

PUBLICATIONS

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

1. **Alzahrani AH**, Skytte MJ, Samkani A, Thomsen MN, Astrup A, Ritz C, Chabanova E, Frystyk J, Holst JJ, Thomsen HS, Madsbad S, Haugaard SB, Krarup T, Larsen TM, Magkos F. Body weight and metabolic risk factors in patients with type 2 diabetes on a self-selected high-protein low-carbohydrate diet. *European Journal of Nutrition* **2021**; 60 (8): 4473-4482 [PMID: 34101004] <https://doi.org/10.1007/s00394-021-02605-0>

2. **Alzahrani AH**, Skytte MJ, Samkani A, Thomsen MN, Astrup A, Ritz C, Frystyk J, Holst JJ, Madsbad S, Haugaard SB, Krarup T, Larsen TM, Magkos F. Effects of a self-prepared carbohydrate-reduced high-protein diet on cardiovascular disease risk markers in patients with type 2 diabetes. *Nutrients* **2021**; 13 (5): 1694 [PMID: 34067585] <https://doi.org/10.3390/nu13051694>

SUMMARY

Overconsumption of energy-dense foods is a growing health problem, and major shifts in dietary patterns have been observed globally over last few decades. The rapid nutrition transition has occurred at a global and regional levels and was accompanied by calls for reducing excess calorie intake to avoid considerable health consequences particularly in people with type 2 diabetes (T2D). Evidence is also mounting regarding the importance of having a good quality diet with optimum macronutrient composition in carbohydrate, fat, protein and fiber. In this regard, several published systematic reviews and meta-analyses of hypocaloric diets in people with T2D did not support any particular macronutrient profile or style over others and therefore, no consensus exists on the exact macronutrient composition of the optimal diet for patients with T2D, particularly in long-term management of T2D.

Recently, our research group assessed the effects of a weight-maintaining carbohydrate-reduced high-protein (CRHP) diet, compared to an isocaloric conventional diabetes (CD) diet, with all food provided to the participants, on several metabolic parameters, cardiovascular risk markers, gut hormone secretion, and satiety in 28 T2D patients who were randomized to each diet for 6 weeks in a crossover design. In that trial, carbohydrates were substituted with protein and fat; and the macronutrient distribution in the CRHP diet were 30/30/40 % of total calories for carbohydrate/protein/fat, respectively. Results demonstrated that a moderate macronutrient shift induced by substituting carbohydrates with protein and fat for 6 weeks, reduced HbA1c and hepatic fat content in weight-stable individuals with T2D. Additionally, the CRHP diet reduced glucose excursions and improved β -cell function, including proinsulin processing, and increased subjective satiety in patients with T2D. Additional beneficial effects were also reported on some risk markers for cardiovascular disease (CVD).

In this thesis, we extended that initial study and aimed to investigate the effects of consuming a self-selected and self-prepared CRHP diet—with appropriate dietetic guidance—on body weight, anthropometric measurements, selected glucoregulatory metabolic parameters and CVD risk factors in patients with T2D over the subsequent 6 months, and to compare these effects to baseline and to those that were reported after the earlier crossover 2×6-week trial, when the CRHP diet was fully provided.

Our results demonstrate that patients with T2D can effectively self-select and self-prepare a CRHP diet and can maintain their body weight for 6 months. The beneficial effects on several

parameters—including anthropometric measurements, metabolic and cardiovascular disease risk factors—that were observed in the initial 2×6-week trial were either maintained or improved over the subsequent 6 months. The CRHP diet lowered fasting glucose and HbA1c concentrations, decreased visceral adipose tissue volume, and decreased liver fat content. The diet also increased fasting insulin and C-peptide concentrations and decreased daily glycaemia; and all these parameters improved compared not only to baseline, but also compared to the end of the crossover trial. With regards to the effects on CVD risk factors, the CRHP diet led to considerable improvements in fasting blood lipid profile and postprandial lipemia; in addition to small improvements in markers of inflammation and diurnal heart rate. No changes in appetite-related gut hormones were observed during the 6-month follow-up period.

These favorable findings have important implications as they suggest that patients with T2D can follow a CRHP diet by themselves in a dietitian-supported setting and gain considerable benefits on metabolic homeostasis and cardiovascular disease risk factors in the absence of major weight loss. Our results are in line with those from another study reporting that patients with T2D are able to sustain a low-carbohydrate high-fat dietary paradigm for 26-53 months, even without support from their primary care physicians.

In conclusion, substitution of dietary carbohydrate by protein and fat has beneficial effects on several metabolic parameters related to glucose hemostasis and CVD risk, which are either maintained or further improved over a 6-month period, when patients with T2D self-select and self-prepare a CRHP diet on their own.

