Analyse comparative de la réponse cardiométabolique à une supplémentation en acide eicosapentaénoïque et docosahexaénoïque

Thèse

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Philosophiæ doctor (Ph. D.)

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Sous la direction de :
Benoît Lamarche, directeur de recherche
Patrick Couture, co-directeur de recherche

Résumé

La famille des acides gras oméga-3 à longue chaîne (AGn3-LC) est composée principalement de deux acides gras qui exercent des effets physiologiques connus, soit les acides eicosapentaénoïque (EPA, C20:5n3) et docosahexaénoïque (DHA, C22:6n3). La grande majorité des études cliniques menées jusqu'à maintenant ont observé l'effet d'un supplément alimentaire qui combine l'EPA et le DHA sous différentes formes et proportions. Alors que les études épidémiologiques tendent à montrer un effet protecteur des AGn3-LC sur le risque cardiovasculaire, la plupart des études cliniques qui ont été menées jusqu'à maintenant tendent à montrer un effet neutre de la supplémentation en AGn3-LC sur le risque d'évènements cardiovasculaires. L'utilisation de suppléments d'AGn3-LC en prévention des évènements cardiovasculaires est donc un sujet plutôt controversé à ce jour. De plus en plus d'évidences suggèrent que l'EPA et le DHA exercent des effets différents sur les facteurs de risque cardiovasculaire. Ainsi, la proportion d'EPA et de DHA contenus dans les suppléments utilisés dans les études pourrait en partie expliquer les résultats plutôt neutres observés quant à l'effet des AGn3-LC sur le risque d'évènements cardiovasculaires. De plus, certaines études ont rapporté une importante variabilité dans la réponse aux AGn3-LC, ce qui suggère que la supplémentation en AGn3-LC pourrait être bénéfique seulement chez une certaine proportion d'individus en ce qui concerne la santé cardiovasculaire.

L'**objectif général** de ce doctorat était de comparer les effets de l'EPA et du DHA sur le risque cardiométabolique. Plus spécifiquement, nous avons comparé l'effet de l'EPA et du DHA sur les lipides sanguins, les biomarqueurs inflammatoires, le métabolisme des lipides, l'expression des gènes impliqués dans le métabolisme des lipides, la variabilité inter- et intra-individuelle dans la réponse des triglycérides et du cholestérol dans les lipoprotéines de faible densité (C-LDL), ainsi que les déterminants de la réponse aux AGn3-LC. Ce doctorat a été rendu possible grâce à une étude clinique randomisée en chassé-croisé et à double insu qui a été menée à l'Institut sur la nutrition et les aliments fonctionnels. Un total de 154 participants ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 10 semaines et séparées par une période libre de 9 semaines. Chaque participant a été supplémenté avec 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Les résultats de ce projet de recherche ont permis d'observer que l'EPA et le DHA exerçaient des effets différents sur les lipides sanguins, certains biomarqueurs inflammatoires, l'Indice Oméga-3, le métabolisme de l'apolipoprotéine B100 des LDL et la taille des LDL. Une importante variabilité inter- et intra-individuelle a également été observée dans la réponse des triglycérides et du cholestérol dans les LDL sériques suite à la supplémentation en EPA et en DHA. Les résultats de ce projet de doctorat supportent l'hypothèse que l'EPA et le DHA pourraient exercer des effets différents sur le risque cardiovasculaire. De plus, l'EPA et/ou le DHA pourraient exercer des effets cardioprotecteurs chez certains individus, mais pourraient aussi exercer des effets neutres ou néfastes sur la santé cardiovasculaire.

Abstract

Most clinical trials to date have used the two main types of long-chain omega-3 polyunsaturated fatty acids (LCn3-PUFAs), docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3) in various forms and proportions. While LCn3-PUFA consumption tends to exert a cardioprotective effect in epidemiological studies, most clinical studies have shown a neutral effect of LCn3-PUFA supplementation on the risk of cardiovascular events and mortality. Thus, the use of LCn3-PUFA to prevent cardiovascular events remains controversial. The inconsistent effect of LCn3-PUFAs on cardiovascular events might be because EPA and DHA have distinct effects on cardiovascular risk. The proportion of EPA and DHA contained in supplements may influence the effects of LCn3-PUFA supplements. Moreover, inter-individual variability in the plasma triglyceride response to LCn3-PUFA supplementation has been fairly well documented in several clinical studies, suggesting that some but not all individuals benefit from DHA and EPA supplementation.

The **general objective** of this thesis was to compare the individual effect of EPA and DHA on cardiometabolic risk. Specifically, we compared the effect of EPA and DHA on blood lipids, markers of inflammation, lipids metabolism, gene expression of lipid metabolism, the inter- and intra-individual variability of triglyceride and low-density lipoprotein cholesterol (LDL-C) response as well as the determinants of the response. A randomized controlled crossover and double-blind trial was conducted at the Institute of Nutrition and Functional Foods. A total of 154 participants were randomized to difference sequences of three experimental phases of 10 weeks and separated with 9-week washouts. Each participant was supplemented with 2,7 g per day of EPA, 2,7 g per day of DHA and 3 g per day of corn oil as the control. The results of this study have shown that EPA and DHA exert differential effects on blood lipids, on some inflammation markers, on the Omega-3 Index, on the LDL-apolipoproptein B100 metabolism and on LDL particle size. An important inter- and intra-individual variability in the triglyceride and the LDL-C response to EPA and DHA has also been observed. Altogether, these observations support the hypothesis that EPA and DHA may exert differential effects on the risk of cardiovascular events. Moreover, data on the variability in the triglyceride and LDL-C response suggest that some but not all individuals may benefit from DHA and EPA supplementation in terms of cardiovascular prevention.

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Liste des abréviations, sigles, acronymes

AA : acide arachidonique
ACC : acétyl-CoA carboxylase
ABCA1 : *ATP-binding cassette 1*
AGn3-LC : acides gras polyinsaturés oméga-3 à longue chaîne
AGn3 : acides gras polyinsaturés oméga-3
AHA : Association américaine du cœur
Apo : apolipoprotéine
ASCEND : *A Study of Cardiovascular Events in Diabetes*
CD36 : *cluster of differentiation 36*
CETP : *cholesteryl ester transfer protein*
C-HDL : cholestérol dans les lipoprotéines de haute densité
C-LDL : cholestérol dans les lipoprotéines de faible densité
COMIT : *Canola Oil Multicenter Intervention Trial*
ComparED : *the Comparing EPA to DHA Study*
COX-2 : cyclooxygénases-2
CRP : protéine C-réactive
D3-Leucine : isotopomère de leucine tri-deutérée
DGAT : diacylglycérol O-acyltransférase
DHA : acide docosahexaénoïque
DPA : acide docosapentaénoïque
ELISA : *enzyme-linked immunosorbent assay*
EPA : acide eicosapentaénoïque
FADS1 : *fatty acid desaturase 1*
FAS : acide gras synthase ou *Fatty Acid Sensor Study*
FCR : taux de catabolisme fractionnel
FINGEN : *Fish Oil Intervention and Genotype*
GPAM : *glycérol-3-phosphate acyltransférase mitochondrial*
HDL : lipoprotéine de haute densité
HMG-CoA réductase : *3-hydroxy-3-methylglutaryl coenzyme A reductase*
HR : rapport de hasards
IC : intervalle de confiance
IDL : lipoprotéine de densité intermédiaire
IL : interleukine
INAF : Institut sur la nutrition et les aliments fonctionnels
JELIS : *Japan EPA Lipid Intervention Study*
LC/MS/MRM : *liquid chromatography-mass spectrometric-multiple reaction monitoring*
LCAT : *lecithin :cholesterol acyltransferase*
LDL : lipoprotéines de faible densité
LOX : lipoxygénase
LPL : lipoprotéine lipase
PCSK9 : proprotéine convertase subtilisine/kexine de type 9
PLA2 : phospholipases A2

PPAR-α : *peroxisome proliferator-activated receptor alpha*
PR : taux de production
REDUCE-IT : *Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial*
RR : risque relatif
SAAM II : *Simulation, Analysis, and Modeling Software II*
SR-A I : *class A scavenger receptor type I*
SR-A II : *class A scavenger receptor type II*
SREBP1c : *sterol regulatory element-binding protein 1c*
TNF-α : facteur de nécrose tumorale alpha
VITAL : *The Vitamin D and Omega-3 Trial*
VLDL : lipoprotéines de très faible densité

À grand-maman

« To be yourself in a world that is constantly trying to make you something else is the greatest accomplishment »
- Ralph Waldo Emerson

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La réalisation des projets sur lesquels j'ai travaillé durant ces trois années et demie de doctorat a nécessité la collaboration de plusieurs personnes, incluant des professionnels, investigateurs, collaborateurs, coordonnateurs et participants. Je remercierai chaque personne impliquée dans les différents projets dans la section avant-propos. La présente section est réservée aux remerciements plus personnels.

J'aimerais d'abord remercier mon directeur de recherche, le Dr Benoît Lamarche, sans qui ce doctorat n'aurait pas vu le jour et sans qui je ne serais pas la personne que je suis aujourd'hui. Depuis le début de mes études graduées (en tant que codirecteur à la maîtrise et même durant mon baccalauréat), Benoît m'a offert un encadrement exemplaire en ce qui concerne mes travaux de recherche. J'aimerais d'ailleurs le remercier pour la confiance qu'il a eue en moi dès le départ. C'est connu, Benoît Lamarche est un professeur et un chercheur très occupé, mais lorsqu'on entre dans son bureau, on se sent important ! En effet, il prend toujours le temps pour ses étudiants qui sont sa motivation et sa priorité au quotidien. Avant d'être un directeur de recherche remarquable, Benoît a d'abord été un mentor exceptionnel. Je me suis toujours sentie considérée autant en tant qu'étudiante qu'en tant que personne. De par ses qualités humaines, il a été pour moi une source d'inspiration dans tous les aspects de ma vie. Benoît, j'aimerais sincèrement te remercier pour ton écoute, ta compréhension, ta sensibilité, ton empathie et ta considération. Je ne pourrai jamais être suffisamment reconnaissante pour ton accompagnement dans mon cheminement personnel et professionnel.

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J'aimerais remercier les Instituts de recherche en santé du Canada et le Fonds de recherche du Québec – Santé pour m'avoir octroyé une bourse de recherche pour la durée de mon doctorat. Cela a grandement contribué à ma motivation et m'a permis de m'investir dans mes études.

Un merci à tous les professeurs, professionnels de recherche et étudiants en nutrition que j'ai eu l'occasion de côtoyer durant mes années à l'Institut sur la nutrition et les aliments fonctionnels et qui selon moi contribuent à rendre l'ambiance de cet institut accueillant et plus qu'agréable. Merci spécialement à Johanne Marin et André J Tremblay pour votre disponibilité pour répondre à mes questions de nature plus « technique ».

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Avant-propos

Cinq articles dont j'ai eu l'occasion de rédiger en tant que premier auteur durant mon doctorat font l'objet de cette thèse aux **chapitres 4, 5, 6, 7** et **8**. La rédaction de ces cinq articles a été possible grâce aux données d'un projet de recherche clinique qui a été mené à l'Institut sur la nutrition et les aliments fonctionnels (INAF) de 2013 à 2015. Je n'ai pas participé à la phase clinique de ce projet de recherche puisqu'elle était terminée au moment de mon arrivée l'INAF en septembre 2015. Brièvement, ce projet avait comme objectif principal de comparer l'effet de la supplémentation en deux types d'acides gras polyinsaturés oméga-3 à longue chaîne, l'acide acide eicosapentaénoïque (EPA, C20 :5n3) et docosahexaénoïque (DHA, C22 :6n3), sur les concentrations plasmatiques de protéine C-réactive. Le devis de l'étude est expliqué en détail dans l'article présenté au chapitre 4. Comme vous pourrez le voir dans le titre de l'article présenté au chapitre 4, le nom de cette étude est *the Comparing EPA to DHA Study*. J'utiliserai l'abréviation ComparED dans cet avant-propos. Tous les articles ont été publiés dans des journaux scientifiques avec révision par les pairs, à l'exception de l'article du chapitre 8 qui est actuellement en préparation.

J'ai divisé les prochains paragraphes en fonction des chapitres qui présentent les articles scientifiques de cette thèse. Je présenterai d'abord ma contribution à l'article et je remercierai et soulignerai la contribution des coauteurs de chacun des articles, ainsi que les personnes qui ont contribué de près ou de loin à ces projets. Si j'oublie de mentionner la contribution de certaines personnes, veuillez noter que cet oubli n'est aucunement volontaire !

De façon plus générale, j'aimerais remercier les participants de cette étude clinique qui a permis de générer une quantité et une qualité de données inestimables. Ce projet a mené à la publication de plusieurs articles et a contribué de façon considérable à l'avancement des connaissances concernant l'effet différentiel de l'EPA et du DHA sur le risque cardiométabolique. J'admire leur volonté, leur courage (surtout pour les études de la cinétique des lipoprotéines) et leur discipline. J'ai éprouvé beaucoup de fierté lorsque j'ai présenté les résultats du projet ComparED dans le cadre de congrès nationaux et internationaux. Je remercie le personnel infirmier de l'Unité d'investigation clinique de l'INAF, Steeve Larouche et Christiane Landry, pour les soins prodigués envers participants. Enfin, j'aimerais une fois de plus remercier le Dr Benoît Lamarche qui est l'investigateur principal du projet de recherche et le responsable de l'approbation finale (et beaucoup plus) de chacun des articles présentés. Merci, Benoît, pour ton calme et ta considération lors de ce marathon de rédaction de trois années et demie !

Chapitre 4

Allaire J, Couture P, Leclerc M, Charest A, Marin J, Lépine MC, Talbot D, Tchernof A, Lamarche B. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study. *The American Journal of Clinical Nutrition 2016* Aug;104(2):280-7.

Cet article présente les résultats principaux de l'étude ComparED. Mes tâches principales auront consisté à l'analyse statistique des données et à l'interprétation des résultats, puis à la rédaction de l'article scientifique en tant que première auteure. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission et les révisions requises par les réviseurs de *the American Journal of Clinical Nutrition* avant que l'article soit accepté pour publication.

Il s'agit d'un projet de recherche qui a été subventionné par les Instituts de recherche en santé du Canada et dont les investigateurs sont le **Dr Benoît Lamarche**, le **Dr Patrick Couture** et le **Dr André Tchernof**. Le Dr Benoît Lamarche est l'investigateur principal du projet et professeur à l'École de nutrition de l'Université Laval. Il m'a conseillé lors de l'analyse statistique et l'interprétation des données et a contribué de façon importante à la rédaction de cet article. Le Dr Patrick Couture est un chercheur-clinicien au Centre hospitalier universitaire de l'Université Laval – CHU de Québec et est affilié à l'INAF. Le Dr André Tchernof est un professeur à l'École de nutrition de l'Université Laval dont l'affiliation principale est le Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie du Québec, mais qui est aussi affilié à l'INAF. Ils ont contribué de façon importante au développement et à la réalisation de l'étude ComparED et à l'interprétation des données.

La phase clinique de l'étude ComparED n'aurait pu être possible sans l'excellent travail d'**Amélie Charest,** nutritionniste et professionnelle de recherche dans l'équipe du Dr Benoît Lamarche. Elle coordonne les études cliniques d'une main de maître. Elle trouve des solutions à chacun des problèmes rencontrés durant la planification et la phase clinique de l'étude et s'assure de la qualité des données recueillies. Elle est le contact principal avec les participants des études. **Myriam Leclerc** est une étudiante à la maîtrise qui contribué à la réalisation de la phase clinique du projet et qui a effectué une partie des analyses au laboratoire. **Johanne Marin** et **Marie-Claude Lépine** ont analysé la presque totalité des échantillons biologiques de ce projet. Le **Dr Denis Talbot,** professeur au Département de médecine sociale et préventive à la Faculté de médecine de l'Université Laval, a contribué de façon importante aux analyses statistiques pour cet article. Il a révisé ma programmation statistique et m'a également fourni du support pour la programmation de l'analyse « en intention de traiter ». Il a été d'une grande disponibilité et générosité pour répondre à mes questions.

Chapitre 5

Allaire J, Harris WS, Vors C, Charest A, Marin J, Jackson KH, Tchernof A, Couture P, Lamarche B. Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid. *Prostaglandins, Leukotrienes & Essential Fatty Acids*. 2017 May;120:8-14.

Cet article présente la comparaison de l'effet de l'EPA et du DHA sur l'Indice Oméga-3. L'Indice Oméga-3 a été développé par le **Dr William S Harris** et son équipe de recherche, incluant sa fille la **Dre Kristina Harris Jackson**. L'Indice Oméga-3 est calculé en effectuant la somme de la proportion d'EPA et de DHA (en %) dans les globules rouges. Mes tâches dans le cadre de ce projet auront principalement consisté à l'élaboration du plan d'analyses, l'analyse statistique des données et l'interprétation des résultats, puis à la rédaction de l'article scientifique en tant que première auteure. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission ainsi que les révisions requises par les réviseurs de *Prostaglandins, Leukotrienes & Essential Fatty Acids* avant que l'article soit accepté pour publication.

Le Dr William S Harris et la Dre Kristina Harris Jackson ont effectué l'analyse de la composition en acides gras des globules rouges de tous les participants de l'étude *ComparED*. La **Dre Cécile Vors**, qui est chercheure postdoctorale dans l'équipe du Dr Benoît Lamarche, a contribué de façon importante à l'interprétation des résultats de cet article ainsi que pour les articles présentés au chapitre 6, 7 et 8.

Chapitre 6

Allaire J, Vors C, Tremblay AJ, Marin J, Charest A, Tchernof A, Couture P, Lamarche B. High-Dose DHA Has More Profound Effects on LDL-Related Features Than High-Dose EPA: The ComparED Study. *The Journal of Clinical Endocrinology & Metabolism*. 2018 Aug 1;103(8):2909-2917.

Cet article présente la comparaison de l'effet de l'EPA et du DHA sur le métabolisme *in vivo* de l'apolipoprotéine B100 et CIII, sur la taille des LDL et sur les concentrations de proprotéine convertase subtilisine/kexine de type 9. Mes tâches dans le cadre de ce projet auront principalement consisté à la modélisation des données de la cinétique *in vivo* de l'apolipoprotéine B100 et CIII dans le logiciel *Simulation, Analysis, and Modeling Software II (*SAAM II) *for tracer and phamacokinetic studies*, à l'analyse statistique des données et à l'interprétation des résultats, puis à la rédaction de l'article scientifique en tant que première auteure. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission ainsi que les révisions requises par les réviseurs de *The Journal of Clinical Endocrinology & Metabolism* avant que l'article soit accepté pour publication. Le **Dr André J Tremblay** a contribué de façon importante à la modélisation des données de cinétique et à l'interprétation des résultats. J'aimerais aussi remercier **Isabelle Kelly** et **Benjamin Nehmé** de la Plateforme de

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Chapitre 7

Allaire J, Vors C, Harris WS, Jackson KH, Tchernof A, Couture P, Lamarche B. Comparing the serum triglyceride response to high-dose supplementation with either DHA or EPA among individuals with increased cardiovascular risk: The ComparED study. Sous presse à *The British Journal of Nutrition*.

Cet article présente la variabilité inter- et intra- individuelle de la réponse des triglycérides sériques à l'EPA et au DHA dans l'étude ComparED. Mes tâches principales auront consisté à l'élaboration du plan d'analyses, l'analyse statistique des données et l'interprétation des résultats, puis à la rédaction de l'article scientifique en tant que première auteure. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission et les révisions requises par les réviseurs de *The British Journal of Nutrition* avant que l'article soit accepté pour publication. Dans le cadre de cet article, le **Dr William S Harris** et la **Dre Kristina Harris Jackson** ont contribué de façon importante à l'interprétation des résultats.

Chapitre 8

Allaire J, Vors C, Harris WS, Jackson KH, Tchernof A, Couture P, Lamarche B. Is the increase in LDL-C after high-dose supplementation with either DHA or EPA consistent, and should we worry about it? The ComparED study. Soumis pour publication dans *The Journal of Nutrition* le 7 mai 2019.

Cet article présente la variabilité inter- et intra- individuelle de la réponse du cholestérol dans les LDL à l'EPA et au DHA dans l'étude ComparED. Mes tâches principales auront consisté à l'élaboration du plan d'analyses, l'analyse statistique des données et l'interprétation des résultats, puis à la rédaction de l'article scientifique en tant que première auteure.

Notes concernant les publications non incluses dans cette thèse :

Dans le cadre de mon doctorat, j'ai eu l'occasion d'écrire deux autres articles scientifiques pour lesquels je suis l'auteure principale, mais qui ne font pas partie de cette thèse.

Allaire J, Vors C, Couture P, Lamarche B. LDL particle number and size and cardiovascular risk: anything new under the sun? *Current Opinion in Lipidology*. 2017 Jun;28(3):261-266.

Cet article présente une revue narrative des plus récentes évidences concernant le lien entre la taille et le nombre de particules LDL et le risque cardiovasculaire. Mes tâches principales auront consisté à la recherche

bibliographique des articles qui ont été inclus dans l'analyse et à la rédaction du manuscrit. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission et les révisions requises par les réviseurs de *Current Opinion in Lipidology* avant que l'article soit accepté pour publication. La **Dre Cécile Vors** a contribué de façon importante à la recherche bibliographique et à la rédaction de l'article. Le **Dr Patrick Couture** et le **Dr Benoît Lamarche** ont contribué de façon importante à la révision de l'article.

Allaire J, Talbot D, Couture P, Tchernof A, Jones PJH, Kris-Etherton P, West SG, Connelly PW, Jenkins DJA, Lamarche B. Assessing the impact of the diet on cardiometabolic outcomes: are multiple measurements post-intervention necessary? *The European Journal of Clinical Nutrition*. 2018 Jul 31.

Cet article présente les résultats d'un projet de type méthodologique. Dans le cadre de ce projet, nous avons combiné les données de deux études cliniques randomisées en chassé-croisé. La première est l'étude ComparED qui a été menée exclusivement à l'INAF. La deuxième est une étude multicentrique dont le nom est *Canola Oil Multicenter Intervention Trial* (COMIT) et qui a été menée à l'INAF et dans des centres de recherche externes. L'objectif principal de l'article était de comparer l'effet de la prise de deux mesures répétées et la prise d'une seule mesure après une supplémentation en DHA sur la taille d'échantillon requise pour mesurer un effet sur une sélection de marqueurs biochimiques du risque cardiométabolique. Mes tâches auront consisté à l'élaboration du plan d'analyses statistiques, aux analyses statistiques, à l'analyse des résultats et à la rédaction du manuscrit. J'ai apporté les modifications à l'article suite à la révision par les coauteurs avant la soumission et les révisions requises par les réviseurs de *The European Journal of Clinical Nutrition* avant que l'article soit accepté pour publication.

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Introduction générale

L'intérêt pour la famille des acides gras polyinsaturés oméga-3 à longue chaîne (AGn3-LC) remonte au début des années 1970 avec les travaux de deux chercheurs danois, Bang et Dyerberg (1). Ces chercheurs ont tenté d'expliquer le faible taux d'incidence de maladies cardiovasculaires chez la population d'Esquimaux du Groenland (1, 2). Ils avaient alors attribué la majeure partie des effets protecteurs observés à leur alimentation riche en poissons gras qui contiennent des quantités importantes d'AGn3-LC (1). Depuis, plusieurs études cliniques de grande envergure ont été menées afin d'évaluer l'effet des AGn3-LC sur la santé cardiovasculaire.

La famille des AGn3-LC comprend principalement deux acides gras qui exercent des effets physiologiques connus, soit les acides eicosapentaénoïque (EPA, C20:5n3) et docosahexaénoïque (DHA, C22:6n3). La grande majorité des études cliniques menées jusqu'à maintenant ont observé l'effet d'un supplément alimentaire qui combine l'EPA et le DHA sous différentes formes et proportions. Alors que les études épidémiologiques tendent à montrer un effet protecteur des AGn3-LC sur le risque cardiovasculaire (3-5), les études cliniques qui ont été menées jusqu'à présent tendent à montrer un effet neutre de la supplémentation en AGn3-LC sur le risque d'évènements cardiovasculaires voire légèrement protecteur sur le risque d'évènements coronariens (6, 7). L'utilisation de suppléments d'AGn3-LC en prévention des évènements cardiovasculaires est donc un sujet plutôt controversé à ce jour. Certains aspects pourraient toutefois expliquer ces résultats plutôt surprenants.

Premièrement, la forme sous laquelle sont administrés les AGn3-LC peut influencer leur absorption, et conséquemment leurs effets dans le corps (8). Deuxièmement, de plus en plus d'évidences suggèrent que l'EPA et le DHA auraient des effets différents sur plusieurs facteurs du risque cardiométabolique (9-12). Après plusieurs années de résultats neutres concernant le lien entre la supplémentation en AGn3-LC et le risque d'évènements cardiovasculaires (7), l'étude REDUCE-IT, dont les résultats ont été publiés en novembre 2018, a montré qu'une supplémentation de 4 g d'EPA par jour diminuait le risque d'évènements cardiovasculaires de 25% en comparaison avec un témoin d'huile minérale (13). Dans cette étude, l'EPA a été choisi plutôt que le DHA puisqu'il a été montré que le DHA augmentait les concentrations sanguines de cholestérol dans les lipoprotéines de faible densité (C-LDL) de façon plus importante que l'EPA (9, 10). Toutefois, le DHA semble avoir des effets bénéfiques sur plusieurs autres facteurs du risque cardiométabolique comparativement à l'EPA (9-12). Enfin, une certaine variabilité dans la réponse des triglycérides sanguins aux AGn3-LC a été rapportée dans quelques études jusqu'à maintenant (14-17). Par exemple, alors que la supplémentation en AGn3-LC est reconnue pour diminuer les concentrations de triglycérides sanguins, une augmentation des triglycérides sanguins est généralement rapportée chez 30% des individus (14-17). Au meilleur de nos connaissances, la variabilité inter- et intra-individuelle dans la réponse cardiométabolique à une supplémentation en EPA et en DHA n'a pas été investiguée jusqu'à maintenant.

Dans le cadre de cette thèse, nous avons comparé l'effet d'une supplémentation en EPA et en DHA sur plusieurs facteurs du risque cardiométabolique, incluant les lipides sanguins, les biomarqueurs inflammatoires, le métabolisme des lipides et l'expression des gènes impliqués dans le métabolisme des lipides. Nous avons également étudié la variabilité inter- et intra- individuelle dans la réponse des triglycérides et du C-LDL, ainsi que les déterminants de la réponse à l'EPA et au DHA.

Cette thèse est divisée en 9 chapitres. Le premier chapitre permet de mieux comprendre la problématique de recherche en présentant brièvement le portrait global des maladies cardiovasculaires, en abordant la prévalence et les facteurs de risque, ainsi que le métabolisme des lipides. Le deuxième chapitre touche principalement le métabolisme des AGn3-LC ainsi que leurs effets sur la santé cardiovasculaire. Enfin, j'aborderai les objectifs du projet ainsi que les hypothèses qui s'y rattachent dans le chapitre 3. C'est dans ce chapitre que j'introduirai les chapitres 4 à 8 qui présentent les articles scientifiques dont les résultats permettent de répondre aux hypothèses de recherche. Enfin, le dernier chapitre présente la discussion et la conclusion générale de cette thèse.

Chapitre 1 Maladies cardiovasculaires

1.1 Définition des maladies cardiovasculaires

Les maladies cardiovasculaires sont considérées comme des maladies chroniques multifactorielles qui se développent graduellement sur plusieurs années. L'expression « maladie cardiovasculaire » englobe plusieurs maladies du cœur et des vaisseaux sanguins incluant entre autres la cardiopathie ischémique, la maladie cérébrovasculaire, la maladie vasculaire périphérique et l'insuffisance cardiaque (18). Les maladies cardiovasculaires se divisent aussi en plusieurs sous-groupes selon l'organe qui est touché par la maladie: les maladies du cœur touchent spécifiquement le cœur (infarctus du myocarde, fibrillation auriculaire, angine, etc.), les maladies cérébrovasculaires touchent spécifiquement les vaisseaux du cerveau alors que les maladies vasculaires périphériques touchent spécifiquement les vaisseaux périphériques du corps (18).

Dans la littérature scientifique, on retrouve le concept d'évènement cardiovasculaire et de décès lié aux maladies cardiovasculaires. L'évènement cardiovasculaire réfère habituellement à un problème de santé d'ordre cardiovasculaire qui a mené à une consultation médicale ou à une hospitalisation. Évidemment, le décès lié à une maladie cardiovasculaire réfère à un décès suite à un évènement cardiovasculaire. Parfois, les évènements cardiovasculaires peuvent inclure les décès suite à un évènement cardiovasculaire. Les évènements cardiovasculaires peuvent aussi être divisés selon la physiopathologie de la maladie. On peut d'abord retrouver les évènements de type hémorragique qui se produisent lors de la rupture d'un anévrisme se trouvant dans la paroi d'un vaisseau sanguin (18). Il y a ensuite les évènements ischémiques qui se produisent lors de l'obstruction partielle ou totale d'un vaisseau sanguin par la plaque d'athérosclérose ou encore par un thrombus. La plaque d'athérosclérose, ou plaque athéromateuse, est une maladie macro-vasculaire qui est caractérisée par une inflammation chronique et par l'accumulation de cholestérol dans la paroi des vaisseaux sanguins (19). Elle est directement associée aux maladies cardiovasculaires (19).

La définition de la catégorie « évènements cardiovasculaires » est plutôt hétérogène entre les études. De façon non exclusive, les évènements cardiovasculaires peuvent inclure le décès par maladie cardiovasculaire, le décès par maladie coronarienne, un infarctus du myocarde, une maladie coronarienne, un accident vasculaire cérébral, de l'angine de poitrine, de l'insuffisance cardiaque, une maladie vasculaire périphérique ou une chirurgie cardiaque.

1.2 Prévalence et incidence des maladies cardiovasculaires

Les maladies du cœur se classent au deuxième rang des causes de décès au Canada et sont la principale cause de décès dans le monde avec 45% du total des décès attribuables à des maladies non transmissibles (20). L'accident vasculaire cérébral se classe au troisième rang des causes de décès au Canada (20). La

prévalence ajustée des maladies cardiovasculaires pour l'âge a légèrement diminué dans les dernières années, passant de 9,3% [intervalle de confiance (IC) à 99% 9,3 à 9,4] en 2005-2006 à 9,0% [IC à 99% 9,0 à 9,1%] en 2015-2016 (21). Néanmoins, le nombre de cas est en constante augmentation avec plus de 14 000 cas s'ajoutant chaque année (21). Le taux d'incidence des maladies cardiovasculaires ajusté pour l'âge a diminué dans les dernières années passant de 11,3% [IC à 99% 11,2 à 11,4%] en 2005-2006 à 8,1% [IC à 99% 8,0 à 8,2%] en 2015-2016 (21). Le taux de mortalité toutes causes confondues parmi les individus atteints d'une maladie cardiovasculaire connue est demeuré plutôt stable entre la période de 2005-2006 à 2015-2016 (2,1% [IC à 99% 2,0 à 2,2%] et 1,9% [IC à 99% 1,8 à 2,0%] respectivement), bien que le nombre de cas augmente d'environ 600 chaque année (21). Les causes de décès les plus fréquentes chez les individus avec une maladie cardiovasculaire connue sont : la maladie cardiovasculaire avec 32,1% des décès, suivi du cancer avec 26,6% et des maladies respiratoires avec 11,0% (18).

La cardiopathie ischémique est définie par l'accumulation de plaque athéromateuse dans les artères coronaires qui peuvent provoquer une insuffisance cardiaque, un accident vasculaire cérébral ou une crise cardiaque (18). Elle représente la maladie du cœur la plus fréquente au Canada et dans le monde, causant presque la moitié des décès liés aux maladies du cœur (18). En 2012-2013, la prévalence de cardiopathie ischémique au Québec était légèrement supérieure à celle du Canada chez les adultes de plus de 20 ans (8,4% [IC à 95% 8,4 à 8,5%] et 8,1% [IC à 95% 8,1 à 8,1%] respectivement) (18). La cardiopathie ischémique se développe environ 10 ans plus tard chez la femme que chez l'homme (18).

Alors que la prévalence et l'incidence des maladies cardiovasculaires ont diminué dans les dernières années au Canada, le taux de mortalité est resté plutôt stable (18). Néanmoins, les maladies cardiovasculaires représentent le groupe de maladies dont le fardeau économique est le plus important au Canada avec 11,7 milliards de dollars en coûts directs annuellement (22).

1.3 Athérosclérose et métabolisme des lipoprotéines

Les lipoprotéines sont des complexes macromoléculaires qui permettent le transport des lipides dans le corps. Le centre des lipoprotéines est composé de triglycérides et d'esters de cholestérol (partie hydrophobe) alors que leur membrane est composée d'apolipoprotéines (apos), de cholestérol non estérifié et de phospholipides (partie hydrophile). Les apos sont essentielles à la structure et à la fonction des lipoprotéines. Le métabolisme des lipoprotéines est intimement lié au développement de la plaque d'athérosclérose.

