er forskellige kostmønstre muligvis relateret til tarmmikrobiotaen på forskellig vis, dog er der stadig få studier som har undersøgt dette i en dansk population.

Det overordnede formål var at undersøge kosten, tarmmikrobiotaen og deres indbyrdes samspil i forhold til kardiometabolisk sundhed. MAX-sub-kohorten blev etableret som en del af Kost, Kræft og Helbred – Næste Generationer (KKH-NG) kohorten for at validere KKH-NG fødevarerfrekvensspørgeskemaet (FFQ); undersøge en kostscore i relation til risikofaktorer for CMD; undersøge den tidsmæssige variation af tarmmikrobiotaen og dens sammenhæng med plantebaserede kostmønstre samt at undersøge de direkte og indirekte effekter af en sund kost (Nordisk- og Middelhavskost) på risikofaktorer for CMD, medieret af tarmmikrobiotaen.

FFQen viste en acceptabel rangering af individer baseret på energi- og næringsstofindtag. Kostscoren var i stand til at kategorisere individer i grupper med henholdsvis usunde, mellem og sunde kostvaner. Sunde kostvaner var associeret med lavere niveauer af flere risikofaktorer for CMD. 39% af de identificerede bakterieslægter viste moderat til god reproducerbarhed (ICC>0,5). Der blev desuden identificeret tarmmikrobiota subgrupper (*Bacteroides*, *Prevotella* 9 og *Ruminococcaceae*). Overholdelse af plantebaserede kostmønstre var forskellig for disse subgrupper. En sund Nordisk- og Middelhavskost var associeret med lavere niveauer af adipositas, men der var ingen indirekte effekt via tarmmikrobiotaen (*Prevotella*-til-*Bacteroides* ratio). Dog var disse kostmønstre associeret med lavere niveauer af dyslipidæmi og hs-CRP og en del af denne effekt var indirekte via adipositas.

Konklusion: KKH-NG FFQen kan anvendes til at rangere individer baseret på deres kostindtag i epidemiologiske studier, og kostscoren fungerer som en god indikator for den samlede kvalitet af kosten. Kostmønstrene var forbundet på forskellig vis med subgrupperne af tarmmikrobiota og specifikke bakterieslægter. Der var en effekt af kosten på risikofaktorer for CMD, dog var denne ikke medieret gennem tarmmikrobiotaen.

## LIST OF PUBLICATIONS

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This doctoral thesis is based on the work contained in the following papers:

- I. **Rostgaard-Hansen AL**, Rosthøj S, Brunius C, Olsen SF, Bjerregaard AA, Cade JE, Tjønneland A, Landberg R, Halkjær J. Relative Validity and Reproducibility of a Web-Based Semi-Quantitative Food Frequency Questionnaire in the Danish Diet, Cancer, and Health—Next Generations MAX Study. *Nutrients*, 2023. DOI: <https://doi.org/10.3390/nu15102389>
- II. **Rostgaard-Hansen AL**, Lau CJ, Halkjær J, Olsen A, Toft U. An updated validation of the Dietary Quality Score: associations with risk factors for cardiometabolic diseases in a Danish population. *European Journal of Nutrition*, 2023. DOI: <https://doi.org/10.1007/s00394-023-03100-4>
- III. **Rostgaard-Hansen AL\***, Esberg A\*, Dicksved J, Hansen T, Pelve E, Brunius C, Halkjær J, Tjønneland A, Johansson I, Landberg R. Gut microbiota composition in the Danish Diet, Cancer, and Health – Next Generations MAX sub-cohort: Temporal variability and association with dietary patterns. Submitted.
- IV. **Rostgaard-Hansen AL**, Grand MK, Esberg A, Rosthøj S, Dicksved J, Hansen T, Pelve E, Brunius C, Halkjær J, Tjønneland A, , Johansson I, Landberg R. Direct and indirect effects of healthy Nordic and Mediterranean diet patterns via gut microbiota on intermediate risk factors for cardiometabolic health. Manuscript.

\*Joint first authorship.

## Published papers not included in the thesis:

Palmnäs M, Brunius C, Shi L, **Rostgaard-Hansen A**, Torres NE, González-Domínguez R, Zamora-Ros R, Ye YL, Halkjær H, Tjønneland A, Riccardi G, Giacco R, Costabile G, Vetrani C, Nielsen J, Andres-Lacueva C, Landberg R (2020). Perspective: Metabotyping-A Potential Personalized Nutrition Strategy for Precision Prevention of Cardiometabolic Disease. *Adv Nutr*, 11(3): p. 524-532.

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## CONTRIBUTION REPORT

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- Paper I:** Agnetha Linn Rostgaard-Hansen (ALRH) designed the study together with co-authors, coordinated the data collection, participated in data processing, conducted the data analyses with supervision, interpreted the data and was responsible for writing the manuscript.
- Paper II:** ALRH designed the study together with co-authors, coordinated the data collection and data preparation, interpreted the data and was responsible for writing the manuscript.
- Paper III:** ALRH designed the study together with co-authors, coordinated the data collection and data preparation, worked in close collaboration with the joint first author who conducted the data analysis, interpreted the data and was responsible for writing the manuscript.
- Paper IV:** ALRH designed the study together with co-authors, coordinated the data collection and data preparation, worked in close collaboration with the second author who conducted the data analysis, interpreted the data and was responsible for writing the manuscript.

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