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CLINICAL RESEARCH STUDY PLAN Version 3/21.10.2019

The Effect of E-EPA on Circulating LDL Particles and Plasma Lipid Metabolism-Regulating Proteins

Abbreviated Title: E-EPA and LDL

Clinicaltrials.gov registration: NCT04152291

Partner Organizations:

1. Wihuri Research Institute, Helsinki, Finland
2. University of Helsinki, HiLipid Lipidomics Unit, HiLife, Helsinki, Finland
3. Minerva Foundation Institute for Medical Research, Helsinki, Finland
4. Karolinska Institute, Stockholm, Sweden

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I. BACKGROUND

Atherosclerosis is a condition in which cholesterol and other lipids transported by low-density lipoprotein (LDL) particles accumulate in the inner layer (intima) of the arterial wall (Boren & Williams, 2016). Cells within the intima secrete various proteases, lipases, and oxidative agents, which can modify LDL particles. These modifications make LDL particles unstable, leading to aggregation (figure 1). Aggregated LDL particles bind tightly to the extracellular matrix of the arterial wall (Öörni *et al*, 1997), further promoting LDL accumulation. Macrophages within the intima can internalize modified LDL particles, triggering an inflammatory response that drives atherosclerosis progression and results in a chronic inflammatory state in the arterial wall (Boren & Williams, 2016).

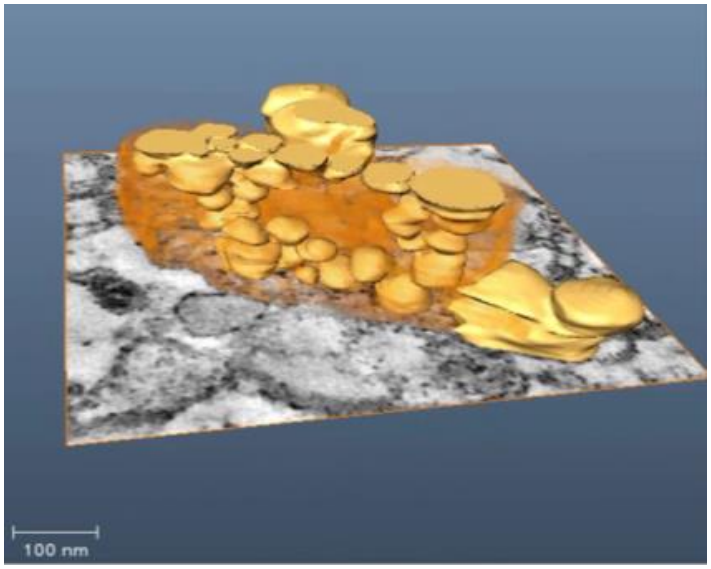

Figure 1. Aggregated LDL-particles in human atherosclerotic plaque.
3D-reconstruction of a human atherosclerotic plaque lipid accumulation based on electron microscopy (Lehti *et al*, 2018).

At the Wihuri Research Institute, a method has been developed to measure LDL particle aggregation propensity (Ruuth *et al.*, 2018). Previous studies demonstrated significant inter-individual variability in LDL aggregation susceptibility, with findings indicating that aggregation-prone LDL predicts future cardiovascular deaths (Ruuth *et al.*, 2018). Our research has also established that the lipid composition of LDL particles regulates their aggregation susceptibility, with LDL particles rich in sphingomyelin being particularly prone to aggregation.

The REDUCE-IT study (Bhatt *et al*, 2019) investigated the effect of eicosapentaenoic acid (EPA; 20:5 n-3) ethyl ester (E-EPA) on blood lipoproteins and cardiovascular disease outcomes. This groundbreaking study demonstrated that a daily dose of 4 g E-EPA significantly reduced cardiovascular events over a 4.9-year follow-up period.