1.3.1 Développement de la plaque d'athérosclérose – un survol

La plaque d'athérosclérose, ou plaque athéromateuse, se développe conséquemment à l'accumulation de cholestérol dans la paroi interne des artères (19). Les artères sont composées de trois couches de tissus.

L'intima est directement en contact avec la lumière de l'artère et est formée d'une fine couche de cellules endothéliales. Elle repose sur une couche de fibres de collagène et de protéoglycanes qui lui confèrent des propriétés élastiques (19). La media est située sous l'intima et est constituée de collagène, d'élastine et de fibres musculaires lisses dans les plus gros vaisseaux (19). L'adventice est composé de tissu conjonctif et de fibres élastiques et est la couche la plus externe de l'artère (19). Loin d'être un tissu inerte, l'endothélium vasculaire exerce un contrôle sur la pression artérielle grâce à son élasticité et à sa capacité à se contracter (vasoconstriction) ou se dilater (vasodilatation). L'endothélium vasculaire peut également produire des médiateurs chimiques de l'inflammation comme des cytokines et participer activement à la formation de la plaque athéromateuse (19). L'athérosclérose est une pathologie complexe qui implique plusieurs mécanismes dont la dysfonction endothéliale, la rétention et la modification des lipoprotéines dans l'endothélium, l'expression de molécules d'adhésion, l'attraction et la transformation de monocytes en macrophages, la formation de cellules spumeuses et la sécrétion de facteurs de croissance (19).

La dysfonction endothéliale est la première étape du développement de l'athérosclérose. L'obésité, le diabète, l'hypertension, le tabagisme, la présence de LDL oxydées et le syndrome métabolique sont quelques-uns des facteurs qui contribuent à cette altération de l'endothélium (23, 24). L'hypercholestérolémie familiale est également un facteur de risque important du développement de l'athérosclérose (24). Chacun de ces facteurs peut amorcer le développement de l'athérosclérose en contribuant au stress oxydatif de l'endothélium vasculaire.

Le stress causé à l'endothélium peut ensuite provoquer une vasoconstriction (par une diminution de la disponibilité de l'oxyde nitrique) et une augmentation de la synthèse de médiateurs de la coagulation et de l'inflammation (19). Cette lésion oxydative entraîne aussi l'augmentation de la perméabilité de l'intima qui permet l'accumulation de lipoprotéines porteuses d'une apo B comme les lipoprotéines de très faible densité (VLDL) ou les LDL. Ces lipoprotéines riches en cholestérol diffusent passivement entre les jonctions des cellules endothéliales et adhèrent à l'intima grâce au complexe formé par l'apo B ou E et les protéoglycanes (19). Les lipoprotéines subissent ensuite des transformations telles qu'une oxydation, une lipolyse ou une protéolyse qui contribuent également à l'inflammation vasculaire (19).

Les modifications des lipoprotéines et de l'apo B qu'elles contiennent stimulent la production de molécules d'adhésion qui attirent les monocytes circulants (19). Les monocytes se transforment en macrophages lors de leur entrée dans l'endothélium. Les récepteurs « scavengers » des macrophages tels que le « *class A scavenger receptor type I/II (SR-A I/SR-A II)* » et le « *cluster of differentiation 36* (CD36) » reconnaissent les lipoprotéines oxydées et les captent par endocytose ou par phagocytose (19, 25). Puisque les macrophages ne sont pas dotés d'un système de régulation du cholestérol, ils accumulent peu à peu les lipoprotéines riches en cholestérol

(25). Par apoptose, les macrophages se transforment ensuite en cellules spumeuses qui sont particulièrement riches en esters de cholestérol et s'accumulent dans la paroi endothéliale (19, 25). L'accumulation des cellules spumeuses dans la paroi endothéliale provoque un soulèvement de la couche unicellulaire de l'intima. Ce soulèvement de l'intima est connu sous le nom de strie lipidique (26). L'accumulation progressive de cholestérol dans les cellules spumeuses aggrave la strie lipidique et peut même entraîner la rupture de la paroi endothéliale. Afin d'empêcher cette complication et de renforcir l'endothélium, les cellules musculaires lisses de la media prolifèrent et migrent sur le dessus de la plaque athéromateuse (26).

La dernière étape du développement de l'athérosclérose est la rupture de la plaque athéromateuse (26). S'ensuit la formation d'un thrombus pour réparer la lésion et l'obstruction du vaisseau sanguin (26). Lorsque le thrombus se forme dans les artères coronaires, l'hypoxie peut entraîner une perte de la fonction d'une partie plus ou moins étendue du muscle cardiaque (18, 26). Comme mentionné précédemment, la cardiopathie ischémique, causée par la plaque athéromateuse, est la maladie du cœur la plus fréquente au Canada et dans le monde, causant presque la moitié des décès liés aux maladies du cœur (18). L'athérosclérose est donc une pathologie qui doit être prise en charge rapidement. Puisque l'athérosclérose est définie comme étant l'accumulation de cholestérol dans les parois des artères, il existe un lien étroit entre les dyslipidémies, le métabolisme des lipoprotéines et les maladies cardiovasculaires.

1.3.2 Métabolisme des lipoprotéines impliquées dans le transport des lipides – un survol

La figure 1.1 présente un résumé du métabolisme des lipides de sources exogènes et endogènes. Les lipoprotéines sont des complexes macromoléculaires dont le rôle est de permettre le transport des lipides (hydrophobes) dans le corps (milieu hydrophile). Brièvement, les chylomicrons sont des lipoprotéines qui sont assemblées dans l'entérocyte à partir des lipides provenant de l'alimentation (c.-à-d. triglycérides, phospholipides, cholestérol et vitamines liposolubles) et des sels biliaires (27, 28). Les chylomicrons sont caractérisés par la présence des apos B48, CIII et E à leur surface (27, 28). Ces lipoprotéines sont riches en triglycérides (80 à 95%) et leur contenu relatif en cholestérol est faible (2 à 7%) (27, 28). Elles sont sécrétées par les cellules intestinales dans le système lymphatique et rejoignent ensuite la circulation sanguine au niveau thoracique (27, 28). Les triglycérides contenus dans les chylomicrons sont hydrolysés par la lipoprotéine lipase (LPL) et les acides gras sont distribués aux tissus périphériques (c.-à-d. les muscles, le tissu adipeux et l'endothélium vasculaire) (27, 28). Les résidus de chylomicrons sont enfin captés par les cellules hépatiques surtout grâce à leur apo E (27, 28).

Les VLDL sont synthétisées par le foie principalement à partir 1- des lipides provenant de l'alimentation qui arrivent au foie via la veine porte, 2- des lipides provenant de l'alimentation qui arrivent au foie via les résidus

de chylomicrons et 3- des acides gras libres provenant du tissu adipeux (27, 28). Les VLDL peuvent également contenir des acides gras synthétisés *de novo* par les cellules hépatiques ainsi que des acides gras qui proviennent des réserves lipidiques du foie (27, 28). Le rôle des VLDL est de transporter les lipides du foie aux tissus périphériques via la circulation sanguine (27, 28). Elles peuvent contenir jusqu'à 80% de triglycérides et contiennent les apos B100, C et E à leur surface (27). L'apo B100 est intégrée aux VLDL lors de la production hépatique et est transmise à ses résidus métaboliques que sont les lipoprotéines de densité intermédiaire (IDL) et les LDL (29). Le récepteur LDL au foie pourrait en partie moduler la dégradation de l'apo B100 dans les hépatocytes et la sécrétion des VLDL par le foie (30, 31). Les triglycérides contenus dans les VLDL sont hydrolysés par la LPL des tissus périphériques (c.-à-d. les muscles, le tissu adipeux et l'endothélium vasculaire), ce qui diminue graduellement leur contenu en triglycérides et leur grosseur, et augmente leur contenu relatif en cholestérol (27). L'hydrolyse des triglycérides contenus dans les VLDL est accélérée par la présence d'apo CII qui augmente l'activité de la LPL (32, 33). Quant à l'apo CIII, elle diminue le catabolisme des VLDL par l'inhibition de l'activité de la LPL (32, 33). L'apo CIII des VLDL pourrait également interférer avec la liaison de l'apo B100 et de l'apo E aux récepteurs hépatiques et entraîner une augmentation de la durée de résidence des VLDL et de leur concentration dans le sang (32, 33). L'hydrolyse des triglycérides contenus dans les VLDL génère les IDL. Une proportion des VLDL, celles de plus grande taille, est directement retirée de la circulation par la liaison de l'apo E ou de l'apo B100 aux récepteurs hépatiques (27). De manière alternative, les lipides contenus dans les VLDL de plus petite taille et dans les IDL sont hydrolysés par la LPL et la lipase hépatique jusqu'à l'obtention d'une lipoprotéine de faible densité riche en cholestérol qu'est la LDL (27). Le temps de résidence des VLDL dans le sang est d'environ 15 minutes.

Les LDL contiennent environ 75% du cholestérol retrouvé dans le sang (27). Grâce à leur apo B100, les LDL se fixent sur les récepteurs spécifiques des LDL pour transférer le cholestérol aux cellules des tissus périphériques (p. ex. glandes surrénales, muscle squelettique, endothélium vasculaire, lymphocytes, gonades et reins) (27). Ils sont ensuite captés par les récepteurs LDL au foie ou dans les tissus périphériques grâce à l'apo B100 (27). Les LDL peuvent résider dans la circulation sanguine jusqu'à trois jours (27). Chez les individus en santé, l'arrivée de cholestérol au foie via les chylomicrons, les VLDL, les IDL, les LDL et les lipoprotéines de haute densité (HDL) régule l'activité de la *3-hydroxy-3-methylglutaryl coenzyme A reductase* (HMG-CoA réductase), l'enzyme limitante de la synthèse de cholestérol dans les cellules hépatiques, et diminue la production de récepteurs LDL (28).

Globalement, les HDL permettent le transport inverse du cholestérol des tissus périphériques jusqu'au foie (28, 34). Ils pourraient diminuer l'inflammation vasculaire grâce à leurs propriétés antioxydantes et leur capacité à éliminer le cholestérol des parois vasculaires (28, 34). L'apo A-1 des HDL peut être produite par le foie et par l'intestin (28, 34). Les HDL sont formés grâce au cholestérol provenant des tissus périphériques, au récepteur

« ATP-binding cassette 1 » (ABCA1) et aux résidus d'hydrolyse des chylomicrons et des VLDL (34). La *lecithin :cholesterol acyltransferase* (LCAT) et la *cholesteryl ester transfer protein* (CETP) jouent un rôle important dans le remodelage des HDL en permettant les échanges du cholestérol entre le HDL et les autres lipoprotéines (28). Les récepteurs ABCA1 et « scavenger » de type B1 (SR-B1) sont des récepteurs qui permettent le transfert ou l'échange du cholestérol entre les tissus périphériques et les HDL (28). Ces récepteurs ont aussi été identifiés comme des joueurs importants dans la phagocytose des LDL oxydées par les macrophages dans l'endothélium et dans la formation des cellules spumeuses (35).

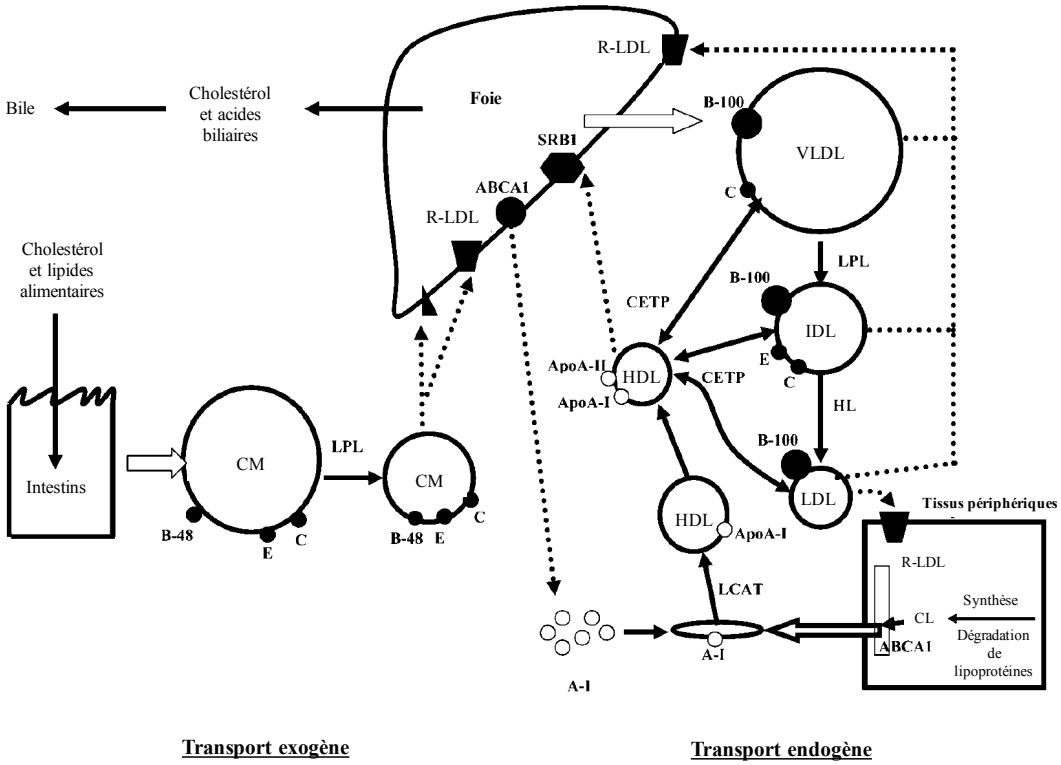

Figure 1.1 Résumé du transport des lipides de sources exogènes et endogènes

Adapté de (29). Traduction libre. Abréviations : ABCA1 : récepteur « *ATP-binding cassette 1* » ; Apo : apolipoprotéine; CETP : *cholesteryl ester transfer protein ;* CL : cholestérol libre; CM : chylomicrons; HDL : lipoprotéine de haute densité; LCAT : *lecithin :cholesterol acyltransferase;* LDL : lipoprotéine de faible densité; LPL : lipoprotéine lipase; R-LDL : récepteur LDL; SRB1 : récepteur « scavenger » de type B1 ; VLDL : lipoprotéine de très faible densité.

La compréhension du métabolisme des lipoprotéines et des mécanismes qui sous-tendent les changements dans les concentrations sanguines de lipides suite à un traitement pharmacologie ou à une intervention nutritionnelle est en grande partie possible grâce aux études de cinétique *in vivo* des lipoprotéines.

1.3.3 Étude de la cinétique des lipoproprotéines

Une étape primordiale de l'étude de la cinétique *in vivo* des lipoprotéines est le marquage endogène des lipoprotéines (29). Bien qu'il existe plusieurs méthodes, le principe consiste généralement à marquer la partie protéique (apo) des lipoprotéines (le tracé) à l'aide d'un marqueur (le traceur) qui 1- n'interfère pas avec le métabolisme normal du tracé, 2- peut être mesuré facilement dans le sang et 3- ne peut pas être synthétisé par le corps (29). Dans le cadre des études cliniques menées à l'INAF, le marquage des apos des lipoprotéines se fait habituellement par l'infusion intraveineuse d'un bolus suivie d'une infusion constante d'isotopomère de leucine tri-deutérée (D3-Leucine), qui est le traceur (36). Cette infusion permet de marquer la leucine dans la partie protéique (apo) des lipoprotéines. Durant une journée type d'étude de la cinétique (15 heures), les participants consomment le 1/30^{e} de leurs besoins énergétiques sous forme de galettes toutes les 30 minutes afin de maintenir un état postprandial stable (« *steady state* ») durant toute la durée de l'étude de cinétique. L'état postprandial stable facilite la modélisation des taux auxquels les lipoprotéines sont synthétisées et catabolisées dans le corps. Lorsque l'état postprandial est atteint, soit trois heures après la première galette, un bolus de D3-Leucine est administré par voie intraveineuse. Après l'injection du bolus, la D3-Leucine s'équilibre rapidement dans la circulation, puis avec les liquides de l'espace interstitiel et le cytoplasme des cellules (29). La D3-Leucine s'incorpore ensuite dans les protéines (incluant les apos) qui sont synthétisées et sécrétées par la cellule à un certain taux, qui est généralement rapporté de façon absolue (PR, taux de production en mg/kg/j), et métabolisées à un certain taux, qui est rapporté de façon relative (FCR, taux de catabolisme fractionnel en « *pools* »/j) (29). La D3-Leucine s'incorpore de la même façon que s'incorporerait la leucine non marquée (29). Une infusion constante de D3-Leucine (à raison de 10 µmol/kg masse corporelle par heure) est ensuite administrée durant les 12 heures suivantes afin de fournir au corps un apport constant en D3-Leucine. Afin de pouvoir mesurer l'enrichissement en D3-Leucine dans les apos, des échantillons sanguins (20 ml) à 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 11, 12 heure(s) sont prélevés via un second cathéter intraveineux.

Trois temps sont utilisés pour estimer la concentration plasmatique moyenne (taille du « *pool* ») des VLDL, IDL et LDL par « *enzyme-linked immunosorbent assay* (ELISA) » pour chaque participant. La taille du « *pool* » est considérée comme étant constante durant l'étude de cinétique puisque le participant est maintenu en état postprandial stable durant les 15 heures. La concentration en D3-Leucine dans les apos des VLDL, des IDL et des LDL est déterminée par « *liquid chromatography-mass spectrometric-multiple reaction monitoring* » (LC/MS/MRM) dans chaque échantillon sanguin. L'enrichissement en D3-Leucine est ensuite calculé de la façon suivante :

$$Enrichissement = \left(\frac{[D3\ Leucine]}{[Leucine] + [D3\ Leucine]}\right)$$

Les valeurs d'enrichissement sont alors transposées sous la forme d'un graphique en fonction du temps pour chaque lipoprotéine (c.-à-d. VLDL, IDL et LDL). La modélisation des valeurs d'enrichissement en fonction du temps est effectuée grâce au logiciel *Simulation Analysis and Modelling Program II* (SAAM II, SAAM Institute, Seattle). Ce type de modélisation permet de représenter les systèmes biologiques sous la forme de plusieurs compartiments reliés entre eux par des constantes de transfert de métabolites. Des modèles mathématiques multicompartimentaux prédéterminés sont utilisés pour déterminer les taux de transfert, les PR et les FCR des apos des VLDL, des IDL et des LDL grâce à des équations mathématiques différentielles. Les données peuvent ensuite être utilisées dans les modèles d'analyses statistiques pour comparer les effets de différentes interventions sur le métabolisme des lipoprotéines.

Comme nous avons pu le constater dans les paragraphes précédents, le métabolisme des lipides est complexe et implique plusieurs types de lipoprotéines ainsi que l'action de plusieurs enzymes et transporteurs. Je discuterai de comment le métabolisme des lipoprotéines et les concentrations sanguines de lipides peuvent influencer le risque de maladies cardiovasculaires dans la prochaine section. De façon non exhaustive, je discuterai également d'autres facteurs de risque cardiovasculaire dont certains marqueurs de l'inflammation systémique.

1.4 Facteurs de risque cardiométabolique

Les facteurs de risque qui contribuent au développement des maladies cardiovasculaires, plus spécifiquement au développement de la plaque d'athérosclérose, peuvent se diviser en plusieurs catégories. Parmi celles-ci, on retrouve entre autres les facteurs de risque traditionnels ou non traditionnels, majeurs ou mineurs, modifiables ou non modifiables et génétiques ou environnementaux.

L'étude cas-témoins INTERHEART menée dans 52 pays chez près de 30 000 participants a permis d'identifier neuf facteurs de risque modifiables qui pouvaient prédire environ 90% du risque de développer une maladie cardiovasculaire (37). Ces facteurs sont le tabagisme, le tour de taille, le diabète de type 2, la tension artérielle, l'apport alimentaire en fruits et en légumes, la consommation d'alcool, le rapport des concentrations sanguines d'apo B et A1, le stress et le niveau d'activité physique (37). L'étude de Framingham menée chez plus de 5 200 participants suivis depuis 1948 est également une étude importante qui a permis de développer un outil de calcul du risque absolu d'évènements cardiaques sur 10 ans (38). Le score est calculé en utilisant la présence de facteurs de risque tels que le sexe, l'âge, la cholestérolémie totale, la concentration de cholestérol dans les HDL (C-HDL), le statut tabagique et la tension artérielle (38). De plus, certaines conditions pathologiques comme la résistance à l'insuline (39) et le diabète de type 2 augmentent considérablement le risque de développer une maladie cardiovasculaire (40, 41).

Encore aujourd'hui, la recherche permet d'identifier de nouveaux facteurs de risque du développement de la plaque athéromateuse et des maladies cardiovasculaires en général. Parmi les facteurs qu'on pourrait qualifier d'en émergence ou plus récemment découverts, on retrouve entre autres la lipoprotéine(a), la proprotéine convertase subtilisine/kexine de type 9 (PCSK9), ainsi que certains facteurs thrombotiques, inflammatoires et génétiques.

Les maladies cardiovasculaires sont des maladies chroniques multifactorielles qui se développent graduellement sur plusieurs années. Dans la section suivante, j'effectuerai une revue non exhaustive des facteurs de risque qui contribuent au développement des maladies cardiovasculaires en discutant spécifiquement des facteurs qui ont été étudiés dans le cadre de ce doctorat.

1.4.1 Facteurs de risque cardiométabolique de type lipidique

Cholestérol total

La concentration de cholestérol total dans le sang, ou cholestérolémie totale, est utilisée dans le calcul du risque absolu de maladie coronarienne de Framingham (38). Il a été montré qu'une augmentation de la cholestérolémie de 1% augmentait le risque coronarien de façon linéaire d'environ 2% (42). Par exemple, les hommes dont le cholestérol plasmatique total est de 6,2 mmol/L sont jusqu'à trois fois plus à risque de décéder de maladies coronariennes que les hommes dont le cholestérol total de 5,2 mmol/L (43).

Cholestérol dans les lipoprotéines de faible densité

Le C-LDL est un facteur de risque important dans le développement des maladies cardiovasculaires (44). Une augmentation de 1 mmol/L a été associée de façon linéaire à une augmentation de 41% et de 24% du risque relatif d'évènements coronariens chez les hommes et les femmes respectivement (45). Le traitement pharmacologique avec des statines permet de diminuer les concentrations de C-LDL et le risque d'évènements coronariens de façon importante (44, 46, 47). Il a été montré que le risque d'évènements coronariens diminuait de 10 à 25% sur 5 ans pour chaque diminution de 1 mmol/L de C-LDL secondaire à un traitement pharmacologique avec des statines (44, 48). Toutefois, malgré l'atteinte des cibles recommandées, la diminution des concentrations de C-LDL permettrait de réduire jusqu'à 60% du risque prédit d'évènements coronariens et de mortalité (49). Ainsi, malgré une diminution significative des concentrations de C-LDL, il subsiste un risque résiduel qui peut être expliqué en partie par d'autres facteurs de risque comme le tour de taille, les triglycérides ou le C-HDL exemple. Il est également bien documenté que la présence de plusieurs facteurs de risque a un effet synergique sur le risque cardiovasculaire (37, 38, 50). C'est pourquoi une évaluation globale de l'état de santé et des habitudes de vie, incluant le tabagisme, l'activité physique et l'alimentation, est primordiale dans l'évaluation clinique des individus à risque d'évènements cardiovasculaires.

Apolipoprotéine B totale

Les concentrations sanguines d'apo B totale reflètent directement le nombre de particules athérogènes (c.-à-d. VLDL et LDL) en circulation, puisqu'une seule apo B se retrouve sur la surface de ces lipoprotéines (51). À noter que ≥90% de l'apo B totale mesurée à jeun dans le sang se trouve sur les LDL (51). Une méta-analyse regroupant les données de sept études cliniques chez des individus traités avec une statine a montré que la réduction d'un écart type dans la concentration sanguine d'apo B100 et de C-LDL était associée à une réduction du risque d'évènements coronariens de 24,4% [IC à 95% 19,2 à 29,2%] et de 20,1% [IC à 95% 15,6 à 24,3%] respectivement (52). La Société canadienne de cardiologie recommande de mesurer les concentrations de C-LDL ou d'apo B totale pour l'évaluation du risque cardiovasculaire et le traitement chez la population à risque d'évènements cardiovasculaires (46). En d'autres mots, le nombre de particules athérogènes (c.-à-d. concentration d'apo B totale) et le C-LDL seraient des prédicteurs importants du risque d'évènements cardiovasculaires.

Apolipoprotéine CIII

L'apo CIII est synthétisée par le foie et est sécrétée dans la circulation sur les VLDL (25). Elle peut être transférée aux HDL durant l'hydrolyse des triglycérides (25). L'apo CIII inhibe l'activité de la LPL dont le rôle est d'hydrolyser les triglycérides contenus dans les VLDL (25). Des concentrations sanguines élevées d'apo CIII sont généralement associées à une diminution du catabolisme des VLDL et donc à une augmentation des concentrations sanguines de VLDL et de triglycérides (25). Jusqu'à maintenant la concentration d'apo CIII, surtout sur les VLDL, a été identifiée comme étant un prédicteur important de la progression des lésions athéromateuses (53, 54) et est généralement associée à un risque plus élevé de maladies ou d'évènements cardiovasculaires (55-58). La présence d'apo CIII sur les VLDL est aussi associée à la formation de LDL petites et denses qui représentent aussi un facteur de risque d'évènements cardiovasculaires (59, 60).

Taille des LDL

Austin et ses collaborateurs ont été les premiers à montrer que la taille des LDL avait une influence sur le risque cardiovasculaire (60). Ils ont observé que les individus qui présentaient une plus grande proportion de LDL petites et denses avaient un risque jusqu'à trois fois plus élevé d'avoir un infarctus du myocarde que les individus qui présentaient une prépondérance de LDL de plus grande taille (60). Cette association serait partiellement expliquée par la faible affinité des récepteurs LDL au foie pour les LDL de petite taille, ce qui entraînerait un temps de résidence prolongé et une augmentation de l'athérogénicité des LDL petites et denses comparativement aux LDL de plus grande taille (61). De par leur petite taille, les LDL petites et denses auraient également plus de facilité à pénétrer l'endothélium vasculaire et seraient plus sujettes à l'oxydation que les LDL

de plus grande taille (61), ce qui contribuerait au développement de la plaque athéromateuse. La prépondérance de LDL petites et denses est associée à la présence d'autres facteurs de risque cardiovasculaire tels que l'hypertriglycéridémie, une concentration sanguine élevée d'apo B totale et une faible concentration de C-HDL (62).

Cholestérol dans les HDL

Des concentrations élevées de C-HDL sont généralement associées à un risque plus faible de maladies cardiovasculaires. Par exemple, dans les études épidémiologiques, une augmentation dans les concentrations de C-HDL de 0,40 mmol/L a été associée de façon linéaire à une diminution de 34% et de 24% du risque d'évènements coronariens chez les hommes et les femmes respectivement (45). Toutefois, les études cliniques ont rapporté que la majorité des inhibiteurs de la CETP, qui ont comme objectif d'augmenter les concentrations de C-HDL, ne réduisent pas le risque d'évènements cardiovasculaires (63, 64). Une augmentation du risque a même été rapportée avec l'utilisation de torcetrapib (65) malgré une modification favorable du bilan lipidique (c.-à-d. une diminution des concentrations de C-LDL et une augmentation des concentrations de C-HDL) (63-65). L'utilisation de l'anacetrapib a entraîné une réduction modérée du risque d'évènements coronariens majeurs de 9% en comparaison avec un témoin (rapport de taux : 0,91 [IC à 95% 0,85 à 0,97]), mais cette diminution était liée à son effet sur les concentrations de C-LDL plutôt que sur le C-HDL (66).

Triglycérides

La concentration élevée de triglycérides à jeun et postprandiale, ou l'hypertriglycéridémie, est associée au risque d'évènements cardiovasculaires (67-70). Dans le cas de la concentration de triglycérides à jeun, cette association n'est généralement pas indépendante des autres facteurs de risque, comme le cholestérol non-HDL, le C-HDL et le tour de taille (67, 69). La concentration sanguine de triglycérides à jeun n'est donc pas une cible de traitement primaire proposée pour réduire le risque d'évènements cardiovasculaires dans les plus récentes lignes directrices de la Société canadienne de cardiologie (46). Néanmoins, une concentration élevée de triglycérides à jeun (c.-à-d. > 1,7 mmol/L) fait partie des critères diagnostiques du syndrome métabolique de plusieurs organisations internationales et canadiennes (71-73). Tel que mentionné précédemment, il peut subsister un risque résiduel d'évènements cardiovasculaires chez les patients traités avec une statine et chez qui les cibles de C-LDL sont atteintes (49). Chez ces patients, les triglycérides sanguins peuvent alors devenir une cible de traitement secondaire dans le but de réduire le risque résiduel d'évènements cardiovasculaires (74). Chez les individus qui présentent des concentrations très élevées de triglycérides sanguins (c.-à-d. > 11,3 mmol/L), le traitement de l'hypertriglycéridémie est important afin de réduire le risque de pancréatite (75). Dans le cas de la concentration de triglycérides en postprandial, elle a été associée à un risque plus élevé risque d'évènements cardiovasculaires dans la *Women's Health Study* (rapport de hasards (HR) du troisième tertile

comparativement au premier tertile de concentrations de triglycérides en postprandial : 1,98 [IC à 95% 1,21 à 3,25]), indépendamment de l'âge, de la pression artérielle, du statut tabagique, du diabète de type 2, de l'indice de masse corporelle, et des concentrations sanguines de cholestérol total, de C-HDL et de protéine C-réactive (CRP) (69). Il a également été observé qu'une concentration élevée de triglycérides en postprandial était associée à une augmentation du risque d'infarctus du myocarde indépendamment de plusieurs facteurs de risque chez les femmes (HR pour une augmentation de 1 mmol/L : 1,20 [IC à 95% 1,05 à 1,37]), mais pas chez les hommes (HR : 1,04 [IC à 95% 0,98 à 1,11]) dans la *Copenhagen City Heart Study* (70).

Cholestérol non-HDL

Le cholestérol non-HDL est calculé en effectuant la soustraction de la concentration sanguine du C-HDL de la concentration de cholestérol total. Il représente la concentration de cholestérol retrouvé dans les lipoprotéines athérogènes (c.-à-d. VLDL et LDL) (76) et est associé au risque de maladies cardiovasculaires (52, 77). Une méta-analyse regroupant les données de sept études cliniques chez des individus traités avec une statine a montré que la réduction d'un écart type dans la concentration sanguine de cholestérol non-HDL et de C-LDL était associée à une réduction du risque d'évènements coronariens de 20,0% [IC à 95% 15,2 à 24,7%] et de 20,1% [IC à 95% 15,6 à 24,3%] respectivement (52).

Proprotéine convertase subtilisine/kexine de type 9 (PCSK9)

La PCSK9 est une enzyme hépatique qui participe à la régulation du métabolisme du cholestérol en se liant au récepteur LDL au foie (78). Elle est majoritairement exprimée au foie, mais également dans les intestins et dans le cerveau (79). On peut également la trouver dans le plasma (79). Brièvement, PCSK9 entraîne la dégradation des récepteurs LDL au foie, ce qui diminue la clairance des LDL et augmente les concentrations sanguines de C-LDL (79). Il a été montré que des mutations de type « gain de fonction » dans le gène codant pour PCSK9 étaient associées à des concentrations de C-LDL élevées et à un risque élevé d'évènements cardiovasculaires (80). À l'opposé, des mutations de type « perte de fonction » sont associées à une hypocholestérolémie et à un faible risque d'évènements cardiovasculaires (80). Tel que mentionné précédemment, même si la diminution des concentrations de C-LDL par un traitement avec des statines a été prouvée comme étant efficace pour diminuer le risque d'évènements coronariens, le risque peut demeurer élevé chez certains patients (49). Pour faire suite à la découverte du rôle de PCSK9 dans le métabolisme du cholestérol, une nouvelle classe de médicaments a été développée pour le traitement de l'hypercholestérolémie, soit les inhibiteurs de PCSK9 (81). Selon la molécule utilisée, il a été montré que les inhibiteurs de PCSK9, ajoutés à un traitement avec des statines, permettaient de réduire les concentrations de C-LDL de 40 à 80% chez les patients avec une hypercholestérolémie (81). Cette diminution des concentrations de C-LDL avec les inhibiteurs de PCKS9 s'est traduite en une diminution du risque d'évènements cardiovasculaires de 17% (risque relatif (RR) : 0,83 [IC à

95% 0,78 à 0,88]) et par une modeste diminution de la mortalité de toutes causes en comparaison avec une statine seule (RR : 0,92 [IC à 95% 0,85 à 1,02]) (82). Il semble donc que les inhibiteurs de PCSK9 aient une valeur ajoutée au traitement avec des statines quant à la prévention d'évènements cardiovasculaires. Toutefois, le coût de ce type de traitement est encore élevé, ce qui le rend peu accessible.