However, longer and larger studies are needed to fully elucidate the long-term efficacy, safety and feasibility of substituting dietary carbohydrates with fat and proteins in patients with T2D in real-life settings. Results of future studies will hopefully help in extending prescription of CRHP diets as an effective and safe nutritional therapeutic strategy for the general T2D population. In the meantime, carbohydrate-restricted diets could be offered to people living with diabetes as part of an individualized management plan.

SUMMARY IN DANISH

Overindtag af energitætte fødevarer er et voksende sundhedsproblem, og store ændringer i kostmønstre er blevet observeret globalt i de sidste par årtier. Dette er blevet ledsaget af opfordringer til at reducere overskydende kalorieindtag for at undgå betydelige sundhedsmæssige konsekvenser, især hos mennesker med type 2-diabetes (T2D). Der er også stigende dokumentation for vigtigheden af at have en god kvalitet af kosten med en optimal sammensætning af makronæringsstofferne kulhydrat, fedt og protein samt kostfibre. Relateret hertil, har flere systematiske reviews og metaanalyser vedrørende hypokaloriske diæter blandt mennesker med T2D dog ikke nogen specifik makronæringsstofprofil, og der eksisterer derfor ingen konsensus om den optimale makronæringsstofsammensætning for patienter med T2D, især i forhold til langsigtet behandling af T2D.

For nyligt vurderede vores forskergruppe virkningerne af en vægtvedligeholdende kulhydrat-reduceret diæt med højt proteinindhold (CRHP) sammenlignet med en isokalorisk konventionel diabetes (CD) diæt - hvor al mad blev leveret til deltagerne - på flere metaboliske parametre, kardiovaskulær risikomarkører, tarmhormonsekretion og mæthed hos 28 T2D-patienter, som blev randomiseret til hver diæt i 6 uger i et crossover-design. I det forsøg blev kulhydrater erstattet med protein og fedt; og makronæringsstoffordelingen i CRHP-diæten blev derfor 30/30/40 % af de samlede kalorier for henholdsvis kulhydrat/protein/fedt. Resultaterne viste at et moderat makronæringsstofsift induceret ved at erstatte kulhydrater med protein og fedt i 6 uger, reducerede HbA1c og leverfedtindhold hos vægtstabile individer med T2D. Derudover reducerede CRHP-diæten glukoseudsving og forbedrede β -cellefunktion, herunder pro-insulinbehandling, og øgede subjektiv mæthed hos forsøgspersonerne. Yderligere gavnlige virkninger blev også rapporteret på nogle risikomarkører for kardiovaskulær sygdom (CVD).

I denne afhandling udvidede vi denne indledende undersøgelse og havde til formål at undersøge virkningerne af at indtage en selvvalgt og selv-tilberedt CRHP-diæt – med passende diætetisk vejledning – på kropsvægt, antropometriske målinger, udvalgte glukoregulatoriske metaboliske parametre og CVD-risikofaktorer hos patienter med T2D over de efterfølgende 6 måneder, og for at sammenligne disse effekter med baseline og dem der blev rapporteret efter det tidligere crossover 2×6-ugers forsøg, hvor CRHP diæten var fuldt ud tilvejebragt.

Vores resultater viser, at patienter med T2D effektivt kan vælge og forberede en CRHP diæt og kan bevare deres kropsvægt i 6 måneder. De gavnlige virkninger på adskillige parametre -

inklusive antropometriske målinger, metaboliske og kardiovaskulære sygdomsrisikofaktorer - der blev observeret i det indledende 2×6-ugers forsøg blev enten opretholdt eller forbedret over de efterfølgende 6 måneder. CRHP-diæten sænkede fastende glukose- og HbA1c-koncentrationer, reducerede visceralt fedtvævsvolumen og nedsatte leverfedtindhold. Diæten øgede også fastende insulin- og C-peptid koncentrationer og reducerede daglig glykæmi; og alle disse parametre forbedredes ikke kun i forhold til baseline, men også sammenlignet med slutningen af crossover-forsøget. Med hensyn til virkningerne på CVD-risikofaktorer førte CRHP-diæten til betydelige forbedringer i fastende blodlipidprofil og postprandial lipæmi; ud over små forbedringer i markører for inflammation og puls. Der blev ikke observeret ændringer i appetitrelaterede tarmhormoner i den 6-måneders opfølgingsperiode.

