Table of Contents

Preface

This PhD thesis is based on two clinical human dietary intervention studies. Study 1 was conducted at the August Krogh Section for Molecular Physiology, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark and study 2 was conducted at Department of Endocrinology, Copenhagen University Hospital Hvidovre, Denmark. The studies were performed in collaboration with Danish Research Centre for Magnetic Resonance, Center for Functional and Diagnostic Imaging and Research, Copenhagen University Hospital Hvidovre, Denmark.

The thesis is based on the following manuscripts:

Amalie London*, Michael M. Richter*, Kim Anker Sjøberg, Nicolai J. Wewer Albrechtsen, Michal Považan, Lylia Drici, Amanda Schaufuss, Lise Madsen, Jannike Øyen, Sten Madsbad, Jens Juul Holst, Gerrit van Hall, Hartwig Roman Siebner, Erik A. Richter, Bente Kiens, Annemarie Lundsgaard, Kirstine Nyvold Bojsen-Møller. *Shared first authorship. The Impact of Short-term Eucaloric Low- and High-Carbohydrate Diet on Liver Triacylglycerol Content in Males with Overweight and Obesity; a Randomized Cross-Over Study. American Journal of Clinical Nutrition of Clinical Nutrition, 2024 June. DOI: 10.1016/j.ajcnut.2024.06.006.

Amalie London, Amanda Schaufuss, Michal Považan, Marie-Louise Dichman, Jasmin Merhout, Carsten Dirksen, Sten Madsbad, Hartwig Roman Siebner, Annemarie Lundsgaard, Andreas Mæchel Fritzen, Bente Kiens, and Kirstine Nyvold Bojsen-Møller. Effects of Acute Iso- and Hypocaloric Carbohydrate Restriction on Liver Fat and Glucose and Lipid Metabolism – a Cross-over Randomized Clinical Trial. Submitted to American Journal of Physiology 22nd of August.

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English summary

Accumulation of liver triacylglycerol (TG) is associated with impaired glucose metabolism evident already at TG levels >1.5-1.85% (1, 2). The regulation of liver TG content is controlled by several factors with accumulating of liver TG occurring when lipid acquisition is outpacing lipid disposal. Lipid acquisition is driven by fatty acid (FA) availability towards the liver from the diet and release of endogenous FA primarily from the adipose tissue. In the liver, de novo lipogenesis (DNL) is contributing with FA synthesis from non-lipid sources. Subsequently, hepatic FA can be esterified to liver TG. Hepatic lipid disposal is regulated by the capacity to oxidate FA in the liver and to promote ketogenesis along with embedding VLDL particles for secretion as VLDL-TG from the liver.

It is well known that weight loss can reduce liver TG content, but previous studies suggest that liver TG content may be modulated within days in response to changes in dietary macronutrient composition and energy provision (3–7), indicating effects independent of weight loss. Furthermore, in individuals with insulin resistance, a hypocaloric diet with low-carbohydrate content was demonstrated to be superior in reducing liver TG content within 48 hours, when compared to a hypocaloric diet with a high-carbohydrate content (3). However, the effects of acute alterations in dietary carbohydrate and fat availability on liver TG content, glucose and lipid metabolism, particularly under conditions of preserved energy balance, are not yet fully understood. Therefore, this thesis primary objective is to examine the effects of acute (within days) alterations in dietary carbohydrate and fat availability on liver TG content, with an emphasis on elucidating the mechanistic insights underlying the associated changes in glucose and lipid metabolism.