1.4.2 Facteurs de risque cardiométabolique de type inflammatoire

L'inflammation systémique chronique exerce un rôle important dans le développement et la progression de la plupart des maladies cardiovasculaires (83-85). Cette inflammation est différente de l'inflammation dite aiguë qui est généralement associée à une réaction physiologique normale de l'organisme contre une agression (86). L'inflammation aiguë entraîne entre autres de la rougeur, de la chaleur et une vasodilatation des vaisseaux sanguins afin de maîtriser la source d'agression (86). Une fois la source d'agression contrôlée, un processus de résolution de l'inflammation s'ensuit avec la sécrétion de molécules inflammatoires et de médiateurs dont le rôle est de protéger les organes, d'activer la réparation des tissus, et d'assurer la clairance des débris engendrés par la réaction inflammatoire (86). L'objectif de cette réaction aiguë est de rétablir l'homéostasie corporelle. Elle est habituellement localisée et temporaire.

L'inflammation chronique résulte généralement d'un état de déséquilibre d'origine pathologique (86). Il existe deux types d'inflammation chronique. Le premier type est une inflammation chronique à composante auto-immune qui est associée à des signes cliniques évidents et accompagnée d'une augmentation importante de biomarqueurs de l'inflammation dans la circulation systémique (86). On retrouve ce type d'inflammation dans les maladies comme l'arthrite rhumatoïde, les maladies inflammatoires de l'intestin ou le psoriasis par exemple. Le second type d'inflammation est celui qui sera abordé dans cette thèse. Il s'agit d'une inflammation chronique dite de « faible intensité » (« *low-grade inflammation* » ou « *subclinical inflammation* ») (86). Elle ne s'accompagne habituellement pas de signes cliniques évidents, mais d'une légère augmentation de la concentration des biomarqueurs de l'inflammation dans le sang.

Le système vasculaire, les cellules immunitaires et le tissu adipeux produisent des cytokines inflammatoires qui incluent entre autres les interleukines (IL-6 et IL-18) et le facteur de nécrose tumorale (TNF-α) (86). Cette sécrétion de cytokines stimule la sécrétion de CRP au foie, un autre biomarqueur non spécifique de l'inflammation (86). Le tissu adipeux peut également sécréter des molécules anti-inflammatoires comme l'adiponectine (87). Bien qu'il existe de nombreux autres biomarqueurs de l'inflammation, les prochains paragraphes traiteront brièvement des biomarqueurs inflammatoires qui ont été étudiés dans le cadre de cette thèse, soit l'IL-6, l'IL-18, le TNF-α, l'adiponectine et la CRP.

Puisque les concentrations de **CRP** peuvent augmenter suite à une multitude de stimuli comme des infections ou des brûlures, la CRP est reconnue comme étant un marqueur non spécifique de l'inflammation. Elle est synthétisée par le foie et ses fonctions principales seraient liées à l'immunité innée et à l'élimination des débris cellulaires (86). Chez un individu en santé, la valeur médiane de CRP a été estimée à 0,8 mg/L alors que dans le cas d'une inflammation aiguë, les valeurs de CRP dans le sang peuvent dépasser 10 mg/L (88). Il est généralement accepté que des valeurs situées entre 1 mg/L et 10 mg/L indiquent un état inflammatoire chronique et de faible intensité (88). Bien que non spécifique, il s'agit du marqueur de l'inflammation le plus étudié (85).

L'**IL-6** et le **TNF-α** sont sécrétés par plusieurs types de cellules incluant les cellules immunitaires, endothéliales, adipeuses et même musculaires (86). Quant à l'**IL-18**, qui est une forme active de cytokine provenant de la famille des cytokines inflammatoires IL-1, elle peut être sécrétée par les cellules immunitaires, endothéliales et rénales (86, 89). L'IL-6 aurait plusieurs rôles dont l'activation de la synthèse de protéines de phase aiguë, la stimulation de la différenciation des lymphocytes B et la libération d'acides gras par le tissu adipeux (86). Elle aurait également un rôle à jouer dans la sensibilité à l'insuline (86). Le TNF-α stimulerait l'apoptose cellulaire et l'expression des molécules d'adhésion cellulaire et pourrait contribuer au développement de la résistance à l'insuline (86). L'IL-18 serait davantage associée à l'inflammation vasculaire et rénale et donc à l'hypertension artérielle (89).

L'**adiponectine** est une hormone sécrétée par le tissu adipeux qui améliore l'utilisation du glucose, la sensibilité à l'insuline et stimule l'oxydation des acides gras au foie et dans les muscles squelettiques (87). Elle a également des propriétés anti-inflammatoires, comme la diminution de l'expression des macrophages de type M1 (pro-inflammatoires) et du facteur de transcription NF-κB (« *nuclear transcription factor kappa B* ») qui active une voie importante de la régulation de la réponse inflammatoire (90). Enfin, l'adiponectine pourrait inhiber la transformation des macrophages en cellules spumeuses dans l'endothélium, ce qui serait protecteur contre le développement de la plaque athéromateuse (91).

Des concentrations anormalement élevées d'IL-6, de TNF-α, de CRP et des concentrations faibles d'adiponectine ont été associées au risque de plusieurs pathologies chroniques telles que l'hypertension (92), le diabète de type 2 (93-97) et les maladies cardiovasculaires (98-102). De plus, il a été montré qu'une intervention pharmacologique avec des anticorps monoclonaux ajoutés au traitement de l'hypercholestérolémie pouvait réduire l'inflammation et diminuer le risque d'évènements cardiovasculaires chez des patients en prévention secondaire (103, 104).

Comme nous avons pu le constater, les maladies cardiovasculaires sont des maladies complexes et multifactorielles. L'évaluation globale des habitudes de vie et de l'état de santé des patients est donc essentielle afin d'optimiser la prise en charge de ces maladies. Il existe actuellement plusieurs options de traitements pharmacologiques pour prévenir le développement de ces maladies chroniques. Toutefois, l'adoption de saines habitudes de vie est primordiale afin de prévenir et de traiter les maladies cardiovasculaires. Parmi les changements d'habitudes de vie les plus importantes, on retrouve la cessation tabagique et l'adoption d'un mode de vie actif et d'une alimentation équilibrée. Comme nous pourrons le constater au prochain chapitre, notre alimentation peut exercer un effet important sur le risque cardiométabolique et de maladies chroniques.

Chapitre 2 Alimentation et maladies cardiovasculaires

2.1 Facteurs nutritionnels en lien avec le risque de maladies cardiovasculaires

L'adoption de saines habitudes de vie, incluant les habitudes alimentaires, est essentielle à l'atteinte et le maintien d'une bonne santé cardiovasculaire tout au long de la vie. Plusieurs facteurs nutritionnels ont été associés au risque (augmenté ou diminué) d'évènements cardiovasculaires jusqu'à maintenant (105). Le tableau 2.1 présente les recommandations qui ont été émises par l'Association américaine du cœur (AHA) afin de réduire le risque d'évènements cardiovasculaires (par une diminution des concentrations sanguines de C-LDL). Les recommandations présentées dans le tableau 2.1 sont appuyées par des évidences scientifiques de qualité élevée (grade A) (105).

Tableau 2.1 Recommandations de l'AHA afin de diminuer les concentrations sanguines de C-LDL sanguines et de réduire le risque d'évènements cardiovasculaires

Recommandations
Améliorer la qualité de l'alimentation en consommant une variété de: - Fruits; - Légumes; - Grains entiers; - Produits laitiers faibles en gras; - Volailles et poissons; - Légumineuses; - Huiles polyinsaturées; - Noix.
Limiter: - La viande rouge; - Les boissons sucrées; - Les sucreries.
Diminuer la quantité d'énergie provenant des acides gras saturés: - Viser un apport entre 5 et 6 % de l'énergie totale.
Diminuer la quantité d'énergie provenant des acides gras trans.

Adapté de (105)**.** Traduction libre.

Les recommandations présentées dans le tableau 2.1 concernent à la fois la consommation d'aliments et la consommation de nutriments. Par exemple, il semble favorable de consommer des noix (aliment), mais il est recommandé de limiter sa consommation d'acides gras saturés (nutriment). Le lien entre les apports alimentaires en différents types d'acides gras, les lipides sanguins et le risque cardiovasculaire, suscite l'attention des chercheurs, des producteurs, des représentants de l'industrie, des institutions gouvernementales,

et de la population en général depuis plusieurs décennies. Le tableau 2.2 présente un résumé des effets des différents types d'acides gras contenus dans les aliments sur les concentrations sanguines de C-LDL et de C-HDL.

Tableau 2.2 Effet des différents types d'acides gras sur les concentrations sanguines de C-LDL et le C-HDL

Acide gras	C-LDL	C-HDL
Saturés	⇑⇑	⇑
Monoinsaturés		
C18 :1*cis* (oléique)	⇓	⇑
Polyinsaturés		
C18 :2 (linoléique)	⇓	⇔
C18 :3 (α-linolénique)	⇓	⇔
C20 :5 et C22 :6 (eicosapentaénoïque et docosahexaénoïque)	⇑⇔	⇑⇔
Trans		
C18 :1*trans* (élaïdique)	⇑⇑	⇔

Adapté de (106). Les changements sont exprimés en comparaison avec des glucides. ⇑ : augmente; ⇔ : pas d'effet; ⇓ : diminue.

Dans le cadre de cette thèse, nous nous sommes intéressés plus spécifiquement à l'effet des acides eicosapentaénoïque (EPA, C20 :5n3) et docosahexaénoïque (DHA, C22 :6n3), deux acides gras oméga-3 polyinsaturés à longue chaîne (AGn3-LC), sur le risque cardiométabolique. Le chapitre 2 sera donc plus spécifiquement consacré aux AGn3-LC et à leurs effets sur le risque cardiométabolique. Tel que présenté dans le tableau 2.2, les AGn3-LC peuvent entraîner une augmentation des concentrations sanguines de C-LDL et de C-HDL ou exercer un effet neutre (c.-à-d. aucun effet). Comme nous pourrons le constater dans les sections suivantes, l'effet des AGn3-LC sur les lipides sanguins dépend en partie de la dose et du type d'acide gras (c.-à-d. EPA ou DHA).

2.2 Acides gras oméga-3

2.2.1 Métabolisme des acides gras oméga-3

Les acides gras polyinsaturés oméga-3 (AGn3) font partie de la famille des acides gras polyinsaturés. Comme leur nom l'indique, ils présentent une double liaison sur le troisième carbone à partir du groupement méthyle (-CH_3) de leur chaîne carbonée. L'acide α-linolénique (C18 :3n3) est un AGn3 essentiel retrouvé dans les sources alimentaires végétales telles que les noix de Grenoble, l'huile de canola, l'huile de soja, les graines de lin, les graines de chia et les graines de chanvre. Tel que présenté à la figure 2.1, le corps humain peut convertir l'acide α-linolénique provenant de l'alimentation en EPA, en acide docosapentaénoïque (DPA, C22 :5n3) et en DHA grâce à l'action de la Δ6-désaturase, la Δ5-désaturase, d'élongases et de la β-oxydation dans le foie (107). Les DHA peut également être reconverti en EPA par β-oxydation (107).

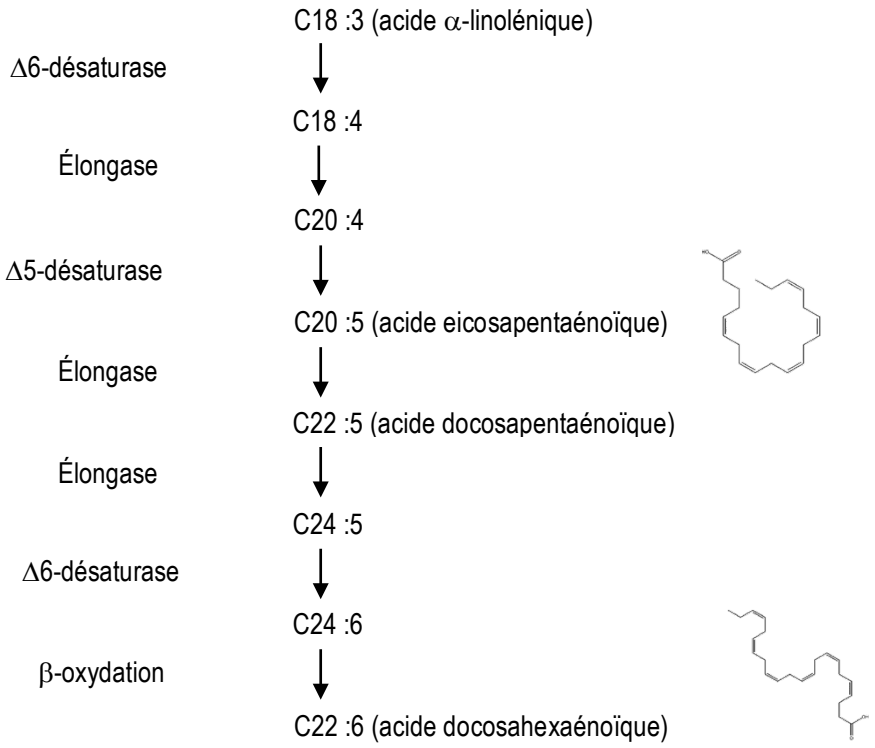

Figure 2.1 Métabolisme *in vivo* des acides gras polyinsaturés oméga-3

Adapté de (107).

Les désaturases sont des enzymes qui permettent de créer une double liaison sur la chaîne carbonée (sur le sixième et sur le cinquième carbone à partir du groupement hydroxyle pour la Δ6-désaturase et la Δ5-désaturase respectivement), alors que les élongases allongent la chaîne carbonée en y ajoutant deux carbones à la fois. Théoriquement l'acide α-linolénique peut être métabolisé en EPA, en DPA et en DHA. Les études ont toutefois montré que la conversion en EPA est de moins de 8% chez les hommes et d'environ 20% chez les femmes (108, 109). La conversion de l'acide α-linolénique en DPA est d'environ 8% chez les hommes et de 6% chez les femmes (108, 109) et la conversion en DHA est de moins de 1% chez les hommes et d'environ 9% chez les femmes (108, 109). Il a également été montré qu'une augmentation de l'apport en EPA et de DHA provenant de l'alimentation diminuait leur synthèse *in vivo* à partir de l'acide α-linolénique, probablement via l'activation du *peroxisome proliferator-activated receptor alpha* (*PPAR-α*) qui inhibe la transcription de l'Δ6-désaturase (110). Une grande proportion de l'acide α-linolénique est aussi utilisée pour produire de l'énergie dans le corps (107). Ainsi, bien qu'ils ne soient pas considérés comme des acides gras essentiels, les AGn3-LC (c.-à-d. EPA, DPA et DHA) retrouvés dans le corps proviennent presque exclusivement de l'alimentation.

2.2.2 Recommandations alimentaires pour les acides gras oméga-3 chez la population en santé

Les recommandations nutritionnelles chez l'adulte pour l'atteinte et le maintien d'une bonne santé sont de 0,6 à 1,2% de l'énergie totale en acide α-linolénique (ce qui correspond à 1,3 et 2,7 g par jour pour un apport de 2000 kilocalories) (111). L'apport alimentaire en acide α-linolénique des Nord-Américains est de 1,1 à 1,6 g par jour (112), ce qui est suffisant selon les recommandations.

Les AGn3-LC se retrouvent principalement dans les produits marins tels que les algues et les poissons « gras » comme le saumon, le maquereau, le hareng et les sardines. Le tableau 2.3 présente la composition en EPA, en DPA et en DHA de différents aliments.

Tableau 2.3 Composition en EPA, DPA et DHA des divers aliments de source marine (par portion de 75 g)

Aliment	EPA (g)	DPA (g)	DHA (g)
Saumon, atlantique, élevage, cuit au four	0,518	0,269	1,093
Sardines, dans l'huile, égouttées	0,355	0,000	0,382
Thon blanc, dans l'eau, égoutté	0,175	0,014	0,472
Crevettes, bouillies	0,101	0,009	0,026
Morue, atlantique, cuite au four	0,003	0,010	0,116

Données tirées du Fichier canadien sur les éléments nutritifs de 2015, Santé Canada.

Comme on peut le constater, les aliments riches en EPA et en DHA contiennent peu de DPA, à l'exception du saumon. Il a d'ailleurs été montré que le DPA retrouvé dans le corps provient presque exclusivement de la conversion de l'EPA en DPA (113). Bien que les effets du DPA sur la santé suscitent de plus en plus d'intérêt en recherche (114), nous nous intéresserons principalement à l'EPA et au DHA dans les prochaines sections.

Les Canadiens atteignent les recommandations nutritionnelles en acide α-linolénique, mais il semblerait que leur statut en AGn3-LC ne soit pas optimal. En effet, selon les données de l'Enquête canadienne sur les mesures de santé de 2012 et 2013, l'Indice Oméga-3 moyen des Canadiens est de seulement 4,5% [IC à 95% 4,2 à 4,8%] (115). L'Indice Oméga-3, développé par Harris et ses collaborateurs, est un indice du statut en EPA et en DHA chez l'humain et a été inversement associé au risque de décès par maladie coronarienne (5, 116, 117). Il est calculé en effectuant la somme de la composition des globules rouges en EPA et en DHA (en % des acides gras totaux) (116). Il a été observé qu'une valeur de 4% ou moins était associée à un risque élevé d'évènements coronariens, une valeur de plus de 4% à moins de 8% à un risque modéré et une valeur de 8% ou plus à un risque faible (116). Par exemple, comparativement à la catégorie de risque élevé (c.-à-d. 4% ou plus), la catégorie de risque faible (c.-à-d. 8% ou plus) a été associée à une diminution du risque de décès par maladie coronarienne jusqu'à 90% (116). Un Indice Oméga-3 d'environ 8% peut être atteint par la consommation de

plus de 0,5 g d'EPA+DHA par jour, ce qui correspond à environ deux portions de 75 g de poisson par semaine, dépendamment du type de poisson (116).

Selon l'Enquête sur la santé dans les collectivités canadiennes de 2015, la consommation moyenne de poisson (incluant le poisson gras, le poisson maigre et les fruits de mer) des adultes canadiens est de 6,1 g par jour, ce qui correspond à moins d'un repas par mois (résultats non publiés). Toujours selon l'Enquête sur la santé dans les collectivités canadiennes de 2015, 62,3% des adultes canadiens consomment **moins deux portions** de poisson par semaine (données non publiées). Environ 5% des adultes canadiens consomment habituellement une portion de poisson **gras** par semaine et 0% consomme habituellement deux portions de poisson **gras** par semaine (données non publiées). Enfin, 12,8% des adultes canadiens consomment habituellement des suppléments d'AGn3-LC contenant de l'EPA et du DHA (données non publiées). À noter qu'il n'y a actuellement pas d'évidence scientifique qui appuie une recommandation de supplémentation en AGn3-LC chez la population en santé afin de réduire le risque de maladies cardiovasculaires (118).

2.2.3 Les différents types de suppléments d'acides gras oméga-3

Les suppléments retrouvés sur le marché et utilisés dans les études peuvent contenir des proportions variables d'EPA et de DHA. Les suppléments peuvent également contenir d'autres composés ainsi que d'autres types d'acides gras en différentes proportions (119). L'EPA est habituellement retrouvé en plus grande proportion que le DHA, majoritairement sous la forme d'un ratio de 3 :2 (119). Les proportions d'EPA et de DHA sont toutefois très variables entre les études. L'EPA et le DHA peuvent aussi se trouver sous différentes formes, les plus fréquentes étant les triglycérides réestérifiés et les esters d'éthyle. Dus à leur grande susceptibilité à l'oxydation, les AGn3-LC sont rarement retrouvés sous la forme d'acides gras libres dans les suppléments (8). Les triglycérides réestérifiés sont plus biodisponibles et donc mieux absorbés que les esters d'éthyle (8). La prise d'un repas simultanément à la prise du supplément augmente la biodisponibilité des esters d'éthyle, mais n'aurait pas d'effet sur la biodisponibilité des triglycérides réestérifiés (8). Il a également été démontré que l'incorporation de l'EPA et du DHA dans la membrane des globules rouges s'effectue de manière dose-dépendante (120).

Bien que la majorité des suppléments retrouvés sur le marché et utilisés dans les études contiennent à la fois de l'EPA et du DHA, de plus en plus d'évidences montrent que ces deux AGn3-LC auraient des effets différents sur les facteurs de risque cardiométabolique (9, 10). Les effets **individuels** de l'EPA et du DHA sous la forme de **suppléments** seront discutés dans les prochaines sections. Leurs effets lorsqu'ils sont consommés **ensemble** sous la forme d'**aliments** ou de **suppléments** seront aussi discutés.

2.3 Acides gras oméga-3 et risque cardiométabolique

Depuis plusieurs années, les AGn3-LC suscitent de l'intérêt grâce à leurs effets potentiellement bénéfiques sur la santé incluant le développement de la maladie d'Alzheimer, les symptômes de la dépression, le cancer et les maladies auto-immunes, pour ne nommer que quelques exemples (121). Dans le cadre de ce doctorat, nous nous sommes principalement intéressés à leurs effets sur le risque cardiométabolique. La figure 2.2 présente quelques mécanismes potentiels par lesquels les AGn3-LC pourraient prévenir le développement des maladies cardiovasculaires.

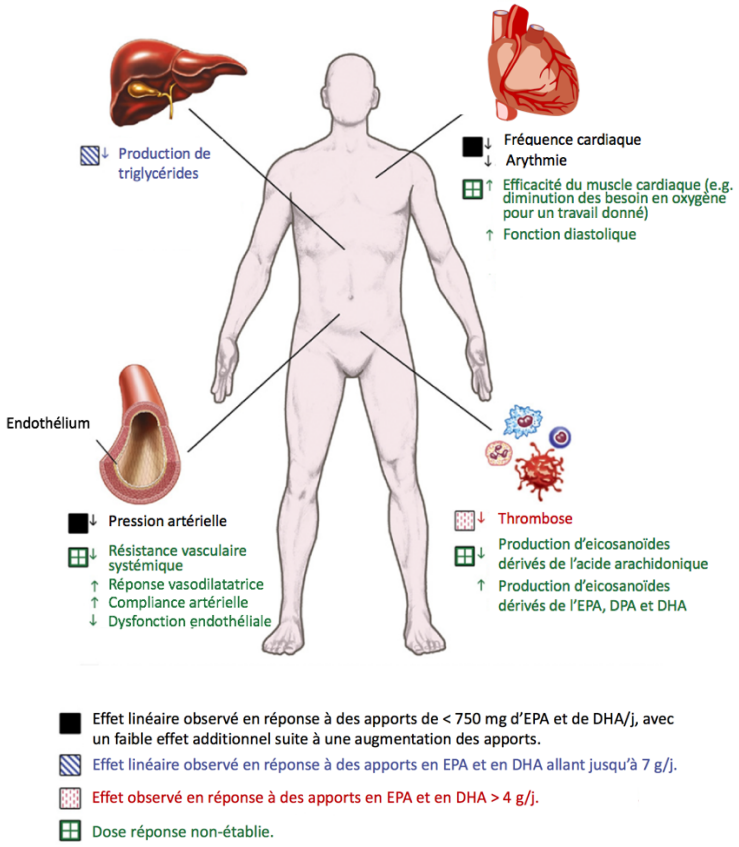

Figure 2.2 Effets des acides gras oméga-3 polyinsaturés à longue chaîne sur la santé cardiométabolique

Adapté de (122). Traduction libre. Abréviations : DHA, acide docosahexaénoïque ; DPA : acide docosapentaénoïque ; EPA, acide eicosapentaénoïque.

De façon générale et non exhaustive, les AGn3-LC diminuent les concentrations sanguines de triglycérides, peuvent diminuer la pression artérielle, réguler le rythme cardiaque et améliorer la fonction endothéliale (122, 123). De plus, la dose administrée peut influencer leurs effets sur le risque cardiométabolique. Par exemple, l'effet antiarythmique et hypotenseur peut être observé à des doses plus faibles (< 750 mg d'EPA et de DHA par jour) sans effet additionnel considérable avec des doses plus élevées, mais l'effet antithrombotique

nécessite des doses de plus de 4 g d'EPA et de DHA par jour. De façon générale, plus la dose est élevée, plus l'effet va être important (123).

L'EPA et le DHA sont des acides gras qui ont un « potentiel actif » important dans le corps. Par exemple, l'EPA et le DHA sont des précurseurs de la synthèse de différents types d'eicosanoïdes dans le corps grâce aux actions des lipoxygénases (5-LOX) et des cyclooxygénases-2 (COX-2) (121). L'EPA permet la synthèse de prostaglandines et de thromboxanes de série 3, de leucotriènes de série 5, et de résolvines de série E (121). Quant au DHA, il permet la synthèse de marésines et de résolvines de type D (121). Les eicosanoïdes sont entre autres des médiateurs de l'inflammation, de la contraction des muscles lisses et de l'agrégation plaquettaire (121). L'EPA et le DHA permettent donc la régulation de plusieurs mécanismes vitaux dans le corps.

Il est important de mentionner que les **suppléments** d'AGn3-LC et les **aliments** riches en AGn3-LC peuvent exercer des effets différents sur la santé et les marqueurs du risque cardiométabolique. Comparativement au supplément, l'aliment contient non seulement des AGn3-LC, mais également d'autres nutriments, vitamines et minéraux qui peuvent exercer des effets additionnels ou synergiques dans le corps. Par exemple, une étude menée chez 53 participants hyperlipidémiques a montré que 2,8 g d'EPA et 1,6 g de DHA par jour sous la forme de poisson gras (250 g de poisson à raison de deux fois par semaine) et 2,5 g d'EPA et 1,7 g de DHA par jour sous la forme d'un supplément permettaient de diminuer les concentrations de cholestérol total, de cholestérol non-HDL et de triglycérides après deux mois (124). Toutefois, à doses égales d'AGn3-LC, cette diminution était plus importante dans le groupe qui consommait du poisson comparativement au groupe qui consommait le supplément (124). Ces résultats suggèrent que les autres nutriments contenus dans le poisson pourraient avoir des effets additionnels ou synergiques à ceux des AGn3-LC dans le corps. Puisque nous nous sommes intéressés à l'effet de la supplémentation dans le cadre de ce doctorat et qu'il y a peu d'études cliniques qui ont observé l'effet des AGn3-LC provenant des aliments, l'effet de la **supplémentation** en AGn3-LC sur la santé cardiométabolique sera discuté dans les prochaines sections. Néanmoins, une attention particulière sera portée à la différence entre l'effet des aliments et des suppléments, surtout dans la partie sur l'effet des AGn3-LC sur les évènements cardiovasculaires et la mortalité.

2.3.1 Acides gras oméga-3 et facteurs de risque cardiométabolique

Facteurs de risque de type lipidique

Le tableau 2.4 présente un résumé des effets des AGn3-LC en comparaison avec d'autres agents hypotriglycéridémiants (c.-à-d. niacine et fibrates) et hypocholestérolémiants (c.-à-d. statines, résines, ezitimibe et inhibiteurs de PCSK9) sur les concentrations sanguines de triglycérides, C-LDL et C-HDL.

Tableau 2.4 Effets de différents agents hypolipémiants sur les concentrations sanguines de triglycérides, C-LDL et C-HDL

Agent	Changement triglycérides (%)	Changement C-LDL (%)	Changement C-HDL (%)
AGn3-LC (EPA+DHA)	⇓ 20-50	⇑/⇔	⇑/⇔
Niacine	⇓ 20-50	⇓ 5-25	⇑ 15-35
Fibrates	⇓ 20-50	⇑/⇓ 0-20*	⇑ 6-20
Statines	⇓ 7-40	⇓ 18-60	⇑ 3-15
Résines	⇑/⇔	⇓ 10-30	⇑ 3-5
Ezetimibe	⇓ 4-11	⇓ 17-22	⇑ 2-5
Inhibiteurs de PCSK9	⇓ 7-17	⇓ 54-74	⇑ 4-9

Adapté de (125-127). *Les fibrates peuvent augmenter le C-LDL chez certains individus hypertriglycéridémiques. ⇑ : augmente; ⇔ : pas d'effet; ⇓ : diminue.

Les AGn3-LC sont reconnus pour diminuer les concentrations sanguines de **triglycérides** jusqu'à 50% et de façon proportionnelle à la dose administrée et aux concentrations de triglycérides de départ (7, 122, 125). L'effet des AGn3-LC sur le métabolisme des lipides n'est pas encore totalement compris, mais les études de la cinétique *in vivo* des lipoprotéines ont montré que la diminution des triglycérides était due à la fois à une réduction du PR l'apo B100 des VLDL et à une augmentation de la conversion de l'apo B100 des VLDL en LDL (128, 129). La figure 2.3 présente les mécanismes potentiels qui permettraient d'expliquer la diminution des triglycérides par les AGn3-LC. La diminution de la production des VLDL au foie pourrait en partie être expliquée par une diminution de l'expression du « *sterol regulatory element-binding protein 1c (SREBP1c)* » (125, 130). Cette diminution de l'expression aurait pour effet de diminuer l'activité de l'acide gras synthase (FAS) et de l'acétyl-CoA carboxylase (ACC) qui sont des enzymes qui participent à la lipogenèse au foie (125, 130). Les AGn3-LC semblent également diminuer l'activité de la diacylglycérol O-acyltransférase (DGAT) qui catalyse l'étape finale de la synthèse de triglycérides au foie (125, 130). La diminution des triglycérides peut également être expliquée par l'activation de PPAR-α, ce qui augmente la β-oxydation des acides gras au foie et réduit la concentration des substrats nécessaires pour la synthèse de triglycérides (125, 131). Les AGn3-LC pourraient également entraîner la dégradation de l'apo B100 nouvellement synthétisée dans le réticulum endoplasmique des hépatocytes (125, 131) en partie par un effet sur le récepteur LDL (30, 31). Les AGn3-LC diminueraient les concentrations d'apo CIII et augmenteraient l'expression et l'activité de la LPL, une enzyme responsable de l'hydrolyse des triglycérides dans les VLDL qui conduit à la production de LDL dans le plasma (125, 132). Cet effet sur la LPL peut expliquer à la fois la diminution des concentrations sanguines de triglycérides et l'augmentation des concentrations de C-LDL parfois observées suite à une supplémentation en AGn3-LC (125, 132). Les AGn3-LC semblent également entraîner la production de VLDL de plus petite taille qui sont précurseurs de LDL comparativement aux VLDL de plus grande taille (133).

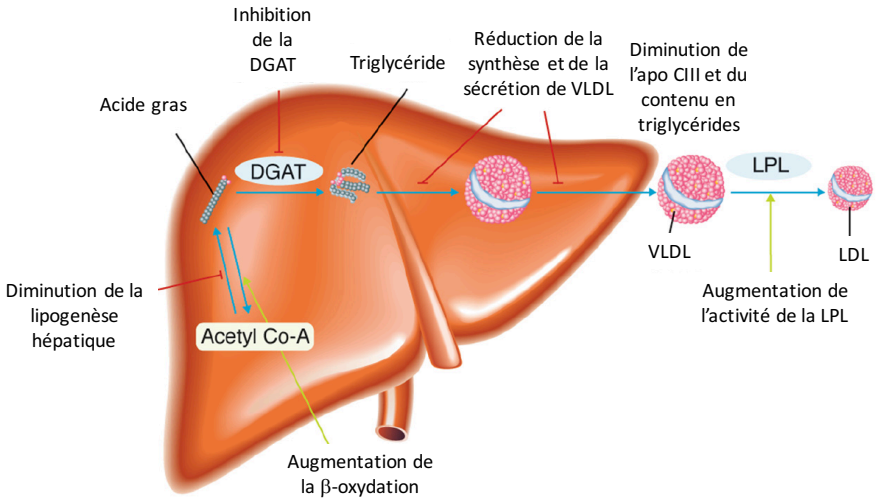

Figure 2.3 Mécanismes sous-jacents à la diminution des concentrations de triglycérides sanguins en réponse à une supplémentation en acides gras oméga-3 polyinsaturés à longue chaîne

Adapté de (134). Traduction libre. Abréviations : apo, apolipoprotéine ; DGAT, diacylglycérol O-acyltransférase ; LDL, lipoprotéine de faible densité ; LPL, lipoprotéine lipase ; VLDL, lipoprotéine de très faible densité.

En ce qui concerne l'effet individuel du DHA et de l'EPA, il a été montré que la diminution des concentrations de triglycérides dans le sang est plus importante en réponse à la supplémentation en DHA qu'en EPA (Δ-6,14 mg/dL (0,069 mmol/L), IC à 95% -2,47 à -9,82 mg/dL) (9, 10). Il y a actuellement très peu d'études qui permettent de comparer les effets de l'EPA et du DHA sur les mécanismes qui sous-tendent ces différences.

