

The PhD thesis is based on the following 4 papers:

Paper I.

Andersen, S.S.H.; Zhu, R.; Kjølbaek, L.; Raben, A. Effect of Non- and Low-Caloric Sweeteners on Substrate Oxidation, Energy Expenditure, and Catecholamines in Humans — A Systematic Review. *Nutrients* **2023**, *15*, 2711. <https://doi.org/10.3390/nu15122711>

Paper II.

Kjølbaek, L.; Manios, Y.; Blaak, E.E.; Martínez, J.A.; Feskens, E.J.M.; Finlayson, G.; **Andersen, S.S.H.;** Reppas, K.; Navas-Carretero, S.; Adam, T.C.; et al. Protocol for a Multicentre, Parallel, Randomised, Controlled Trial on the Effect of Sweeteners and Sweetness Enhancers on Health, Obesity and Safety in Overweight Adults and Children: The SWEET Project. *BMJ Open* **2022**, *12*, <https://doi:10.1136/bmjopen-2022-061075>

Paper III.

Andersen, S.S.H.; Kjølbaek, L.; Halford, J.C.G; Harrold, J.A; Raben, A. Acute and Prolonged Effects of Sweeteners and Sweetness Enhancers on Postprandial Substrate Oxidation, Energy Expenditure, Glucose and Insulin in Humans, a SWEET Sub-Study. Ready for submission; intended for submission to the journal *Obesity*.

Paper IV.

Andersen, S.S.H.; Raben, A.; Halford, J.C.G; Harrold, J.A; Kjølbaek, L. Acute and Prolonged Effects of Sweeteners and Sweetness Enhancers on Postprandial Appetite Sensations, Palatability and Ad Libitum Energy Intake in Humans, a SWEET Sub-Study. In preparation; intended for submission to the journal *Appetite*.

Contributions to international conferences:

Raben, A.; Kjølbaek, L.; **Andersen, S.S.H.**; Martinez, A.; Blaak, E.; Halford, J.; Finlayson, G.; Harrold, J.; Feskens, E.; Hodgkins, C. E.; Monique M Raats, M.M.; Moshoyianni, H.; Lam, T.; Alamo, M.D.; Voegler, T.; Manios, Y. Sweeteners and Sweetness Enhancers – SWEET: Prolonged Effects on Health, Obesity, and Safety. Protocol for a 2-Year Multi-center European Intervention Study. Abstract for the *Nordic Nutrition Conference*, **December 2020**. Accepted as an E-poster.

Raben, A.; Kjølbaek, L.; **Andersen, S.S.H.**; Martinez, A.; Blaak, E.; Halford, J.; Finlayson, G.; Harrold, J.; Feskens, E.; Hodgkins, C. E.; Monique M Raats, M.M.; Moshoyianni, H.; Lam, T.; Alamo, M.D.; Voegler, T.; Manios, Y. Sweeteners and Sweetness Enhancers – SWEET: Prolonged Effects on Health, Obesity, and Safety. Protocol for a 2-year Multi-center European Intervention Study. Abstract for the *European and International Congress on Obesity*, **September 2020**. Accepted for an oral E-presentation.

Previous publication:

Andersen, S.S.H.; Heller, J.M.F.; Hansen, T.T.; Raben, A. Comparison of Low Glycaemic Index and High Glycaemic Index Potatoes in Relation to Satiety: A Single-Blinded, Randomised Crossover Study in Humans. *Nutrients* **2018**, *10*, 1726.

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Summary

The global rise in overweight and obesity is of major concern due to the associated risk of e.g. type 2 diabetes and cardiovascular diseases. To combat this, reducing the consumption of foods and drinks with added sugar is advocated for, as these products often have low nutritional value and mainly provide energy. This has led to an increased use of non- and low-caloric sweeteners (NCS/LCS), allowing a variety of products to maintain palatability without the associated energy and glycemic impact. However, the use of NCS and LCS as a means to prevent overweight and obesity is highly debated, as they have been proposed to potentially lead to weight gain by e.g. increasing appetite and inducing insulin resistance. As a group, NCS and LCS can be defined as sweeteners and sweetness enhancers (S&SEs).