Previous research at Wihuri Research Institute has shown that dietary modifications affecting LDL lipid composition can influence its aggregation propensity (Ruuth *et al.*, 2018). The introduction of plant oils into the diet has been associated with decreased sphingomyelin levels in LDL particles and a significant reduction in aggregation susceptibility. Additionally, intake of alpha-linolenic acid (ALA, 20:3 n-3) (10g/day) or fatty fish (containing approximately 1g of n-3 fatty acids, including

EPA and docosahexaenoic acid (DHA, 22:6 n-3)) led to changes in LDL lipid composition. However, as these modifications were relatively minor, no significant changes in aggregation susceptibility were observed ((Manninen *et al*, 2018) and unpublished results).

II. OBJECTIVES

This research plan builds upon our previous studies investigating LDL particle modifications in the early stages of atherosclerosis and plaque formation. The primary objective of this study is to determine whether dietary supplementation with E-EPA influences LDL aggregation propensity, particle binding to proteoglycans, or LDL composition. Additionally, plasma or serum samples will be analysed for factors associated with lipid metabolism.

III. STUDY IMPLEMENTATION AND TIMELINE

Study Population

The study aims to recruit 40-70 normolipidemic healthy individuals aged 18-65 years. The study will last for 35 days. The E-EPA-supplementation will last for 28 days, followed by a seven-day washout period. Blood samples are taken before (day 0), during day (day 7), after (day 28) the E-EPA-supplementation and after the washout period (day 35). At day 0 visit, participants will undergo a health examination (inclusive an interview, health questionnaire, anthropometric measurements, blood pressure measurement) and blood withdrawal. The blood will undergo laboratory analyses including complete blood count and key metabolic biomarkers (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apoB-100, apoA-I, and Lp(a), vitamin D3, and blood glucose).

Individuals with exceptionally low LDL aggregation susceptibility will be excluded, as further investigation of their LDL particles would not be meaningful in the present context. Additional exclusion criteria include pregnancy, known blood coagulation disorders, LDL cholesterol >5 mmol/L, triglycerides >3 mmol/L, regular use of anti-inflammatory drugs, and fish allergy.

Supplementation

We will follow the dosing model used in the REDUCE-IT (Bhatt *et al.*, 2019) study, administering 2 grams of pure E-EPA twice daily with meals. Pure E-EPA is not commercially available in Finland, and thus a supplement containing 650 mg of E-EPA and 12.5 µg of vitamin D3 per capsule will be employed. The supplement will be provided as three capsules to be consumed in the morning and three in the evening, providing a daily total of 3.9 g of E-EPA and 75 µg of vitamin D3.

Based on previous studies, vitamin D3 is not expected to influence the primary outcome variables. The internationally accepted maximum tolerable daily intake of vitamin D3 for long-term use is 100 µg. In Finland, the estimated daily dietary intake of vitamin D3 ranges between 10-20 µg, suggesting that overall vitamin D3-dosage will remain well within safe limits even with the increased intake provided by the supplement. Notably, the participants will be instructed not to use other (commercial) vitamin D supplements during the study.

Research Methods

Blood samples will be analyzed for key parameters related to lipid metabolism and cardiovascular health, including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apoB-100, apoA-I, and Lp(a). Additionally, vitamin D3 and blood glucose levels will be measured. All blood samples will be processed under anonymized identifiers.

Plasma lipoproteins will be isolated via ultracentrifugation. The LDL particle aggregation propensity will be determined using a method developed in our laboratory (Figure 2), employing a dynamic light scattering-based microplate reader (Dyna Pro Plate Reader II). Furthermore, the lipid composition of lipoprotein samples will be analyzed using a method optimized for LDL particle analysis at the University of Helsinki's HiLipid lipidomics unit in collaboration with Associate Professor Reijo Käkelä. The ability of LDL particles to bind to proteoglycans will be assessed by measuring plasma lipoprotein binding to immobilized proteoglycans isolated from the human aorta, a method developed in our laboratory (Ahmed *et al*, 2018).

