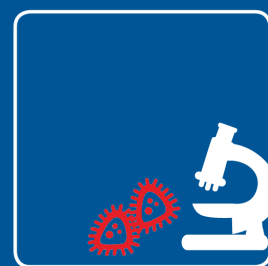


Bente Kiens:

SMIL – Specielle sundhedsforbedrende fedtsyrer i mælk

SMIL – Special milk lipids for health



Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

Dansk: Specielle sundhedsforbedrende fedtsyrer i mælk

English: SMIL - Special Milk Lipids for Health

2. Project manager

Bente Kiens, Professor

Institut for Idræt og Ernæring, Københavns Universitet

August Krogh Bygningen, Universitetsparken 13,

2100 København Ø

+45 28751619

bkiens@nexs.ku.dk

3. Other project staff

Lars Wiking, Institut for Fødevarer , Aarhus Universitet, Lars.wiking@food.au.dk

Zheng Guo, Institut for Bio- og Kemiteknologi , Aarhus Universitet, guo@bce.au.dk

Andreas Fritzen, Institut for Biomedicin, Panum, Københavns Universitet, 42633359, amfritzen@sund.ku.dk

Maximilian Kleinert, German Institute of Human Nutrition (DiFe), Germany, Maximilian.kleinert@dife.ge

4. Source of funding

Milk Levy Fund

Arla – Food for Health

Novo Nordisk Fonden

5. Project period

Project period with DDRF funding: January 2021 – December 2024

Total project period, if sub-project within a larger project: January 2020- June 2025

6. Project summary

Dansk:

Formål

Formålet har været at skabe en ny mejeriingrediens rig på mellemkædede fedtsyrer (MCFA'er) og vurdere dens potentielle sundhedsmæssige fordele i mennesker; herunder effekter på insulinfølsomhed, vægtkontrol og risikofaktorer for hjerte-kar-sygdomme. Derudover har formålet været at identificere fysiologiske og molekylære mekanismer bag samt biomarkører for effekter af MCFA-indtagelse.

Resultater

Vi har udviklet en ny metode til at berige mælkefedt med MCFA. Ved at kombinere tør- eller solventfraktionering af mælkefedt med efterfølgende kortvejs-destillation (SPD) har vi opnået en berigelse af MCFA fra 10,1 % til 20,2 % sammenlignet med naturligt mælkefedt. For kortkædede fedtsyrer (C4-C6) steg indholdet fra 6,9 % til 15 %. Kombinationen af SPD med tør- eller solventfraktionering førte ikke til en væsentlig yderligere berigelse af MCFA. Brug af mælkefedt fra Jersey-køer gav en let højere MCFA-koncentration i destillatet. Destillatet udgjorde ca. 8-12 % af udgangsmaterialet. Ud over MCFA-berigelse resulterede SPD også i en betydelig stigning i E-vitaminindholdet i destillatet, fra 2,5 mg/100 g i mælkefedt til 47-63 mg/100 g fedt. Smelte- og krystallisationspunkter samt fast-fedtindhold blev analyseret i alle fraktioner, og som forventet havde destillatet lavere smeltepunkt pga. af de kortere fedtsyrer.

Retentater fra kortvejsdestillationen (SPD) er blevet analyseret. I dette projekt er retentatet en sidefraktion, men udgør ~90 % af massen. Samlet set var retentatet beriget i fedtsyrerne C18:0 og C18:1-trans og reduceret i fedtsyrer med kædelængder på C4-14 sammenlignet med mælkefedt.

I en opskaleret opsætning blev der produceret 12 kg fødevarer godkendt destillat (kaldet MSFAT) med et MCFA-indhold på 14,7 % til forsøg med mennesker. Derudover blev 1,5 kg destillat produceret fra Jersey-mælk til forsøg med mus med et MCFA-indhold på 20 %.

Vores proof-of-concept-studier på mus viste, at indtagelse af MSFAT øgede cirkulerende niveauer af ketonstoffer, sænkede blodsukkerniveauet og undertrykte fødeindtagelsen (skabte mæthed).

Hos mennesker resulterede indtagelse af MSFAT i øgede cirkulerende ketonstoffer, en tendens til at reducere ($p = 0,07$) postprandiale glukosestigninger og en akut stigning i energiomsætning. Vores resultater viser, at mejeriprodukter beriget med MSFAT kan have betydelige metaboliske fordele.

