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Abstract

The pharmacological effect of growth differentiation factor 15 (GDF15) on suppressed appetite has undergone scientific scrutiny. However, this effect may be driven by nausea in rodents through the GDNF family receptor α-like (GFRAL), located in the hindbrain. In contract, less is known about the endogenous effect of plasma GDF15 levels on appetite suppression. In **Study 1**, the aim was to investigate the relationship between exercise-induced endogenous plasma GDF15 levels and appetite suppression. We found that plasma GDF15 levels increased after one bout of exercise in four independent exercise studies and reached "pathological levels" when the exercise bout lasted beyond 2 hours. In mice subjected to forced treadmill running, we observed comparable increase in plasma GDF15 levels as in humans immediately following exercise. However, we did not observe any suppression of appetite in wild type (WT) nor GFRAL knockout (KO) mice in the hours following exercise, suggesting that endogenous GDF15 levels may not be sufficient to suppress appetite.

Metformin, a blood-glucose-lowering medication, has been reported to increase endogenous plasma GDF15 levels and to decrease body weight by reducing appetite in pre-diabetic and type 2 diabetic individuals. In **Study 2**, we aimed to investigate whether there is a relationship between elevated metformin-induced endogenous plasma GDF15 levels and body weight loss and appetite reduction. We found that metformin treatment increased plasma GDF15 levels in both human and mice. When we administrated metformin to diet-induced obese (DIO) WT, DIO GFRAL KO and DIO GDF15 KO mice, we observed a reduction in body weight and appetite. This effect was independent of the GDF15-GFRAL pathway, in contrast to previous findings. Our data suggest that the lowering effect of metformin on body weight is independent of GDF15, however, further research is needed to elucidate the underlaying mechanisms of the effect of metformin on energy balance.

Fibroblast growth factor 21 (FGF21) has been extensively studied in rodents for its pharmacological effect on energy expenditure. Moreover, endogenous plasma FGF21 levels have been shown to increase in mice fed a protein-restricted diet, which was accompanied by increased energy expenditure. In **Study 3**, we aimed to investigate whether humans exhibit a similar phenotype when subjected to a prolonged eucaloric protein-restricted diet. We found that a protein-restricted diet high in carbohydrate or fat increased fasting plasma FGF21 levels in healthy young men. Moreover, we found that a protein-restricted diet necessitated an increase in energy intake to maintain body weight. These findings suggest that a protein-restricted diet has the potential to prevent weight gain and the

development of obesity by increasing energy utilization in humans, perhaps via increased FGF21 secretion. Further research is required to fully understand the underlaying mechanisms responsible for the need to increase energy intake to maintain body weight when ingesting a protein-restricted diet.

Resumé (dansk)

Den farmakologiske effekt af growth differentiation factor 15 (GDF15) på appetitundertrykkelse er velundersøgt. Denne effekt kan dog være drevet af kvalme hos gnavere gennem GDNF-familie receptor α-like (GFRAL) lokaliseret i baghjernen. Modsat vides der ikke meget omkring den endogene effekt af plasma GDF15 niveauer på appetitundertrykkelse. I **studie 1** var formålet at undersøge sammenhængen mellem træningsinduceret endogen plasma GDF15 niveauer og appetitundertrykkelse. Vi fandt, at plasma GDF15 niveauer steg efter akut arbejde i fire uafhængige træningsstudier og nåede "patologiske niveauer", når det akutte arbejde varede mere end 2 timer. I mus, som blev udsat for tvunget løb på løbebånd, observerede vi en sammenlignelig stigning i plasma GDF15 niveauer som hos mennesker umiddelbart efter arbejde. Vi observerede dog ingen undertrykkelse af appetitten i wild type (WT) eller GFRAL-knockout (KO) mus i timerne efterfulgt arbejdet, hvilket tyder på, at endogen GDF15 niveauer muligvis ikke er tilstrækkelige til at undertrykke appetitten.