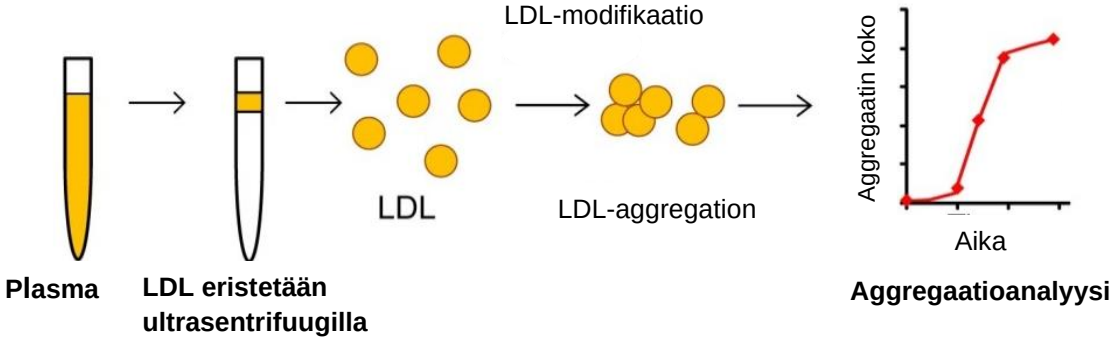

Figure 2. LDL Particle Aggregation Analysis.
LDL is isolated from blood samples using ultracentrifugation. The LDL particles are modified under precisely controlled conditions, with modification induced by enzymatic treatment. This modification leads to strong LDL aggregation, which is monitored by measuring aggregate size using a dynamic light scattering-based method. The method has been developed, optimized, and standardized in our laboratory (Ruuth *et al*, 2018).

Phospholipid transfer protein (PLTP) and cholesterol ester transfer protein (CETP) activities will be assessed using radiometric assays (Groener *et al*, 1986; Jauhiainen & Ehnholm, 2005) and Paraoxonase 1 (PON-1) activity by a chromogenic method (Kleemola *et al*, 2002). Lecithin-cholesterol acyltransferase (LCAT) activity will be measured by following formation of radiolabeled cholesterol esters (Jauhiainen & Dolphin, 1986).

Angiopoietin-like proteins (ANGPTL) 3, 4, and 8 will be analyzed using specific ELISA assays (Robciuc *et al*, 2010; Tikka *et al*, 2017).

Resolvin levels will be measured in collaboration with the Karolinska Institute in Stockholm. The analysis will be conducted using mass spectrometry optimized for lipid mediators.

Implementation

The responsible physician of the study is Professor Petri T. Kovanen (MD, PhD) from the Wihuri Research Institute. Study participants will visit the Wihuri Research Institute, where blood samples will be collected under his supervision. In addition, Professor Kovanen will personally conduct the baseline health checkups. During these visits, participants will have the opportunity to discuss the study with the physician or the Principal Investigator, Dr. Katariina Öörni. Breakfast will be provided for the participants after each visit.

Blood samples accrued during the study will be processed and analysed at the Wihuri Research Institute, the HiLipid Lipidomics Unit, the Minerva Foundation Institute for Medical Research, and the Karolinska Institutet in Stockholm.

Professor Petri Kovanen, Associate Professor Katariina Öörni, and BSc Lauri Äikäs will inform participants about the study and collect signed consent forms prior to enrollment to the study. Bioanalyst Maija Atuegwu will execute blood withdrawals. BSc Lauri Äikäs will carry out LDL particle isolation, lipidomic analyses, aggregation analyses, and plasma enzyme measurements under the supervision of Associate Professors Katariina Öörni, Matti Jauhiainen, and Reijo Käkelä and Dr. Hildur Arnardottir at the Karolinska Institutet. All senior team members will participate in data analysis.

Timeline and Reporting

Participant recruitment will begin once ethical approval and research permits have been granted. Recruitment will be conducted via internal mailing lists at the University of Helsinki and Biomedicum Helsinki. Laboratory analyses will take place between 2019 and 2020, with data analysis and reporting occurring within the same timeframe. The results will be published in international scientific journals, with a target of 1-2 original research articles. Findings will also be presented at international conferences. BSc Lauri Äikäs will include part of the results in his master's thesis. All research team members will contribute to study planning, data evaluation, and reporting.