Undersøgelsen viste også, at indtagelse af kommercielt tilgængelige mellemkædede triacylglyceroler (MCT) hos mennesker øgede ketogenesen og stofskiftet samt sænkede blodsukkerniveauet over 5 timer. Den hypoglykæmiske virkning af MCT-indtagelsen var ledsaget af en samtidig midlertidig stigning i plasma-insulin og glukagonniveauer. Effekterne på ketogenese, stofskifte og glykæmi blev bevaret hos personer med fedme og opretholdt efter 8 dages daglig tilskud.

Sideløbende har vi blotlagt de fysiologiske effekter af indtagelse af MCFA i både slanke og personer med overvægt – både akutte effekter og effekter af længevarende MCFA-supplementering til kosten. Dette viser, at der er positive effekter på glukosestofskiftet og energibalancen ved indtagelse af mellemkædede fedtsyrer – og det ses både i slanke og personer med overvægt – og både akut og ved længevarende indtagelse af MCFA.

Endeligt har vi ved brug af transgene dyr identificeret nogle af de molekylære mekanismer der underligger de fysiologiske effekter af mellemkædede fedtsyrer ved bl.a. at identificere to hormoner frigivet fra leveren, som synes at være involveret i dels den mæthedsinducerende effekt og dels en sænkende effekt af leverfedtindholdet ved indtagelse af mellemkædede fedtsyrer.

Sammenfatning

Resultaterne understreger de sundhedsmæssige fordele ved mellemkædede fedtsyrer hos både slanke og metabolisk udfordrede individer efter dagligt tilskud, også ved indtagelse af destillat udvundet fra mælk. Desuden afslører vi nye

aspekter af biologien omkring mellemkædede fedtsyrer og giver indsigt i, hvordan disse fedtsyrer fremkalder fysiologiske effekter hos mennesker.

English:

The aim has been to create a novel dairy ingredient rich in medium-chain fatty acids (MCFAs) and assess its potential health benefits in humans, including insulin sensitivity, body weight control and cardiovascular risk factors. Furthermore, the aim has been to identify mechanisms and biomarkers for MCFA action

Results: By combining dry or solvent fractionation of milk fat and subsequent short-path distillation (SPD) we have obtained an enrichment of MCFA (medium-chain fatty acids) from 10.1 to 20.2% compared to native milk fat. For short-chain fatty acids (C4-6), we increased from 16.9% to 15%. Combining SPD with dry or solvent fractionation did not lead to significant enrichment of MCFA. Using milk fat from Jersey cows yielded a slightly higher MCFA content in the distillate. The distillate represents approximately 8-12% of the starting material (milk fat). In addition to MCFA enrichment, SPD also resulted in a significant increase in Vitamin E content in the distillate, achieving 47-63 mg/100g fat compared to 2.5 mg/100g in milk fat. Melting and crystallization points, as well as solid fat content, were analyzed in all fractions.

Retentates from the Short-path distillation (SPD) has been analyzed, which in this project is the side-stream but occupy ~90% of the mass. Overall, the retentate was enriched in the fatty acids, C18:0 and C18:1-trans, and reduced in the fatty acids of chain-lengths of C4-14 compared to milk fat.

12 kg of food-grade distillate (named MSFAT) with an MCFA content of 14.7% was produced in an upscaled setup for human trials. Additionally, 1.5 kg of distillate was produced from Jersey milk for mouse trials, with an MCFA content of 20%.

Our proof-of-concept studies in mice demonstrated that intake of MSFAT increased circulating ketone bodies, reduced blood glucose levels, and suppressed food intake.

In humans, ingestion of MSFAT resulted in increased circulating ketone bodies, trended to attenuate ($p = 0.07$) postprandial glucose excursion, and acutely elevated energy expenditure. Our findings show that milk products enriched with MSFAT may hold significant metabolic advantages.

The study also revealed that consumption of commercial available medium-chain triacylglycerol (MCT) in humans increased ketogenesis and metabolic rate while lowering blood glucose levels over 5 h. The hypoglycemic action of MCT intake was accompanied by a concomitant transient increase in plasma insulin and glucagon levels. The effects on ketogenesis, metabolic rate, and glycemia were preserved in individuals with obesity and sustained after 8 days of daily supplementation.

