Sluttrapportering til FHF, prosjektnummer 901592 – Helseeffekt av ketolinsyre (i mus og i mennesker)

Dato: 26.03.25

1. Sammendrag

Norsk sammendrag:

Flerumettede fettsyrer har en kjent beskyttende effekt mot kardiovaskulær sykdom, men sammenhengen mellom langkjedede enumettede fettsyrer og kardiovaskulær risiko er mindre kjent. I denne studien var målet å undersøke effekten av supplement med ketolinsyre på kardiovaskulære risikofaktorer i en preklinisk modell og en human intervensjon. Mus som fikk supplement med ketolinsyre utviklet mindre aterosklerose sammenlignet med mus som fikk en høyfett diett, en diett beriket med flerumettede fettsyrer, eller en diett beriket med makrellolje. Videre viste vi at supplement med ketolinsyre hadde en reduserende effekt på både pro- og antiinflammatoriske cytokiner i plasma. Dette så vi også for diettene beriket med flerumettede fettsyrer eller en diett beriket med makrellolje. Supplement med ketolinsyre ga økte nivåer av ketolinsyre, som reflekterer dietten, samt økte nivåer av omega-3-fettsyrene eikosapentaensyre (EPA) og dokoheksaensyre (DHA) i plasma og lever. Videre så vi at supplement med ketolinsyre til friske humane individer ga økte nivåer av EPA, DHA og ETA i plasma og av ETA i røde blodceller; i tillegg til økt ketolinsyre, både i plasma og celler. Disse funnene tyder på en beskyttende effekt av ketolinsyre på utvikling av aterosklerose, mulig gjennom produksjon av andre gunstige fettsyrer og via anti-inflammatoriske mekanismer. Videre viser den humane studien at tilskudd med ketolinsyre inkorporeres og kan utføre sine potentielt gunstige effekter i humane blodceller.

English summary:

Polyunsaturated fatty acids have a known protective effect against cardiovascular disease, while the relationship between long-chain monounsaturated fatty acids and cardiovascular risk is less understood. This study aimed to investigate the effect of cetoleic acid supplementation on cardiovascular risk factors in a preclinical model and a human intervention. Mice supplemented with cetoleic acid developed less atherosclerosis than mice fed a high-fat diet, a diet enriched with polyunsaturated fatty acids, or a diet enriched with mackerel oil. Furthermore, we showed that cetoleic acid supplementation had a reducing effect on both pro- and anti-inflammatory cytokines in plasma. This was also observed for diets enriched with polyunsaturated fatty acids or mackerel oil. Cetoleic acid supplementation resulted in increased levels of cetoleic acid, reflecting the diet, as well as increased levels of omega-3 fatty acids EPA and DHA in plasma and liver. Additionally, we found that cetoleic acid supplementation in healthy individuals led to increased levels of EPA, DHA, and ETA in plasma and ETA in red blood cells, in addition to increased cetoleic acid in both plasma and cells. These findings suggest a protective effect of cetoleic acid on the development of atherosclerosis, possibly through the production of other beneficial fatty acids and via anti-inflammatory mechanisms. Furthermore, the human study shows that cetoleic acid supplementation is incorporated and can exert its potentially beneficial effects in human blood cells.

2. Innledning

Intake of fish has been shown in several epidemiological studies to have a health beneficial effect and regular consumption of omega-3 polyunsaturated fatty acids (PUFA) as fish or fish oils are associated with protection against cardiovascular disease [1, 2]. The fatty acid composition of different fish species varies greatly. In addition to EPA and DHA, certain fish species as the North Atlantic herring, capelin, mackerel and sand eel contains high levels of long-chain monounsaturated fatty acids (LC-MUFA), i.e., 22:1n-11, cetoleic acid [3]. This is in contrast South American fish oils which have higher concentration of the known long-chain marine polyunsaturated fatty acids, but less of the cetoleic acid. Compared with EPA and DHA, limited information is available on the health benefits of marine-derived LC-MUFA, including cetoleic acid, particularly in regard to cardiovascular disease (CVD). It is a promising new area of research that may lead to new insights into the health benefits of a different component of fish oils.