Funding

The study is funded through research grants to Associate Professors Katariina Öörni and Matti Jauhiainen.

Table 1. Cost Estimates

Expenses	Combined
Salaries	
Master's thesis worker's salary – including overheads	15 600 €
Analytics:	
LDL aggregation: 10 €/sample, 200 samples	2 000 €
Lipoprotein isolation: 12.5 €/sample, 200 samples	2 500 €
Lipid extraction and mass spectrometry analyses	5 000 €
Enzymatic analyses	2 000 €
ELISA-analyses	2 500 €

Other costs:	
E-EPA capsules	2 000 €
Breakfast for the participants	500 €
Combined	**32 100€**

IV. ETHICAL CONSIDERATIONS

The study adheres to Good Clinical Practice (GCP) guidelines, the study plan, and applicable regulations. The study plan aligns with the research objectives and provides sufficient guidance for the research staff to conduct the study appropriately. The research methods used are well-established.

Participants will provide four fasting blood samples over a five-week period. These samples will be analysed for LDL cholesterol, HDL cholesterol, apoB-100, apoA-I, Lp(a), and triglyceride levels. Blood pressure measurements will also be taken during study visits. Participants will receive a summary of these measurements after analyses have been concluded. Prior to each blood sample collection, participants will complete a 24-hour dietary record. As the results related to lipoprotein aggregation and lipidomics remain at an experimental and theoretical level, their clinical significance cannot be assessed at an individual level, and therefore, individual reports on these results will not be provided to participants. However, participants may access study findings after the study has concluded and the results have been published.

E-EPA has not been associated with harmful side effects. Since fish oil-based E-EPA does not contain fish protein, it is generally safe for use; however, due to the high dosage, individuals with fish allergies will not be included in the study. EPA has blood-thinning effects, making anticoagulant medication a contraindication for participation. In the REDUCE-IT trial, 2.3% of E-EPA users reported joint pain, compared to 1% in the placebo group. The amount of vitamin D3 administered in the study exceeds Finland’s daily recommended intake but remains well below the EU's upper safe limit of 100 µg, which has been shown to have no adverse effects. Participants taking daily vitamin D3 supplements will be requested to discontinue their use minimum one week prior to commencing the study. Vitamin D3 levels in the blood will be monitored for potential side effects.

Participants may withdraw from the study or revoke their consent at any point prior to its conclusion without any repercussions. Any data collected before consent withdrawal will be used if deemed necessary for the accuracy and reliability of overall study results.
Personal data will be collected in accordance with current data protection regulations. Participants will provide standard personal information, such as name, date of birth, and health-related details, via the consent form. The risk associated with processing this data is assessed as low, and risk assessments will continue throughout the maintenance of the registry. Data protection rights will be upheld during data storage.

Participant samples will be blinded so that only the responsible researcher can link them to personal information. Results or data containing personal identifiers will never be made publicly available. Personal data and study-related documents will be securely stored at the Wihuri Research Institute in locked archive cabinets and password-protected computers. All members of the research team

have been trained in proper data handling. The Principal Investigator will maintain a data registry in compliance with applicable privacy laws.

Participant-specific data will remain confidential and will not be shared with third parties or included in medical records. Sample material containing DNA will not be stored; instead, it will be properly disposed of after sample processing.

V. SCIENTIFIC SIGNIFICANCE

This is an exploratory clinical study utilizing advanced lipid analysis techniques to investigate how E-EPA affects the properties of low-density lipoproteins. Specifically, the study examines changes in LDL lipid composition, its ability to bind to proteoglycans, and its susceptibility to aggregate. Additionally, the study provides insights into how E-EPA influences key plasma enzymes and lipid transfer proteins that regulate lipid metabolism. These findings are significant for assessing the risk of atherosclerosis and coronary artery disease.