Two human dietary intervention studies were performed. In study 1, we implemented a randomized cross-over design to evaluate the effect of five days low-carbohydrate, high-fat (LC) diet and five days high-carbohydrate, low-fat (HC) diet during eucaloric conditions in males with overweight or obesity. Liver TG content was measured by ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS). A low dose hyperinsulinemic euglycemic clamp, including the use of stable glucose isotope tracer, was applied to measure changes in hepatic glucose production and hepatic insulin sensitivity. Liver TG content was reduced by 35% after the LC diet concurrently with decreased fasting plasma TG, improvements

in the hepatic insulin sensitivity index (HISI) and higher fasting plasma beta-hydroxybutyrate levels. Plasma C16:1n-7, representing the desaturated end product of the DNL, was lower along with a lower respiratory exchange ratio (RER) measured by indirect calorimetry. Thus, indicating that the lipid acquisition was decreased by lower DNL, while the lipid disposal was increased by increased whole body FA oxidation and ketogenesis after the LC diet. In contrast, no changes were observed in liver TG content, fasting plasma TG, hepatic glucose production or hepatic insulin sensitivity after the HC diet. The RER was however increased after HC indicating a lower whole body fat oxidation. During both LC and HC diets peripheral insulin sensitivity was unchanged.

In study 2, we investigated the effects of a two-day matched absolute carbohydrate restriction under two different dietary conditions: very-low-calorie conditions (VLCD) and a low-carbohydrate, high-fat diet (LCHF) that maintained isocaloric conditions. The participants enrolled were males and post-menopausal females who were overweight or obese. This study aimed to decipher the distinct impacts of calorie deficit and carbohydrate restriction, and to separate the effects of low carbohydrate intake from those of low carbohydrate intake combined with increased fat intake. Liver TG content in this study was lower after LCHF but not after VLCD, when compared with after two days of a control diet (CON), respectively. Whole body fat oxidation tended to be higher after LCHF and was higher after VLCD along with higher plasma beta-hydroxybutyrate indicative of increased ketogenesis after both LCHF and VLCD, when compared with after the control diet. Despite lower fasting plasma C-peptide, insulin, glucose and homeostatic model assessment for insulin resistance (HOMA2-IR) after LCHF and VLCD, the postprandial plasma glucose concentrations were higher when reintroducing a carbohydrate-rich mixed meal, when compared with the response after CON. Concurrently, the insulinogenic index (IGI), was lower following LCHF and VLCD indicating an impairment in the initial beta-cell response to the carbohydrate rich mixed meal after two days of carbohydrate restriction as either LCHF or VLCD.

Together the findings from study 1 and study 2 demonstrate that liver TG content can be lowered within days after consuming a low-carbohydrate diet supplemented with fat to maintain eucaloric or isocaloric conditions – the LC diet (study 1) and the LCHF diet (study 2). The lower liver TG content could be ascribed to lower lipid acquisition via lowering of DNL and increased lipid disposal through

increased ketogenesis. Both the LC and LCHF diet encompassed favorable fasting parameters for glucose and lipid metabolism. Furthermore, after LC (study 1) it was demonstrated that hepatic insulin sensitivity in the liver was improved together with preserved peripheral insulin sensitivity. However, when reintroducing a liquid mixed meal after two days of LCHF (study 2) the postprandial glucose concentrations were higher when compared with after CON which could be attributed to an impaired initial beta-cell response (lower IGI). In conclusion, a low-carbohydrate, high-fat diet lowers liver TG content after only two-four days and improves glucose and lipid metabolism in the fasted state. However, acute dietary carbohydrate restriction also leads to higher postprandial plasma glucose concentrations and impaired initial beta-cell response when reintroducing carbohydrates.

Dansk resume (Danish summary)

Akkumulering af triacylglycerol (TG) i leveren er forbundet med forringet glukosemetabolisme allerede ved lever TG niveauer >1,5-1,85% (1, 2). Leverens TG-indhold reguleres af flere faktorer, og lever TG akkumuleres, når lipidoptagelsen overstiger lipidafskaffelsen. Lipidoptagelse i leveren er afhængig af tilgængeligheden af fedtsyrer (FA) fra kosten og frigivelse af endogene fedtsyrer, som primært stammer fra lipolyse i fedtvæv. I leveren bidrager de novo lipogenese (DNL) med nydannelse af fedtsyrer. Fedtsyrer kan efterfølgende esterficeres i leveren til TG. Leverens lipidafskaffelse reguleres af kapaciteten til at oxidere fedtsyrer og fremme ketogenese og kapaciteten til dannelse af VLDL-partikler, som secerneres fra leveren til blodbanen.