In parallel, we have revealed the physiological effects of MCFA intake in both lean and overweight individuals – both acute effects and effects of long-term MCFA supplementation to the diet. This shows that there are positive effects on glucose metabolism and energy balance when consuming medium-chain fatty acids – and this is seen in both lean and overweight individuals – and both acutely and with long-term intake of MCFA.

Finally, using transgenic animals, we have identified some of the molecular mechanisms underlying the physiological effects of medium-chain fatty acids by, among other things, identifying two hormones released from the liver that appear to be involved in both the satiety-inducing effect and a lowering effect on liver fat content when consuming medium-chain fatty acids.

In summary, these findings underscore the health benefits of medium-chain fatty acids in lean as well as in metabolically compromised individuals after daily supplementation also when consuming distillate extracted from milk. Moreover, we uncover novel aspects of medium-chain fatty acid- biology, providing insights into how these fatty acids orchestrate physiological effects in humans.

7. Project aim

Dansk: Formålet med projektet var at skabe en ny mejeri-ingrediens rig på mellemkædede fedtsyrer (MCFAs) og vurdere dens potentielle sundhedsmæssige fordele hos mennesker, herunder effekter på insulinfølsomhed, kropsvægtkontrol og kardiovaskulære risikofaktorer. Ydermere har målet været at identificere fysiologiske og molekylære mekanismer og biomarkører for effekter af indtagelse af MCFA.

English: The aim has been to create a novel dairy ingredient rich in medium-chain fatty acids (MCFAs) and assess its potential health benefits in humans, including insulin sensitivity, body weight control and cardiovascular risk factors. Furthermore, the aim has been to identify physiological and molecular mechanisms and biomarkers for MCFA action.

8. Background for the project

Medium-chain fatty acids (MCFAs) refer to fatty acids which consist of 8-12 carbon atoms. MCFAs make up ~10% of the fat in milk and intake of MCFAs has been shown to improve insulin sensitivity and increase energy expenditure in humans. It was, however, previously assessed that dairy products enriched for MCFAs did not induce benefits on weight loss and insulin levels in individuals with obesity. However, in that study, milk was enriched for MCFAs by feeding cows a low-fat diet only and the mean intake of MCFAs was increased from 6.9 to 8.5 g/day. It is plausible that an increase of 1.6 g of MCFAs per day is insufficient to trigger metabolic adaptations. The idea was therefore to produce a milk fat fraction with a high and optimized content of MCFA and secure the quality and functionality of the fraction. The next step was to test this milk-fat fraction for physiological and metabolic benefits in mice and humans.

Therefore, we established two interconnected work packages (WP). In **WP1**, Aarhus University (FOOD and ENG), we aimed at combining for the first time raw milk selection, dry fractionation and short-path distillation, to generate milk lipid fractions with >2-3 fold higher MCFA content than regular milk fat. After in-depth MS/MS lipidomics-based characterization, kilogram amounts of this novel MCFA-rich milk fat were produced for testing in humans. In **WP2**, Copenhagen University (NEXS) investigated the mechanisms by which MCFAs improve health in rodents and humans – including an evaluation of which MCFAs are most potent in inducing the health beneficial effects. Furthermore, the novel MCFA-rich milk fat (produced in WP1) will be tested and elucidate whether this distillate has health beneficial effects on energy turnover, glucose metabolism and insulin tolerance in humans – and also in mice to get a more mechanistic approach.

Collectively, we are combining state-of-the-art technology and biology to create a new dairy product that has the potential to become a new nutritional ingredient in a healthy diet for the population and to help combat obesity and type 2 diabetes.

9. Sub-activities in the entire project period

We have produced a milk fat fraction with a high and optimized content of MCFA for delivering to WP2 and secure the quality and functionality of the fraction. AU FOOD has collected milk, separated milk fat, performed pre-fractionation, and examined quality and functionality. AU ENG has conducted short-path distillation and has produced the amount (several kilograms of dairy fat) needed for human studies. AU has also studied the functionality of both fractions, and the oxidative stability of the retentate.