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Deviations from the original study plan:

Statistical analysis plan

This study is a **single-arm, longitudinal nutritional intervention study with a repeated-measures design**, investigating the effects of icosapent ethyl on blood composition and functional parameters in healthy, normolipidemic volunteers. A total of 38 participants were recruited and screened for inclusion and exclusion criteria based on an initial blood draw. Eligible participants provided a baseline blood sample at Day 0 before receiving the intervention. Participants consumed the supplement daily for four weeks (Days 1–28), with blood samples collected at Days 7 and 28. Following the intervention period, participants underwent a 7-day washout phase, after which a final blood sample was collected at Day 35. No control or placebo group was included in the study, as each participant served as their own control through repeated within-subject comparisons over time.

1. Statistical Methods

The statistical methods employed in this study include tests for normality, group differences, analysis of variance, correlation analysis, time series clustering, principal component analysis (PCA), linear mixed modeling, hierarchical clustering, distance calculations, sparse partial least squares-discriminant analysis (sPLS-DA), uniform manifold approximation projection (UMAP), and machine learning models. Specific methods include:

- **Normality Testing:** D'Agostino-Pearson omnibus K2 test.
- **Group Differences:** Paired multiple t-tests (LIMMA) on log2-transformed data, with false discovery rate (FDR) correction.
- **Analysis of Variance:** One-way ANOVA with post hoc analysis (Benjamini, Krieger, and Yekutieli FDR correction).
- **Correlation Analysis:** Spearman's correlation with exact p-values for small datasets and t-ratio derived p-values for larger datasets.
- **Time Series Clustering:** VSClust fuzzy c-means clustering with k-nearest neighbors (kNN) imputation for missing values.
- **PCA:** Performed on log2-normalized lipid abundances, using centering and scaling.
- **Linear Mixed Modeling:** Implemented using lmer in the lme4 package, with time as a fixed effect and subject as a random effect.
- **Hierarchical Clustering:** Ward's method applied to Euclidean distance matrices from PCA scores.
- **Distance Calculations:** Euclidean distances calculated from PCA scores to assess intra- and inter-subject variability.
- **sPLS-DA:** Conducted via MetaboAnalyst 6.0 with nested cross-validation.
- **UMAP:** Dimensionality reduction using the umap package with predefined parameters.
- **Machine Learning:** XGBoost regression models trained on lipidomic and clinical data using nested cross-validation.

No formal interim analysis was planned.

2. Sample Size and Power Justification

The study initially planned to enrol 40–70 participants, with a total of 72 recruited and 68 enrolled. Due to COVID-19 restrictions, only 38 participants completed the study. A formal power calculation was not conducted, and sample size selection was based on feasibility and the exploratory nature of the study. The final analysis will include only the 38 participants who completed the study.

A sample size assessment was conducted post hoc to evaluate whether the study had sufficient statistical power to detect changes in LDL aggregation, triglycerides, and EPA incorporation. This assessment was based on observed variability in the data and repeated-measures ANOVA. The observed effect size was computed using partial eta-squared (η^2p). The effect size was then converted to Cohen's f and used to estimate the required sample size for 80% power. The results indicated that for the primary outcomes, the study had sufficient power to detect moderate-to-large effects, with a required sample size of approximately 16 participants for the observed effect sizes.

3. Level of Significance

A two-tailed significance level of 0.05 was used throughout the study. Multiple hypothesis testing corrections were applied using the false discovery rate (FDR) method where appropriate.

4. Criteria for Termination of the Trial

The trial would be terminated if more than 10% of the participants encountered adverse effects related to the intervention.

5. Handling of Missing Data

A complete case analysis approach was used for analyses requiring full datasets, such as UMAP, PCA, and machine learning analyses. Within these complete cases, missing values were handled using k-nearest neighbors (kNN) imputation where required. However, for single-variable analyses, all available data were used, and missing values were omitted rather than imputed.