Les évidences provenant des méta-analyses montrent qu'une supplémentation en AGn3-LC influencerait peu ou pas les concentrations sanguines de **C-LDL** (7). Toutefois, cet effet neutre serait probablement dû au fait que les suppléments d'AGn3-LC contiennent habituellement une plus grande proportion d'EPA que de DHA. En effet, le DHA augmente les concentrations de C-LDL comparativement à l'EPA (Δ+4,63 mg/dL (0,12 mmol/L), IC à 95% +2,15 à+ 7,10 mg/dL) (9, 10). L'EPA aurait des effets plutôt neutres ou augmenterait légèrement les concentrations de C-LDL dans le sang (9, 10). Le DHA tend à diminuer les concentrations sanguines d'apo CIII comparativement à l'EPA, ce qui pourrait en partie expliquer la plus grande diminution des concentrations de triglycérides et également contribuer à la formation de particules LDL de plus grande taille avec le DHA (11, 135, 136). Le DHA et l'EPA auraient des effets similaires sur l'activité de la LPL (137). Il a été observé chez l'animal que le DHA, mais pas l'EPA, diminuait l'expression des récepteurs LDL au foie, mais les études chez les humains montrent des résultats plutôt hétérogènes (138).

Les AGn3-LC semblent pouvoir légèrement augmenter les concentrations de **C-HDL** dans le sang (7), mais cette augmentation serait plus importante avec le DHA qu'avec l'EPA (Δ+3,74 mg/dL (0,097 mmol/L), IC à 95% +2,42 à +5,05 mg/dL) (9, 10). Il y a actuellement très peu d'études qui permettent de comparer les effets de l'EPA et du DHA sur les mécanismes qui sous-tendent ces différences.

Selon une étude clinique menée chez 92 femmes en santé, une supplémentation de 2,2 g d'AGn3-LC par jour durant 12 semaines diminuerait les concentrations sanguines de **PCSK9** comparativement à l'huile de carthame (139). Des analyses secondaires de l'étude *Canola Oil Multicenter Intervention Trial (COMIT)* menée chez 54 participants à risque d'évènements cardiovasculaires a montré que la consommation d'une huile de canola enrichie en DHA diminuait les concentrations sanguines de PCSK9 comparativement à une huile de canola régulière (140). Des études supplémentaires sont nécessaires pour mieux comprendre les mécanismes sous-jacents à ces changements.

Facteurs de risque de type inflammatoire

L'EPA et le DHA présents dans le corps permettent la production d'eicosanoïdes qui sont entre autres des médiateurs de l'inflammation (141). L'EPA et le DHA pourraient donc exercer des effets anti-inflammatoires dans le corps. L'EPA et le DHA exerceraient également leurs effets anti-inflammatoires en prenant la place de l'acide arachidonique (AA, C20 :4n6) dans la membrane des cellules (122). L'AA est un acide gras polyinsaturé oméga-6 qui permet la production d'eicosanoïdes ayant des effets pro-inflammatoires (141). Les résultats d'une méta-analyse publiée en 2014 comprenant les données de 68 études cliniques randomisées menées chez plus de 4000 individus ont montré que les AGn3-LC diminuaient les concentrations sanguines de CRP, d'IL-6 et de TNF-α chez des individus en santé et avec une maladie chronique telle qu'une maladie coronarienne ou une hypercholestérolémie (142). Les AGn3-LC augmenteraient également les concentrations sanguines d'adiponectine (143). Peu d'études ont été menées afin de comparer les effets de l'EPA et du DHA sur les marqueurs de l'inflammation systémique jusqu'à maintenant. Mori et ses collaborateurs ont observé qu'une supplémentation de 4 g d'EPA ou de DHA par jour (esters d'éthyle, pureté de 96%) durant 6 semaines n'avait pas d'effet sur les concentrations de CRP, d'IL-6 et de TNF-α chez 59 individus avec un diabète de type 2 en comparaison avec un témoin d'huile d'olive (11). De façon similaire, Tsunoda et ses collaborateurs n'ont pas observé d'effet d'une supplémentation de 1,8 g par jour de DHA ou d'EPA durant six semaines sur les concentrations sanguines de CRP en comparaison avec un témoin d'huile d'olive chez 50 participants en santé (12). Plusieurs facteurs peuvent expliquer cette discordance entre l'effet observé avec l'EPA et le DHA combiné comparativement à leur effet individuel. La dose utilisée, la durée de l'intervention, la taille de l'échantillon et l'état de santé des participants sont quelques facteurs qui peuvent influencer la réponse à une supplémentation en EPA et en DHA.

2.3.2 Acides gras oméga-3 et risque d'évènements cardiovasculaires et de mortalité

Les études épidémiologiques montrent que la consommation de poisson et d'AGn3-LC est associée à une faible diminution du risque d'évènements coronariens (3, 4) et de mortalité de toutes causes (144). Il existe peu d'évidences concernant l'effet d'une intervention nutritionnelle visant à augmenter l'apport en AGn3-LC par l'alimentation (c.-à-d. poissons et fruits de mer ou produits enrichis) sur le risque de mortalité cardiovasculaire et de toutes causes (7). Basé sur le peu de données disponibles provenant d'études cliniques, une augmentation de l'apport en AGn3-LC par l'alimentation ne semble pas associée au risque de mortalité cardiovasculaire (RR : 0,95 [IC à 95% 0,52 à 1,71]) et toutes causes (RR : 0,90 [IC à 95% 0,60 à 1,35]), et au risque d'évènements cardiovasculaires (RR : 1,13 [IC à 95% 0,86 à 1,49]) (7). Selon les données de plus de 80 000 participants, la consommation d'AGn3-LC sous la forme d'une supplémentation ne semble pas associée au risque de mortalité de toutes causes (RR : 0,92 [IC à 95% 0,92 à 1,01]), mais à un risque plus faible de mortalité cardiovasculaire comparativement à un témoin ou à une dose plus faible d'AGn3-LC (RR : 0,94 [IC à 95% 0,88 à 0,99]) (7). La consommation d'AGn3-LC sous la forme d'un supplément ne semble pas associée au risque d'évènements cardiovasculaires (RR : 0,97 [IC à 95% 0,91 à 1,02]), indépendamment de la dose (7). De façon générale, la consommation d'AGn3-LC toutes formes confondues serait associée à une diminution du risque d'évènements coronariens (RR : 0,93 [IC à 95% 0,89 à 0,98]), mais pas au risque de mortalité par maladie coronarienne (RR : 0,93 [IC à 95% 0,79 à 1,09]) ni au risque d'accident vasculaire cérébral (RR : 1,06 [IC à 95% 0,96 à 1,16]) (7).

Depuis la publication des plus récentes méta-analyses sur le sujet, les résultats de deux études cliniques de grande envergure sur l'effet de la supplémentation en AGn3-LC sur le risque d'évènements cardiovasculaires ont été publiés. D'abord, l'étude ASCEND (*A Study of Cardiovascular Events in Diabetes*) menée chez 15 341 participants avec un diabète de type 2, mais sans maladie cardiovasculaire au départ, a montré qu'une supplémentation de 840 mg d'AGn3-LC (460 mg d'EPA + 380 mg de DHA) par jour n'avait pas d'effet sur le risque d'évènements cardiovasculaires comparativement à un témoin d'huile d'olive (rapport de taux : 0,97 [IC à 95% 0,87 à 1,08]) (145). La supplémentation en AGn3-LC a néanmoins diminué la mortalité par maladie coronarienne de 19 % (rapport de taux : 0,81 [IC à 95% 0,67 à 0,99]) et par maladie vasculaire de 18% (rapport de taux : 0,82 [IC à 95% 0,68 à 0,98]) comparativement au témoin (145).

La deuxième est l'étude VITAL (*The Vitamin D and Omega-3 Trial*) qui a été menée chez 25 871 participants sans maladie cardiovasculaire au départ (146). Les participants ont été assignés de façon aléatoire à 840 mg d'AGn3-LC (460 mg d'EPA + 380 mg de DHA) par jour ou à un témoin d'huile d'olive (146). La supplémentation en AGn3-LC n'a pas eu d'effet sur le risque d'évènements cardiovasculaires (HR : 0,92 [IC à 95% 0,80 à 1,06]) et sur le décès par maladie cardiovasculaire (HR : 0,96 [IC à 95% 0,76 à 1,21]) comparativement au témoin (146). Toutefois, la supplémentation en AGn3-LC a diminué le risque d'infarctus du myocarde de 28%(HR : 0,72

[IC à 95% 0,59 à 0,90]), le risque de revascularisation coronarienne de 22% (HR : 0,78 [IC à 95% 0,63 à 0,95]) et le risque de décès suite à un infarctus du myocarde de 50% (HR : 0,50 [IC à 95% 0,26 à 0,97]) comparativement au témoin (146). Ces deux études montrent qu'à faible dose (< 1 g par jour), la supplémentation en AGn3-LC pourrait surtout avoir des effets protecteurs sur les évènements coronariens en prévention primaire. Il est important de considérer que ces catégories d'évènements ne représentent pas les issues principales pour lesquelles ces deux études ont été élaborées. Néanmoins, ces résultats vont dans le sens des études précédentes qui ont montré qu'une supplémentation en AGn3-LC pourrait prévenir les évènements coronariens.

Actuellement, deux études cliniques ont observé l'effet d'une supplémentation en EPA sur le risque d'évènements cardiovasculaires et/ou de mortalité. La première est la *Japan EPA Lipid Intervention Study* (JELIS) (147) et la deuxième est l'étude *Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial* (REDUCE-IT) (13). Aucune étude n'a encore documenté l'effet d'une supplémentation en DHA sur le risque d'évènements cardiovasculaires ou de mortalité jusqu'à maintenant. L'étude JELIS est une étude randomisée contrôlée dont l'objectif était d'observer l'effet de l'ajout de 1,8 g d'EPA par jour (esters d'éthyle, 6 x 300 mg, pureté de >98%) à un traitement de statine en comparaison avec un traitement de statine seulement chez 18 645 sujets avec une hypercholestérolémie (147). L'ajout d'EPA a été associé à une diminution de 19% du risque d'évènements coronariens totaux en comparaison avec l'utilisation d'une statine seule dans cette étude (HR : 0,81 [IC à 95% 0,69 à 0,95]) (147). L'ajout d'EPA n'a pas eu d'effet sur le risque de mortalité par maladie coronarienne (HR : 0,94 [IC à 95% 0,57 à 1,56]) ni de mortalité de toutes causes (HR : 1,09 [IC à 95% 0,92 à 1,28]) en comparaison avec la statine seule (147). La réduction du risque d'évènements coronariens totaux semble donc en grande partie due à la diminution du risque d'évènements coronariens n'ayant pas causé la mort (147). Les auteurs ont également observé que l'effet bénéfique de l'EPA additionné au traitement de statine était plus important en prévention secondaire qu'en prévention primaire (147). La diminution de la concentration du C-LDL était similaire dans les deux groupes à l'étude (-25%) alors que la concentration de triglycérides a diminué de façon plus importante dans le groupe EPA comparativement au groupe témoin (Δ9% et 4% respectivement, P<0,0001 entre les groupes) (147). Cette observation suggère que la diminution du risque d'évènements cardiovasculaires dans le groupe EPA est indépendante de la diminution des concentrations de C-LDL. Il est important de noter que cette étude a été menée chez des Japonais dont la consommation de poisson peut être jusqu'à cinq fois plus élevée qu'en Amérique du Nord et chez qui la maladie coronarienne est plutôt rare (147, 148). Ces résultats ne peuvent donc pas être généralisés aux Canadiens.

L'étude REDUCE-IT est une étude randomisée contrôlée et à double insu dont l'objectif était d'observer l'effet d'une supplémentation de 4 g par jour d'EPA (esters d'éthyle, 2 x 2 g, pureté de >98%) en comparaison avec un témoin d'huile minérale chez plus de 8000 participants (5785 en prévention secondaire et 2394 en prévention

primaire) avec des concentrations sanguines de triglycérides élevées et traités avec une statine (13). Cette étude a permis d'observer une diminution du risque d'évènements cardiovasculaires de 25% (HR : 0,75 [IC à 95% 0,68 à 0,83]) et une diminution du risque de décès par maladie cardiovasculaire de 20% dans le groupe EPA (HR : 0,80 [IC 95% 0,66 à 0,98]) comparativement au groupe témoin (13). Le risque de décès de toutes causes était similaire entre les deux groupes à l'étude (HR : 0,87 [IC 95% 0,74 à 1,02]) (13). De façon similaire à ce qui a été observé dans l'étude JELIS, l'effet bénéfique de la supplémentation en EPA était plus important en prévention secondaire qu'en prévention primaire dans cette étude (13). Les auteurs ont observé une plus grande diminution de la concentration sanguine de triglycérides dans le groupe EPA comparativement au groupe témoin (-18,3% et +2,2% respectivement ; P<0,001 entre les groupes) (13). Ils ont également noté une légère augmentation de la concentration de C-LDL (+3,1%) dans le groupe EPA (13). Cette augmentation était toutefois plus faible que celle observée dans le groupe témoin (+10,2% ; P<0.001 entre les groupes) (13). La diminution du risque cardiovasculaire n'était pas associée aux changements dans les concentrations sanguines de triglycérides dans le groupe EPA (13). Cette observation supporte l'hypothèse qu'en plus de moduler les lipides sanguins, les AGn3-LC exercent des effets bénéfiques sur la santé cardiovasculaire par d'autres mécanismes.

Plusieurs raisons permettent d'expliquer la disparité entre les résultats ou l'effet neutre des AGn3-LC sur le risque d'évènements cardiovasculaires qui a été rapporté dans les études menées jusqu'à maintenant. Parmi celles-ci se trouvent entre autres la dose et le type d'AGn3-LC. Les résultats des études JELIS et REDUCE-IT suggèrent que l'EPA et le DHA pourraient avoir des effets différents sur le risque d'évènements cardiovasculaires et de mortalité. De plus, il est important de considérer que lorsqu'ils sont administrés ensemble, les doses respectives d'EPA et de DHA sont habituellement plus faibles que lorsqu'ils sont administrés seuls, ce qui peut atténuer leurs effets. Enfin, la variabilité dans la réponse aux AGn3-LC pourrait également influencer les résultats observés dans les études cliniques qui ont été menées jusqu'à maintenant. La variabilité dans la réponse au traitement est un concept reconnu en pharmacologie (149) et de plus en plus rapporté dans la réponse à l'activité physique (150). Quelques équipes de recherche se sont intéressées à la variabilité dans la réponse aux AGn3-LC jusqu'à maintenant.

2.3.3 Variabilité dans la réponse des triglycérides et du C-LDL à une supplémentation en acides gras oméga-3

Les AGn3-LC sont reconnus pour réduire les concentrations sanguines de triglycérides. Ils pourraient aussi, dans certains cas, augmenter les concentrations de C-LDL. Or, la réponse des triglycérides et du C-LDL à une supplémentation en AGn3-LC peut varier de façon importante d'un individu à l'autre. La figure 2.4 illustre la variabilité interindividuelle dans la réponse des concentrations sanguines de triglycérides et de C-LDL à une supplémentation de 3 g d'EPA + DHA par jour durant six semaines chez 55 hommes en santé avec des concentrations de C-LDL et de triglycérides élevées (151). Malgré une diminution moyenne observée de 35%

dans les concentrations de triglycérides, les auteurs ont observé une variation interindividuelle allant de -114% à +62% (152). Un phénomène similaire a été observé pour la réponse du C-LDL avec une augmentation moyenne de 7%, mais une variabilité interindividuelle allant de -49% à +87% (152).

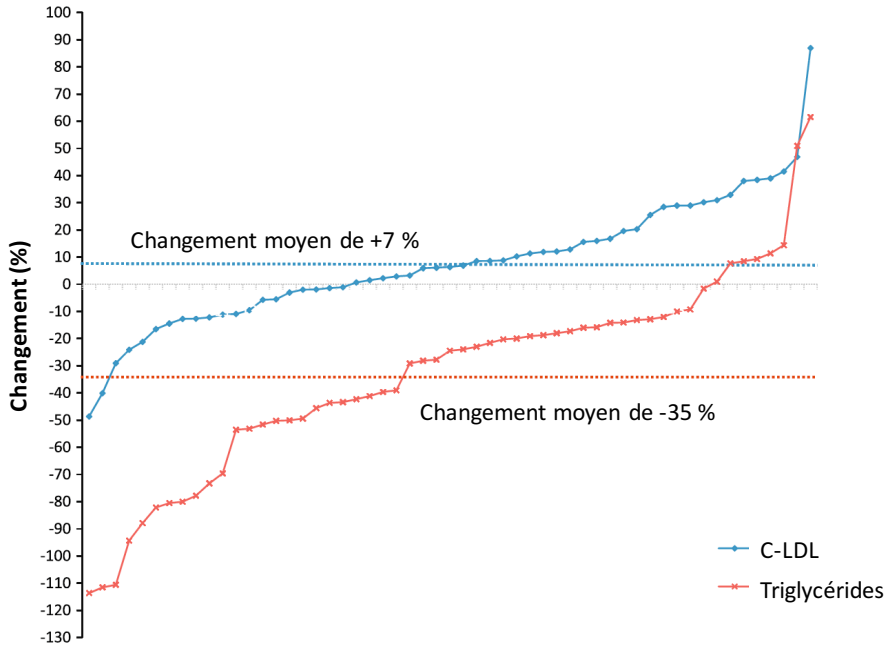

Figure 2.4 Changement (%) dans les concentrations plasmatiques de C-LDL (bleu) et de triglycérides (rouge) en réponse à une supplémentation de 3 g par jour d'EPA + DHA durant six semaines chez 55 hommes

Tirée de (152). Chaque point représente un participant et la réponse est triée pour le C-LDL et les triglycérides afin d'illustrer la variabilité interindividuelle pour chaque marqueur. La ligne pointillée représente le changement moyen pour chaque marqueur.

Une variabilité similaire dans la réponse des triglycérides à une supplémentation en AGn3-LC a été observée dans deux autres études, soit l'étude *Fish Oil Intervention and Genotype* (FINGEN) et l'étude *Fatty Acid Sensor* (FAS) (16). L'étude FAS a été menée afin d'identifier les déterminants de la réponse des triglycérides à six semaines de supplémentation avec 3 g par jour d'AGn3-LC (1,9 à 2,2 g d'EPA + 1,1 g de DHA) chez 208 individus en santé (16, 17). Les auteurs ont observé une diminution moyenne de la concentration de triglycérides d'environ 16% suite à la supplémentation en AGn3-LC en comparaison avec les valeurs de triglycérides au départ (17). Ils ont également observé une augmentation des concentrations de triglycérides chez 28,8% des participants (16). L'étude FINGEN a été menée afin d'identifier les déterminants dans la réponse des triglycérides à une supplémentation en AGn3-LC (0,7 et 1,8 g d'EPA + DHA par jour) durant huit semaines chez 312 individus en santé (15). Les auteurs ont rapporté une augmentation des concentrations de triglycérides sanguins chez 31% des participants malgré une diminution moyenne d'environ 11% (14). Au meilleur de nos connaissances, aucune étude n'a observé la variabilité inter- et intra- individuelle à l'EPA et au DHA

lorsqu'administrés séparément. Certains déterminants de la réponse des triglycérides et du C-LDL à une supplémentation en AGn3-LC ont été identifiés jusqu'à maintenant.

Déterminants de la réponse des triglycérides et du C-LDL à une supplémentation en acides gras oméga-3

Des variations génétiques dans les gènes impliqués dans le métabolisme des lipides ont été associées à la variabilité dans la réponse des triglycérides à une supplémentation en AGn3-LC. Des variations génétiques dans les gènes du *PPAR-*α (153, 154), du *glycérol-3-phosphate acyltransférase mitochondrial 3 et 4* (*GPAM, GPAT3* et *GPAT4*) (155), de la *fatty acid desaturase 1* (*FADS1*, qui code pour la Δ5-désaturase) (16) et des *phospholipases A2* (*PLA2*) (156) pourraient être en partie responsables de la variabilité dans la réponse des triglycérides à une supplémentation en AGn3-LC. En effet, selon un score de risque génétique calculé à partir des données de l'étude FAS, les variations génétiques permettraient d'expliquer près de 50% de la variance dans la réponse des triglycérides à une supplémentation en AGn3-LC dans un modèle ajusté pour l'âge, le sexe et l'indice de masse corporelle (157). De façon intéressante, ce score de risque génétique a également montré que l'âge, le sexe et l'indice de masse corporelle permettaient d'expliquer 0,04%, 0% et 0,21% de la variabilité dans la réponse des triglycérides respectivement (données non publiées de (157)). De plus, ni l'âge, le sexe, l'indice de masse corporelle et le génotype de l'apo E ont été identifiés comme des déterminants de la réponse des triglycérides à une supplémentation en AGn3-LC dans l'étude FAS (17). De façon similaire, le génotype de l'apo E ne semblait pas non plus associé à la réponse des triglycérides suite à une supplémentation de 3,7 g par jour de DHA + 3,3 g par jour d'EPA chez 38 hommes en santé (158). Rappelons que l'apo E des VLDL permet entre autres leur clairance au foie (27). La concentration sanguine de triglycérides avant l'intervention a été identifiée comme un déterminant important de la réponse des triglycérides aux AGn3-LC (159).

En ce qui concerne la réponse du C-LDL, le DHA (3,7 g par jour durant quatre semaines), mais pas l'EPA (3,3 g), augmenterait les concentrations sanguines de cholestérol total et de C-LDL chez les individus avec le génotype de l'apo E4 comparativement au génotype de l'apo E3 et E2 (151, 158). Le génotype E4 semble également associé à la production de VLDL de petite taille en réponse à une supplémentation en DHA (14). D'un autre côté, aucune interaction entre le génotype de l'apo E et l'effet d'une supplémentation de 0,7 à 1,8 g d'AGn3-LC par jour n'a été observée dans l'étude FINGEN (15). Des différences en ce qui concerne la dose d'EPA et de DHA et les caractéristiques des participants à l'étude peuvent en partie expliquer les différences dans les résultats de ces études.

Jusqu'à maintenant, certaines caractéristiques génotypiques et phénotypiques permettent en partie de prédire la réponse des triglycérides et du C-LDL à une supplémentation en AGn3-LC. Au meilleur de nos connaissances, aucune étude n'a été menée afin d'identifier les déterminants de la réponse inter- et intra- individuelle des

concentrations de triglycérides et de C-LDL à l'EPA et au DHA lorsqu'administrés individuellement. Puisque la variabilité dans la réponse à l'EPA et au DHA peut en partie influencer l'effet d'une supplémentation en AGn3-LC sur le risque cardiovasculaire, des études supplémentaires sont nécessaires afin de mieux caractériser la réponse à l'EPA et au DHA, ainsi que pour identifier les différents types de répondeurs.

2.3.4 Supplémentation en acides gras oméga-3 chez la population à risque d'évènements cardiovasculaires

Enfin, considérant les effets des AGn3-LC sur le risque d'évènements coronariens, l'AHA recommande un supplément de ≥ 1g par jour en AGn3-LC en prévention secondaire (tableau 2.5). Chez les individus qui présentent des concentrations de triglycérides élevées (2,3 à 5,6 mmol/L ou 205 à 500 mg/dL) ou très élevées (≥ 5,6 mmol/L ou 500 mg/dL), une supplémentation entre 2 à 4 g d'AGn3-LC par jour est recommandée dans le but de prévenir un évènement cardiovasculaire ou une pancréatite.

Tableau 2.5 Recommandations en suppléments d'acides gras oméga-3 polyinsaturés à longue chaîne selon l'AHA

Indication	Recommandation
Pour ↓ le risque de mortalité par maladie cardiovasculaire chez ceux qui ont une maladie cardiovasculaire connue	≥ 1g EPA+DHA par jour
Pour ↓ le risque de mortalité par maladie cardiovasculaire et de réhospitalisation pour un évènement cardiovasculaire après un arrêt cardiaque	≥ 1g EPA+DHA par jour
Pour ↓ les concentrations de triglycérides dans le sang (surtout pour la prévention de pancréatite si des concentrations ≥ 5,6 mmol/L ou 500 mg/dL)	2 à 4 g EPA+DHA par jour

À partir de (118). Traduction libre.

En ce qui concerne les effets secondaires potentiels, la supplémentation en AGn3-LC n'est généralement pas associée au risque de complications de type hémorragique (RR : 1,06 [IC à 95% 0,78 à 1,41]) et aux problèmes gastro-intestinaux nécessitant une hospitalisation (RR : 1,75 [IC à 95% 0,53 à 5,79]) (7). Concernant les effets secondaires possibles, les suppléments d'AGn3-LC peuvent augmenter le risque de présenter de la nausée (RR : 1,76 [IC à 95% 1,25 à 2,48]) (7). Les données suggèrent que les suppléments d'AGn3-LC peuvent aussi entraîner des douleurs abdominales (RR : 1,10 [IC à 95% 1,25 à 2,48]) et de la diarrhée (RR : 1,15 (IC à 95% 0,92 à 1,43]) (7). Néanmoins, la supplémentation en AGn3-LC est habituellement considérée comme un traitement sécuritaire qui entraîne peu d'effets secondaires importants.

Chapitre 3 Objectifs et hypothèses

L'effet d'une supplémentation en AGn3-LC sur le risque cardiovasculaire et les mécanismes sous-jacents aux changements dans les facteurs de risque ne sont pas encore totalement compris. Plusieurs facteurs peuvent influencer la réponse à une supplémentation en AGn3-LC. D'abord, de plus en plus d'études tendent à montrer que l'EPA et le DHA exercent des effets différents sur plusieurs facteurs de risque cardiovasculaire. Ensuite, la dose d'EPA et de DHA est un déterminant important de la réponse à la supplémentation en AGn3-LC. Enfin, une variabilité interindividuelle considérable dans la réponse des triglycérides et du C-LDL a été rapportée en réponse à une supplémentation en AGn3-LC. Toutefois, la variabilité inter- et intra- individuelle à l'EPA et au DHA, ainsi que les déterminants de la réponse des triglycérides et du C-LDL sont jusqu'à maintenant inconnus.

L'**objectif général** de ce doctorat était de comparer les effets de l'EPA et du DHA sur le risque cardiométabolique. Plus spécifiquement, nous avons comparé l'effet de l'EPA et du DHA sur les lipides sanguins, les biomarqueurs inflammatoires, le métabolisme des lipides, l'expression des gènes impliqués dans le métabolisme des lipides, la variabilité inter- et intra- individuelle dans la réponse des triglycérides et du C-LDL, ainsi que les déterminants de la réponse aux AGn3-LC.

L'objectif de l'article présenté au **chapitre 4** était de comparer les effets de l'EPA et du DHA sur les marqueurs inflammatoires et les lipides sanguins. L'hypothèse était que 2,7 g par jour de DHA a un effet plus important sur les marqueurs inflammatoires et les lipides sanguins que 2,7 g par jour d'EPA. Nous avions également émis l'hypothèse que l'EPA induit une réponse plus importante chez les hommes alors que le DHA induit une réponse plus importante chez les femmes.

L'objectif de l'article présenté au **chapitre 5** était de comparer les effets de l'EPA et du DHA sur l'Indice Oméga-3. L'hypothèse était que le DHA augmente l'Indice Oméga-3 de façon plus importante que l'EPA.

L'objectif de l'article présenté au **chapitre 6** était de comparer les effets de l'EPA et du DHA sur le métabolisme de l'apo B100 et de l'apo CIII, sur la taille des LDL et sur les concentrations sanguines de PCSK9. L'hypothèse était que le DHA induit des changements favorables sur la taille des LDL comparativement à l'EPA, et que l'EPA et le DHA ont des effets différents sur le métabolisme de l'apo B100 et de l'apo CIII.

L'objectif de l'article présenté au **chapitre 7** était de comparer la réponse des triglycérides sanguins à une supplémentation en EPA et en DHA, ainsi que les prédicteurs de cette réponse. L'hypothèse était que le DHA diminue les triglycérides chez un plus grand nombre d'individus que l'EPA. Nous avions également émis l'hypothèse que les individus présentant une diminution plus importante de triglycérides sont ceux avec des concentrations de triglycérides plus élevées au départ.

L'objectif de l'article présenté au **chapitre 8** était de comparer la réponse du C-LDL sanguin à une supplémentation en EPA et en DHA, ainsi que les prédicteurs de cette réponse. L'hypothèse était que le DHA augmente les concentrations de C-LDL chez une plus grande proportion d'individus que l'EPA. Nous avions également émis l'hypothèse que les individus présentant une augmentation plus importante des concentrations de C-LDL sont ceux avec des concentrations plus élevées de C-LDL au départ.

Chapitre 4 Comparaison de l'effet de l'EPA et du DHA sur les lipides sanguins et les marqueurs inflammatoires : une étude clinique randomisée, en chassé-croisé et à double insu

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L'article présenté dans ce chapitre s'intitule: A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study.

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Résumé

Jusqu'à maintenant, la majorité des études qui ont observé l'effet des acides gras polyinsaturés oméga-3 à longue chaîne chez les humains ont utilisé des suppléments qui combinent les acides eicosapentaénoïque (EPA) et docosahexaénoïque (DHA) en différentes formes et proportions. L'objectif de cette étude était de comparer les effets de l'EPA et du DHA (sous la forme de triglycérides réestérifiés ; pureté à 90%) sur les marqueurs inflammatoires (objectif principal) et les lipides sanguins (objectif secondaire) chez des hommes et des femmes à risque cardiovasculaire. Un devis randomisé contrôlé en chassé-croisé et à double insu a été utilisé pour répondre à ces objectifs. Un total de 48 hommes et 106 femmes ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Chaque phase expérimentale était d'une durée de 10 semaines et les phases étaient séparées par une période libre de 9 semaines. Comparativement à l'EPA, la supplémentation en DHA a mené à une diminution plus importante d'interleukine (IL) 18 (-7,0% ± 2,8% vs -0,5% ± 3,0%, respectivement ; P = 0,01) et à une augmentation plus importante d'adiponectine (3,1% ± 1,6% vs -1,2% ± 1,7%, respectivement ; P < 0,001). L'effet du DHA et de l'EPA sur la CRP (-7,9% ± 5,0% vs -1,8% ± 6,5%, respectivement ; P = 0,25), l'IL-6 (-12,0% ± 7,0% vs -13,4% ± 7,0%, respectivement ; P = 0,86) et le facteur de nécrose tumorale alpha (-14,8% ± 5,1% vs -7,6% ± 10,2%, respectivement ; P = 0,63) était similaire. Comparativement à l'EPA, le DHA a mené à une diminution plus importante des triglycérides (-13,3% ± 2,3% vs -11,9% ± 2,2%, respectivement ; P = 0,005) et du ratio cholestérol total sur le cholestérol dans les HDL (-2,5% ± 1,3% vs 0,3% ± 1,1%, respectivement ; P = 0,006) et une augmentation plus importante du cholestérol dans les HDL (7,6% ± 1,4% vs -0,7% ± 1,1%, respectivement ; P < 0,0001) et du cholestérol dans les LDL (6,9% ± 1,8% vs 2,2% ± 1,6%, respectivement ; P = 0,04). L'augmentation du cholestérol dans les LDL avec le DHA comparativement à EPA était plus importante chez les hommes que chez les femmes (P interaction traitement × sexe = 0.046). Cette étude a montré que le DHA avait des effets plus importants sur plusieurs facteurs de risque cardiovasculaire que l'EPA. Des études supplémentaires sont nécessaires afin de comparer si ces différences influencent le risque d'évènements cardiovasculaires à long terme.

Abstract

Background: To date, most studies on the anti-inflammatory effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans have used a mixture of the 2 fatty acids in various forms and proportions. Objectives: We compared the effects of EPA supplementation with those of DHA supplementation (re-esterified triacylglycerol; 90% pure) on inflammation markers (primary outcome) and blood lipids (secondary outcome) in men and women at risk of cardiovascular disease. Design: In a double-blind, randomized, crossover, controlled study, healthy men (n = 48) and women (n = 106) with abdominal obesity and low-grade systemic inflammation consumed 3 g/d of the following supplements for periods of 10 wk: 1) EPA (2.7 g/d), 2) DHA (2.7 g/d), and 3) corn oil as a control with each supplementation separated by a 9-wk washout period. Primary analyses assessed the difference in cardiometabolic outcomes between EPA and DHA. Results: Supplementation with DHA compared with supplementation with EPA led to a greater reduction in interleukin-18 (IL-18) (-7.0% ± 2.8% compared with -0.5% ± 3.0%, respectively; P = 0.01) and a greater increase in adiponectin (3.1% ± 1.6% compared with -1.2% ± 1.7%, respectively; P < 0.001). Between DHA and EPA, changes in CRP (-7.9% ± 5.0% compared with -1.8% ± 6.5%, respectively; P = 0.25), IL-6 (-12.0% ± 7.0% compared with -13.4% ± 7.0%, respectively; P = 0.86), and tumor necrosis factor-α (-14.8% ± 5.1% compared with -7.6% ± 10.2%, respectively; P = 0.63) were NS. DHA compared with EPA led to more pronounced reductions in triglycerides (-13.3% ± 2.3% compared with -11.9% ± 2.2%, respectively; P = 0.005) and the cholesterol:HDL-cholesterol ratio (-2.5% ± 1.3% compared with 0.3% ± 1.1%, respectively; P = 0.006) and greater increases in HDL cholesterol (7.6% ± 1.4% compared with -0.7% ± 1.1%, respectively; P < 0.0001) and LDL cholesterol (6.9% ± 1.8% compared with 2.2% ± 1.6%, respectively; P = 0.04). The increase in LDL-cholesterol concentrations for DHA compared with EPA was significant in men but not in women (P-treatment × sex interaction = 0.046). Conclusions: DHA is more effective than EPA in modulating specific markers of inflammation as well as blood lipids. Additional studies are needed to determine the effect of a long-term DHA supplementation per se on cardiovascular disease risk.