We have in KU NEXS performed human studies evaluating health beneficial effects of an acute and long-term intake of MCT vs LCT in lean men and men with obesity. We have also elucidated the effects of the acute intake of the distillate in humans as well as in mice.

Mice studies have been performed in order to elucidate the mechanisms behind the beneficial health effects of MCT.

10. Deviations

Originally one task was to feed humans the novel MCFA-rich fat produced in WP1 (High MCFA HFD) in a hypercaloric high fat diet including 15% fat from the novel distillate for three days versus three days on a eucaloric, mixed diet (50 energy % (E) carbohydrate, 35E% Fat, 15E% Protein) (CON). The aim was then to measure different health-related parameters following the diet intervention. However, it turned out that it was not possible to produce the amount of distillate needed also due to the low yield from the extraction. In-stead we have performed acute studies in lean and obese humans and mice with the distillate.

11. Project results

A series of experiments combining dry or solvent fractionation of milk fat and subsequent short-path distillation (SPD) have been conducted. SPD alone resulted in an enrichment of MCFA (medium-chain fatty acids) from 6.9% to 15% compared to native milk fat. For short-chain fatty acids (C4-6), we increased from 10.1% to 20.2%. Combining SPD with dry or solvent fractionation did not lead to significant enrichment of MCFA. Using milk fat from Jersey cows yielded a slightly higher MCFA content in the distillate. The distillate represents approximately 8-12% of the starting material (milk fat).

In addition to MCFA enrichment, SPD also resulted in a significant increase in Vitamin E content in the distillate, achieving 47-63 mg/100g fat compared to 2.5 mg/100g in milk fat. Melting and crystallization points, as well as solid fat content, were analyzed in all fractions.

12 kg of food-grade distillate with an MCFA content of 14.7% was produced in an upscaled setup for acute human trials. Additionally, 1.5 kg of distillate was produced from Jersey milk for mouse trials, with an MCFA content of 20%.

Retentates from the Short-path distillation (SPD) has been analyzed, which in this project is the side-stream but occupy ~90% of the mass. While our previous work described the potential health benefits of the distillate rich in short- and medium-chain fatty acids, the present study examined the application potential and oxidative stability of the retentate fraction. Both lab- and a production scale retentates were produced and tested. Overall, retentate was enriched in the fatty acids, C18:0 and C18:1-trans, and reduced in the fatty acids of chain-lengths of C4-14 compared to milk fat (MF). Differential scanning calorimetry and oscillatory rheology analyses showed early nucleation and network formation for the retentates compared to the MF. Moreover, the retentate had higher solid fat content. At high and low supercooling, the hardness during storage for 14 days was similar for MF and retentate. The formation of peroxides and color changes during storage were unaffected by fat type.

These results are all in accordance with the set goals.

In accordance with the set goals, we have performed a large part of testing of physiological and metabolic effects of MCFA in general in humans and underlying mechanisms in transgenic mice.

In humans, we have elucidated the postprandial physiological and metabolic effects of MCFA, obtained from commercial oils, both acutely and again after 8 days intake. This was compared with intake of energy-matched triacylglycerol consisting of long-chain fatty acids (long-chain triacylglycerols, LCT) using a randomized cross-over design. Besides

studying lean men ($n = 8$) we also performed these studies in men with obesity ($n = 8$). The study revealed that consumption of medium-chain triacylglycerol (MCT) increased ketogenesis and metabolic rate while lowering blood glucose levels over 5 h. The hypoglycemic action of MCT intake was accompanied by a concomitant transient increase in plasma insulin and glucagon levels. The effects on ketogenesis, metabolic rate, and glycemia were preserved in individuals with obesity and sustained after 8 days of daily supplementation. Lipidomic plasma analysis in lean individuals ($n = 4$) showed that a part of the ingested MCT bypasses the liver and enters the systemic circulation as medium-chain fatty acids (MCFAs). The findings suggest that MCFAs, along with ketone bodies from the liver, may act as signaling molecules and/or substrates in the peripheral tissues, thereby contributing to the effects of MCT intake. In summary, these findings underscore the health benefits of MCT in metabolically compromised individuals after daily supplementation. Moreover, we uncover novel aspects of MCFA biology, providing insights into how these fatty acids orchestrate physiological effects in humans.