Previous randomized controlled trials (RCTs) have explored the impact of NCS and/or LCS on e.g. appetite and glucose regulation in humans. Overall, no adverse effects of NCS and/or LCS have been observed. The impact of S&SEs on the other side of the energy balance equation, substrate oxidation and energy expenditure, has not been extensively investigated. Consequently, the objective of this PhD project was to assess the potential effects of S&SEs on substrate oxidation, energy expenditure and appetite – with fat oxidation being the primary outcome.

Paper I systematically reviewed the existing literature assessing the impact of NCS and LCS on substrate oxidation, energy expenditure, and catecholamines compared to caloric sweeteners, water, and various doses and types of NCS and LCS. The systematic review found that drinks and meals with NCS or LCS resulted in higher fat and lower carbohydrate oxidation compared to caloric sweeteners under non-isoenergetic conditions; a finding attributed to the extra carbohydrate load in the interventions with caloric sweeteners. The results for energy expenditure were inconsistent. No other conclusions could be made due to insufficient and inconsistent data. The systematic review showed a need for further studies using isoenergetic comparators (e.g. water) and of longer duration.

Paper II presented the 1-year European multicenter RCT assessing prolonged effects of S&SEs on health, obesity, and safety: The SWEET WP3 study. The trial was conducted as a part of this PhD project.

Paper III and **IV** presented the design, conduction and results of an RCT sub-study including participants from SWEET WP3. The objective of the sub-study was to investigate the acute effects of a mixture of acesulfame potassium and cyclamate versus water on postprandial substrate oxidation and energy expenditure, glucose and insulin concentrations, and appetite at baseline, after a 2-month weight loss period, and after a 4-month weight loss maintenance period with either S&SEs or sugar in the diet. The results of the sub-study showed no differences between the 2 groups in fasting or postprandial fat oxidation, carbohydrate oxidation, energy expenditure, glucose, or insulin on or across the 3 test days ($p>0.05$). However, the S&SEs group rated lower prospective consumption, and desire to eat something sweet across the 3 test days after the test drink with acesulfame potassium and cyclamate compared to the Sugar group consuming water ($p<0.05$).

Overall, data from the papers included in this thesis find evidence to suggest that NCS have similar effects as water on substrate oxidation and energy expenditure both acutely and in the longer-term. Regarding appetite, **Paper IV** suggests that acesulfame potassium and cyclamate may induce sensory-specific satiety both acutely and after regular exposure to S&SEs. This could suggest that NCS may be beneficial in relation to certain aspects of appetite regulation. For LCS, less data are in general available, and thus the strength of evidence in this thesis lies with the NCS. However, based on the findings in **Paper I**, it can be concluded that LCS show higher fat and lower carbohydrate oxidation when compared to caloric sweeteners under non-isoenergetic conditions. Thus, compared to caloric sweeteners, LCS do not seem to exert any effect on substrate oxidation beyond what can be expected based on their energy load and metabolism. However, more research is needed for both NCS and LCS across all outcomes. These studies should incorporate an isoenergetic comparator, a range of S&SEs, modes of administration, and should be conducted over a minimum of 1 year.

Sammendrag (Danish summary)

Den globale stigning af overvægt og fedme vækker bekymring på grund af den medfølgende risiko for bl.a. type-2-diabetes og kardiovaskulære sygdomme. En strategi til at bekæmpe denne udvikling er at reducere indtaget af fødevarer og drikkevarer med tilsat sukker, da disse produkter ofte har en lav næringsværdi og dermed primært tilfører energi. Dette har medført et øget forbrug af kaloriefri og -reducerede sødemidler. Disse sødemidler gør det muligt at bevare den søde smag i forskellige produkter, mens energiindhold og glykæmisk påvirkning reduceres eller helt udelades. Brugen af kaloriefri og -reducerede sødemidler til at forebygge overvægt og fedme er meget omdiskuteret, da det er blevet foreslået, at de kan have en vægtøgende effekt ved f.eks. at stimulere appetit og inducere insulinresistens.

Randomiserede humane studier, der har undersøgt virkningen af kaloriefri og/eller -reducerede sødemidler i relation til f.eks. appetit- og glukoseregulering, finder overordnet set ikke negative effekter. I forhold til substratoxidation og energiforbrænding er effekten af kaloriefri og -reducerede sødemidler ikke blevet undersøgt i omfattende grad. Derfor var formålet med dette ph.d.-projekt at vurdere potentielle effekter af kaloriefri og -reducerede sødemidler på substratoxidation, energiforbrænding og appetit - med fedtoxidation som det primære undersøgelsesformål.