Title page

A randomized crossover head-to-head comparison of EPA and DHA supplementation to reduce systemic inflammation in men and women: the ComparED Study

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Short title: EPA, DHA and inflammation in men and women

Abbreviations: Apo B: apolipoprotein B; CHD: coronary heart disease; C: cholesterol; CRP: C-reactive protein; CVD: cardiovascular disease; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FSH: follicle-stimulating hormone; HDL: high-density lipoprotein; IL: interleukin; INAF: Institute of Nutrition and Functional Foods; ITT: intent-to-treat; LCn-3PUFA: long-chain omega-3 polyunsaturated fatty acids; LDL: low-density lipoprotein; MetS: metabolic syndrome; NFκB: nuclear factor κB; RCT: randomized controlled trial; TG: triglycerides; TNF: tumor necrosis factor.

Clinical Trial Registry number and website: http://www.clinicaltrials.gov (NCT01810003).

Introduction

Subclinical inflammation is now recognized as a key etiological factor in the development of atherosclerosis leading to cardiovascular disease (CVD) (1, 2). There is a growing body of literature suggesting that long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), primarily eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), may attenuate the pro-inflammatory state associated with obesity and metabolic syndrome (MetS) (3). In that regard, a number of mechanisms supporting the purported anti-inflammatory effects of LCn-3PUFA have been proposed. These include inhibition of the pro-inflammatory nuclear factor κB (NFκB) in various tissues through a series of metabolic cascades involving activation of peroxisome proliferator activated receptor (PPAR)-γ and several other signaling proteins (4).

A recent meta-analysis of randomized controlled trials (RCT) substantiated the anti-inflammatory effect of LCn-3PUFA supplementation, as evidenced by significant reductions in plasma C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentrations (5). This analysis was based on data from 68 RCTs and 4601 individuals with or without chronic non-auto-immune diseases such as dyslipidemia, obesity, type 2 diabetes and MetS. The meta-analysis revealed significant research gaps pertaining to the effects of LCn-3PUFA on inflammation. Most importantly, almost all of the RCTs available so far have either used a mix of EPA and DHA in various ratios or have investigated only one of the two LCn-3PUFA. This is not a trivial issue considering that DHA appears to be more potent than EPA in modulating plasma lipid levels (6). Whether EPA and DHA have similar or different effects on markers of inflammation remains unknown. Other significant shortcomings include the fact that almost all of the available studies to date were not designed to investigate the impact of EPA and/or DHA on inflammation as a primary outcome and were also based on sample sizes that may have been to small to yield robust results. Finally, whether the efficacy of EPA and DHA to modulate inflammation is influenced by sex remains speculative. Addressing these gaps has important public health implications, considering that EPA+DHA supplements are broadly and indiscriminately recommended in American Heart Association guidelines for the management of triglycerides (TG) for coronary heart disease risk prevention (7).

The *Comparing EPA to DHA* (ComparED) Study is a double blind, randomized, crossover, controlled study designed to compare the anti-inflammatory effects of EPA and DHA in individuals with abdominal obesity and subclinical inflammation. As a secondary objective, we compared the impact of EPA and DHA on plasma lipids as well as verified if the response to EPA and DHA is similar in men and women. We hypothesized that DHA is more potent than EPA in modulating inflammatory markers and plasma lipid concentrations. Based on evidence suggesting that platelet aggregation is more responsive to EPA in men and to DHA in women (8), we also hypothesized that EPA supplementation induces a greater anti-inflammatory response than DHA in men, while women are more responsive to supplementation with DHA.

Subjects and Methods

Study design: This study used a double-blind randomized, controlled crossover design with three treatment phases: 1- EPA, 2- DHA, 3- control. Each treatment phase had a median duration of 10 weeks. The median washout time between treatments was 9 weeks. Participants were randomized to one of six treatment sequences stratified by sex using an in-house computer program. Allocations to treatments were concealed to participants as well as study coordinators and lab technicians throughout the study. Codes were unconcealed after all primary statistical analyses were completed. Participants were supplemented with three identical 1 g capsules of >90% fish oils per day providing: 2.7 g/d EPA, 2.7 g/d DHA and 0 g/d EPA+DHA (3 g of corn oil control). Supplements were provided by Douglas Laboratories as re-esterified triacylglycerol. Participants were instructed to maintain a constant body weight during the course of the study. They were also counselled on how to exclude fatty fish meals (including salmon, tuna, mackerel, and herring), fish-oil supplements, flax products, walnuts, and omega-3–enriched products during the study. Vitamin supplements and natural health products were allowed at a stable dose. Alcohol consumption was permitted during the study with intakes not exceeding one or two serving(s) (12-15 g alcohol) per day, but was forbidden over the four days that preceded blood draws. Subjects were also instructed to maintain their usual physical activity except for the four days that preceded blood sampling at the various stages of the study, during which they were asked not to engage in any form of vigorous physical activity.

Study population: The *a priori* defined eligibility criterion was to have MetS as per the IDF definition (9). However, this criterion was modified two months into recruitment, due to unforeseen difficulties in achieving the intended sample size using such criteria (eligibility rate 2.4% on the basis of 170 screens). The eligibility criteria were modified to having abdominal obesity using the IDF sex specific cut-offs ($\geq$80 cm for women, $\geq$94 cm for men) (9), in combination with a screening plasma CRP concentration >1 mg/L but <10 mg/L. Subjects had to be healthy otherwise. These new criteria were consistent with the primary aim of the study, which was to compare the impact of EPA and DHA supplementation on markers of inflammation. Subjects were recruited at the Institute of nutrition and functional foods (INAF) via the media (newspaper, radio) and electronic newsletters. Subjects had to be aged between 18 and 70 years and have stable body weight for at least three months prior to randomization. Among pre-menopausal women, only those with a regular menstrual cycle for the last three months (25-35 days) were included. Follicle-stimulating hormone (FSH) measurement was performed when needed to confirm the premenopausal status (FSH <25 IU/L) (10). Women using contraceptive agents were eligible. Use of contraceptive agents was documented and adjusted for if required (see below). Evidence suggests that phases of the menstrual cycle have little effects on markers of inflammation (11) and therefore collections of samples were not adjusted to menstrual cycle. Exclusion criteria were plasma CRP >10 mg/L at screening, extreme dyslipidemias such as familial hypercholesterolemia, having a personal history of CVD (coronary heart disease (CHD), cerebrovascular disease or peripheral arterial disease), taking medications or

substances known to affect inflammation (e.g. steroids, binging alcohol), and use of LCn-3PUFA supplements within two months of study onset. Postmenopausal women using hormone replacement therapy at stable dose were included (12). All participants signed an informed consent document approved by local Ethics Committees at the beginning of the study and the study protocol was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003).

Anthropometry: Anthropometric measures including waist and hip circumferences were obtained according to standardized procedures (13).

Risk factor assessment: Plasma CRP concentrations were measured using the Behring Latex-Enhanced highly sensitive assay on the Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer as described previously (14). Other pro- and anti-inflammation markers were measured using commercial ELISA kits for the human form of the cytokine: IL-6, TNF-α (R&D Systems #HS600B and #HSTA00D, Minneapolis, MN), IL-18 (MBL International #7620, Woburn, MA) and adiponectin (B-Bridge International #K1001-1, Santa Clara, CA). Serum total cholesterol (C), TG and high-density lipoprotein (HDL)-C were assessed on a Roche/Hitachi Modular (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's specifications and using proprietary reagents. Plasma low-density lipoprotein (LDL-C) concentrations were calculated using the Friedewald Equation. Total plasma apolipoprotein B (apo B) concentrations were measures using the Alerchek (#A70102, Springvale, ME) commercial ELISA kit. Coefficients of variations for each analyte are shown in Supplemental Table 1. Total C, LDL-C, HDL-C, TG, apo B, CRP, IL-6 and adiponectin were measured twice on consecutive days at the end of each treatment to reduce variation and hence increase statistical power. The mean of the two measurements were used in the analyses. Treatment-specific baseline values were measured once. All personnel involved in the measurements of the study outcomes were blinded to treatments.

Compliance: Compliance to supplementation was assessed by counting supplements that were returned to study coordinators. Subjects on any given treatment with a compliance <80% were considered as non-compliant and therefore excluded from analyses. EPA and DHA content in plasma phospholipids was also used as another proxy of compliance in a randomly selected subsample of participants (n=30, 15 women and 15 men). The EPA and DHA content of plasma phospholipids were measured before and after treatments as described previously (15). The fatty acid composition of plasma phospholipids was expressed as a percentage of all fatty acids. Concurrent use of medication during the experimental protocol was tracked using checklists. Participants were asked to notify the physician in charge of the clinical aspects of the study before initiating any medication. Variations in dietary habits during the intervention was monitored using a validated quantitative web-based food

frequency questionnaire (web-FFQ) at the end of each treatment phase (16). Usual physical activity was monitored using a 3-day validated physical activity journal (17).

Sample size calculation: A priori sample size calculations indicated that n=150 would allow us to detect a minimal difference of 10% in plasma CRP concentrations when comparing any two treatments with a power of 81% and $p<0.01$ (two-tailed) (18). CRP was used as the primary outcome measure for sample size calculations because it is considered a key variable for the assessment of the inflammatory status in clinical practice (19). A 10% reduction in plasma CRP was considered to be of clinical relevance based on several epidemiological studies having shown a linear relationship between CRP and risk of CHD (19). Power to detect a significant treatment*sex interaction was estimated using the GLMPower procedure in SAS with treatment, sex and treatment*sex as main effects. Based on standard deviation (SD) estimates (35 %) and considering a sample size of 150 equally distributed evenly between men and women, the power was 80% to detect a significant treatment*sex interaction ($p<0.05$) for a reduction in plasma CRP vs. control that is 10% or greater with treatment "A" (no change with treatment "B") in men and 10% or greater change with treatment "B" (no change with treatment "A) in women. The minimal detectable difference between treatments in plasma CRP within each sex was 11.5% (power=80%, $p<0.05$ two-tailed, n=75 in each group).

Statistical analyses: Differences in study outcomes between treatments were assessed using the MIXED procedure for repeated measures in SAS (v9.4, Cary, NC), with treatment, sex and treatment*sex interaction (when significant) as fixed effects and subject as the random effect with covariance matrix. In the case of the CRP analysis per se, missing values were attributed when the mean of the two consecutive post-treatment CRP values was >10 mg/L. The change vs. the control treatment (post treatment EPA minus control and DHA minus control) was used as the dependent variable in all analyses as per the a priori defined analytical plan (20). The comparison of the change vs. control treatment (post treatment EPA minus control vs. DHA minus control) was calculated by the MIXED procedure and reflects the comparison between EPA and DHA effects directly. Specific treatment effect was calculated by testing change vs. the control treatment (post treatment EPA minus control and DHA minus control) to the null hypothesis by the LSMEANS statement in the MIXED procedure. Potential confounders of the outcome measure response to treatment, mainly obesity/body fat status, age, use of contraceptive agents (premenopausal women), menopausal status, energy and nutrient intake and sequence of treatments were considered by integrating interaction terms with the main treatment effect into the MIXED models. Results from analyses based on the most parsimonious models (i.e. retaining only the variables that contributed significantly to variations in any given study outcome) are shown. All primary statistical analyses were undertaken in a blinded fashion, using study codes for each treatment. The skewness in the distribution of all study outcomes was considered and data were log-transformed when required. Since the MIXED procedure is robust to missing data, primary analyses were conducted using a MIXED modeling approach without multiple

imputation for missing data (21). In secondary analyses, data were analysed using an intent-to-treat (ITT) approach, with multiple imputation of missing data (10% of all missing values). Using the MI procedure in SAS, we imputed missing variables values 10 times, thereby creating 10 imputed datasets. Statistical modeling from the 10 imputed database was conducted using the MINIALIZE procedure. Comparisons between treatment effects vs. control (significant vs. not significant) were similar for all variables in the ITT analysis, with small differences in the magnitude of the significance of the effects for IL-18 and adiponectin (slightly larger P values with the ITT approach, see Supplemental Table 2). Hence, only results based on the MIXED modeling approach are presented.

Results

Subjects' baseline characteristics

Figure 1 represents the Consolidated Standards of Reporting Trials (CONSORT) flow diagram of this study (22). The study was initiated on April 3, 2013 and was completed on June 19, 2015. Of the 173 eligible men and women, a total of 154 were randomized to treatment sequences, 138 completed at least one treatment phase and 123 completed all three phases. The characteristics at the screening visit of the 138 subjects included in the analyses are shown by sex (n=42 for men, n=96 for women) in Table 1. Sixty-three % of women were postmenopausal, of which 25% were receiving hormonotherapy. Seventy-five percent of pre-menopausal women were using contraceptive agents. As per the inclusion criteria, all subjects had a high waist circumference ($\geq$94 cm for men and $\geq$80 cm for women) and an elevated plasma CRP concentration as a group, but were healthy otherwise. There was no difference in the baseline characteristics of participants between treatments (Supplemental Table 3) indicating no residual effects of the previous supplementation phase on the next one on these outcomes. We found marginal differences concerning dietary intakes of fibers, proteins, EPA and DHA as well as a significant difference in alcohol consumption between treatments (Supplemental Table 4). Such differences in nutrient intake had no impact on study outcomes ($p>0.05$) and thus were not included in the final MIXED models.

Compliance to treatments and side effects

The mean compliance to supplementation during each treatment phase based on returned capsules was high (control: 97±5%; EPA: 97±6%; DHA: 96±5%) and not significantly different between treatments (Kruskal-Wallis test, $p=0.17$). Treatment-specific data of participants with a compliance <80% for each phase (control n=2, EPA n=1, DHA n=1) were considered as missing in the analyses. The plasma phospholipid fatty acid composition during the study is detailed in Supplemental Table 5. The phospholipid fatty acid profile tracked well with each supplementation phases, also reflecting high compliance to treatments. Changes in the plasma phospholipid concentrations of EPA, DPA and DHA after each treatment were similar between males and females, with the exception of plasma DPA levels, which were higher among males than among females after EPA supplementation (2.56±0.11% vs. 2.10±0.07%, $p=0.01$). There was no difference in the frequency of self-reported side effects between treatments (Supplemental Table 6).

There was no difference between EPA, DHA and control in post-treatment body mass index, waist circumference, % of fat and android fat (data not shown). Table 2 presents the absolute change in study outcomes after EPA and DHA vs. control while Figure 2 depicts treatment-specific changes in relative terms vs. control.

Inflammation markers

Compared with control, EPA decreased plasma IL-6 concentrations (-13.4%, Table 2, Figure 2) but had no significant effect on other inflammation markers. Compared with control, DHA supplementation significantly decreased plasma CRP (-7.9%), IL-6 (-12.0%), IL-18 (-7.0%) and TNF-α (-14.8%) and increased adiponectin concentrations (+3.1%). The reduction in plasma IL-18 and the increase in plasma adiponectin concentrations were significantly greater with DHA than with EPA (p between ΔDHA and ΔEPA of 0.002 and <0.001, respectively). There was no significant treatment*sex interaction in the response of inflammation markers to EPA and DHA.

Blood lipids

Compared with control, EPA supplementation significantly decreased plasma TG (-11.9%) and increased LDL-C concentrations (+2.2%) but had no significant impact on other lipid variables (Table 2, Figure 2). On the other hand, supplementation with DHA compared with control increased total C (+3.8%), LDL-C (+6.9%), total apo B (+4.5%) and HDL-C (+7.6%) and reduced plasma TG (-13.3%) and the C/HDL-C ratio (-2.5%). Changes vs. control in plasma LDL-C, HDL-C, TG as well as in the C/HDL-C ratio with DHA were higher in magnitude than with EPA (Table 2, Figure 2).

Finally, there was a significant treatment*sex interaction (p=0.0455) in the LDL-C response to EPA and DHA (Figure 3). While DHA supplementation significantly increased LDL-C concentrations in both men and women compared with control, this increase in LDL-C was significantly greater with DHA than with EPA among men (12.5% vs. 5.1%) but not among women (4.4% vs. 3.0%).

Discussion

To the best of our knowledge, this is the first study designed specifically to compare head-to-head the impact of EPA and DHA on inflammation markers as a primary outcome in both men and women. Data suggest that DHA is more effective than EPA in attenuating systemic inflammation and modulating plasma lipid risk factors in healthy individuals with abdominal obesity and subclinical systemic inflammation.

LCn-3PUFA and inflammation

The present study addresses key research gaps pertaining to the effect of LCn-3PUFA on inflammation markers. Previous RCTs on the topic have yielded inconsistent results due to a number of experimental and methodological factors (5, 23). First and foremost, most of the available RCTs in healthy subjects or at risk for CVD investigated the impact of LCn-3PUFA on inflammation markers as secondary outcomes, not as primary outcome. In the meta-analysis by Li *et al*. (5), only one study provided a head-to-head comparison of EPA and DHA on inflammation markers among the 68 RCTs reviewed. This study in patients with type-2 diabetes showed no significant effect of EPA or DHA supplementation (4 g/d for 6 weeks) on CRP, IL-6 and TNF-α concentrations but was based on a sample of only 25 patients per group (24). Two more recent studies have compared EPA and DHA directly (<2 g/d), showing no significant impact on CRP and pro-inflammatory cytokines (25, 26). These parallel arm studies comprised fewer than 20 subjects per group and were therefore also clearly underpowered to yield robust results. This emphasizes that studies so far, in almost all cases, were not adequately designed to investigate inflammation specifically.

In that context, results from this large RCT provide novel and meaningful information. Supplementation with EPA (2.7 g/d) for 10 weeks decreased plasma IL-6 concentrations but had little effect on other inflammation markers compared with control. On the other hand, supplementation with DHA (2.7 g/d) for 10 weeks decreased plasma CRP, IL-6, IL-18 and TNF-α concentrations and increased adiponectin concentrations compared with control. Variations in plasma IL-18 and adiponectin concentrations with DHA were greater than with EPA. Also of note, the reduction in plasma CRP concentrations with DHA was almost four-fold greater in magnitude than with EPA, although this difference did not reach statistical significance. These data confirm the indirect evidence from the meta-analysis by Li *et al.* (5), suggesting that the anti-inflammatory effects of LCn-3PUFA seen in earlier studies may have in fact been attributable to DHA. Results from a meta-analysis of 13 RCTs suggest a modest increase in plasma adiponectin concentrations with LCn-3PUFA supplementation (27), but our data indicate that this effect may be attributable more specifically to DHA.

Mendelian randomization studies have indicated that increased plasma CRP levels are unlikely to be even a modest causal factor for CVD (28). This does not rule out the importance of inflammation in the etiology of

atherosclerosis and resulting CVD (2). Data from the JUPITER trial has shown that statin-treatment of patients with low LDL-C levels but with subclinical inflammation (CRP>2 mg/L) was indeed highly effective in reducing the risk of vascular events, myocardial infarction and stroke (29). Data from the present study indicated that DHA was effective in reducing plasma concentrations of several inflammation markers (IL-6, IL-18 and TNF-α) in addition to CRP. IL-18 is expressed in human atheroma where it influences the expression of adhesion molecules, chemokines, cytokines and matric metalloproteinases. IL-6 is involved in the acute-phase response by inducing the production of CRP and other inflammatory markers in the liver. Expression of IL-6 is also stimulated in smooth muscle cells by circulating IL-18 and other cytokines (30). EPA enhances the synthesis of the E-series resolvins while DHA leads to the production of the D-series resolvins, in addition to enhancing the synthesis of protectins and maresins, all of which may have different anti-inflammatory properties. While relatively well characterized in cell and animal models (31), the potentially distinct contributions of resolvins from EPA and protectins/maresins from DHA on inflammation have not yet been well characterized *in vivo* in humans (4, 23).

While a supplementation with a mixture of EPA and DHA may activate the PPARα pathway in both sexes, the NF-κB pathway appears to be activated in men only (32), suggesting that men and women may respond differently to EPA and DHA supplementation. Our results are not consistent with a sex-specific anti-inflammatory response to EPA and DHA.

LCn-3PUFA and plasma lipids

A meta-analysis of RCTs having compared the impact of different doses of EPA and DHA (>96% pure) on blood lipids has recently been published (6). Among the 21 studies included in this meta-analysis, 10 studies compared EPA to a control, 17 studies compared DHA to a control and only six studies compared EPA and DHA directly. Results from our own study are consistent with specific analyses of these six EPA vs. DHA head-to-head comparison studies in showing significantly greater reduction in plasma TG and significantly greater increases in plasma LDL-C and HDL-C with DHA than with EPA. The fact that the LDL-C-raising effect of DHA seems to be more pronounced in men than in women deserves further investigation. Interestingly, we found that the increase in total plasma apo B after DHA supplementation was half that of LDL-C, which suggests an increase in LDL particle size with DHA as well (33). This assumption needs to be verified by proper measures of change in LDL particle size with EPA and DHA.

This study has several strengths but also some limitations that need to be outlined. This is to the best of our knowledge the largest crossover design study having compared head-to-head the impact of EPA and DHA on inflammatory markers as a primary outcome. The repeated measures after treatment reduced the intra-individual variability of the results and hence increased statistical power. The use of corn oil as a control may have blunted

the impact of EPA and DHA on some of the study outcomes. However, many previous RCTs used various vegetable oils as a control and to that extent, our study design is similar to previous studies on this topic (5). Compared to baseline values, corn oil supplementation decreased total C and LDL-C concentrations but had no effect on other markers in this study (data not shown). Whether plasma levels of EPA and DHA have returned to baseline values after the washout periods cannot be assessed since they were measured only post treatment in a subset of participants. However, concentrations of blood lipids and inflammatory markers were similar at baseline among the three treatments (Supplemental Table 3) and absence of a significant "sequence by treatment" interaction on study outcomes provide convincing evidence of no residual effects or carryover effects of a treatment on the subsequent one. The 20% dropout rate is on the high side but remains acceptable for a crossover study of a total duration of 46 weeks. Also, the number of subjects eligible for statistical analyses was lower than *a priori* anticipated but we were able to detect effects sizes that were slightly larger than what we had expected. Inflammation markers are known to be sensitive to acute immune challenges. However, sensitivity analyses excluding values higher than the 95e percentile for each risk factor had no impact on the results (not shown), making the results quite robust. The use of MIXED models vs. an ITT approach for the analysis of data from RCTs is a controversial issue. Nevertheless, both methods yielded almost identical results, supporting the robustness of the experimental data.

Data from this carefully controlled RCT indicate that DHA supplementation at a dose of approximately 3 g/d for 10 weeks has more potent anti-inflammatory effects than a similar dose of EPA in men and women at risk for MetS. These are important new data as most available studies on the anti-inflammatory effects of LCn-3PUFA have been undertaken using mixtures of various ratios of EPA and DHA. Consistent with previous studies, DHA is also more potent than EPA in modulating lipid risk factors. The extent to which such differences in the lipid-lowering and anti-inflammatory effects of EPA and DHA translate into meaningful differences in terms of cardiovascular risk prevention remains unclear and need to be investigated in the future.

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The authors' responsibilities were as follows: BL, PC and AT designed research. PC was responsible for the screening and medical supervision of the study participants. ML, AC, JM and MCL conducted the research. JA performed statistical analyses and wrote the manuscript. DT critically revised statistical analysis methods of the manuscript. BL had primary responsibility for final content. All authors critically revised the manuscript and contributed intellectually to its development, provided final approval of the submitted manuscript, had full access to all of the data in the study, took responsibility for the integrity of the data and the accuracy of the data in the analysis, affirm that the article is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

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References

1. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. Nat Rev Cardiol. 2009;6(6):399-409.

2. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009;54(23):2129-38.

3. Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis. 2008;197(1):12-24.

4. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015;1851(4):469-84.

5. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. PLoS One. 2014;9(2):e88103.

6. Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. Curr Atheroscler Rep. 2011;13(6):474-83.

7. Vannice G, Rasmussen H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. J Acad Nutr Diet. 2014;114(1):136-53.

8. Phang M, Sinclair AJ, Lincz LF, Garg ML. Gender-specific inhibition of platelet aggregation following omega-3 fatty acid supplementation. Nutr Metab Cardiovasc Dis. 2012;22(2):109-14.

9. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366(9491):1059-62.

10. Landgren BM, Collins A, Csemiczky G, Burger HG, Baksheev L, Robertson DM. Menopause transition: Annual changes in serum hormonal patterns over the menstrual cycle in women during a nine-year period prior to menopause. J Clin Endocrinol Metab. 2004;89(6):2763-9.

11. Bell HK, Bloomer RJ. Impact of serum estradiol on postprandial lipemia, oxidative stress, and inflammation across a single menstrual cycle. Gend Med. 2010;7(2):166-78.

12. Karim R, Stanczyk FZ, Hodis HN, Cushman M, Lobo RA, Hwang J, Mack WJ. Associations between markers of inflammation and physiological and pharmacological levels of circulating sex hormones in postmenopausal women. Menopause. 2010;17(4):785-90.

13. Airlie, Lohman T, Roche A, Martorel R. Standardization of anthropometric measurements. The Airlie (VA) Concensus Conference. Champaign, Ill: Human Kinetics; 1988. p. 39-80.

14. Pirro M, Bergeron J, Dagenais GR, Bernard PM, Cantin B, Despres JP, Lamarche B. Age and duration of follow-up as modulators of the risk for ischemic heart disease associated with high plasma C-reactive protein levels in men. Arch Intern Med. 2001;161(20):2474-80.

15. Kroger E, Verreault R, Carmichael PH, Lindsay J, Julien P, Dewailly E, Ayotte P, Laurin D. Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. Am J Clin Nutr. 2009;90(1):184-92.

16. Labonte ME, Cyr A, Baril-Gravel L, Royer MM, Lamarche B. Validity and reproducibility of a web-based, self-administered food frequency questionnaire. European Journal of Clinical Nutrition. 2012;66(2):166-73.

17. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. Am J Clin Nutr. 1983;37(3):461-7.

18. Richard C, Couture P, Desroches S, Lamarche B. Effect of the Mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity (Silver Spring). 2013;21(1):51-7.

19. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R, Francis GA, Poirier P, Lau DC, Grover S, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Can J Cardiol. 2013;29(2):151-67.

20. Bland JM, Altman DG. Best (but oft forgotten) practices: testing for treatment effects in randomized trials by separate analyses of changes from baseline in each group is a misleading approach. Am J Clin Nutr. 2015;102(5):991-4.

21. Press R. A mixed model approach for intent-to-treat analysis in longitudinal clinical trials with missing values. 2009.

22. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. Int J Surg. 2011;9(8):672-7.

23. Myhrstad MC, Retterstol K, Telle-Hansen VH, Ottestad I, Halvorsen B, Holven KB, Ulven SM. Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors. Inflamm Res. 2011;60(4):309-19.

24. Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, Beilin LJ. Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. Free Radic Biol Med. 2003;35(7):772-81.

25. Azizi-Soleiman F, Jazayeri S, Eghtesadi S, Rajab A, Heidari I, Vafa MR, Gohari MR. Effects of pure eicosapentaenoic and docosahexaenoic acids on oxidative stress, inflammation and body fat mass in patients with type 2 diabetes. Int J Prev Med. 2013;4(8):922-8.

26. Tsunoda F, Lamon-Fava S, Asztalos BF, Iyer LK, Richardson K, Schaefer EJ. Effects of oral eicosapentaenoic acid versus docosahexaenoic acid on human peripheral blood mononuclear cell gene expression. Atherosclerosis. 2015;241(2):400-8.

27. von Frankenberg AD, Silva FM, de Almeida JC, Piccoli V, do Nascimento FV, Sost MM, Leitao CB, Remonti LL, Umpierre D, Reis AF, et al. Effect of dietary lipids on circulating adiponectin: a systematic review with meta-analysis of randomised controlled trials. Br J Nutr. 2014;112(8):1235-50.

28. Collaboration CRPCHDG, Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, Shah T, Engert JC, Clarke R, Davey-Smith G, et al. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ. 2011;342:d548.

29. Ridker PM. The JUPITER trial: results, controversies, and implications for prevention. Circ Cardiovasc Qual Outcomes. 2009;2(3):279-85.

30. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem. 2008;54(1):24-38.

31. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: An update. Biochim Biophys Acta. 2010;1801(12):1260-73.

32. Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P, Lemieux S, Barbier O, Vohl MC. Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population. J Nutr Biochem. 2013;24(1):54-61.

33. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, Beilin LJ. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin

Tables

Table 4.1 Characteristics at screening of subjects randomized into study (n=138)

	Men (n=42)	Women (n=96)
Age, years	57 ± 12	50 ± 16
BMI, kg/m^2	30 ± 4	29 ± 4
Waist circumference, cm	107 ± 10	98 ± 9
SBP, mm HG	118 ± 14	110 ± 12
DBP, mm HG	77 ± 9	71 ± 7
Total C, mmol/L	4.9 ± 0.8	5.5 ± 0.9
LDL-C, mmol/L [1]	2.9 ± 0.7	3.2 ± 0.8
HDL-C, mmol/L	1.3 ± 0.3	1.7 ± 0.4
C/HDL-C ratio	4.0 ± 1.0	3.5 ± 1.0
TG, mmol/L	1.5 ± 0.8	1.4 ± 0.7
CRP, mg/L	2.9 ± 1.9	3.7 ± 2.5
Fasting glucose, mmol/L	5.5 ± 1.0	5.2 ± 0.8
n with MetS (%)	11 (26)	16 (17)

Data are presented as means ± SD unless stated otherwise.
[1] n = 41 in men due to a missing value.
BMI: body mass index; C: cholesterol; CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MetS: metabolic syndrome; TG: triglycerides.

Table 4.2 Changes vs. control in post-treatment inflammation markers and blood lipids with EPA and DHA

	Control [1]	Δ EPA vs. control [1]	P value [2,4]	Δ DHA vs. control [1]	P value [2,4]	P value Δ EPA vs. Δ DHA [3,4]
Inflammation markers						
CRP, mg/L [5,6]	3.02 ± 0.14	-0.05 ± 0.14	0.45	-0.23 ± 0.14	0.02	0.25
IL-6, pmol/L [5]	1.61 ± 0.16	-0.21 ± 0.10	0.03	-0.19 ± 0.10	0.01	0.86
IL-18, pmol/L [5]	271.7 ± 12.6	-2.12 ± 6.29	0.38	-18.15 ± 6.25	0.002	0.01
TNF-α, pmol/L [5]	1.35 ± 0.14	-0.11 ± 0.10	0.10	-0.20 ± 0.05	0.01	0.63
Adiponectin, mg/L [5]	7.03 ± 0.46	-0.08 ± 0.12	0.14	0.22 ± 0.12	0.047	<0.001
Blood lipids						
Total C, mmol/L	5.16 ± 0.08	-0.03 ± 0.05	0.62	0.15 ± 0.05	0.001	<0.001
LDL-C, mmol/L	2.99 ± 0.07	0.07 ± 0.04	0.046	0.16 ± 0.04	<0.0001	0.04
HDL-C, mmol/L	1.54 ± 0.04	-0.01 ± 0.02	0.48	0.11 ± 0.02	<0.0001	<0.0001
C/HDL-C [5]	3.55 ± 0.09	0.01 ± 0.04	0.86	-0.10 ± 0.05	<0.001	0.006
Apo B, g/L [5]	1.31 ± 0.04	0.01 ± 0.02	0.46	0.03 ± 0.02	0.02	0.16
TG, mmol/L [5]	1.38 ± 0.06	-0.16 ± 0.03	<0.0001	-0.25 ± 0.04	<0.0001	0.005

Values are unadjusted means ± SEM for the control and unadjusted mean change (Δ) ± SEM vs. control for EPA and DHA.

[1] n =125 for control, 121 for the EPA and 123 for DHA changes (Δ) from the control due to missing data.

[2] P values for the EPA and DHA change vs. the control in the outcome, as determined by the LSMEANS statement in the MIXED models.

[3] P values for the comparison between EPA and DHA change vs. the control in the outcome, as determined the MIXED models.

[4] Adjustment for potential covariates (sex, age, weight, waist circumference, menopausal status, value on control treatment, treatment-specific baseline value, sequence of treatment as well as nutrient intakes) was considered only when these covariates were found to be significant at P < 0.05 in the MIXED models.