6. Deviations from the Original Statistical Plan

The study deviated from the original protocol in the following ways:

- **Sample Size Reduction**: The original plan aimed for 40–70 participants, but only 38 completed the study due to COVID-19 restrictions. The final analysis includes only these 38 participants under a per-protocol approach rather than an intention-to-treat model.

- **Changes in Outcome Assessments**: Some planned secondary outcomes, including enzyme activity assays (PLTP, CETP, PON-1, LCAT) and ELISA assays for ANGPTLs and resolvins, were not conducted due to COVID-19-related constraints.

    - **Changes in study collaborators:** Due to Covid-19 travel restrictions and delays, the international collaboration with Karolinska Institute was halted and Professor Magnus Bäck and PhD Hildur Arnardottir removed from the research team.

- **Expanded Statistical Methods:** The study incorporated additional statistical methods, including machine learning (XGBoost), time series clustering (VSClust), hierarchical clustering, UMAP, and sPLS-DA, which were not in the original protocol.

- **Data Handling Approach:** The original plan allowed for analysis of partially completed cases, but the final study adopted a complete case analysis approach, excluding participants with missing time points.

- **Stopping Criteria Introduced:** The original plan did not specify stopping rules. The final study introduced a stopping rule stating that if >10% of participants experienced adverse effects, the study would be terminated.

These modifications were made to accommodate study constraints while maintaining scientific rigor.

7. Selection of Subjects for Analysis

The final analysis will include only the 38 participants who completed the study, following a per-protocol approach. Participants with missing time points will not be included in the analysis.

8. Direct Access to Source Data/Documents

The study sponsor will ensure that the investigator(s)/institution(s) permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents. The source data will not be made publicly available in any repository due to legislation on anonymity. Data is stored on secure backup media and deposited on a secure university server for long-term storage and controlled access.

9. Quality Control and Quality Assurance

The study adhered to standardized protocols for data collection, processing, and statistical analysis to ensure data integrity and reliability. Quality control measures were implemented to maintain consistency and accuracy in data handling.

10. Ethical Considerations

Written informed consent was obtained from all participants. The study adhered to the ethical guidelines of the Declaration of Helsinki and was approved by an appropriate ethics committee.

11. Data Handling and Record Keeping

All study data were securely stored in a regulatory-compliant manner, with participant identifiers removed to maintain confidentiality. Data processing was performed using GraphPad Prism v10.1.2 and RStudio (Version 2023.12.1, build 402, Posit Software, PBC).

12. Financing and Insurance

The study is sponsored by the Wihuri Research Institute, a private, foundation-funded academic institution. Wihuri Research Institute is supported by the Jenny and Antti Wihuri Foundation. Additional funding for this study was received through grants from private foundations and the Research Council of Finland. Funding for this study was solely for academic research, with no commercial interests involved. All study participants were insured by the Wihuri Research Institute.

13. Publication Policy

The publication policy follows the predefined guidelines agreed upon by study investigators. The study findings will be published in gold/green open-access peer-reviewed journals, ensuring transparency and reproducibility.

14. Supplements

Further details regarding statistical methodologies, exploratory analyses, and supplementary findings can be found in the Supplementary Methods section of the final study report.

Information for participants of E-EPA study

Name of the study

The effect of E-EPA on circulating lipoproteins and plasma proteins related to lipid metabolism

We kindly ask you to participate in our study in which we study eicosapentaenoic acid (EPA), an omega-3 fatty acid, and its effect on circulating LDL-particle composition and other cardiovascular disease factors.

Participation is completely voluntary

Participation in this study is fully voluntary. You may stop the study or withdraw your approval at any point without further notice. This will not impact your future access to any medical treatment.

Please read this document carefully! If you have any questions, you can contact the research doctor or the principal investigator, whose contact details are found at the end of this document, on page 4. If you decide to participate in the study, we will ask you to sign the agreement on the last page of the document.