Metformin, en blodsukkersænkende medicin, er blevet rapporteret til at øge endogene plasma GDF15 niveauer samt reducere kropsvægten via reduceret appetit hos prædiabetiske og type 2-diabetiske individer. I **Studie 2** var formålet at undersøge, om der er en sammenhæng mellem forhøjet merformin-induceret endogene plasma GDF15 niveauer, kropsvægttab og appetitreduktion. Vi fandt, at metformin behandling øgede plasma GDF15 niveauer i både mennesker og mus. Da vi administrerede metformin til diæt-induceret overvægtige (DIO) WT, DIO GFRAL KO og DIO GDF15 KO mus, observerede vi en reduktion i kropsvægt og appetit. Denne effekt var uafhængig af GDF15-GFRAL-vejen, i modsætning til tidligere fund. Vores data tyder på, at den sænkende effekt of metformin på kropsvægt og appetit er uafhængig af GDF15, men yderligere forskning er nødvendigt for at belyse de underliggende mekanismer for effekten af metformin på energibalancen.

Fibroblast growth factor 21 (FGF21) er blevet grundig undersøgt i gnavere for dens farmakologiske effekt på energiforbrug. Desuden har endogene plasma FGF21 niveauer vist sig a stige i mus fodret med en proteinrestriktiv diæt samtidig med øget energiomsætning. I **Studie 3** var formålet at undersøge, om mennesker udviser lignende fænotype, når de indtager en langvarig eukalorisk proteinrestriktiv diæt. Vi fandt, at en proteinrestriktiv diæt høj på kulhydrat eller fedt øgede faste plasma FGF21 niveauer hos raske unge mænd. Desuden fandt vi, at en proteinrestriktiv diæt nødvendiggjorde en stigning i energiindtaget for at opretholde kropsvægten. Disse resultater tyder på,

at en proteinrestriktiv diæt har potentiale til at forhindre vægtøgning og udvikling of fedme ved at øge energiudnyttelsen hos mennesker, måske via øget FGF21 sekretion. Yderligere forskning er påkrævet for fuldt ud at forstå de underliggende mekanismer, der er ansvarlige for behovet for at øge energiindtaget for at opretholde kropsvægten, når en proteinrestriktiv diæt indtages.

List of manuscripts

This PhD thesis is based on three manuscripts: two published and one ready for submission. They are referred to as Study 1-3.

Study 1

Pharmacological but not physiological GDF15 suppresses feeding and the motivation to exercise

Nature Communications, 2021, Feb 15;12(1):1041

Anders B. Klein*, **Trine S. Nicolaisen***, Niels Ørtenblad, Kasper D. Gejl, Rasmus Jensen, Andreas M. Fritzen, Emil L. Larsen, Kristian Karstoft, Henrik E. Poulsen, Thomas Morville, Ronni E. Sahl, Jørn W. Helge, Jens Lund, Sarah Falk, Mark Lyngbæk, Helga Ellingsgaard, Bente K. Pedersen, Wei Lu, Brian Finan, Sebastian B. Jørgensen, Randy J. Seeley, Maximilian Kleinert, Bente Kiens, Erik A. Richter & Christoffer Clemmensen.

Study 2

The GDF15-GFRAL pathway is dispensable for the effects of metformin on energy balance

Cell Reports, 2022, Aug 23;40(8):111258

Anders B. Klein*, **Trine S. Nicolaisen***, Kornelia Johann*, Andreas M. Fritzen, Cecilie V. Mathiesen, Cláudia Gil, Nanna S. Pilmark, Kristian Karstoft, Martin B. Blond, Jonas S. Quist, Randy J. Seeley, Kristine Færch, Jens Lund, Maximilian Kleinert & Christoffer Clemmensen.

Study 3

Protein-restricted diet increases circulating FGF21 levels and necessitating an increase in energy intake to maintain body weight in healthy human individuals

Manuscript in preparation

Trine S. Nicolaisen, Kim A. Sjøberg, Anne-Marie Lundsgaard, Andreas M. Fritzen, Aslak E. Lyster, Jakob K. Jensen, Daniel Haas, Matilde E. Madsen, Casper k. Nielsen, Mads Bloch-Ibenfeldt**,** Nicolai J. Wewer Albrechtsen, Adam J. Rose, Natalie Krahmer, Christoffer Clemmensen*,* Erik A. Richter & Bente Kiens.