[5] Log-transformed data were used in these analyses due to skewness of the distributions of post-treatment values.

[6] n = 117 for control, 110 for the EPA and n=111 for DHA changes (Δ) from the control due to exclusions because CRP >10 after treatment phase.

Apo B: apolipoprotein B; C: cholesterol; CRP: C-reactive protein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; HDL: high-density lipoprotein; IL: interleukin; LDL: low-density lipoprotein; TNF: tumor necrosis factor; TG: triglycerides.

Supplemental Table 4.1 Methods used to assess each cardiometabolic variable and coefficients of variations

		Coefficient of variations	
Variable	**Method**	**Inter-assay (%) [1]**	**Biological (%) [2]**
CRP	Nephelometry	3.3	16.2
IL-6	ELISA	7.8	18.3
TNF-α	ELISA	8.3	ND
IL-18	ELISA	7.5	ND
Adiponectin	ELISA	5.2	9.2
Total C	Nephelometry	2.0	3.6
LDL-C	Calculated [3]	-	4.9
HDL-C	Nephelometry	3.0	4.0
Apo B	ELISA	8.0	7.3
TG	Nephelometry	3.5	11.7

[1] Based on the manufacturer's data.
[2] Mean biological variation calculated based two consecutive measures at the end of each treatment. Data from all treatments have been combined. There is no difference in biological variations between treatments (not shown).
[3] Calculated using the Friedewald equation.
Apo B: apolipoprotein B; C: cholesterol; CRP: C-reactive protein; HDL: high-density lipoprotein; IL: interleukin; LDL: low-density lipoprotein; ND: not determined; TNF: tumor necrosis factor; TG: triglycerides.

Supplemental Table 4.2 Difference in change of Omega-3 Index (O3I) after EPA and DHA supplementation, vs. control in men and women (%)

	Δ EPA vs. control	P value [2,4]	Δ DHA vs. control	P value [2,4]	P value [3,4]
Inflammation markers					
CRP, mg/L [5,6]	-0.09 ± 0.15	0.14	-0.28 ± 0.14	0.01	0.42
IL-6, pmol/L [5]	-0.24 ± 0.10	0.009	-0.23 ± 0.04	0.003	0.76
IL-18, pmol/L [5]	4.57 ± 7.41	0.56	-13.22 ± 7.16	0.15	0.049
TNF-α, pmol/L [5]	-0.12 ± 0.09	0.09	-0.20 ± 0.05	0.006	0.62
Adiponectin, mg/L [5]	-0.13 ± 0.16	0.14	0.15 ± 0.15	0.48	0.03
Blood lipids					
Total C, mmol/L	-0.03 ± 0.04	0.50	0.16 ± 0.04	<0.001	<0.0001
LDL-C, mmol/L	0.07 ± 0.04	0.044	0.17 ± 0.04	<0.0001	0.02
HDL-C, mmol/L	-0.02 ± 0.02	0.39	0.11 ± 0.02	<0.0001	<0.0001
Apo B, g/L [5]	0.00 ± 0.02	0.77	0.04 ± 0.02	0.02	0.077
TG, mmol/L [5]	-0.19 ± 0.03	<0.0001	-0.24 ± 0.03	<0.0001	0.03

Values are unadjusted means ± SEM for the control and unadjusted mean change (Δ) ± SEM vs. control for EPA and DHA. Using the MI procedure in SAS, we imputed missing variables values 10 times, thereby creating 10 imputed datasets. Statistical modeling from the 10 imputed database was conducted using the MINIALIZE procedure in SAS.

[1] n =138 for the EPA and DHA changes (Δ) from the control.

[2] P values for the EPA and DHA change vs. the control in the outcome, as determined by the LSMEANS statement in the MIXED models.

[3] P values for the comparison between EPA and DHA change vs. the control in the outcome, as determined the MIXED models.

[4] Adjustment for potential covariates (sex, age, weight, waist circumference, menopausal status, value on control treatment, treatment-specific baseline value, sequence of treatment as well as nutrient intakes) was considered only when these covariates were found to be significant at $P < 0.05$ in the MIXED models.

[5] Log-transformed data were used in these analyses due to skewness of the distributions of post-treatment values.

[6] 283 observations (out of 2720) were excluded because CRP >10 mg/L after treatment phase.

Apo B: apolipoprotein B; C: cholesterol; CRP: C-reactive protein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; HDL: high-density lipoprotein; IL: interleukin; LDL: low-density lipoprotein; TNF: tumor necrosis factor; TG: triglycerides.

Supplemental Table 4.3 Comparison of treatment-specific baseline values

	Control [1]	EPA [1]	DHA [1]	P value [2]
Waist circumference, cm	100.4 ± 10.3	100.6 ± 11.0	100.7 ± 11.7	0.57
BMI, kg/m^2	29.3 ± 4.1	29.3 ± 4.3	29.4 ± 4.4	0.45
SBP, mmHg	115.9 ± 12.1	115.3 ± 13.4	116.2 ± 13.2	0.69
DBP, mmHg	70.0 ± 9.2	69.7 ± 8.3	70.1 ± 9.6	0.87
Total C, mmol/L [3]	5.29 ± 0.95	5.25 ± 0.91	5.24 ± 0.87	0.55
LDL-C, mmol/L [3]	3.12 ± 0.80	3.07 ± 0.80	3.05 ± 0.78	0.25
HDL-C, mmol/L [3]	1.55 ± 0.41	1.57 ± 0.54	1.54 ± 0.40	0.76
C/HDL-C [3,4]	3.61 ± 1.03	3.76 ± 1.69	3.61 ± 1.00	0.35
Apo B, g/L [4]	1.34 ± 0.40	1.38 ± 0.44	1.35 ± 0.41	0.63
TG, mmol/L [3,4]	1.34 ± 0.62	1.45 ± 0.88	1.41 ± 0.63	0.13
CRP, mg/L [4,5]	3.40 ± 2.70	5.09 ± 13.08	3.99 ± 3.69	0.58
IL-6, pmol/L [4]	1.64 ± 2.72	3.51 ± 20.78	1.78 ± 2.11	0.24
IL-18, pmol/L [4]	269.7 ± 146.4	274.1 ± 143.8	271.8 ± 139.0	0.94
TNF-α, pmol/L [4]	1.20 ± 0.80	1.47 ± 2.55	1.28 ± 1.02	0.49
Adiponectin, mg/L [4]	7.16 ± 5.48	6.97 ± 5.48	7.02 ± 5.16	0.35

[1] n = 125, 128 and 133 for control, EPA and DHA respectively.

[2] P values for the comparison between ΔEPA and ΔDHA, as determined by MIXED models.

[3] n = 127 for EPA, due to missing value for one subject.

[4] Analyses were performed on log-transformed data to normalize the skewed distribution.

[5] n = 124 for control, due to missing value for one subject.

Apo B: apolipoprotein B; BMI: body mass index; C: cholesterol; CRP: C-reactive protein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; HDL: high-density lipoprotein; IL: interleukin; LDL: low-density lipoprotein; TNF: tumor necrosis factor; TG: triglycerides.

Supplemental Table 4.4 Self-reported food intakes of subjects during each treatment phase

	Control [1]	EPA [1]	DHA [1]	P value [2]
Energy, kCal	2374 ± 867	2316 ± 925	2407 ± 864	0.37
Carbohydrates, % of energy	48.4 ± 7.5	48.4 ± 6.6	48.1 ± 7.2	0.42
Fibers, g	29.1 ± 11.9	27.7 ± 11.7	29.3 ± 13.4	0.05
Proteins, % of energy	16.8 ± 2.7	17.3 ± 3.0	17.2 ± 2.6	0.06
Proteins, g	97.8 ± 34.7	98.9 ± 38.9	101.9 ± 35.7	0.30
Fat, % of energy	35.4 ± 6.6	35.2 ± 5.9	35.5 ± 6.0	0.71
SFA, % of energy	12.1 ± 3.0	12.4 ± 3.3	12.3 ± 2.8	0.30
MUFA, % of energy	14.3 ± 3.1	14.0 ± 2.7	14.2 ± 2.9	0.48
PUFA, % of energy	6.4 ± 1.8	6.2 ± 1.6	6.2 ± 1.6	0.46
EPA, mg [3]	21.2 ± 17.9	18.4 ± 16.2	23.3 ± 25.8	0.05
DHA, mg [3]	108.5 ± 93.1	95.8 ± 89.1	115.3 ± 127.9	0.07
Alcohol, % of energy	2.5 ± 2.7	2.1 ± 2.2	2.2 ± 2.8	0.09
Alcohol, g [3]	8.2 ± 8.9	6.7 ± 7.1	7.8 ± 11.2	0.02

Daily food intakes were assessed by a validated web-based, self-administered food-frequency questionnaire as outlined in the methods' section. Data are presented as means ± SD.

[1] n = 108, 110 and 114 for the control, EPA and DHA respectively due to missing data.

[2] P values for the comparison between control, EPA and DHA, as determined by MIXED models.

[3] Analyses were performed on log-transformed data to normalize the skewed distribution.

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

Supplemental Table 4.5 Plasma phospholipid fatty acid composition after supplementation with control, EPA and DHA for 10 weeks

	% of fatty acids			
	Control [1]	EPA [1]	DHA [1]	P value [2]
Total SFA	44.45 ± 1.11	45.05 ± 1.06	44.83 ± 0.94	0.04
Myristic acid, 14:0	0.37 ± 0.09	0.40 ± 0.08	0.36 ± 0.09	0.22
Pentadecanoic acid, 15:0	0.20 ± 0.04	0.20 ± 0.03	0.20 ± 0.05	0.48
Palmitic acid, 16:0	26.37 ± 1.41	26.54 ± 1.75	26.63 ± 1.45	0.41
Heptadecanoic acid, 17:0	0.38 ± 0.05	0.39 ± 0.05	0.40 ± 0.07	0.065
Stearic acid, 18:0	13.62 ± 1.29	13.92 ± 1.38	13.49 ± 1.10	0.02
Total cis-MUFA	12.90 ± 0.94	12.47 ± 0.90	12.52 ± 1.01	0.04
Oleic acid, 18:1 n9	8.30 ± 0.75	7.93 ± 0.81	7.97 ± 0.87	0.046
Total trans-MUFA	0.10 ± 0.09	0.14 ± 0.14	0.11 ± 0.09	0.47
Total cis-PUFA n6	34.29 ± 1.48	28.01 ± 2.15	28.86 ± 1.89	<0.0001
Linoleic acid, 18:2 n6	19.76 ± 2.08	16.76 ± 2.07	18.00 ± 1.83	<0.0001
Arachidonic acid, 20:4 n6	10.62 ± 1.55	8.56 ± 0.98	7.87 ± 1.02	<0.0001
Total cis-PUFA n3	5.84 ± 0.92	11.92 ± 2.40	11.13 ± 1.56	<0.0001
Alpha-linoleic acid, 18:3 n3	0.24 ± 0.08	0.21 ± 0.07	0.22 ± 0.08	0.29
EPA, 20:5 n3	1.09 ± 0.33	6.01 ± 1.95	2.1 ± 0.61	<0.0001
Docosapentaenoic acid, 22:5 n3	0.94 ± 0.23	2.24 ± 0.54	0.58 ± 0.12	<0.0001
DHA, 22:6 n3	3.45 ± 0.58	3.32 ± 0.47	8.08 ± 1.30	<0.0001

Values are means ± SD. Total % does not add up to 100% because not all fatty acids are shown in this table.
[1] n = 30 for control, EPA and DHA.
[2] P values for the comparison between control, EPA and DHA, as determined by MIXED models.
DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; n3: omega-3; n6: omega-6; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

Supplemental Table 4.6 Self-reported side effects during control, EPA and DHA supplementation

Side effect	Control n (%)	EPA n (%)	DHA n (%)	P value [1]
Headache	21 (17)	26 (20)	25 (19)	0.76
Anxiety	28 (22)	26 (20)	27 (20)	0.90
Tiredness	23 (18)	26 (20)	26 (20)	0.93
Negative thoughts	15 (12)	9 (7)	10 (8)	0.31
Loss of balance	19 (15)	19 (15)	10 (8)	0.11
Pain in the joints	27 (22)	17 (14)	26 (20)	0.27
Fast heart beat	18 (14)	15 (12)	18 (14)	0.81
Decreased appetite	17 (14)	25 (20)	15 (11)	0.15
Abdominal/stomach pain	24 (19)	22 (17)	23 (18)	0.89
Nausea	15 (12)	20 (16)	13 (10)	0.34
Vomiting	4 (3)	8 (6)	5 (4)	0.46
Constipation	18 (14)	28 (22)	29 (22)	0.22
Diarrhea	27 (22)	31 (24)	33 (25)	0.85

N and % of subjects who reported an increase in the frequency of symptoms during each phase.
[1] Determined by the Chi-Square test.
DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid.

Figures

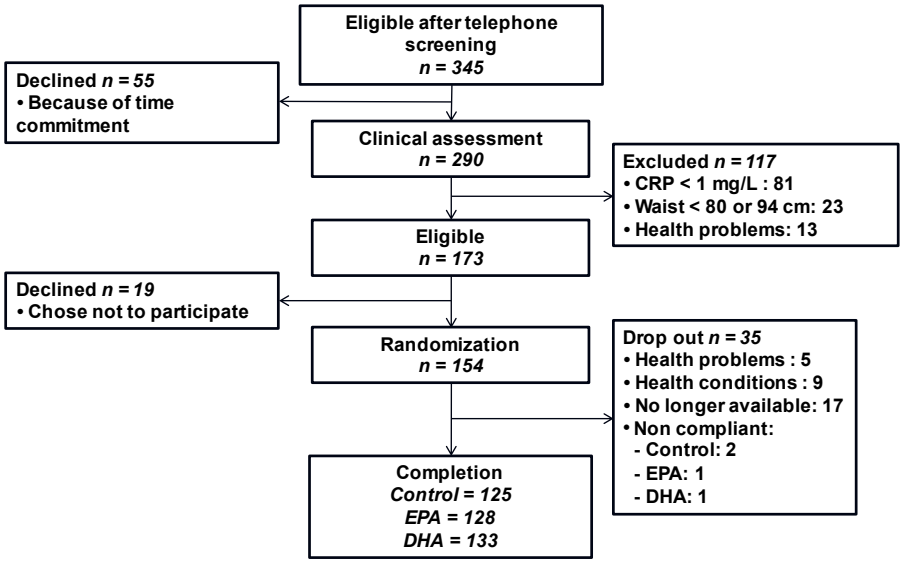

Figure 4.1 CONSORT chart of study subjects

After a telephone screening of 786 subjects, 451 did not meet inclusion criteria. Out of 345 subjects eligible, 55 declined because of time commitment and 290 underwent a clinical assessment. Out of 173 eligible subjects, 154 were randomized. Completion rate varies by phases because of the randomized crossover nature of the study.

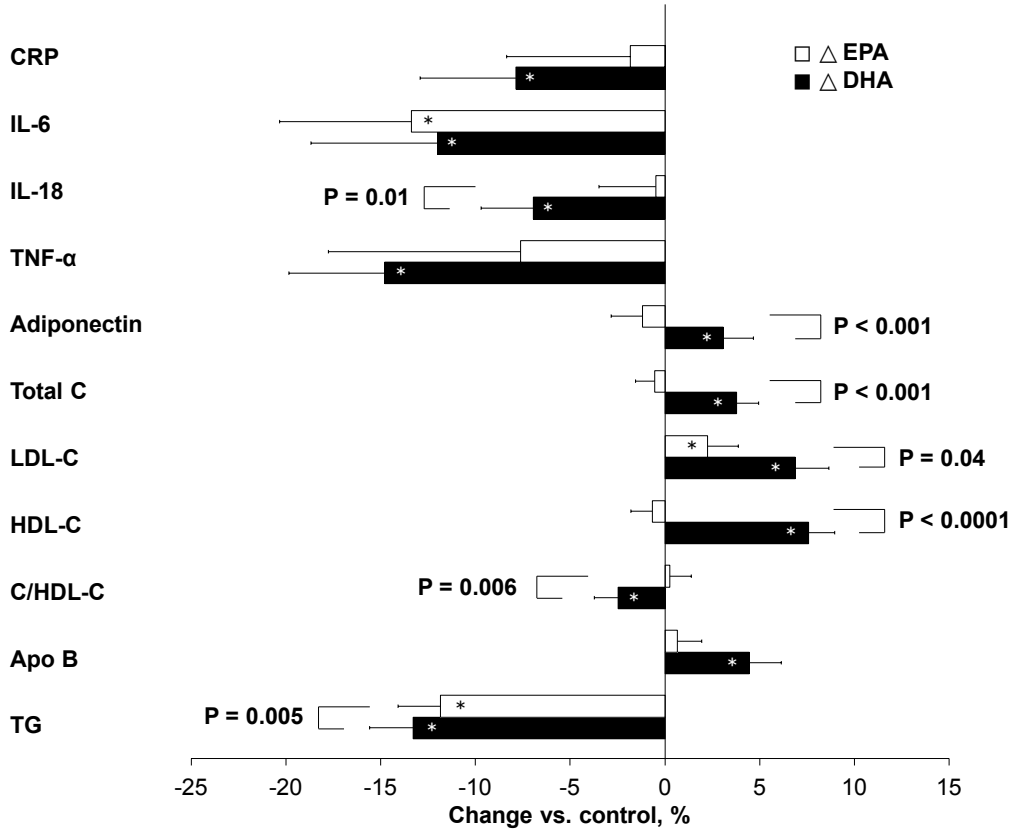

Figure 4.2 Changes vs. control in post-treatment inflammation markers and blood lipids with EPA and DHA

Values are mean ± SEM. * P <0.05 for the treatment-specific effect vs. control for ΔEPA and ΔDHA, corresponding to the ones shown in Table 2 and determined by MIXED models. n = 121 for the EPA and 123 for DHA changes (Δ)from the control due to missing data. n = 110 for the EPA and n=111 for DHA changes (Δ) from the control due to exclusions because CRP >10 after treatment phase. Apo B: apolipoprotein B; C: cholesterol; CRP: C-reactive protein; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; HDL: high-density lipoprotein; IL: interleukin; LDL: low-density lipoprotein; TNF: tumor necrosis factor; TG: triglycerides.

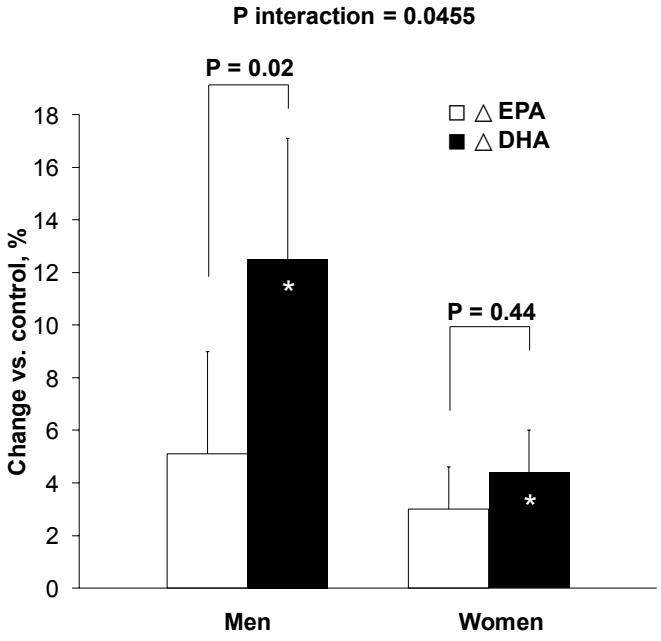

Figure 4.3 Changes vs. control in post-treatment LDL-C with EPA and DHA by sex

Values are mean ± SEM. * P <0.05 for the treatment-specific effect vs. control for EPA and DHA as determined by MIXED models. n = 36 for the EPA and 37 for DHA changes (∆) from the control for men and n = 88 for the EPA and 89 for DHA changes (∆)from the control for women due to missing data. C: cholesterol; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; LDL: low-density lipoprotein.

Chapitre 5 Comparaison de l'effet de l'EPA et du DHA sur l'Indice Oméga-3

Janie Allaire, William S Harris, Cécile Vors, Amélie Charest, Johanne Marin, Kristina Harris Jackson, André Tchernof, Patrick Couture, Benoît Lamarche

L'article présenté dans ce chapitre s'intitule: Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid.

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Lien pour accéder à l'article : https://linkinghub.elsevier.com/retrieve/pii/S0952-3278(17)30030-3.

Résumé

L'objectif de cette étude était de comparer les effets de l'EPA et du DHA sur l'Indice Oméga-3 chez des hommes et des femmes avec une obésité abdominale et une inflammation systémique de bas grade. Un devis randomisé contrôlé en chassé-croisé et à double insu a été utilisé pour répondre à ces objectifs. Un total de 48 hommes et 106 femmes ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Chaque phase expérimentale était d'une durée de 10 semaines et les phases étaient séparées par une période libre de 9 semaines. Dans cette étude, la supplémentation en DHA a mené à une augmentation plus importante de l'Indice Oméga-3 que l'EPA (+5,6% pour le DHA comparativement au témoin, P<0,0001 et +3.3% pour l'EPA comparativement au témoin, P<0,0001 ; DHA vs EPA, P<0,0001). Cet effet différentiel de l'EPA et du DHA sur l'Indice Oméga-3 et l'impact de cette différence sur le risque d'évènements cardiovasculaires à long terme mérite d'être étudié dans de futures études cliniques de grande envergure.

Abstract

Background: The objective of this study was to compare the effects of EPA and DHA supplementation on the Omega-3 Index (O3I) in men and women with abdominal obesity and subclinical inflammation. **Methods:** In a double-blind controlled crossover study, 48 men and 106 women were randomized to a sequence of three treatment phases: 1- 2.7 g/d of EPA, 2- 2.7 g/d of DHA, and 3- 3 g/d of corn oil. The 10-week treatment phases were separated by nine-week washouts. **Results:** The increase in the O3I after DHA (+5.6% vs. control, $P<0.0001$) was significantly greater than after EPA (+3.3% vs. control, $P<0.0001$; DHA vs. EPA, $P<0.0001$). **Conclusions:** The increase in the O3I is greater with high dose DHA than with high dose EPA. The extent to which such differences between EPA and DHA in increasing the O3I relates to long-term cardiovascular risk needs to be investigated in the future.

Title page

Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid

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Abbreviations: BMI: body mass index; CHD: coronary heart disease; CRP: C-reactive protein; CVD: cardiovascular diseases; C: cholesterol; DHA: docosahexaenoic acid; DPA : docosapentaenoic acid ; ELOVL2: ELOVL fatty acid elongase 2 ; ELOVL5: ELOVL fatty acid elongase 5; EPA: eicosapentaenoic acid; FADS1: fatty acids desaturase 2 or delta-5 fatty acids desaturase; FADS2: fatty acids desaturase 2 or delta-6 fatty acids desaturase ; GAPDH : glyceraldehyde 3-phosphate dehydrogenase; HDL : high-density lipoprotein; INAF: Institute of nutrition and functional foods; LCn3-PUFA: long-chain omega-3 polyunsaturated fatty acid ; LDL:

low-density lipoprotein; MetS: metabolic syndrome; MUFA: monounsaturated fatty acid ; O3I : Omega-3 Index; PUFA : polyunsaturated fatty acid ; RBC: red blood cell ; SFA: saturated fatty acid ; TG: triglyceride.

Clinical Trial Registry number and website: http://www.clinicaltrials.gov (NCT01810003).

Introduction

Considerable research has been conducted to determine the association between long-chain polyunsaturated omega-3 fatty acids (LCn3-PUFAs) consumption and cardiovascular risk. LCn3-PUFAs modulate a variety of cardiometabolic risk factors such as blood lipids, blood pressure, thrombosis and inflammation.[1] Fatty fish and supplements, often combining eicosapentaenoic and docosahexaenoic acids (EPA and DHA), are the main dietary sources of LCn3-PUFAs. There is emerging evidence suggesting that EPA and DHA exert different effects on blood lipids and inflammation markers.[2,3] However, such evidence is limited, most studies to date having assessed these effects using a mixture of EPA and DHA in different forms and proportions. Little is known with regard to the specific effects of EPA and DHA on metabolic pathways and biological processes underlying cardiometabolic health in humans.

The fatty acid composition of cell membranes influences their physico-chemical properties and, ultimately, organ functions.[1,4] The Omega-3 Index (O3I), which is calculated as the relative content of red blood cell (RBC) membranes as EPA plus DHA, reflects the phospholipid LCn3-PUFA composition of major organs,[4] including cardiac tissue.[4,5] A high O3I (8-12%) has been associated with a lower risk of coronary heart disease (CHD) and coronary mortality in epidemiological studies.[6,7] Supplementation with EPA+DHA is recommended by various health agencies including the American Heart Association for secondary CHD prevention or management of plasma triglycerides (TG).[8] Yet, whether EPA and DHA have distinct effect on the O3I is currently unknown. Considering that the O3I is modifiable by diet,[5] studies are required to compare the effects of different LCn3-PUFAs on this promising clinical tool for the management of diet-related CHD risk.

The objective of this study was to compare the effects of high doses of re-esterified EPA and DHA on the O3I using a randomized double-blind controlled crossover study design, in men and women with abdominal obesity and subclinical inflammation. We hypothesized that the O3I increases more with DHA than with EPA. Based on previous studies of fatty acid metabolism in men and women,[9] we also hypothesized that the increase in the O3I with both EPA and DHA is greater among women than among men.

Patients and Methods

Study design: This analysis is based on data from a double-blind randomized, controlled crossover study with three treatment phases (1- EPA, 2- DHA and 3- corn oil as control), for which the primary outcome was the change in C-reactive concentrations (CRP) concentrations. Details of the study design and results of primary analyses have been published previously.[10] Briefly, each treatment phase had a median duration of 10 weeks and were separated by a nine-week washout. Randomization of participants to one of six treatment sequences was performed using an in-house computer program and was stratified by sex. Participants were supplemented with three identical 1 g capsules of >90% purified LCn3-PUFA per day providing either 2.7 g/d EPA or 2.7 g/d DHA. Corn oil was used as a control (0 g/d EPA+DHA). LCn3-PUFA supplements were formulated as re-esterified TG and provided by Douglas Laboratories. Participants were instructed to maintain a constant body weight during the course of the study. They were also counseled on how to exclude fatty fish (including salmon, tuna, mackerel, and herring), other LCn3-PUFA supplements, flax products, walnuts, and LCn3-PUFA-enriched products during the three study phases.

Study population: Primary eligibility criteria were to have abdominal obesity based on the International Diabetes Federation sex specific cut-offs (≥80 cm for women, ≥94 cm for men)[11] in combination with a screening plasma CRP concentration >1 mg/L but <10 mg/L. Subjects had to be otherwise healthy. Adult subjects (18 and 70 years of age) were recruited at the Institute of Nutrition and Functional Foods (INAF). Body weight had to be stable for at least three months prior to randomization. Exclusion criteria were plasma CRP >10 mg/L at screening, extreme dyslipidemias such as familial hypercholesterolemia, having a personal history of cardiovascular diseases (CVD) (CHD, cerebrovascular disease or peripheral arterial disease), taking medications or substances known to affect inflammation (e.g. steroids, binging alcohol), and use of LCn3-PUFA supplements within two months of study onset. All participants signed an informed consent document approved by local Ethics Committees at the beginning of the study and the study protocol was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003).

Anthropometry: Anthropometric measures including waist and hip circumferences were obtained according to standardized procedures.[12] Body composition was measured by Dual-energy X-ray absorptiometry (GE Healthcare, Madison, WI).

Dietary habits: Food intakes during each phase was monitored using a validated quantitative web-based, self-administered food frequency questionnaire at the end of each treatment phase.[13]

Risk factor assessment: Serum total cholesterol (C), TG and high-density lipoprotein (HDL)-C were assessed on a Roche/Hitachi Modular (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's

specifications and using proprietary reagents. Plasma low-density lipoprotein (LDL)-C concentrations were calculated using the Friedewald Equation. Total C, LDL-C, HDL-C and TG were measured twice on consecutive days at the end of each treatment. The mean of the two measurements were used in the analyses. Treatment-specific baseline values were measured once. All personnel involved in the measurements of the study outcomes were blinded to treatments. Metabolic syndrome (MetS) was defined using the International Diabetes Federation criteria.[11]

RBC membrane fatty acids measurements: RBC membrane fatty acid composition was measured at baseline and at the end of each treatment phase. RBC membrane was analyzed by OmegaQuant Analytics, LLC, (Sioux Falls, South Dakota, United States) according to the Omega-3 Index® methodology as modified from Harris *et al.*[14] Fatty acid methyl esters were generated from erythrocytes by transesterification with boron trifluoride and analyzed by gas chromatography. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristics of RBCs. Each fatty acid is expressed as a weight percent of total identified fatty acids after a response factor correction (based on calibration curves) was applied to each fatty acid. The O3I represents the sum of EPA and DHA expressed as a percent of total RBC fatty acids.[14] The RBC composition at baseline and after each phase is presented in Supplemental Table 1. Baseline RBC composition for men and women is presented in Supplemental Table 2.

Gene expression of polyunsaturated fatty acid metabolism: Fasting fresh blood was collected in PAXgene Blood RNA tubes (Becton Dickinson, Canada) after each treatment in a subsample of 44 randomly selected participants for gene expression analyses. RNA was isolated using a PAXgene RNA-kit according to manufacturer's instructions (Qiagen, Canada). Quantity of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and total RNA quality was assayed on an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Reverse transcription was performed on 1.5-2 µg total RNA. cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based Realtime PCR quantification using the LightCycler 480 (Roche Diagnostics, Mannheim, DE). The genes targeted were ELOVL fatty acid elongase 2 and 5 (ELOVL2 and ELOVL5) and fatty acid desaturases, delta-5 and delta-6 (FADS1 and FADS2). Sequence primers and gene description are available in the Supplemental Table 3. Values were normalized to expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Statistical analyses: Differences in RBC membrane fatty acid composition between treatments were assessed using the MIXED procedure for repeated measures in SAS (v9.3, Cary, NC), with treatment as fixed effect and a compound symmetry or autoregressive covariance matrix to account for within-subject correlations. The change vs. the control treatment (post treatment EPA minus control and post treatment DHA minus control) was

used as the dependent variable in all analyses.[15] To be included in the analyses, subjects had to have completed the control phase plus at least one of the two treatment phases. Using this approach, the main treatment effect in the models reflected the direct comparison of EPA and DHA. Adjustment for multiple comparisons was not necessary as the main treatment effect had only two levels. In the same model, the change vs. control for each treatment was tested against the null hypothesis by the LSMEANS statement in the MIXED procedure. Potential confounders of the outcome measure response to treatment, mainly body mass index, age, sex, energy intake were added to models and results from analyses based on the most parsimonious models (i.e. retaining only the variables that contributed significantly to variations in any given study outcome) are shown. We considered sex, sequence of treatments and baseline values interaction by adding interaction terms with the main treatment effect into the model statement in the MIXED procedure. The skewness in the distribution of model residuals was considered and data were log-transformed when required. Correlations were tested using Pearson's correlations coefficients.

Results

Subjects' characteristics: Of the 173 eligible men and women, 154 were randomized to treatment sequences. Characteristics of the 154 randomized participants at screening are shown by sex in Table 1. A total of 123 participants have completed both the DHA and the control phases, and 121 participants have completed both the EPA and the control phases.

Fatty acid composition of RBC membranes: Figure 1 shows the individual O3I response to EPA and DHA supplementation. The O3I increased with EPA and DHA in all but one participant. Self-reported compliance for this participant was high (93%). Both EPA (+3.3%, P<0.0001) and DHA supplementation (+5.6%, P<0.0001) significantly increased the O3I in RBC membranes compared with control (Table 2). DHA led to a greater increase in the O3I than EPA in all participants (Figure 1). Thus, the increase in the O3I with DHA was significantly greater than with EPA (P<0.0001). The correlation between the change in O3I with EPA and with DHA (vs. control) was r=0.65 (P<0.0001). DPA levels in RBC increased after EPA (+2.5%, P<0.0001) but decreased after DHA (-0.8%, P<0.0001) compared with control (EPA vs. DHA, P<0.0001, Table 2).

The increase in O3I was not statistically different between men and women after EPA (+3.1% and +3.4% respectively) and after DHA (+5.7% and +5.6% respectively) (Figure 2). However, the difference between DHA and EPA in increasing the O3I tended to be higher in men than in women (+2.6% vs. +2.2% respectively, P for the treatment-by-sex interaction = 0.054). This apparent difference between men and women was independent of baseline EPA (P=0.11) and DHA (P=0.19) levels as well as of the baseline O3I (P=0.12, not shown).

The change in the O3I was inversely correlated with the corresponding change in TG after both EPA and DHA (r=-0.21, P=0.017 and r=-0.24, P=0.0076 respectively) and correlated positively with the change in LDL-C after DHA only (r=0.19, P=0.039 for DHA and r=-0.02, P=0.86 for EPA). Changes in the O3I with EPA and DHA did not correlate significantly with changes in other biomarkers, including inflammation markers. Finally, there was no carryover effect of any treatment on the O3I (not shown).