Executor of the study

This study is carried out by Wihuri Research Institute and the medical doctor in charge is Professor Petri Kovanen. Docent Katariina Öörni is head of personal data registry of this study and is responsible that personal data is handled according to local laws and regulation.

The goal of the study

Cardiovascular diseases cause the number one killer both in Finland and globally. The most important factor causing the disease is cholesterol, which is carried in the LDL particles accumulating in the artery walls. Therefore, LDL-cholesterol is called the “bad cholesterol”. In our recent studies we have discovered that in addition to the amount of LDL particles in circulation, also the composition and quality of the particles have an impact on the development of cardiovascular diseases.

LDL particle composition is affected by dietary fats. Polyunsaturated fatty acids, so called “soft fats” are beneficial to your health and especially omega-3 fatty acid EPA has been found to lower the risk of fatal cardiovascular diseases. To further understand the health benefits of EPA we are in this study focusing on the effects of EPA on LDL particle composition and other cardiovascular disease factors.

Background

EPA is a polyunsaturated omega-3 fatty acid that is necessary for normal health and derived from a diet containing fatty fish and food oils. In this study we use clinically manufactured ethyl esterified EPA capsules. Capsules contain EPA fatty acids and D3-vitamin. In an international study REDUCE-IT, was found that consumption of 4 grams of EPA daily reduced cases of fatal cardiovascular diseases by 25% in 5-year median follow up. In this study we use the same dosage.

EPA is an important precursor from which several signal molecules that reduce inflammation, resolvins, are formed. Resolvins have been shown to reduce the development of cardiovascular diseases. We will measure resolvins from the blood samples. In addition, we will also investigate if

EPA affects lipid metabolism enzymes such as LCAT, CETP, PLTP and PON-1 which all affect the LDL particle composition.

Aggregating LDL in the artery walls is crucial for the development of atherosclerosis. Previous studies show that the LDL particles bind to proteoglycans and other components of the extracellular matrix in the inner layer of the artery wall where LDL particles start to bind to each other and aggregate. LDL particle composition impacts the aggregation susceptibility and hence the development of atherosclerosis. Dietary fats again affect the composition of LDL particles. In this study we examine, how impactful the consumption of EPA is at modifying LDL particle composition and the possible reduction in the LDL particle aggregation susceptibility or binding to proteoglycans extracted from human aorta.

Participation in the study

40-70 voluntary healthy participants, who agree to consume 4 grams daily of E-EPA are recruited to the study. Capsules are taken twice a day (3 in the morning and 3 in the evening) for 4 weeks. Each capsule contains 650mg of E-EPA which will add up to a daily dosage of 3,9 grams. Capsules should be ingested with fat-containing food. During the study period participants are expected to keep up their usual lifestyle and diet. Supplements or the usage of a medication, like cholesterol lowering statins, should remain the same during the study period. If you are pregnant, breastfeeding or planning pregnancy during the study period, you cannot participate in the study. Also, substantially high amount of LDL-cholesterol (>5 mmol/l) or triglycerides (> 3 mmol/l) that we test in our laboratory, will lead to exclusion from participation.

Participation in the study requires 4 visits to Wihuri Research Institute's laboratory. During each visit we will collect a blood sample, which is similar to one taken during doctor's appointment (approximately 20 ml). Before giving the sample, an overnight (8h) fasting is required. Blood samples are collected before the study and after weeks 1 and 4 and 1 week after the end of the diet at week 5. After the 4th blood sample at week 5, your participation in the study ends. All blood sampled are coded and handled anonymously. We also ask you to fill a food dairy from the previous day before each blood sampling. Breakfast will be provided after each visit. At the first visit you will meet with a medical doctor and you blood pressure, blood glucose, weight, height and waist are measured, and you are asked to fill a questionnaire regarding some common factors regarding cardiovascular disease. We measure plasma LDL-cholesterol, HDL-cholesterol, triglycerides and D-vitamin and each participant will get an evaluation of these results after the end of the study.