* Shared first-author

Publication list

Greater molecular capacity for glucose uptake in adipose tissue and skeletal muscle of women compared with men

Resubmitted to Biology of Sex Differences

Trine S. Nicolaisen, Andreas M. Fritzen, Kim A. Sjøberg, Christian S. Carl, Erik A. Richter, Bente Kiens & Anne-Marie Lundsgaard

The anorectic and thermogenic effects of pharmacological lactate in male mice are confounded by treatment osmolarity and co-administered counterions

Nature Metabolism, 2023 April 13, 10.1038

Jens Lund, Alberte Wollesen Breum, Cláudia Gil, Sarah Falk, Frederike Sass, Marie Sophie Isidor, Oksana Dmytriyeva, Pablo Ranea-Robles, Cecilie Vad Mathiesen, Astrid Linde Basse, Olivia Sveidahl Johansen, Nicole Fadahunsi, Camilla Lund, **Trine Sand Nicolaisen**, Anders Bue Klein, Tao Ma, Brice Emanuelli, Maximilian Kleinert, Charlotte Mehlin Sørensen, Zachary Gerhart-Hines & Christoffer Clemmensen

Divergent Roles of α5 and β4 Nicotinic Receptor Subunits in Food Reward and Nicotine-induced Weight Loss in Male Mice

Endocrinology, 2022 Jul 1;163(7)

Alberte Wollesen Breum, Sarah Falk, Charlotte Sashi Aier Svendsen, **Trine Sand Nicolaisen**, Cecilie Vad Mathiesen, Uwe Maskos & Christoffer Clemmensen

Thyroid hormone receptor α in skeletal muscle is essential for T3-mediated increase in energy expenditure

FASEB J. 2020, Nov:34(11):15480-15491

Trine S. Nicolaisen[*], Anders B. Klein[*], Oksana Dmytriyeva, Jens Lund, Lars R. Ingerslev, Andreas M. Fritzen, Christian S. Carl, Anne-Marie Lundsgaard, Mikkel Frost, Tao Ma, Peter Schjerling, Zachary Gerhart-Hines, Frederic Flamant, Karine Gauthier, Steen Larsen, Erik A. Richter, Bente Kiens & Christoffer Clemmensen

Pharmacological targeting of α3β4 nicotinic receptors improves peripheral insulin sensitivity in mice with diet-induced obesity

Diabetologia, 2020 Jun;63(6):1236-1247

Sigrid Jall, Meri De Angelis, Anne-Marie Lundsgaard, Andreas M. Fritzen, **Trine S. Nicolaisen**, Anders B. Klein, Aaron Novikoff, Stephan Sachs, Erik A. Richter, Bente Kiens, Karl-Werner Schramm, Matthias H. Tschöp, Kerstin Stemmer, Christoffer Clemmensen, Timo D.-Müller & Maximilian-Kleinert

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Molecular Metabolism, 2020 May; 35: 100949

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Glucometabolic consequences of acute and prolonged inhibition of fatty acid oxidation

Journal of Lipid Research, 2020 Jan; 61(1): 10-19

Anne-Marie Lundsgaard, Andreas M. Fritzen, **Trine S. Nicolaisen**, Christian S. Carl, Kim A. Sjøberg, Steffen H. Raun, Anders B. Klein, Eva Sanchez-Quant, Jakob Langer, Cathrine Ørskov, Christoffer Clemmensen, Matthias H. Tschöp, Erik A. Richter, Bente Kiens & Maximilian Kleinert

Mechanisms Preserving Insulin Action during High Dietary Fat Intake

Cell Metabolism 2019, Jan 8;29(1):50-63

Anne-Marie Lundsgaard, Jacob B. Holm, Kim A. Sjøberg, Kirstine N. Bojsen-Møller, Lene S. Myrmel, Even Fjære, Benjamin A.H. Jensen, **Trine S. Nicolaisen**, Janne R. Hingst, Sine L. Hansen, Sophia Doll, Philip E. Geyer, Atul S. Deshmukh, Jens J. Holst, Lise Madsen, Karsten Kristiansen, Jørgen F.P. Wojtaszewski, Erik A. Richter & Bente Kiens

* Shared first-author