The main goal of the present study was to perform state-of-the art *human and animal intervention* studies *to investigate health-effects* of cetoleic acid derived from North Atlantic fish oils in comparison with fish oils low in cetoleic acid. The present study aims to elucidate the health effects after consumption of fish oil rich in cetoleic acid both with regard to effects on cardiometabolic risk markers including plasma lipids and lipid class distribution but also on other markers of health such as inflammatory markers.

3. Problemstilling og formål

The overall objectives were:

- To investigate the effects of fish oil enriched in cetoleic acid compared to a fish oil low in cetoleic acid, both oils balanced to the same EPA + DHA content, in a human pilot study and preclinical intervention studies with regard to cardiometabolic risk markers including plasma lipids and lipid class distribution and inflammatory markers.
- To further elucidate the underlying mechanisms by using targeted metabolomics lipidomics and transcriptomics before and after the intervention to further understand the health benefits and the mechanisms of action.

Primary end points:

- Mouse study: degree of atherosclerosis
- Human study: EPA and DHA in plasma and red blood cells

The added value of these data to the society is substantial. Today, North Atlantic fish oils are only to a relatively limited extent used for human nutrition, but primarily used for animal feeds. Also, byproducts from the North Atlantic Mackerel have only to a limited extent been utilized in production of fish oil, possibly also due to lack of knowledge regarding potential human health effects. In Norway, we have large and easily accessible volumes of these fish, which gives the possibility ***for effective production and regular deliverability***. Moreover, the added value of this project does not only relate to the huge economic benefit. Today, environmental and sustainability issues are important priorities within the society. There is a trend towards consumers preferring locally sourced products and that the waste relating to the utilization of the products is as low as possible ("eat the whole animal"). By documenting the health beneficial effects of human consumption of fish oil produced from byproducts from one of the species herring, capelin, mackerel and small sand eel, this project will contribute to ***an enormous release of value-added for society*** by utilizing this as of today un-used potential.

4. Prosjekt-gjennomføring

Mouse study:

ApoE KO mice (atherosclerosis prone mouse model) were subjected to a feeding experiment in which one group received a standard high fat diet (HFD), known to accelerate development of atherosclerosis and the other groups received a HFD enriched with cetoleic acid or mackerel oil or a diet enriched in polyunsaturated fatty acids (PUFA), with the same level of EPA/DHA as for the cetoleic and mackerel diet, but without cetoleic acid. After eight weeks of feeding the mice were sacrificed and atherosclerosis quantified. Plasma and liver lipid levels were measured, and circulating inflammatory markers assessed with MSD multiplex. We also performed RNA sequencing on livers.

Human study:

In the human study, 49 healthy adults were randomized to receive either oil rich in cetoleic acid or control oil. After a three-week run-in period receiving the control oil, the participants were randomized to receive capsules of either control oil or oil rich in cetoleic acid (800 -900 mg/day), with similar amounts of n-3 fatty acids (1100-1200 mg/day) (EPA, Docosapentaenoic acid (DPA), DHA, and ALA) for 4 weeks. Plasma and red blood cell fatty acids content after intervention was measured.

5. Oppnådde resultater, diskusjon og konklusjon

Mouse study: Throughout the study period there was no differences in body weight, or liver and adipose tissue weight between the diet groups. The mice receiving a diet enriched with cetoleic acid developed significant less atherosclerosis as compared to mice receiving a normal HFD and PUFA diet, as assessed by *en face*. There was no significant difference in atherosclerosis between mice fed a HFD and mice fed the PUFA or mackerel diet (Figure 1).