Gene expression of polyunsaturated fatty acid metabolism: There was no significant difference in the expression of *ELOVL2* and *ELOVL5* and *FADS1* and *FADS2* in whole blood cells between EPA and DHA (Table 3). EPA supplementation increased the expression of *ELOVL2* by 24% compared with control (P=0.0084).

Discussion

A high O3I, which reflects a relatively high content of EPA and DHA in the membranes of RBCs, has been associated with a lower risk of CHD and mortality in observational studies.[6,7] While there is emerging evidence suggesting that DHA may be more potent than EPA in modifying cardiometabolic risk,[2,3] their respective impacts on the O3I have not been thoroughly examined. To the best of our knowledge, this is the first randomized double-blind controlled crossover trial to show that the increase in O3I is significantly greater after supplementation with high dose DHA (2.7 g/d) than with a comparable dose of EPA.

Grimsgaard *et al.*[16] found in a parallel study that the increase in serum phospholipid EPA (+4.7%) was greater after supplementation with 3.8 g/d of ethyl ester EPA for 7 weeks than the corresponding increase of serum phospholipid DHA (+3.2%) after a supplementation of with 3.6 g/d of ethyl ester DHA. More recently, Tsunoda *et al.*[17] have shown than the increase in EPA (+1.9%) in peripheral blood mononuclear cells after supplementation with 1.8 g/d of EPA compared to an olive oil control was similar to the corresponding increase in DHA (+2.0%) after 1.8 g/d of DHA supplementation. Authors of these two studies did not report the changes in O3I with EPA and DHA supplementation. Our data further indicated that the relative content of DPA in RBC was increased after EPA and reduced after DHA compared with control. This is consistent with results from Tsunoda *et al.*[17] This increase in DPA levels after supplementation with EPA was also consistent with the observed increase in the expression of *ELOVL2,* which catalyzes the elongation of EPA to DPA. Since DPA is not accounted for in the calculation of the O3I, "disappearance" of EPA in RBC through *in vivo* elongation to DPA does in large part explain the smaller increase in O3I with EPA compared with DHA in our study. In fact, an index based on the sum of EPA, DPA and DHA in RBCs would have increased more after high dose supplementation with EPA than after supplementation with a corresponding dose of DHA (+5.8% vs. +4.8% respectively, P<0.0001, not shown).

Levels of DPA in RBCs have been inversely associated with TG and CRP concentrations in healthy adults following supplementation with LCn3-PUFAs (EPA+DPA+DHA),[18] suggesting that DPA may also be partly responsible for some of the biological and cardioprotective effects attributed to LCn3-PUFA.[19] Lower levels of DPA in serum or plasma have been associated with greater risk of acute myocardial infarction,[20] total mortality,[21] cardiovascular mortality[21] and nonfatal myocardial infarction.[22] DPA concentrations in different tissues (total plasma lipids, plasma or RBC phospholipids and adipose tissue) have also been inversely associated with fatal coronary heart disease in a recent meta-analysis of 19 epidemiological studies (total N=45,637).[23] On the other hand, data from the present study indicate that the correlation between the O3I based on EPA and DHA and the sum of EPA+DPA+DHA in RBC is 0.97 after DHA supplementation, 0.93 after EPA supplementation and 0.92 after the control (not shown, P<0.0001 for all). Such strong correlations suggest little added contribution of DPA to the predictive value of the O3I. Consistent with this, addition of DPA did not significantly improve the mortality

risk prediction of the O3I in the Women's Health Initiative Memory Study.[24] Although a growing body of evidence suggests that DPA may be a highly bioactive compound, studies are needed to dissect out its contribution to CHD risk from that of EPA and DHA, which are also strongly correlated with CHD risk.[25]

ELOVL and FADS are the enzymes responsible of the elongation of EPA to DPA and DPA to DHA. Grimsgaard *et al.*[16] estimated the change in the activities of the FADS1 and FADS2 after seven weeks of DHA (3.6 g/d), EPA (3.8 g/d) or corn oil (4 g/d) supplementation, using ratios of phospholipid fatty acids proportions in serum. Estimated FADS2 activity was increased while estimated FADS1 activity was decreased after DHA supplementation compared with baseline. Supplementation with EPA increased estimated FADS1 activity but had no impact on FADS2 activity compared with baseline.[16] In our study, supplementation with high dose EPA or DHA had no significant impact on either *FADS1* or *FADS2* gene expression in whole blood cells compared with the control corn oil treatment. It is not entirely surprising that estimated FADS1/2 activities based on fatty acids ratios and analysis of gene expression of these enzymes in whole blood cells yielded inconsistent results among existing studies, considering the complex relationship with actual activities of the corresponding enzymes.

EPA levels in RBC increased significantly after high dose supplementation with DHA. This is consistent with data having demonstrated a dose-dependent relationship between EPA levels in blood and the dose of supplemented DHA, which most likely attributable to retro-conversion of DHA into EPA.[26] The fatty acid content of RBC are expressed as a relative weight of all identified fatty acids. It is therefore difficult to assess if the reduction in the proportion of DPA after DHA supplementation is due to further retro-conversion to EPA, or to an increase in the proportion of other fatty acids.

In vivo conversion rate of ALA to EPA and to DHA is estimated at 8-20% and 0.5-9% respectively,[27] with higher rates among women of reproductive age than among men.[9] The regulatory effect of estrogens combined with a lower muscle mass may result in a smaller proportion of ALA being channelled towards beta-oxidation in women, and thus a greater proportion of ALA being converted into EPA and DHA.[9] This suggests potential sex-dependent difference in the response to dietary LCn3-PUFA. While the effects EPA and DHA supplementation on DPA and DHA levels were similar among men and women in the present study (not shown), women compared with men accumulated more EPA in their RBC after supplementation with EPA (+4.0% vs. +3.5%, respectively, P=0.0002, not shown). Our study further showed that the increase in the O3I with EPA and DHA tended to be higher in men than in women. Self-reported fish consumption and compliance were similar between men and women (not shown) and thus differences in the O3I response to EPA and DHA are unlikely to be explained by these factors. This apparent sex-dependent difference in the response to EPA and DHA

supplementation and consequences in terms of cardiovascular risk in men and women needs further investigation.

Albert *et al.*[7] have shown that men in the top quartile of the whole blood EPA+DPA+DHA distribution in the Physicians' Health Study (corresponding to an average O3I of 6.9%)[6] had a 90% lower risk of sudden cardiac death than men in the lowest quartile (corresponding to an average O3I of 3.8%)[6]. In the present study, the O3I after DHA was 2.3% greater than after EPA supplementation. Data from epidemiological studies would therefore suggest a greater benefit of DHA compared with EPA on risk of CVD.[10,28,29] However, the greater increase in LDL-C concentrations with high dose DHA compared to EPA also needs to be factored in when assessing the impact of LCn3-PUFA on CVD risk.[30] We have shown that the reduction in serum TG, interleukin-18 and the total cholesterol/HDL-C ratio, as well as the increase in serum HDL-C, adiponectin and LDL-C were significantly more important after high dose supplementation with DHA than after EPA supplementation.[10] The extent to which the greater improvements in inflammation markers and in HDL-C with DHA compared with EPA counterbalances the potential risk associated with higher LDL-C is uncertain.[10] While changes in the O3I predicted concurrent changes in TG after both DHA and EPA, this was not the case for the change in LDL-C, which correlated with changes in the O3I only after DHA. Correlation analyses also revealed that the change in the O3I was a poor predictor of the response of other cardiometabolic risk factors to EPA and DHA. Very few studies thus far have assessed and compared the individual contributions of EPA and DHA to the association between the O3I and CVD risk, as well as to its overall predictive value. Additional intervention studies are therefore needed to determine potential benefits of a long term DHA vs. EPA supplementation on the O3I as it relates to cardiovascular outcomes.

Our use of a crossover study design had many advantages including attenuation of residual confounding and increase in statistical power. The large sample size of this carefully controlled randomized trial also provided power to detect small changes in RBC composition. The composition of RBCs tracked well with each supplementation phase, reflecting high compliance to treatments and high quality data. One risk of crossover design studies pertains to potential carryover effects of treatments. However, we found no evidence of carryover effects on changes in the O3I after EPA and DHA supplementation, indicating that washout plus intervention periods were of sufficient duration to eliminate the effect of preceding treatments (data not shown). The effects of EPA and DHA on the O3I were compared to 3 g of corn oil as control, which slightly decreased EPA and DPA relative content in RBC (-0.07% and -0.11% respectively, $P<0.05$, not shown), but had no effect on DHA content (+0.15%, $P=0.16$, not shown) and on the O3I (+0.01%, $P=0.66$, not shown) compared with baseline. Most previous controlled studies on LCn3-PUFA have also used low doses of vegetable oils as a control and to that extent, our study design is similar to previous ones on this topic.[3]

Conclusions

Data from this randomized double-blind crossover study shows that the increase in the O3I is numerically greater after supplementation for 10 weeks with 2.7 g/d of DHA than after 2.7 g/d of EPA. This difference may simply reflect the fact that a proportion of EPA is elongated into DPA, which is not included in the O3I calculation. Data also suggested that the O3I may increase slightly more in men than in women after DHA supplementation. Additional studies are needed to better understand how such differences in the net change in O3I after supplementation with high dose DHA compared with EPA relate to the risk of cardiovascular outcomes.

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Disclosures

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References

1. Calder PC. Mechanisms of action of (n-3) fatty acids. *J Nutr.* 2012;142(3):592S-599S.

2. Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. *Curr Atheroscler Rep.* 2011;13(6):474-483.

3. Li K, Huang T, Zheng J, et al. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. *PLoS One.* 2014;9(2):e88103.

4. Fenton JI, Gurzell EA, Davidson EA, et al. Red blood cell PUFAs reflect the phospholipid PUFA composition of major organs. *Prostaglandins Leukot Essent Fatty Acids.* 2016;112:12-23.

5. Harris WS, Sands SA, Windsor SL, et al. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation.* 2004;110(12):1645-1649.

6. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med.* 2004;39(1):212-220.

7. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med.* 2002;346(15):1113-1118.

8. Vannice G, Rasmussen H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. *J Acad Nutr Diet.* 2014;114(1):136-153.

9. Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care.* 2004;7(2):137-144.

10. Allaire J, Couture P, Leclerc M, et al. Randomized, crossover, head-to-head comparison of EPA and DHA supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA Study. *Am J Clin Nutr.* 2016.

11. Alberti KG, Zimmet P, Shaw J, et al. The metabolic syndrome--a new worldwide definition. *Lancet.* 2005;366(9491):1059-1062.

12. Airlie, Lohman T, Roche A, et al. Standardization of anthropometric measurements. *The Airlie (VA) Concensus Conference*. Champaign, Ill: Human Kinetics; 1988:39-80.

13. Labonte ME, Cyr A, Baril-Gravel L, et al. Validity and reproducibility of a web-based, self-administered food frequency questionnaire. *European Journal of Clinical Nutrition.* 2012;66(2):166-173.

14. Harris WS, Pottala JV, Vasan RS, et al. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. *J Nutr.* 2012;142(7):1297-1303.

15. Bland JM, Altman DG. Best (but oft forgotten) practices: testing for treatment effects in randomized trials by separate analyses of changes from baseline in each group is a misleading approach. *Am J Clin Nutr.* 2015;102(5):991-994.

16. Grimsgaard S, Bonaa KH, Hansen JB, et al. Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am J Clin Nutr.* 1997;66(3):649-659.

17. Tsunoda F, Lamon-Fava S, Asztalos BF, et al. Effects of oral eicosapentaenoic acid versus docosahexaenoic acid on human peripheral blood mononuclear cell gene expression. *Atherosclerosis.* 2015;241(2):400-408.

18. Skulas-Ray AC, Flock MR, Richter CK, et al. Red blood cell docosapentaenoic acid (DPA n-3) is inversely associated with triglycerides and c-reactive protein (CRP) in healthy adults and dose-dependently increases following n-3 fatty acid supplementation. *Nutrients.* 2015;7(8):6390-6404.

19. Bowen KJ, Harris WS, Kris-Etherton PM. Omega-3 Fatty Acids and Cardiovascular Disease: Are There Benefits? *Curr Treat Options Cardiovasc Med.* 2016;18(11):69.

20. Oda E, Hatada K, Katoh K, et al. A case-control pilot study on n-3 polyunsaturated fatty acid as a negative risk factor for myocardial infarction. *Int Heart J.* 2005;46(4):583-591.

21. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Ann Intern Med.* 2013;158(7):515-525.

22. Sun Q, Ma J, Campos H, et al. Blood concentrations of individual long-chain n-3 fatty acids and risk of nonfatal myocardial infarction. *Am J Clin Nutr.* 2008;88(1):216-223.

23. Del Gobbo LC, Imamura F, Aslibekyan S, et al. omega-3 Polyunsaturated Fatty Acid Biomarkers and Coronary Heart Disease: Pooling Project of 19 Cohort Studies. *JAMA Intern Med.* 2016;176(8):1155-1166.

24. Harris W.S.; Pottala JVE, M.E.; Margolis, K.E.; Manson, J.E.; Wang, L.; Brasky, T.M.; Robinson, J.G. Red blood cell polyunsaturated fatty acids and mortality in the women's health initiative memory study. *J Clin Lipidol.* 2017, in press.

25. Kaur G, Guo XF, Sinclair AJ. Short update on docosapentaenoic acid: a bioactive long-chain n-3 fatty acid. *Curr Opin Clin Nutr Metab Care.* 2016;19(2):88-91.

26. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr.* 2006;83(6 Suppl):1467S-1476S.

27. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids.* 2006;75(3):161-168.

28. Brinton EA, Mason RP. Prescription omega-3 fatty acid products containing highly purified eicosapentaenoic acid (EPA). *Lipids Health Dis.* 2017;16(1):23.

29. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis.* 2007;193(1):1-10.

Tables

Table 5.1 Characteristics at screening of subjects randomized into the study (n=154)

	Men (n=48)	Women (n=106)
Age, years	57 ± 12	50 ± 16
BMI, kg/m^2	30 ± 4	29 ± 4
Waist circumference, cm	107 ± 10	98 ± 9
Total C, mmol/L	4.9 ± 0.7	5.5 ± 0.9
LDL-C, mmol/L[1]	3.0 ± 0.7	3.2 ± 0.8
HDL-C, mmol/L	1.3 ± 0.3	1.7 ± 0.4
C/HDL-C ratio	4.0 ± 0.9	3.5 ± 1.0
TG, mmol/L	1.5 ± 0.8	1.4 ± 0.7
n with MetS (%)	25 (12)	17 (19)

Data are presented as means ± SD unless stated otherwise.

[1] n = 47 in men due to a missing value.

BMI: body mass index; C: cholesterol; CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MetS: metabolic syndrome; TG: triglycerides.

Table 5.2 Changes in proportions of fatty acids in red blood cell after EPA and DHA supplementation, vs. control

	Control % of total FA	Change with EPA vs. control[1]	Change with DHA vs. control[1]	P-value EPA vs. DHA[2]
Total SFA	40.13 ± 0.07	+0.16 ± 0.04**	+0.12 ± 0.06*	0.56
Myristic acid, 14:0	0.28 ± 0.01	+0.01 ± 0.01	-0.02 ± 0.01**	<0.001
Palmitic acid, 16:0	21.77 ± 0.10	+0.10 ± 0.05*	+0.22 ± 0.05***	0.038
Stearic acid, 18:0	17.04 ± 0.08	+0.04 ± 0.05	-0.09 ± 0.05*	0.020
Total cis-MUFA	16.27 ± 0.09	-0.20 ± 0.05***	-0.35 ± 0.06***	<0.01
Oleic acid, 18:1, n9	14.92 ± 0.08	-0.11 ± 0.05*	-0.26 ± 0.05***	<0.01
Total trans-MUFA	0.69 ± 0.01	+0.00 ± 0.01	-0.02 ± 0.01*	0.016
Total PUFA n6	33.56 ± 0.13	-5.75 ± 0.14***	-4.54 ± 0.11***	<0.0001
Linoleic acid, 18:2 n6	11.79 ± 0.13	-1.55 ± 0.08***	-0.71 ± 0.08***	<0.0001
Arachidonic acid, 20:4 n6	15.53 ± 0.11	-2.56 ± 0.08***	-2.39 ± 0.08*	0.016
Total PUFA n3	9.36 ± 0.11	+5.79 ± 0.14***	+4.78 ± 0.11***	<.0001
Alpha-linoleic acid, 18:3 n3	0.18 ± 0.00	-0.01 ± 0.00**	-0.01 ± 0.00	0.48
EPA, 20:5 n3	0.89 ± 0.02	+3.93 ± 0.09***	+0.61 ± 0.03***	<0.0001
DPA, 22:5 n3	2.94 ± 0.04	+2.50 ± 0.05***	-0.81 ± 0.04***	<0.0001
DHA, 22:6 n3	5.35 ± 0.09	-0.63 ± 0.09***	+4.99 ± 0.11***	<0.0001
Omega-3 Index	6.23 ± 0.10	+3.30 ± 0.12***	+5.60 ± 0.12***	<0.0001

Data are presented as means ± SEM. Total % does not add up to 100% because not all individual fatty acids are shown in this table.

[1] P values for the EPA and DHA change vs. the control treatment, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models. * P<0.05, ** P<0.01, *** P<0.001. N = 123 for DHA, 121 for EPA, 125 for control.

[2] Main treatment P values for the comparison between EPA and DHA change vs. control, as determined by the main treatment effect in the mixed models. The mixed model for the main effect comparing ΔEPA and ΔDHA is based on n=123 observations, with ΔEPA data excluded for 2 participants, due to low compliance.

Covariates (sex, age, self-reported energy intake and baseline fatty acid proportions in red blood cells) was included in the mixed models only when they were found to be significant at P < 0.05 in the mixed models.

DHA : docosahexaenoic acid; DPA: docosapentaenoic acid; EPA : eicosapentaenoic acid; MUFA: monounsaturated fatty acid; PUFA : polyunsaturated fatty acid; SFA: saturated fatty acid.

Table 5.3 Gene expression after EPA and DHA supplementation, vs. control (N=44)

	Gene expression					
Gene	**Control**	**△EPA vs. control**	**P-value EPA vs. control[1]**	**△DHA vs. control**	**P-value EPA vs. control[1]**	**P-value EPA vs. DHA[2]**
FADS1	445.8 ± 17.0	-15.3 ± 14.7	0.33	-16.6 ± 15.8	0.29	0.92
FADS2[3]	1026.5 ± 114.5	-21.4 ± 46.9	0.36	-5.0 ± 40.5	0.52	0.76
ELOVL2[3]	13.3 ± 1.3	+3.1 ± 1.7	**0.0084**	+2.4 ± 1.8	0.21	0.23
ELOVL5	9701.5 ± 281.3	+491.4 ± 357.4	0.11	+423.6 ± 297.4	0.17	0.85

For each gene, expression is presented as no. of copies of mRNA normalized for the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. Values are expressed as means ± SEM. Bold indicates P<0.01.

[1] P values for the EPA and DHA change vs. the control in the outcome, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models.

[2] Main treatment P values for the comparison between EPA and DHA change vs. the control in the outcome, as determined by the main treatment effect in the mixed models.

[3] Log-transformed data were used in these analyses due to skewness of the distributions of post-treatment values.

Adjustment for potential covariates (sex, age, energy intake and baseline fatty acid proportions) was considered only when these covariates were found to be significant at P < 0.05 in the mixed models.

ELOVL2: ELOVL fatty acid elongase 2; ELOVL5: ELOVL fatty acid elongase 5; FADS1: fatty acids desaturase 2 or delta-5 fatty acids desaturase; FADS2: fatty acids desaturase 2 or delta-6 fatty acids desaturase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

Supplemental Table 5.1 Proportions of fatty acids in RBC at baseline and after control, EPA and DHA phases

		% of total fatty acids			
		Baseline	**Control**	**EPA**	**DHA**
Total SFA		40.17 ± 0.07	40.13 ± 0.07	40.32 ± 0.07	40.27 ± 0.07
	Myristic acid, 14:0	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.27 ± 0.01
	Palmitic acid, 16:0	21.72 ± 0.09	21.77 ± 0.10	21.88 ± 0.10	21.99 ± 0.09
	Stearic acid, 18:0	17.14 ± 0.07	17.04 ± 0.08	17.10 ± 0.08	16.95 ± 0.08
Total cis-MUFA		16.28 ± 0.08	16.27 ± 0.09	16.08 ± 0.09	15.99 ± 0.09
	Oleic acid, 18:1, n9	14.95 ± 0.08	14.92 ± 0.08	14.83 ± 0.08	14.74 ± 0.08
Total trans-MUFA		0.71 ± 0.01	0.69 ± 0.01	0.70 ± 0.01	0.68 ± 0.01
Total PUFA n6		33.46 ± 0.14	33.56 ± 0.13	27.84 ± 0.16	29.09 ± 0.13
	Linoleic acid, 18:2 n6	11.27 ± 0.12	11.79 ± 0.13	10.26 ± 0.11	11.08 ± 0.13
	Arachidonic acid, 20:4 n6	15.81 ± 0.11	15.53 ± 0.11	13.00 ± 0.12	13.16 ± 0.11
Total PUFA n3		9.39 ± 0.12	9.36 ± 0.11	15.05 ± 0.16	13.99 ± 0.14
	Alpha-linoleic acid, 18:3 n3	0.18 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.17 ± 0.00
	EPA, 20:5 n3	0.96 ± 0.03	0.89 ± 0.02	4.75 ± 0.10	1.47 ± 0.03
	Docosapentaenoic acid, 22:5 n3	3.05 ± 0.04	2.94 ± 0.04	5.42 ± 0.06	2.13 ± 0.03
	DHA, 22:6 n3	5.19 ± 0.08	5.35 ± 0.09	4.71 ± 0.07	10.21 ± 0.12
Omega-3 Index		6.15 ± 0.10	6.23 ± 0.10	9.46 ± 0.13	11.69 ± 0.14

Data are presented as means ± SEM. Total % does not add up to 100% because not all individual fatty acids are shown in this table. DHA : docosahexaenoic acid; DPA: docosapentaenoic acid; EPA : eicosapentaenoic acid; MUFA: monounsaturated fatty acid; PUFA : polyunsaturated fatty acid; SFA: saturated fatty acid

Supplemental Table 5.2 Baseline proportions of fatty acids in RBC for men and women (% of total fatty acids)

	Men (n=42)	Women (n=96)
Total SFA	40.29 ± 0.74	40.12 ± 0.91
Myristic acid, 14:0	0.26 ± 0.08	0.28 ± 0.09
Palmitic acid, 16:0	21.51 ± 0.79	21.81 ± 1.08
Stearic acid, 18:0	17.51 ± 0.67	16.98 ± 0.83
Total cis-MUFA	16.51 ± 1.18	16.18 ± 0.87
Oleic acid, 18:1, n9	15.24 ± 1.11	14.82 ± 0.82
Total trans-MUFA	0.70 ± 0.15	0.71 ± 0.11
Total PUFA n6	33.47 ± 1.54	33.46 ± 1.61
Linoleic acid, 18:2 n6	11.32 ± 1.50	11.25 ± 1.32
Arachidonic acid, 20:4 n6	15.64 ± 1.34	15.89 ± 1.26
Total PUFA n3	9.04 ± 1.18	9.54 ± 1.43
Alpha-linoleic acid, 18:3 n3	0.18 ± 0.05	0.18 ± 0.04
EPA, 20:5 n3	0.90 ± 0.22	0.98 ± 0.35
Docosapentaenoic acid, 22:5 n3	3.08 ± 0.40	3.04 ± 0.47
DHA, 22:6 n3	4.87 ± 0.96	5.34 ± 0.96
Omega-3 Index	5.77 ± 0.01	6.32 ± 1.17

Data are presented as means ± SD. Total % does not add up to 100% because not all individual fatty acids are shown in this table. DHA : docosahexaenoic acid; DPA: docosapentaenoic acid; EPA : eicosapentaenoic acid; MUFA: monounsaturated fatty acid; PUFA : polyunsaturated fatty acid; SFA: saturated fatty acid.

Supplemental Table 5.3 Sequence primers and gene descriptions

Gene Symbol	Description	GenBank	Size (pb)	Primer sequence 5'→3' S/AS
FADS1	Homo sapiens fatty acid desaturase 1	NM_013402	140	CACCCAGCTCCAGGCC ACATG/GCACCAGGGGA GCCACTTTGT
FADS2	Homo sapiens fatty acid desaturase 2	NM_004265	234	GCCTTTGTCCTTGCTAC CTCTC/GCCCAGAACAA ACACGTGCAG
ELOVL2	Homo sapiens ELOVL fatty acid elongase 2 (ELOVL2)	NM_017770	135	GGGGCTGTGGATAGGA TTGTTC/AACGGGCAGC CAGATTTGTAC
ELOVL5	Homo sapiens ELOVL fatty acid elongase 5 (ELOVL5)	NM_021814	151	CGCACCGCAGGAGAAT CAGAT/TCGAGGCATGG TGGTAGACGT
GAPDH	Homo sapiens glyceraldehyde-3-phosphate dehydrogenase	NM_002046	194	GGCTCTCCAGAACATCA TCCCT/ACGCCTGCTTCA CCACCTTCTT

ELOVL2: ELOVL fatty acid elongase 2 ; ELOVL5: ELOVL fatty acid elongase 5; FADS1: fatty acids desaturase 2 or delta-5 fatty acids desaturase; FADS2: fatty acids desaturase 2 or delta-6 fatty acids desaturase.

Figures

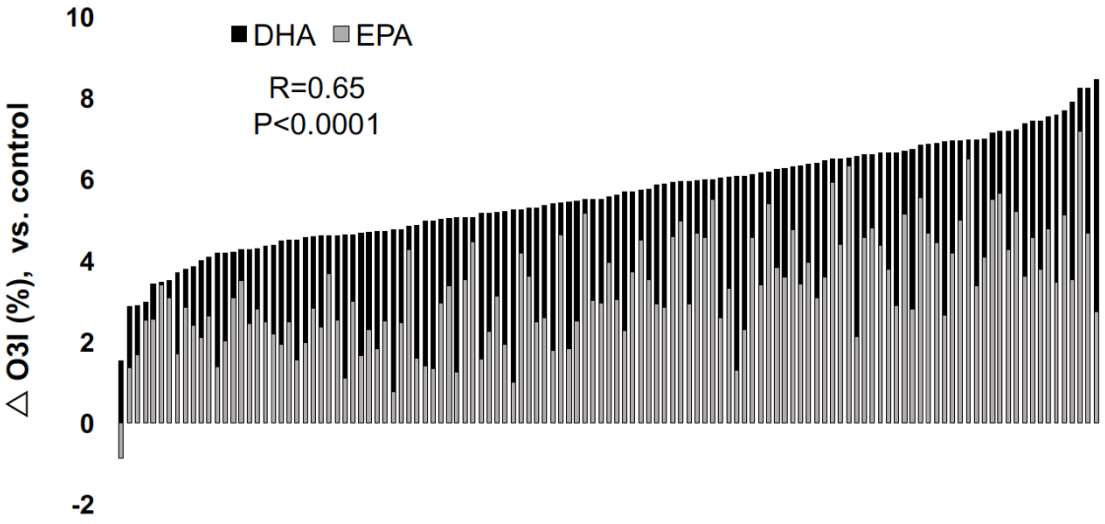

Figure 5.1 Individual variation in changes of the Omega-3 Index (O3I) after EPA and DHA supplementation, vs. control

Each bar represents the change (%) in the O3I after EPA or DHA supplementation for one study participant. Data are sorted to show the range of variation and both EPA (grey) and DHA (black) are presented in the same column for one subject. The O3I increased more with DHA than with EPA in all individuals. Data of two participants were not presented here because having both completed the DHA phase only.

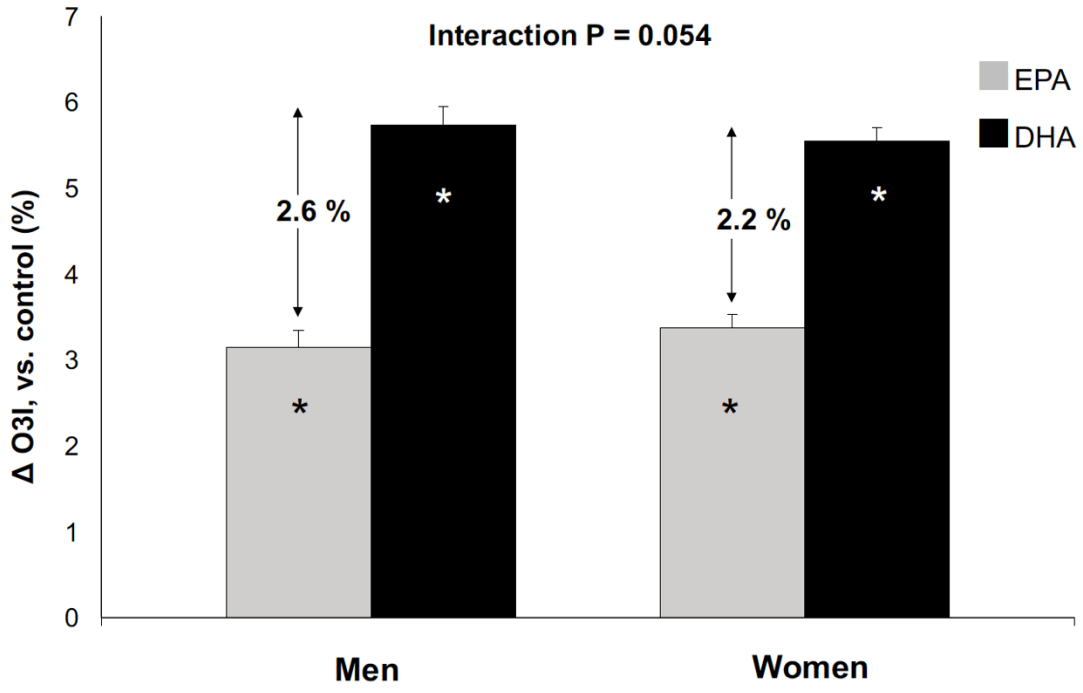

Figure 5.2 Difference in change of Omega-3 Index (O3I) after EPA and DHA supplementation, vs. control in men and women (%)

Values are means ± SEM. * P <0.0001 for the treatment-specific effect vs. control for ΔEPA and ΔDHA. The increase in O3I was higher after DHA than after EPA supplementation for both men and women. We found no difference between men and women response to EPA and DHA supplementation.

Chapitre 6 Comparaison de l'EPA et du DHA sur le métabolisme de l'apo B100, de l'apo CIII des VLDL, sur la taille des LDL et sur PCSK9

Janie Allaire, Cécile Vors, André J Tremblay, Johanne Marin, Amélie Charest, André Tchernof, Patrick Couture, Benoît Lamarche

L'article présenté dans ce chapitre s'intitule: High-dose DHA has more profound effects on LDL-related features than high-dose EPA: The ComparED study

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Résumé

La supplémentation en DHA augmente de façon plus importante les concentrations sériques de C-LDL que l'EPA. Les mécanismes sous-jacents à ces différences sont actuellement inconnus. L'objectif de cette étude était de comparer les effets de l'EPA et du DHA sur les changements phénotypiques des LDL et le métabolisme de l'apolipoprotéine B100 et CIII chez des hommes et des femmes à risque cardiovasculaire. Un total de 48 hommes et 106 femmes ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Chaque phase expérimentale était d'une durée de 10 semaines et les phases étaient séparées par une période libre de 9 semaines. Des études *in vivo* de la cinétique des lipoprotéines avec bolus et une infusion constante d'isotopomère de leucine tri-deutérée (D3-Leucine) ont été menées dans un sous-échantillon de participants (n=19). Comparativement à l'EPA, le DHA a augmenté la taille moyenne des LDL (+0,7 Å, P<0,001) et a diminué la proportion des LDL de petite taille (-3,2%, P<0,01). L'EPA et le DHA ont diminué les concentrations de proprotéine convertase subtilisine/kexine de type 9 de façon similaire (-18,2% vs -25,0% respectivement, P<0,0001 comparativement au témoin). Comparativement à l'EPA, le DHA a augmenté le taux de production (+9,4%, P=0,03) ainsi que taux de catabolisme fractionnel (+11,4%, P=0,008) de l'apo B-100 des LDL. Cette étude a montré qu'une supplémentation de 2,7g par jour de DHA augmente le métabolisme des LDL et contribue à la production de LDL de plus grande taille comparativement à 2,7 g par jour d'EPA. Des études supplémentaires sont nécessaires pour comprendre les mécanismes qui sous-tendent ces effets.