Det er velkendt, at vægttab kan reducere leverens TG-indhold, men tidligere studier viser, at leverens TG-indhold kan moduleres inden for få dage ved ændringer i kostens indhold af makronæringsstoffer og energi (3–7), hvilket indikerer effekter der er uafhængige af vægttab. Desuden er det i individer med insulinresistens demonstreret, at en hypoenergetisk kost med lavt kulhydratindhold, er mere effektiv til at reducere leverens TG-indhold inden for 48 timer sammenlignet med en hypoenergetisk kost med højt kulhydratindhold (3). Effekterne af akutte ændringer i kostens kulhydrat- og fedttilgængelighed på leverens TG-indhold og glukose- og lipidmetabolisme, især under betingelser med bevaret energibalance, er dog endnu ikke fuldt forstået. Derfor er det primære formål med denne ph.d.-afhandling at undersøge effekterne af akutte (inden for dage) ændringer i kostens kulhydrat- og fedttilgængelighed på leverens TG-indhold med fokus på at afdække fysiologiske ændringer, der ligger til grund for ændringer i glukose- og lipidmetabolismen.

Der blev udført to kost-interventionsstudier. I studie 1 blev et randomiseret crossover-design studie udført med det formål at estimere effekten af fem dage med en lav-kulhydrat, høj-fedt (LC) kost og fem dage med en høj-kulhydrat, lav-fedt (HC) kost under euenergetiske forhold hos mænd med overvægt eller svær overvægt. Leverens TG-indhold blev målt ved ^{1}H-magnetisk resonansspektroskopi (^{1}H-MRS). En lavdosis hyperinsulinæmisk euglykæmisk clamp, med brug af stabile glukose sporstoffer, blev anvendt til at måle ændringer i hepatisk glukoseproduktion og hepatisk insulinfølsomhed. Leverens TG-indhold blev reduceret med 35% efter LC kosten og samtidig

fandt vi nedsat faste plasma-TG koncentrationer, forbedringer i hepatisk insulinfølsomhed og højere faste plasma beta-hydroxybutyrat koncentration. Plasma C16:1n-7 koncentrationen, som repræsenterer det desaturerede slutprodukt af DNL, og respiratorisk udvekslingsforhold (RER) målt ved indirekte kalorimetri var lavere. Dette indikerer, at reduceret DNL fører til lavere lipiddannelse i leveren, og at lipidafskaffelsen steg som følge af øget fedtsyre-oxidation og ketogenese. Der blev ikke observeret ændringer i leverens TG-indhold, faste plasma-TG niveauet, hepatisk glukoseproduktion eller hepatisk insulinfølsomhed efter HC kosten. RER var dog øget efter HC kosten, hvilket indikerer en lavere helkrops fedtoxidation. Efter både LC og HC kosten var den perifere insulinfølsomhed uændret.