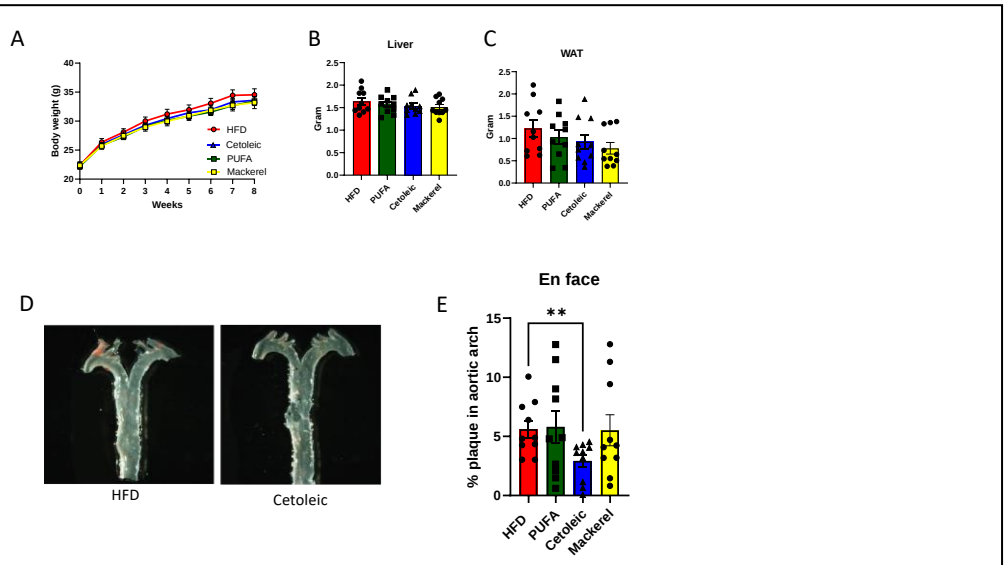

Figure 1. Data show A) Body weight, B) Liver weight, C, White adipose tissue (WAT) weight, D) Atherosclerosis in aortic arch by *en face*, E) Quantification of atherosclerosis in aortic arch after different dietary interventions. HFD: high fat diet, PUFA: polyunsaturated fatty acids. **p<0.01

Further, supplementation with cetoleic acid did not affect plasma levels of total cholesterol, triglycerides, non-esterified fatty acids (NEFA) or glucose (Figure 2). However, levels of insulin and C-peptide were

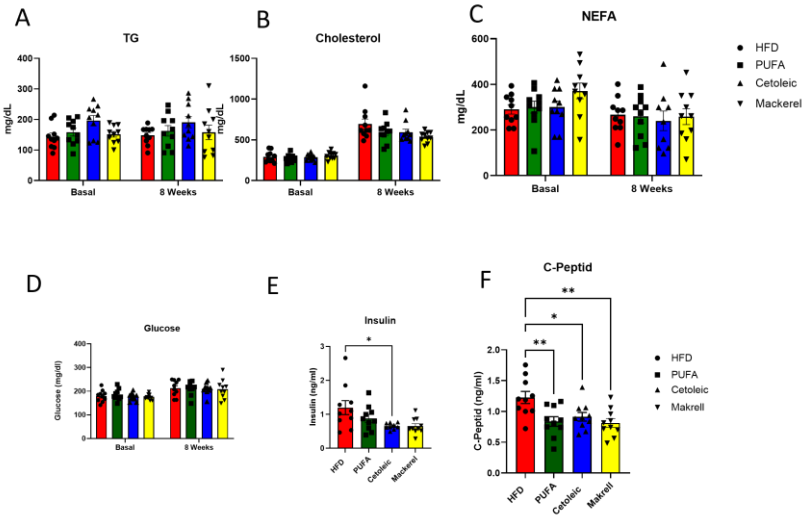

Figure 2. Plasma levels of A) triglycerides (TG), B) total cholesterol, C) non-esterified fatty acids (NEFA), D) glucose, E) insulin and F) C-peptide after dietary interventions. HFD: high fat diet, PUFA: polyunsaturated fatty acids. *p<0.05, **p<0.01

significantly reduced after supplementation with cetoleic acid, as compared to HFD. For C-peptide, this was also true for the PUFA and Mackerel group.

Looking at inflammatory markers in plasma; mice receiving cetoleic acid had significant reduced levels of circulating anti-inflammatory IL-10 as well as the pro-inflammatory cytokines IL-1β and TNF as compared to mice receiving a HFD. Both the PUFA and mackerel diet reduced levels of IL-1β to a similar level of the diet with cetoleic acid, however did not significantly reduce levels of TNF. PUFA reduced IL-10 levels to the same degree as a diet with cetoleic acid (Figure 3).