Disse positive resultater har vigtige implikationer, da de tyder på, at patienter med T2D kan følge en CRHP-diæt alene i en diætiststøttet opsætning og opnå betydelige fordele på metabolisk homøostase og risikofaktorer for kardiovaskulære sygdomme i fravær af større vægttab. Vores resultater er på linje med dem fra en anden undersøgelse, der rapporterer, at patienter med T2D er i stand til at opretholde en kosttilgang med lavt kulhydrat og fedtindhold i 26-53 måneder, selv uden støtte fra deres primære læger.

Som konklusion har substitution af kostens kulhydrat med protein og fedt gavnlige effekter på adskillige metaboliske parametre relateret til glukose homøostase og CVD-risiko, som enten opretholdes eller forbedres yderligere over en 6-måneders periode, når patienter med T2D på egen hånd forberedte en CRHP diæt.

Imidlertid er længere og større undersøgelser nødvendige for fuldt ud at belyse den langsigtede effektivitet, sikkerhed og gennemførlighed af at erstatte kulhydrater med fedt og proteiner hos patienter med T2D. Resultaterne af fremtidige undersøgelser vil forhåbentlig hjælpe med at udvide tilbuddet af CRHP-diæter som en effektiv og sikker ernæringsterapeutisk strategi for den generelle T2D-population. I mellemtiden kunne kulhydrat-begrænsede diæter tilbydes til personer, der lever med diabetes som en del af en individuel håndteringsplan.

TABLE OF CONTENTS

PREFACE	3
ACKNOWLEDGEMENTS	5
PUBLICATIONS	6
SUMMARY	7
SUMMARY IN DANISH	9
ABBREVIATIONS	11
TABLE OF CONTENTS	15
INTRODUCTION AND BACKGROUND	18
<i>Epidemiology of type 2 diabetes mellitus</i>	18
Prevalence of diabetes and magnitude of the problem	18
Worldwide trends of diabetes	19
Trends of diabetes in various geographical regions and countries	19
Trends of diabetes in different age groups	21
Trends of diabetes in men and women	21
Trends of diabetes in different ethnic groups	22
<i>Pathophysiology of type 2 diabetes</i>	22
Pathophysiology of Prediabetes.....	27
<i>Diagnosis</i>	27
<i>Etiology/ Risk factors of type 2 diabetes</i>	29
Overweight and obesity	29
Diet quality	33
Physical inactivity.....	40
Genes	41
Other lifestyle risk factors: Sleep, Alcohol, Tobacco, Caffeine, Stress	42
<i>Diabetes complications and glycemic control</i>	45
<i>Management of Type 2 Diabetes</i>	50
Medications.....	50
Bariatric surgery	53
Physical activity.....	54
Nutritional management and dietary recommendations for people with T2D	55
Low-fat diets	57
The Mediterranean diet.....	58
Low glycemic index diets.....	60
Carbohydrate-restricted diets	61
Systematic reviews and meta-analyses of carbohydrate-restricted diets.....	62
Long-term RCTs assessing the effects of carbohydrate-restriction in T2D	66

Eucaloric, weight-maintaining carbohydrate-restricted diets	67
Safety of carbohydrate-restricted diets	70
RESEARCH GAPS.....	74
AIM AND OBJECTIVES OF THE STUDY	76
<i>The aim of the study.....</i>	76
<i>The objectives of the study.....</i>	76
METHODS	78
<i>Study design and participants.....</i>	78
<i>The endpoints of the main study</i>	80
<i>Dietary intervention.....</i>	80
<i>Body fat distribution</i>	84
<i>Metabolic testing</i>	84
<i>IVGTT.....</i>	84
<i>CGM.....</i>	85
<i>MMT.....</i>	86
<i>Blood sample analysis.....</i>	87
<i>Postprandial and Diurnal Metabolite Responses.....</i>	88
<i>Diurnal Blood Pressure and Heart Rate.....</i>	88
<i>Statistical analysis.....</i>	89
RESULTS	90
<i>Patient Characteristics and Self-Prepared Diet.....</i>	90
<i>Changes in body weight.....</i>	92
<i>Changes in body fat distribution</i>	92
<i>Diurnal Blood Pressure and Heart Rate Responses.....</i>	95
<i>Changes in Medications.....</i>	95
DISCUSSION.....	101
<i>Body fat distribution and glucose homeostasis.....</i>	102
<i>Cardiovascular disease risk factors</i>	106
<i>Appetite-related gut hormones.....</i>	108
<i>Compliance to CRHP diet in a free-living setting.....</i>	109
PERSPECTIVES	110
STRENGTHS AND LIMITATIONS.....	111
CONCLUSIONS	112

REFERENCES.....	113
APPENDIX.....	127
<i>Paper I.....</i>	<i>128</i>
<i>Paper II.....</i>	<i>136</i>