We have also been able to demonstrate that within just eight days of daily MCT intake an impressive 38% increase in basal, fasting plasma EPO concentrations ($7.19 \pm 1.14 \text{ mIU} \cdot \text{ml}^{-1}$ to $9.91 \pm 1.25 \text{ mIU} \cdot \text{ml}^{-1}$) was observed. This unveils a novel physiological stimulus of circulating EPO concentrations in humans, potentially offering a new dietary approach to counter anemia in cardiovascular diseases.

We have elucidated novel molecular mechanisms behind MCFA effects in mice. We have shown that MCFA high-fat diet (HFD) feeding improves glucose tolerance, enhances glucose clearance into brown adipose tissue, and prevents high-fat diet-induced hepatic steatosis in wildtype mice. These benefits were associated with increased liver expression of CREBH target genes (Apoa4 and Apoc2), including Fgf21. Both acute and chronic intake of dietary MCFAs elevated circulating FGF21. Augmented hepatic Fgf21 mRNA following MCFA HFD intake was accompanied by higher levels of active hepatic CREBH; and MCFA-induced hepatic Fgf21 expression was blocked in mice lacking Crebh. Notably, while feeding male and female Fgf21 wildtype mice MCFA HFD resulted in reduced liver triacylglycerol (TG) levels, this liver TG-lowering effect was blunted in Fgf21 knockout mice fed MCFA HFD. The reduction in liver TG levels observed with MCFA HFD was independent of weight loss. This collectively showed that dietary MCFAs reduce liver fat accumulation via activation of a CREBH-FGF21 signaling axis.

We were also able to demonstrate that hepatic and small intestinal expression of Gdf15 and circulating GDF15 increased after ingestion of MCFAs, while intake of typical dietary long-chain fatty acids (LCFAs) had no effect. Plasma GDF15 levels also increased in the portal vein with MCFA intake, indicating that in addition to the liver, the small intestine contributes to the rise in circulating GDF15. Acute oral provision of MCFAs decreased food intake over 24 h compared with a LCFA-containing bolus, and this anorectic effect required the GDF15 receptor, GFRAL. Moreover, sub-chronic oral administration of MCFAs reduced body weight over 7 days, an effect that was blunted in mice lacking either GDF15 or GFRAL. This collectively demonstrated that we have identified ingestion of MCFAs as a novel nutritional approach that increases circulating GDF15 in mice and have revealed that the GDF15-GFRAL axis is required for the full anorectic effect of MCFAs.

In accordance with the set goals, we have made studies in humans and mice using the milk fat with a 2-fold enrichment of medium- and short-chain fatty acids (referred to as MSFAT/distillate).

Our proof-of-concept studies in mice demonstrated that intake of MSFAT increased circulating ketone bodies, reduced blood glucose levels, and suppressed food intake.

In humans, ingestion of MSFAT resulted in increased circulating ketone bodies, trended to attenuate ($p = 0.07$) post-prandial glucose excursion, and acutely elevated energy expenditure. Our findings show that milk products enriched with MSFAT may hold significant metabolic advantages.

12. The relevance of the results, including relevance for the dairy industry

The present project provides new technology to enrich MCFAs in milk fat and demonstrates that milk products enriched with MCFAs appears to hold significant metabolic advantages and have potential to increase the value of milk fat products by introducing new scalable processing technologies. This opens exciting avenues for the dairy industry.

By elucidating novel metabolic effects of MCFA intake and involved underlying physiological and molecular mechanisms uncovering mode-of-action for the health benefits of MCFAs, we further create potential for changing the dietary guidelines and provide novel pharmaceutical targets for metabolic disease prevention and/or treatment.

Interestingly, since we, thoroughly, investigated the retentate (side stream) from the present SPD technology and show that it has similar functionality as native milk, and thereby is expected to be able to be used as ingredients like native milk fat, we further believe we have found a technology that can be used to enrich milk fat to improve health and at the same time in a sustainable way be able to use the rest products from the production to further commercial and relevant consumable actions.