I **Artikel I** blev den eksisterende litteratur, der har evalueret substratoxidation, energiforbrænding og katekolaminer i relation til kaloriefri og -reducerede sødemidler, systematisk gennemgået. **Artikel I** viste, at drikkevarer og måltider med kaloriefri og -reducerede sødemidler resulterede i øget fedtoxidation og lavere kulhydratoxidation sammenlignet med sukker; en konklusion, der blev tilskrevet det ekstra kulhydrat- og deraf energiindhold i sukkerinterventionerne. Resultaterne for energiforbrænding viste uoverensstemmelser. Der kunne ikke drages yderligere konklusioner på grund af utilstrækkelig og uoverensstemmende data. **Artikel I** pegede på et behov for yderligere forsøg, hvor interventionskosten matches ift. kulhydrat- og energiindhold samt et behov for studier af længere varighed.

Artikel II præsenterede det 1-årige europæiske randomiserede multicenter forsøg SWEET WP3. Dette havde til formål at undersøge de langvarige effekter af kaloriefri og -reducerede

sødemidler i relation til sundhed, fedme og sikkerhed. Undersøgelsen blev gennemført som en del af dette ph.d.-projekt.

Artikel III og IV præsenterede design, udførelse og resultater af et sub-studie med deltagere fra SWEET WP3. Formålet med sub-studiet var at undersøge de akutte effekter af en testdrik med acesulfam kalium og cyclamat i relation til faste- og postprandial substratoxidation, energiforbrænding, insulin- og glukosekoncentrationer samt appetit sammenlignet med vand. Dette blev undersøgt ved baseline, efter en 2-måneders vægttabsperiode og efter en 4-måneders vægtvedligeholdelsesperiode med enten kaloriefri og -reducerede sødemidler eller sukker i kosten. Sub-studiet fandt ingen forskelle mellem de 2 interventionsgrupper ift. faste- og postprandial substratoxidation, energiforbrænding, insulin- og glukosekoncentrationer på tværs af de 3 testdage ($p > 0,05$). Ift. appetit viste sub-studiet, at sødemiddelgruppen gav en lavere vurdering af appetitscorerne forventet fødevarerindtag og lyst til at spise noget sødt efter indtagelse af testdrikken med acesulfam kalium og cyclamat på tværs af de 3 testdage sammenlignet med sukkergruppen, der indtog vand ($p < 0,05$).

Samlet set indikerer data fra artiklerne i denne afhandling, at kaloriefri sødemidlers påvirkning af substratoxidation og energiforbrænding, både akut og på længere sigt, er tilsvarende den neutrale påvirkning fra vand. Ift. appetit antyder **Artikel IV**, at acesulfam kalium og cyclamat kan inducere sensorisk specifik mæthed både akut og efter regelmæssigt indtag af kaloriefri og -reducerede sødemidler. Dette kunne indikere, at kaloriefri sødemidler kan forbedre snarere end hæmme visse aspekter af appetitregulering. Ift. kaloriereducerede sødemidler er datamængden generelt mindre, hvorfor styrken i denne afhandling skal findes i behandlingen af data for kaloriefri sødemidler. Dog kan det på baggrund af resultaterne i **Artikel I** konkluderes, at kaloriereducerede sødemidler viser øget fedtoxidation og lavere kulhydratoxidation sammenlignet med sukker under forhold, hvor sukkerinterventionen indeholdt mere energi. Således synes kaloriereducerede sødemidler ikke at påvirke substratoxidation ud over hvad der kan forventes på baggrund af deres energiindhold og metabolisme. Generelt er der behov for mere forskning i både kaloriefri og -reducerede sødemidler ift. alle de parametre, der er undersøgt i denne afhandling. Fremtidige studier bør baseres på interventioner med standardiseret energi- og kulhydratindhold og bør inkludere en bred vifte af forskellige kaloriefri og -reducerede sødemidler indtaget i forskellige sammenhænge og over en periode på minimum 1 år.

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