LDL particles are isolated from the blood samples and their composition, aggregation susceptibility and binding affinity to proteoglycans is measured. Also, previously mentioned enzymes and resolvins are measured. Insights from these results can be given to you after the study is concluded.

Possible side effects from E-EPA

Healthy participants have very low risk for any side effects. A possible joint pain has been reported during longer periods of E-EPA diet.

Exclusion criteria

1. Because E-EPA causes blood thinning, one should not normally take E-EPA without consulting a doctor beforehand. Therefore, the usage of blood thinning medicine or

increased risk (e.g. family history) of problems with blood coagulation prohibits from participating in the study.

2. The capsules are manufactured from fish oils; hence they should not contain fish proteins, but there may be small amounts of fish protein as an impurity and people with fish allergy should be careful regarding fish oil products in general and only start with small doses. Therefore, having a fish allergy prohibits participation in the study.

Financial expenses and clarifications

E-EPA capsules and any procedures regarding the study are free of charge. You will not be paid to participate in the study, but breakfast is provided after each visit. All participants are insured.

The study is financed by Wihuri Research Institute. Principal doctor and other personnel are not given extra payment for conducting the research. Docent Katariina Öörni and Professor Petri Kovanen have applied for a patent regarding LDL particle aggregation susceptibility measurement protocol.

Personal data handling

Personal data is handled according to European Union legislation (GDPR). All samples are handled with code numbers from which a code registry is formed. Code registry is protected by a password and the personal details of the participants are only known by the persons conducting the research. Coded blood samples are preserved in the research facility's freezers, and they are mainly analysed by researcher Lauri Äikäs. Your identity will not be revealed when the results of the study are presented.

This study and the handling of the personal data is based on following decrees. Also, an approval from ethical committee of Helsinki and Uudenmaan hospital district has been given for conducting this study.

- General Data Protection Regulation (GDPR) 2016/679, 6th article sections 1a), b), c) and e) and 9th article sections 3 a), g), i) and j)
- Law regarding scientific research 1999/488

Additional information

If you have any questions regarding the study, we encourage you to be in touch with the principal investigator or rest of the research personnel. With them you may discuss at any time regarding possible concerns related to the study.

Contact:

Principal investigator

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Principal Doctor

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Approval for the study

I have been asked to participate in the study "*The effect of E-EPA on circulating lipoproteins and plasma proteins related to lipid metabolism*".

I have read the document meant for the participants of the study and have discussed with the principal investigator or other responsible personnel. I have been given enough information about the study and the included personal data collection and handling in the study. I have also been informed verbally and have been given answers to all my questions related to the study. Explanation was given to me by _____ (name of the person). I have had enough time to consider participation in the study.

I understand that participating in the study is completely voluntary. I am aware that I may withdraw from the study at any time without further notice and that it won't affect my doctor-patient relationship or future healthcare. I am also aware that personal and other data gathered from me up to this point may still be used in the study if it is necessary for method validation, quality or safety.

I have read the "personal data handling in the code registry"- document and I understand what my personal data is being used for. I understand that the data I give is being coded into a blinded study registry and a separate code registry is formed. Code registry may be accessed by only the key personnel in the Wihuri Research Institute's research group. Responsible person of the code registry is Docent Katariina Öörni.

I give my consent to gather my personal data. I am aware of the personal data gathered and of my rights related to my personal data, such as being able to withdraw my consent at any point. I have been informed on how I may apply my rights and given contact information required for this purpose. I have been given contact information to supervising authority, whom I may contact if I feel that my personal data has been misused or my rights violated.

I give my consent to be contacted in the future, if validating the research results requires it.

With my signature I confirm that I am 18-65-year-old and that I take voluntarily part in the study described in the document. I am aware that my personal data may be handled during an inspection by a national or international supervision agency or by an independent agent providing quality control on behalf of the study provider.

_____ _____

Signature ***Date***

_____ _____

Print name ***Date of birth or social security number***

_____

Address

Phone number_____ Email _____

Acceptor of the written approval

_____ _____

Acceptors signature ***Date***

_____

Print name