13. Communication and knowledge sharing about the project

Artikler i internationale tidsskrifter:

- 1) Kanta JM, Amendt L, Johann K, Holm S, Lundsgaard A, Lund A, Jähnert M, Schürmann A, Clemmensen C, Kiens B, Fritzen AM, Kleinert M. Dietary Medium-Chain Fatty Acids Reduce Food Intake via the GDF15-GFRAL Axis. *Molecular Metabolism*. Aug; 74:101760. IF2022: 8.568.
- 2) Kanta JM, Lundsgaard AM, Sarah Louise Armour, Jesper Havelund, Jakob G. Knudsen, Nils Færgeman, Richter EA, Kleinert M, Fritzen AM, Kiens B. Inductions of ketogenesis, postprandial thermogenesis and blood glucose lowering by medium-chain triacylglycerols are preserved in obesity and after prolonged supplementation. *Am J Physiol Endocrinol Metab*. 2025 Jan 1;328(1):E1-E20. doi: 10.1152/ajpendo.00234.2024. Epub 2024 Oct 23. PMID: 39441241
- 3) Kanta JM, Lundsgaard AM, Kleinert M, Fritzen AM, Kiens B. Induction of Erythropoietin by dietary Medium-Chain triacylglycerol in Humans. *Am J Physiol, Endocrinol Metab*. 2025, DOI: [10.1152/ajpendo.00415.20244](https://doi.org/10.1152/ajpendo.00415.20244).
- 4) Ye Cao Y, Araki M, Nakagawa Y, Amendt L, Lundsgaard A, Kanta JM, Holm S, Johann K, Jacobsen JCB, Jähnert M, Schürmann A, Kiens B, Clemmensen C, Shimano H, Fritzen AM, Kleinert M. Dietary Medium Chain Fatty Acids activate CREBH-FGF21 to Lower Liver Fat. *Mol Metabolism*, 2024.
- 5) S. Mathiasen R. Frydenberg, M. Kleinert, Z. Guo, B. Kiens, L. Wiking (2024). Novel milk fat fractions for health and functionality obtained by short-path distillation. *LWT* 201, 11625.
- 6) S. Mathiasen, J. Kanta, R. Frydenberg, A. Lundsgaard, Z. Guo, A. Fritzen, B. Kiens, L. Wiking & M. Kleinert (2024) Novel Methodology to Enrich Medium- and Short-Chain Fatty Acids in Milk Fat to Improve Metabolic Health. *Food & Function* 15, 7951-60.
- 7) S. Mathiasen, Z. Guo, A. Fritzen, B. Kiens M. Kleinert & L. Wiking. Material properties and stability of milk fat retentate fraction from shortpath distillation. *Int. Dairy Journal* (submitted and revised).

Indlæg ved faglige kongresser, symposier etc.:

Mathiasen S: [Novel milk fat fractions for health and functionality obtained by short path distillation](#), 17 sep. 2023, [19th Euro Fed Lipid Congress](#).

Wiking L: Healthier milk fat fractions through cow feeding or by volatility-based enrichment process, ADSA 2024 West Palm Beach

Fritzen AM: Introduction to medium-chain fatty acids – dairy fat for Health. Arla Food for Health conference. November 2021.

Fritzen AM: SMiL: Special Milk Lipids for Health. Arla Food for Health conference. November 2024. Fritzen AM: Ketogenic fatty acids – an alternative source to ketosis. Danish Cardiovascular Academy Conference: Ketosis: Metabolic Insights and Cardiovascular Effects. October 2024.

14. Contribution to master and PhD education

3 phd-students, 5 master students and bachelor students have been a part of this project and thereby the present project has contributed substantially to education of new scientists.

15. New contacts/projects

The present project has opened for further collaboration between Arla Amba and the investigators. Arla has provided funding for a new project related to the present project. Not only contains dairy products MCFA, but also short-chain fatty acids (SCFA), such as butyrate and propionate, and in the SPD-enrichment of MCFA in milk fat in the present project, a similar upregulation of SCFA were present. Interestingly, the investigators in the present project and Arla Amba share an interest in further understanding the biology and physiological effects of SCFA intake in humans. Thus, a project investigating the physiological and metabolic effects of acute and chronic intake of propionate and butyrate has recently been established, which can be considered as a direct result of the scientific progress and networking between Arla and the investigators during this project.