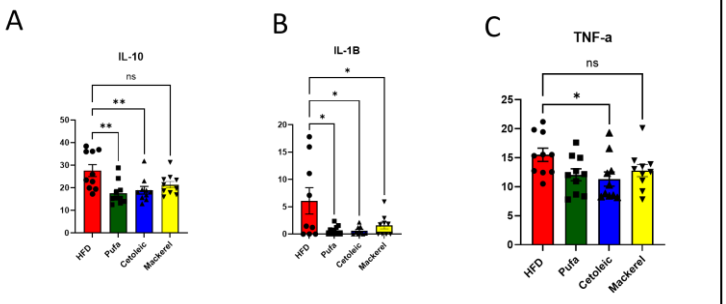

Figure 3. Plasma levels of interleukin (IL) 10, IL-1β and TNF after dietary interventions. HFD: high fat diet, PUFA: polyunsaturated fatty acids. *p<0.05, **p<0.01

We further looked at liver and plasma fatty acid composition. As shown in Figure 4A, there were comparable levels of liver triglycerides between the diet groups. The mice receiving cetoleic acid had significantly increased levels of cetoleic acid both in plasma and liver, illustrating that the feeding experiment increased availability of this fatty acid. Levels were also increased in mice receiving a diet with mackerel oil, however to a lesser degree than for those receiving cetoleic acid (Figure 4B and C). Further, liver and plasma levels of Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was increased in mice receiving PUFA diet, cetoleic acid and mackerel oil, as compared to HFD.

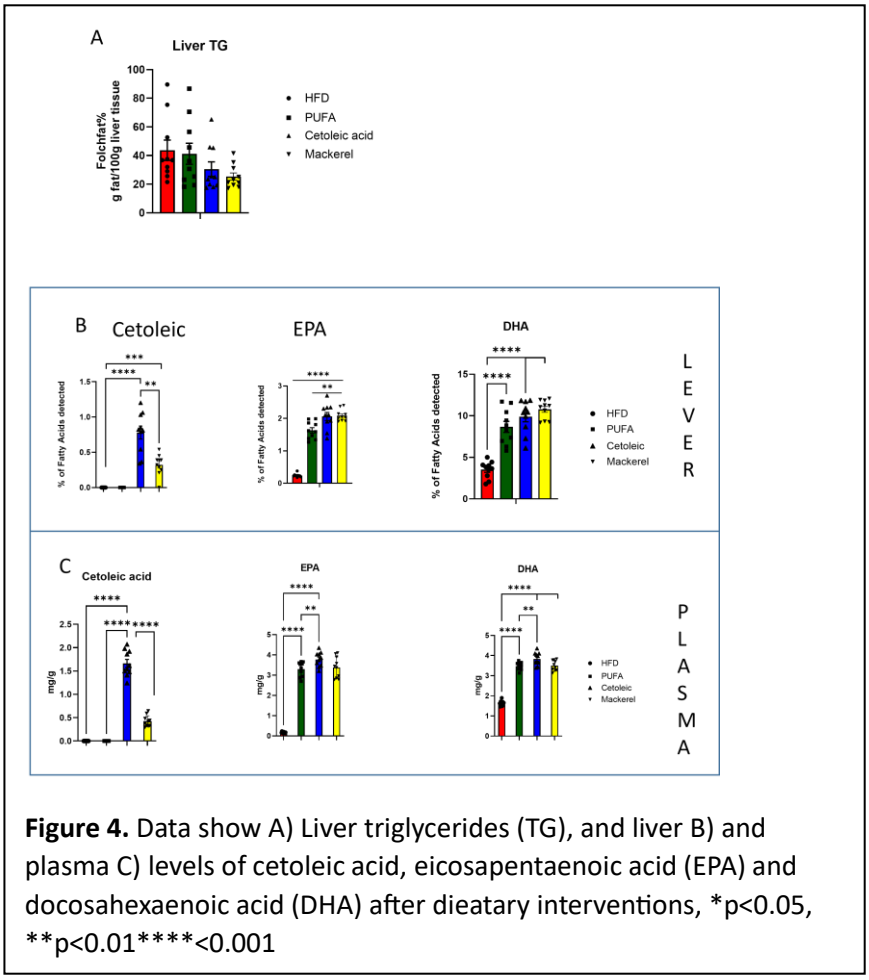

Figure 4. Data show A) Liver triglycerides (TG), and liver B) and plasma C) levels of cetoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) after dieatary interventions, *p<0.05, **p<0.01****<0.001