Studie 2 undersøgte effekterne af en to-dags matchet absolut kulhydratsrestriktion under to forskellige kostbetingelser: meget lav-energetisk (VLCD) og en lav-kulhydrat, høj-fedt kost (LCHF), der opretholdte isoenergetiske forhold. Deltagerne, der blev inkluderet, var mænd og post-menopausale kvinder, med overvægt eller svær overvægt. Dette studie havde til formål at afdække de forskellige virkninger af energiunderskud og kulhydratrestriktion og at adskille effekterne af lav kulhydratindtagelse alene fra dem der er betinget af lavt kulhydratindtagelse kombineret med øget fedtindtagelse. Leverens TG-indhold i dette studie var lavere efter LCHF, men ikke efter VLCD sammenlignet med efter to dage med en kontrolkost (CON). Helkrops fedtoxidation tenderede til at være højere efter LCHF og var højere efter VLCD, og der var højere ketogenese i faste (målt som faste plasma beta-hydroxybutyrat) efter både LCHF og VLCD sammenlignet med efter CON. På trods af lavere faste plasma C-peptid-, insulin-, glukose koncentrationer og insulin resistens index; *homeostatic model assesment for insulin resistance* (HOMA2-IR) efter LCHF og VLCD, var der højere postprandiel plasma glukosekoncentration efter indtagelse af et kulhydratrigt blandet måltid. Samtidig var insulinogenisk indeks (IGI) lavere efter LCHF og VLCD, hvilket indikerer et lavere initielt beta-celle respons efter indtagelse af et kulhydratrigt blandede måltid, forudgået af to dage med lavt kulhydratindtag som enten LCHF eller VLCD.

Samlet viser resultaterne fra studie 1 og studie 2, at leverens TG-indhold kan sænkes inden for to til fire dage med indtagelse af en kulhydratreduceret kost suppleret med fedt for at opretholde

euenergetiske eller isoenergetiske forhold, henholdsvis LC-kosten (studie 1) og LCHF-kosten (studie 2). Det lavere lever TG-indhold kan tilskrives en lavere lipiddannelse betinget af nedsat DNL og en øget lipidafskaffelse via øget ketogenese. Både LC- og LCHF-kostregimerne påvirkede fasteparametre for glukose- og lipidmetabolisme i en gunstig retning, og efter LC (studie 1) fremgik det, at insulinfølsomheden i leveren var forbedret og insulinfølsomheden perifert var bevaret. Ud fra data fra studie 2 fremgår det, at når kulhydrater i form af et flydende blandet måltid blev reintroduceret efter to dage med LCHF (studie 2), var glukosestigningerne i blodet højere sammenlignet med efter en kontrolkost, hvilket delvist kan tilskrives et nedsat initielt beta-celle respons (lavere IGI). Samlet finder studie 1 og studie 2 at en kulhydratrestrikteret fedtrig kost sænker leverens triglyceridindhold efter blot to til fire dage og forbedrer glukose- og lipidmetabolismen i fastende tilstand. Dog fører akut kulhydratrestriktion også til højere postprandielle glukosekoncentrationer og et nedsat initielt beta-cellerespons når kulhydrater reintroduceres.

Overall aim and hypothesis

This thesis focuses on the short term (within days) effects of dietary carbohydrate and fat availability in the regulation of liver TG (triacylglycerol) content during both eucaloric and hypocaloric energy provision. Furthermore, it explores the impact of carbohydrate availability on lipid and glucose metabolism in the fasted state, during a hyperinsulinemic euglycemic clamp and in the postprandial state during a liquid mixed meal test.

Study 1: The role of short-term eucaloric changes in dietary carbohydrate and fat availability on liver triacylglycerol content, glucose and lipid metabolism in male individuals living with overweight or obesity.

The hypothesis was that consuming a eucaloric low-carbohydrate, high-fat diet for five days would reduce liver TG content (primary outcome), decrease hepatic glucose production (HGP), decrease fasting plasma TG concentration and increase insulin clearance when compared to pre-intervention. On the contrary we hypothesized that the opposite effects would occur after a eucaloric high-carbohydrate, low-fat diet.

Study 2: The impact of two days matched dietary carbohydrate restriction during hypocaloric and isocaloric conditions on liver triacylglycerol content, fasting and postprandial glucose and lipid metabolism in males and post-menopausal females with overweight or obesity.

Our hypothesis was that both the hypocaloric low-carbohydrate diet and the isocaloric low-carbohydrate diet would lead to lower liver TG content compared with after two days of a control diet, but might affect postprandial plasma glucose, insulin and TG concentrations differently when carbohydrates and fat were reintroduced as a liquid mixed meal.