We also performed RNA sequencing on liver samples from mice receiving the different diets. Comparing the groups fed HFD enriched with mackerel oil and cetoleic acid we see some clear differences in gene expression, also with relevance for the atherosclerotic process, as illustrated in the pathway plot (Figure 5). Interestingly, in liver from mice receiving cetoleic acid, several relevant pathways were regulated, including "regulation of smooth muscle cells", "activation of matrix metalloproteinases" and "chemotaxis", as compared to livers from mice receiving mackerel oil.

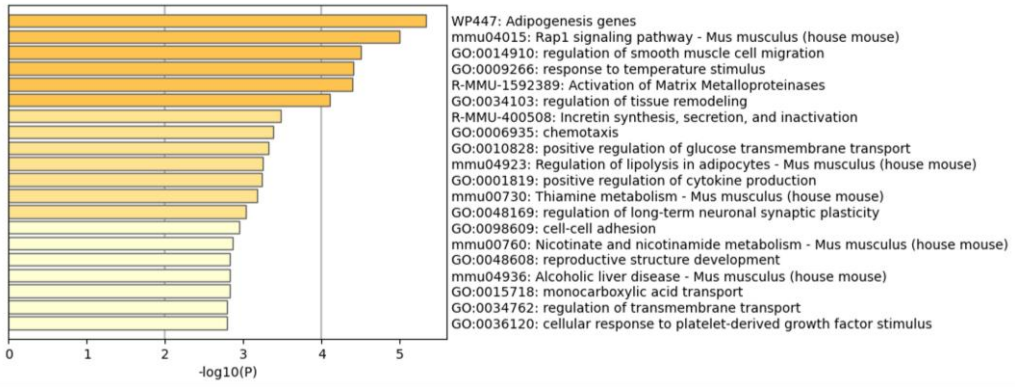

Figure 5. Transcriptome data from mouse livers after dietary interventions, GO-terms comparing mice receiving diet enriched in cetoleic acid to mackerel oil.

Human study: After 4 weeks of intervention, plasma and red blood cell lipids were quantified. In the subjects receiving cetoleic acid, plasma and red blood cell levels of cetoleic acid (22:1n=11) and Eicosatetraenoic acid (ETA) (20:4 n=3) were increased. Further, plasma levels of EPA (20:5 n=3) and DPA (22:5 n=3) were increased, as compared to subjects receiving a control oil.

Overall discussion:

In sum, these data show that both in mice and humans, a diet enriched in cetoleic acids provide availability of cetoleic acid and promote an endogenous production of omega-3 fatty acid EPA and DHA; as illustrated in liver and plasma in the mice, and in plasma of the human subjects. These data illustrate a potential beneficial and atheroprotective effect of a diet enriched in cetoleic acid. In mice, the diet enriched in cetoleic acid resulted in reduced atherosclerosis, which could possibly be mediated through anti-inflammatory effects of this fatty acid, as illustrated by reduced levels of inflammatory cytokines in mice receiving a diet enriched cetoleic acid. Further, the mice had, as mentioned, increased levels of EPA and DHA which have known atheroprotective effects. The transcriptome data also point to regulatory effect on gene pathways of relevance to atherosclerosis; including “regulation of smooth muscle cells”, “activation of matrix metalloproteinases” and “chemotaxis”; which could also explain the beneficial effect on atherogenesis in the mice receiving cetoleic acid. For instance, vascular smooth muscle cells are important players in development of atherosclerosis [4], and if cetoleic acid can affect the functionality of these cells, this can be an important contributor to the atheroprotective effect of this fatty acid. Further studies are however needed to elucidate this association.

In the human study, increased levels of ETA after supplementation with cetoleic acid is of particular interest, as this omega-3 acid is only to a small degree described in the literature. The elevated levels in our study suggest increased conversion to this fatty acid after dietary intake of cetoleic acid; a previously unknown effect of cetoleic acid. ETA is shown to have anti-inflammatory effects [5], supporting our hypothesis of an anti-inflammatory effect of cetoleic acid. However, further studies need to be conducted to the determine if ETA is involved in the atheroprotective effects of cetoleic acid, as shown in our mouse model. It is further of great importance that we were able to show incorporation of cetoleic in red blood cells from human subjects subjected to supplementation, as this illustrates that cetoleic acid supplementation can be incorporated in the cells and thus exert its potential atheroprotective functions in humans. To our knowledge these findings are the first to illustrate this in human subjects and is an important step-stone for testing of cetoleic acid supplementation in patients with increased risk of disease to delineate the causal association between cetoleic acid and cardiometabolic disease, also in humans.

Conclusion: In this FHF project (#901592), we highlight the positive health effects of cetoleic acid, a long-chain monounsaturated fatty acid (LC-MUFA), observed in both mice and humans. In addition to these health benefits, our findings could significantly impact the food industry, as fish enriched with cetoleic acid are readily available in Norway. Furthermore, byproducts from North Atlantic mackerel, which have been underutilized, represent a sustainable resource. This approach could also have substantial economic and environmental benefits. However, additional research on the positive effects following human consumption is still required.

6. Hovedfunn – engelsk

- Supplementation with cetoleic acid reduce development of atherosclerosis in mice.
- Supplementation with cetoleic acid increase levels of EPA and DHA in liver and plasma in mice with subsequently decrease in inflammatory plasma levels of TNF, IL-1β and IL-10.
- Healthy human subjects receiving oil rich in cetoleic acid capsules had increased content of cetoleic acid, as well as ETA in plasma and red blood cells after intervention, and increased plasma levels of EPA and DPA, ETA and cetoleic acid.

Hovedfunn – norsk

- Tilskudd med ketolinsyre reduserer utvikling av aterosklerose i mus.
- Tilskudd med ketolinsyre øker nivåene av EPA og DHA i lever og plasma hos mus, samt reduksjon i inflammasjonsmarkørene TNF, IL-1β og IL-10 i plasma.
- Ketolinsyre-tilskudd økte nivåer av ketolinsyre og ETA i plasma og røde blodceller, og økte nivåer av EPA, DPA, ETA og ketolinsyre i plasma hos friske individer.

7. Referanser

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2. König, A., et al., *A Quantitative Analysis of Fish Consumption and Coronary Heart Disease Mortality.* American Journal of Preventive Medicine, 2005. **29**(4): p. 335-346.
3. Yang, Z.-H., B. Emma-Okon, and A.T. Remaley, *Dietary marine-derived long-chain monounsaturated fatty acids and cardiovascular disease risk: a mini review.* Lipids in health and disease, 2016. **15**(1): p. 201-201.
4. Grootaert, M.O.J. and M.R. Bennett, *Vascular smooth muscle cells in atherosclerosis: time for a re-assessment.* Cardiovascular Research, 2021. **117**(11): p. 2326-2339.
5. Gagnon, K.J., et al., *5-lipoxygenase-dependent biosynthesis of novel 20:4 n-3 metabolites with anti-inflammatory activity.* Prostaglandins Leukot Essent Fatty Acids, 2018. **138**: p. 38-44.

8. Leveranser

- Dr. Sverre Holm holdt presentasjon hos Pelagia i Bergen 09.01.2023 for FHF, Pelagisk Faggruppe i forkant av Pelagisk Arena

- Prof. Bente Halvorsen holdt foredrag Internasjonalt Pelagisk Møte i København, 2021

- Jevnlige møter med referansegruppe

- Presentert prosjektet internt ved forskjellige anledning på UiO/OUS

- Genert ny kunnskap som vil danne bakgrunn for klinisk studie med ketolinsyre til pasienter med økt kardiometabolsk risiko.