Abstract

Context: Supplementation with high dose of docosahexaenoic acid (DHA) increases serum LDL-cholesterol (C) concentrations more than high dose eicosapentaenoic acid (EPA). Mechanisms underlying this difference are unknown. **Objective**: To examine the phenotypic change in LDL and mechanisms responsible for the differential LDL-C response to EPA and DHA supplementation in men and women at risk for cardiovascular disease. **Design, setting, participants and intervention:** In a double-blind controlled crossover study, 48 men and 106 women with abdominal obesity and subclinical inflammation were randomized to a sequence of three treatment phases: 1- 2.7 g/d of EPA, 2- 2.7 g/d of DHA, and 3- 3 g/d of corn oil. All supplements were provided as 3x1 g capsules for a total of 3 g/d. The 10-week treatment phases were separated by nine-week washouts. **Main outcome measure**: *In vivo* kinetics of apolipoprotein (apo) B-100-containing lipoproteins were assessed using primed-constant infusion of deuterated leucine at the end of each treatment in a subset of participants (n=19). **Results:** Compared with EPA, DHA increased LDL-C concentrations (+3.3%, P=0.038) and mean LDL particle size (+0.7 Å, P<0.001) and reduced the proportion of small LDL (-3.2%, P<0.01). Both EPA and DHA decreased proprotein convertase subtilisin/kexin type 9 concentrations similarly (-18.2% vs. -25.0%, P<0.0001 vs. control). Compared with EPA, DHA supplementation increased both LDL apo B-100 fractional catabolic rate (+11.4%, P=0.008) and production rate (+9.4%, P=0.03). **Conclusions:** This study shows for the first time that supplementation with high-dose DHA increases LDL turnover and contributes to larger LDL particles compared with high-dose EPA.

Title page

High-dose DHA has more profound effects on LDL-related features than high-dose EPA: The ComparED study

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Abbreviations: Apo : apolipoprotein; C: cholesterol; CETP: cholesterylester transfer protein; CRP: C-reactive protein; CVD: cardiovascular disease; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FCR: fractional catabolic rate; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; IDL: intermediate-density lipoprotein; INAF: Institute of Nutrition and Functional Foods; LCn3-PUFA: long-chain omega-3 polyunsaturated fatty acid; LDL: low-density lipoprotein; LPL: lipoprotein lipase; PCSK9: proprotein convertase subtilisin/kexin type 9; PR: production rate; PS: pool size; sdLDL: small and dense low-density lipoprotein; TG: triglycerides; TRL: triglyceride-rich lipoprotein; VLDL: very-low density lipoprotein.

Clinical Trial Registry number and website: NCT01810003 (http://www.clinicaltrials.gov).

Introduction

Despite having favorable effects on serum triglyceride (TG) concentrations, cardiac arrhythmia, platelet aggregation, heart rate, blood pressure and inflammation,[1] the extent to which long-chain omega-3 fatty acid (LCn3-PUFA) supplementation prevents cardiovascular disease (CVD) remains controversial.[2] The inconsistent effect of LCn3-PUFAs supplementation on CVD risk may be due to the fact that docosahexaenoic acid (DHA) and eicosapentaenoic acid are also known to increase LDL-cholesterol (C) concentrations.[1] The extent to which different LCn3-PUFAs have distinct effects on CVD risk also remains questionable as previous studies have shown that DHA is more potent than EPA in modulating cardiovascular risk factors, including raising LDL-C concentrations.[3,4] To our knowledge, no study has yet examined how DHA and EPA influence the mechanisms underlying their differential impact on several LDL features including on LDL-C concentrations, and hence on CVD risk.

It is stressed that the cholesterol content of LDL represents only one of several features of this complex lipoprotein, which is heterogeneous in size, charge and protein content.[5,6] Different immunochemically-defined LDL subclasses are thought to have distinct metabolism and atherogenecity.[6] Specifically, small and dense LDL (sdLDL) consists largely of apolipoprotein (apo) CIII containing lipoproteins that originate from the remodeling in plasma of apo CIII rich VLDL.[7] SdLDL have also been consistently associated with an increased risk of developing CVD, independent of LDL-C concentrations.[8] Data from a limited number of trials have suggested that unlike EPA, high-dose DHA increases LDL particle size.[9] In other studies, LCn3-PUFAs supplementation (4 g/d) in hypertriglyceridemic men had no significant impact on features of the LDL size phenotype, but this may have been due to the fact that DHA and EPA were given in combination.[10,11] Although the clinical relevance of potential differences between DHA and EPA in modulating LDL particle size is unclear, data suggest that both LCn3-PUFA may differentially influence LDL metabolism.

Previous studies have investigated the effect of LCn3-PUFAs-rich diet or supplementation on the kinetic of apo B-100-containing lipoproteins. Ooi *et al*.[12] have shown that a high-fish diet providing 1.23 g/d of EPA and DHA reduced TG-rich lipoprotein (TRL) apo B-100 concentration and production rate (PR) compared with a low-fish diet in elderly men and women with moderate hyperlipidemia. They have also shown that the high-fish diet decreased TRL apo B-100 direct catabolism, rechannelling TRL towards conversion into LDL, hence increasing LDL PR. These data have been reproduced in other studies that have used fish oils as supplements.[13-17] Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates cholesterol metabolism by degrading cellular LDL-receptors, hence blunting the clearance of LDL from the circulation.[18] Interestingly, LCn3-PUFAs have been shown to decrease PCSK9 concentrations in several studies,[19,20] which should in theory be associated with increased LDL clearance and hence, reduced serum LDL-C. To our knowledge, no study has yet compared the

impact of high-dose supplementation with DHA and with EPA on the kinetics of apo B-100-containing lipoproteins and PCSK9 as key determinants of LDL-C concentrations.

The objective of this study was therefore to examine the mechanisms underlying the differential effect of DHA and EPA supplementation on LDL features including LDL-C concentrations in men and women at risk for cardiovascular disease. Specifically, we compared the impact of high doses of DHA and of EPA on the intravascular kinetics of apo B-100-containing lipoproteins, VLDL apo C-III, LDL particle size distribution and PCSK9 levels. We hypothesized that DHA has favorable effects on LDL size features compared to EPA, and that changes in intravascular kinetics of LDL are also different between DHA and EPA, thereby partly explaining the different effect of the two LCn3-PUFA on serum LDL-C concentrations.

Material and Methods

Study design: Details of the study design have been previously published.[4] Briefly, this study used a double-blind randomized, controlled crossover design with three treatment phases: 1- DHA, 2- EPA, 3- corn oil as control. Each treatment phase had a median duration of 10 weeks separated by nine-week washouts. Participants were randomized to one of six treatment sequences and were supplemented with three identical 1 g capsules of >90% purified LCn3-PUFA per day providing either 2.7 g/d DHA or 2.7 g/d EPA. Corn oil was used as a control (0 g/d DHA+EPA). LCn3-PUFA supplements were formulated as re-esterified TG and provided by Douglas Laboratories. Participants were instructed to maintain a constant body weight during the course of the study and were counseled on how to exclude fatty fish (including salmon, tuna, mackerel, and herring), other LCn3-PUFA supplements, flax products, walnuts, and LCn3-PUFA-enriched products during the three study phases. The primary outcome of the study was the change in C-reactive protein (CRP) with DHA and EPA.[4] All participants signed an informed consent document approved by local Ethics Committees at the beginning of the study and the study protocol was registered at ClinicalTrials.gov (NCT01810003) on March 4th, 2013.

Study population: Primary eligibility criteria were to have abdominal obesity based on the International Diabetes Federation sex specific cut-offs (≥80 cm for women, ≥94 cm for men)[21] in combination with a screening plasma CRP concentration >1 mg/L but <10 mg/L. Subjects had to be otherwise healthy. Adult subjects (18 and 70 years of age) were recruited at the Institute of Nutrition and Functional Foods (INAF). Body weight had to be stable for at least three months prior to randomization. Exclusion criteria were plasma CRP >10 mg/L at screening, extreme dyslipidemias such as familial hypercholesterolemia, having a personal history of CVD (coronary heart disease, cerebrovascular disease or peripheral arterial disease), taking medications or substances known to affect inflammation (e.g. steroids, binging alcohol), and use of LCn3-PUFA supplements within two months of study onset. Individuals on lipid-lowering drugs for more than 1 month were eligible.

Anthropometry*:* Anthropometric measures including waist and hip circumferences were measured according to standardized procedures before and after each study phase.[22] Body weight was measured before each kinetic protocol.

Compliance*:* Compliance to supplementation was assessed by counting supplements that were returned to study coordinators by participants.[4] DHA and EPA content in red blood cells was also used as another proxy of compliance in all participants.[23]

Laboratory Analyses: Blood samples were collected after a 12-h overnight fast on two consecutive days at the end of each treatment phase. The mean of the two measurements were used in the analyses for LDL features and blood glucose. Total apo B100, apo CIII and PCSK9 and insulin concentrations were measured once after

each treatment phase. Serum Total apo B100, apo CIII and PCSK9 concentrations were measured using the commercial ELISA kits from Alerchek Inc. (#A70102, Springvale, ME, USA), Assaypro LLC (#EA8133-1, St-Charles, MO, USA) and CircuLex (#CY-8079, Nagano, Japan) respectively. Serum LDL-C concentrations were calculated using the Friedewald Equation. Non-denaturing 2%-16% polyacrylamide gradient gel electrophoresis was used to characterize various features of the LDL particle size phenotype,[24] including LDL peak particle size and mean LDL particle size as well as the proportion of LDL in the various size categories. Fasting blood glucose levels were measured by colorimetry and insulin concentrations by electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA). Finally, homeostatic model assessment of insulin resistance (HOMA-IR) was measured using the formula developed by Matthews *et al.*[25] All personnel involved in the measurements of the study outcomes were blinded to treatments.

Experimental protocol for In Vivo Stable Isotope Kinetics: Kinetic studies using primed-constant infusion of deuterated leucine were performed at the end of each treatment in a sub-sample of participants. Participants in the kinetic studies were recruited as part of the general recruitment process in the project, until an N of 20 was reached. The participants underwent a primed-constant infusion of L-[5,5,5-D_3] leucine while kept in a constant fed state to determine the kinetics of apo B100. Starting at 07:00h, the subjects received one small standardized snack every half hour for 15h, each containing 1/30th of their estimate daily food intake, based on the Harris-Benedict equation[26], with 15% of calories from proteins, 45% from carbohydrates and 40% from fat. Snacks were the same for each treatment. At 10:00h, with two intravenous lines in place (one for the infusate and one for blood sampling), L-[5,5,5-D_3] leucine (10 μmol/kg body weight) was injected as an intravenous bolus and then by continuous infusion (10 μmol/kg body weight per hour) over a 12-h period. Blood samples (24 ml) were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 11 and 12 h. See online data supplements for sample processing, laboratory measurements, analysis of lipoprotein PR and fractional catabolic rate (FCR).

Statistical analyses*:* Differences between treatments were assessed using the MIXED procedure for repeated measures, with treatment as fixed effect and a compound symmetry matrix to account for within-subject correlations (SAS, v9.4, Cary, NC). The change versus the control treatment (post treatment DHA minus control and EPA minus control) was used as the dependent variable in all analyses. The main treatment effect in the mixed models reflected the direct comparison of DHA and EPA, and was considered the primary analysis. Adjustment for multiple comparisons was not necessary as the main treatment effect had only two levels (DHA and EPA). In the same model and as secondary analyses, the change vs. control for each treatment was tested against the null hypothesis by the LSMEANS statement. The skewness in the distribution of model residuals was considered and data were log-transformed when required. Wilcoxon signed-rank tests were also performed to test for the difference in the change from control after DHA and EPA supplementation with similar results as generated by the mixed models (data not shown). Spearman's coefficient correlations among changes in apo

B100- and apo CIII-containing lipoprotein kinetic parameters, PCSK9 levels, LDL-C, LDL-apo B100 and LDL size were computed.

Results

Data from one participant who was sick during the first kinetic study test were excluded from analyses. Baseline characteristics of the 19 participants who completed at least one kinetic sub-study and of the participants of the whole sample who completed at least one study phase are presented in **Table 1**. Characteristics of the sub-sample were similar to those of the whole group, with the exception that there were proportionally more females in the kinetic sub-study. Among participants of the kinetic sub-study, one participant completed only one phase and two participants completed two study phases. The mean compliance based on the returned capsules was > 95% for all study phases (not shown). Among the 154 participants randomized to treatment sequences, 12 participants were taking statins. None were taking other lipid lowering drugs. Pharmacotherapy remained unchanged in all participants throughout the study in this crossover trial. None of the kinetic sub-study participants were taking lipid-lowering drugs. Three subjects had type 2 diabetes and one subject had type 1 diabetes among all participants, but none of those were included in this sub-study.

LDL particle size and PCSK9

Blood lipids and LDL particle size features before the control phase and after the three treatments are presented in **Table 2** for all participants as well as for the sub-study group only. Treatment-specific baseline values before DHA and EPA were essentially identical to values measured before the control treatment and therefore are not presented. In all participants, DHA increased mean LDL particle size more than EPA (compared with control, DHA: +0.32 Å, EPA: -0.41 Å; DHA vs. EPA: P<0.0001) as well as LDL peak particle size (compared with control, DHA: +0.58 Å, EPA: -0.32 Å; DHA vs. EPA: P<0.0001). The change in the proportion of sdLDL was also significantly different between EPA and DHA (compared with control, DHA: -1.10%, EPA: +2.10%; EPA vs. DHA: P<0.002). Both EPA and DHA decreased PCSK9 concentrations similarly (compared with control, DHA: -25.0 ng/mL, EPA: -18.2 ng/mL; DHA vs. EPA: P=0.19). Changes in these cardiometabolic outcomes with DHA and EPA were generally similar in direction and magnitude among participants of the sub-study. However, only the difference between the change in PCSK9 concentrations after DHA and EPA compared to control remained statistically significant in the sub-sample.

Kinetic studies

Both DHA and EPA tended to increase VLDL-apo B100 FCR similarly compared with control (DHA: +21%, EPA: +19%; DHA vs. EPA: P=0.73, **Table 3**). However, EPA tended to increase VLDL apo B100 direct catabolism more than DHA (compared with control, DHA: -3%, EPA: +22%; DHA vs. EPA: P=0.10), while changes in VLDL to LDL apo B100 conversion rates were similar after DHA and EPA (compared with control, DHA: +8%, EPA: +7%; DHA vs. EPA: P=0.44). LDL apo B100 FCR was significantly lower after EPA supplementation than after

DHA (compared with control, DHA: 0%, EPA: -10%; DHA vs. EPA: P=0.008). On the other hand, DHA increased LDL apo B100 PR compared with EPA (compared with control, DHA: +2%, EPA: -7%; DHA vs. EPA: P=0.027).

As shown in **Table 4**, the increase in LDL-C concentrations after DHA or EPA did not correlate with variations in LDL apo B100 FCR or PR. On the other hand, variations in LDL apo B100 pool size correlated with change in LDL apo B100 PR after EPA (rs=0.63, P=0.013) and with variations in LDL apo B100 FCR after DHA (rs=-0.52, P=0.04) and PCSK9 concentration after DHA (rs=0.64, P<0.01).

Both DHA and EPA decreased VLDL apo CIII FCR similarly compared with control (DHA: -11%, EPA: -7%, DHA vs. EPA: P=0.60) while the reduction in VLDL apo CIII PR tended to be greater after DHA than after EPA (compared with control, DHA: -12%, EPA: +1%, DHA vs. EPA: P=0.09). The change in VLDL apo CIII FCR was inversely correlated with the change in PCSK9 concentration after EPA (rs=-0.58, P=0.019, not shown), and with the change in LDL apo B100 FCR after DHA (rs=0.54, P=0.030, not shown). The change in VLDL apo CIII PR was inversely correlated with LDL apo B100 FCR and PR (rs=-0.57 and -0.53, P<0.05, not shown) and positively associated with the change in PCSK9 concentrations after DHA (rs=0.54, P=0.021, not shown). We did not find any correlation between changes in TG and LDL-C and between changes in VLDL apo CIII FCR and LDL apo B100 PR after DHA nor EPA (not shown).

Discussion

To the best of our knowledge, this is the first study demonstrating the mechanisms underlying the differential effects of DHA and EPA supplementation on LDL-C and other features of LDL in men and women with abdominal obesity, subclinical inflammation and at risk for CVD. The present study suggests that high-dose DHA increases LDL particle size and modifies LDL apo B100 and VLDL apo CIII kinetics compared with EPA. Although DHA and EPA reduce PCSK9 concentration similarly, the relationships between PCSK9, LDL-C and LDL apo B100 concentrations were different between DHA and EPA.

We have previously shown that the magnitude of the reduction in TG and increase in LDL-C after DHA was greater than after EPA supplementation.[4] Previous *in vivo* kinetic studies have documented the effects of LCn3-PUFAs, either as a dietary supplement or as part of a LCn3-PUFAs-rich diet, on apo B100-containing lipoprotein metabolism.[27,28] Those studies have shown that LCn3-PUFAs reduce TG concentrations primarily by reducing the endogenous production of VLDL apo B100 and by increasing the VLDL to LDL apo B100 conversion rate.[27,28] LCn3-PUFAs have also been shown to increase the clearance of LDL apo B100.[27,28] On the other hand, Ooi *et al.*[12] found that a high-fish diet (containing 1.23 g EPA+DHA/d) increases LDL apo B100 production by 32% and concomitantly decreases LDL apo B100 clearance by 44% compared with untreated baseline values. This disproportionate reduction in LDL apo B100 clearance may partly explain why LDL-C concentrations increase after LCn3-PUFAs supplementation. Because the changes in LDL-C and TG concentrations are greater with DHA supplementation than after EPA supplementation,[4] we hypothesized that DHA compared with EPA induces a greater reduction in VLDL apo B100 production and a greater VLDL to LDL apo B100 conversion rate, hence resulting in a greater increase in LDL apo B100 production. Accordingly, DHA compared with EPA differentially influenced LDL apo B100 production and clearance rates, but these differences were not related to the differential effects of DHA and EPA on LDL-C concentrations. DHA and EPA equally increased VLDL to LDL apo B100 conversion and VLDL apo B100 FCR. These data suggest that metabolic pathways not involving apo B100 *per se* may be responsible for the differential effects of DHA and EPA on LDL-C concentrations and LDL size. It is possible that DHA and EPA differentially influence apo B:C:E ratios on VLDL, which may in turn contribute to differences in LDL-C concentrations and LDL size seen between DHA and EPA.[6,7] Zheng *et al.*[7] have shown that apo CIII containing VLDL are the major precursor of LDL particles. Hence, the suppression of apo CIII PR with DHA may also explain to some extent its impact on LDL particle size. The fact that total VLDL particles were converted more rapidly to IDL after DHA than after EPA (**Table 3**), and that VLDL-apo CIII levels also tended to decrease with DHA compared with EPA, is consistent with this hypothesis.

We also hypothesized that DHA and EPA supplementation modulates LDL particle size differently because the increase in LDL-C concentration after DHA was almost 2-fold greater in magnitude than the increase in total apo B concentration.[4] Accordingly, DHA supplementation slightly increased mean LDL particle size and decreased

the proportion of sdLDL compared with EPA supplementation. This observation is consistent with data from a few studies, which have shown that DHA, but not EPA, is associated with larger LDL.[9,29] This increase in LDL particle size after DHA may be attributed, at least partly, to the greater reduction in serum TG compared with EPA. Indeed, serum TG is an important metabolic determinant of the sdLDL phenotype through a series of metabolic transformation of the LDL particles that involve lipases and cholesteryl ester transfer protein (CETP).[30] However, very few studies have compared the effect of DHA and EPA on enzyme activities. Supplementation with LCn3-PUFA has been shown to have inconsistent effects on CETP activity[31] and may increase lipoprotein lipase (LPL) activity through upregulation of it expression[31] but may have no impact on hepatic lipase activity.[31] More studies investigating these pathways in response to DHA and EPA supplementations are needed.

The increase in LDL size with DHA compared with EPA may also be explained in part by a decrease in apo CIII secretion from the liver.[32] DHA may reduce the apo C-III production through the regulation of the fork-head box O transcription factor (FOX)O1 and carbohydrate response element-binding protein.[33,34] Apo CIII inhibits the binding of apo B to hepatic apo B/E receptor and the lipoprotein lipase activity.[32,35] In a small parallel study, supplementation with EPA alone tended to increase apo CIII concentrations while DHA tended to decrease apo CIII-containing lipoprotein concentrations.[36] Consistent with this, we have shown that high dose EPA also tended to increase VLDL apo CIII mass while DHA tended to decrease VLDL apo CIII mass in comparison with control, although differences did not reach statistical significance. DHA also tended to decrease VLDL apo CIII PR compared with EPA, but the difference also did not reach the statistical significance. Changes in VLDL apo CIII metabolism were correlated with changes in LDL apo B100 metabolism after DHA, but not after EPA, supporting a differential effect of EPA and DHA on VLDL apo CIII and apo B100 metabolism. This apparent reduction in apo CIII production in the liver after DHA supplementation may explain the enhanced conversion of VLDL to LDL apo B100, as well as the formation of larger LDL particles compared with EPA. Individuals with a preponderance of sdLDL have consistently been shown to be at increased risk of myocardial infarction and CVD compared with individuals with a greater proportion of larger LDL particles,[37] but the extent to which the opposite effects of DHA on both LDL-C and LDL particle size modify CVD risk is unknown.

The reduction in PCSK9 concentrations observed after DHA and EPA supplementation is consistent with data from the few available studies on this topic. A recent randomized controlled parallel study in 92 pre- and post-menopausal women has shown that supplementation with 2.2 g/d of marine oil decreased plasma PCSK9 concentrations by 11.4% in premenopausal and by 9.8% in post-menopausal women compared with baseline.[19] Post-hoc analyses of The Canola Oil Multicenter Intervention Trial have also shown that PCSK9 concentration was lower after DHA-enriched canola oil than after regular canola oil supplementation.[20] In the present study, DHA and EPA both reduced serum PCSK9 levels equally compared with control. Although PCSK9 concentrations usually correlate with LDL-C concentrations, variations in PCSK9 levels explain less than 8% of

the LDL-C variance.[38] Furthermore, PCSK9 concentrations may not fully reflect PCSK9 activity.[38] Therefore, the increase in LDL-C after DHA and EPA despite a decrease in PCSK9 concentrations is not entirely unexpected. On the other hand, changes in PCSK9 was positively correlated with changes in LDL apo B100 concentrations and negatively correlated with changes in LDL apo B100 FCR after DHA, but not after EPA, hence suggesting that PCSK9 may be partly involved in explaining the differential effects of DHA and EPA supplementation on the metabolic fate of the LDL particle.

This study has several strengths and limitations. A number of studies have examined the effect of LCn3-PUFAs-rich diet or as a supplement combining EPA and DHA in various forms and proportions on apo B100-containing lipoprotein kinetics.[27,28] To the best of our knowledge, this is the first study to compare head-to-head the effect of high-dose EPA and DHA on apo B100-containing lipoprotein kinetics. The use of a randomized crossover study design reduced the inter-individual variability of the results. Baseline characteristics of the kinetic subsample were similar to the whole study cohort and the compliance was high in all phases of the study.[4] The analyses of the change in blood lipids, LDL particle size and PCSK9 concentrations in the sub-study kinetic sample were conducted in fewer participants, hence influencing statistical power. Estimates of small kinetic pool sizes have relatively high coefficients of variations and small changes in kinetic parameters are difficult to assess. The observed effects of DHA and EPA on serum lipids, including LDL-C, may have resulted from changes in kinetics that may have been too subtle to be detected with this sample size. Corn oil was chosen as a control because of the relatively neutral effects of n6-PUFA on inflammation makers,[39] which were the primary outcome of the trial.[4] Supplementation with the control n6-PUFA rich corn oil decreased total cholesterol (-0.12 mmol/L, P=0.001), LDL-C (-0.13 mmol/L, P=0.003) and mean LDL size (-0.22 Å, P=0.02) compared with control-specific baseline levels. However, results were similar when change from DHA/EPA-specific baseline values were considered. Specifically, the increase in LDL-C and the reduction in TG with DHA compared to baseline values were significantly greater than those seen with EPA (for LDL-C +0.11 vs. 0.00 mmol/L, for TG vs. -0.26 vs. -0.20 mmol/l, all P<0.01). The increase from treatment-specific baseline values in LDL particle size (+0.21 Å vs. -0.71 Å) and the reduction in the proportion of sdLDL (-1.36 % vs. +2.8 %) were also greater with DHA than with EPA (all P<0.01, not shown). Because very few studies have documented the impact of n6-PUFA on apo B100- and apo CIII-containing lipoprotein metabolism, it is difficult to assess how the use of corn oil as the control treatment has impacted the kinetic study data.[27,28,40]

In conclusion, the differential effects of DHA and EPA supplementation on LDL-C concentrations may not be accounted for by differences in the regulation of apo B100 containing lipoprotein metabolism, and may involve other pathways that influence LDL particle size. The extent to which the greater increase in LDL-C with DHA compared with EPA, associated with larger LDL particles, influences CVD risk is unknown. Further studies are

needed to better understand changes in other metabolic factors following EPA and DHA supplementation, including the expression of different genes involved in lipid metabolism.

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Conflicts of interest

BL is Chair of Nutrition at Laval University, which is supported by private endowments from Pfizer, La Banque Royale du Canada and Provigo-Loblaws. BL has received funding in the last 5 years from the Canadian Institutes for Health Research, the Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada (Growing Forward program supported by the Dairy Farmers of Canada (DFC), Canola Council of Canada, Flax Council of Canada, Dow Agrosciences), Dairy Research Institute, Dairy Australia, Merck & Co, Inc., Pfizer and Atrium Innovations for which Douglas Laboratories manufacture and market omega-3 supplements. BL is an Advisory Board member of the Canadian Nutrition Society, the Conseil pour les initiatives de progrès en alimentation and has served as Advisory Expert for the Saturated Fat panel of Heart and Stroke Foundation of Canada. BL has received honoraria from the International Chair on Cardiometabolic risk, DFC and the World Dairy Platform as invited speaker in various conferences.

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References

1. Bradberry JC, Hilleman DE. Overview of omega-3 Fatty Acid therapies. *P T.* 2013;38(11):681-691.

2. Hooper L, Thompson RL, Harrison RA, Summerbell CD, Moore H, Worthington HV, Durrington PN, Ness AR, Capps NE, Davey Smith G, Riemersma RA, Ebrahim SB. Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database Syst Rev.* 2004(4):Cd003177.

3. Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. *Curr Atheroscler Rep.* 2011;13(6):474-483.

4. Allaire J, Couture P, Leclerc M, Charest A, Marin J, Lepine MC, Talbot D, Tchernof A, Lamarche B. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study. *Am J Clin Nutr.* 2016;104(2):280-287.

5. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res.* 2002;43(9):1363-1379.

6. Alaupovic P. The concept of apolipoprotein-defined lipoprotein families and its clinical significance. *Curr Atheroscler Rep.* 2003;5(6):459-467.

7. Zheng C, Khoo C, Ikewaki K, Sacks FM. Rapid turnover of apolipoprotein C-III-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions. *J Lipid Res.* 2007;48(5):1190-1203.

8. Musunuru K, Orho-Melander M, Caulfield MP, Li S, Salameh WA, Reitz RE, Berglund G, Hedblad B, Engstrom G, Williams PT, Kathiresan S, Melander O, Krauss RM. Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. *Arterioscler Thromb Vasc Biol.* 2009;29(11):1975-1980.

9. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, Beilin LJ. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr.* 2000;71(5):1085-1094.

10. Oelrich B, Dewell A, Gardner CD. Effect of fish oil supplementation on serum triglycerides, LDL cholesterol and LDL subfractions in hypertriglyceridemic adults. *Nutr Metab Cardiovasc Dis.* 2013;23(4):350-357.

11. Thomas TR, Smith BK, Donahue OM, Altena TS, James-Kracke M, Sun GY. Effects of omega-3 fatty acid supplementation and exercise on low-density lipoprotein and high-density lipoprotein subfractions. *Metabolism.* 2004;53(6):749-754.

12. Ooi EM, Lichtenstein AH, Millar JS, Diffenderfer MR, Lamon-Fava S, Rasmussen H, Welty FK, Barrett PH, Schaefer EJ. Effects of Therapeutic Lifestyle Change diets high and low in dietary fish-derived FAs on lipoprotein metabolism in middle-aged and elderly subjects. *J Lipid Res.* 2012;53(9):1958-1967.

13. Nestel PJ, Connor WE, Reardon MF, Connor S, Wong S, Boston R. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J Clin Invest.* 1984;74(1):82-89.

14. Bordin P, Bodamer OA, Venkatesan S, Gray RM, Bannister PA, Halliday D. Effects of fish oil supplementation on apolipoprotein B100 production and lipoprotein metabolism in normolipidaemic males. *Eur J Clin Nutr.* 1998;52(2):104-109.

15. Huff MW, Telford DE. Dietary fish oil increases conversion of very low density lipoprotein apoprotein B to low density lipoprotein. *Arteriosclerosis.* 1989;9(1):58-66.

16. Fisher WR, Zech LA, Stacpoole PW. Apolipoprotein B metabolism in hypertriglyceridemic diabetic patients administered either a fish oil- or vegetable oil-enriched diet. *J Lipid Res.* 1998;39(2):388-401.

17. Chan DC, Watts GF, Mori TA, Barrett PH, Redgrave TG, Beilin LJ. Randomized controlled trial of the effect of n-3 fatty acid supplementation on the metabolism of apolipoprotein B-100 and chylomicron remnants in men with visceral obesity. *Am J Clin Nutr.* 2003;77(2):300-307.

18. Tavori H, Rashid S, Fazio S. On the function and homeostasis of PCSK9: reciprocal interaction with LDLR and additional lipid effects. *Atherosclerosis.* 2015;238(2):264-270.

19. Graversen CB, Lundbye-Christensen S, Thomsen B, Christensen JH, Schmidt EB. Marine n-3 polyunsaturated fatty acids lower plasma proprotein convertase subtilisin kexin type 9 levels in pre- and postmenopausal women: A randomised study. *Vascul Pharmacol.* 2016;76:37-41.

20. Rodríguez-Pérez C, Ramprasath VR, Pu S, Sabra A, Quirantes-Piné R, Segura-Carretero A, Jones PJ. Docosahexaenoic acid attenuates cardiovascular risk factors via a decline in proprotein convertase subtilisin/kexin type 9 (PCSK9) plasma levels. *Lipids.* 2016;51(1):75-83.

21. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. *Lancet.* 2005;366(9491):1059-1062.

22. Airlie, Lohman T, Roche A, Martorel R. Standardization of anthropometric measurements. *The Airlie (VA) Concensus Conference*. Champaign, Ill: Human Kinetics; 1988:39-80.

23. Allaire J, Harris W, Vors C, Tchernof A, Couture P, Lamarche B. Docosahexaenoic Acid is More Effective than Eicosapentaenoic Acid in Increasing the Omega-3 Index Measured in Red Blood Cell Membranes. *The FASEB Journal.* 2017;31(1 Supplement):146.143-146.143.

24. St-Pierre A, Ruel I, Cantin B, Dagenais G, Bernard P-M, Després J-P, Lamarche B. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. *Circulation.* 2001;104(19):2295-2299.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.

26. Harris JA, Benedict FG. *A biometric study of basal metabolism in man.* Carnegie institution of Washington; 1919.

27. Lamarche B, Couture P. Dietary fatty acids, dietary patterns, and lipoprotein metabolism. *Curr Opin Lipidol.* 2015;26(1):42-47.

28. Ooi EM, Watts GF, Ng TW, Barrett PH. Effect of dietary Fatty acids on human lipoprotein metabolism: a comprehensive update. *Nutrients.* 2015;7(6):4416-4425.

29. Kelley DS, Siegel D, Vemuri M, Mackey BE. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr.* 2007;86(2):324-333.

30. Lagrost L, Gandjini H, Athias A, Guyard-Dangremont V, Lallemant C, Gambert P. Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects. *Arterioscler Thromb.* 1993;13(6):815-825.

31. Oscarsson J, Hurt-Camejo E. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. *Lipids Health Dis.* 2017;16(1):149.

32. Kawakami A, Yoshida M. Apolipoprotein CIII links dyslipidemia with atherosclerosis. *J Atheroscler Thromb.* 2009;16(1):6-11.

33. Jump DB, Botolin D, Wang Y, Xu J, Demeure O, Christian B. Docosahexaenoic acid (DHA) and hepatic gene transcription. *Chem Phys Lipids.* 2008;153(1):3-13.

34. Chen YJ, Chen CC, Li TK, Wang PH, Liu LR, Chang FY, Wang YC, Yu YH, Lin SP, Mersmann HJ, Ding ST. Docosahexaenoic acid suppresses the expression of FoxO and its target genes. *J Nutr Biochem.* 2012;23(12):1609-1616.

35. Homma Y, Ohshima K, Yamaguchi H, Nakamura H, Araki G, Goto Y. Effects of eicosapentaenoic acid on plasma lipoprotein subfractions and activities of lecithin:cholesterol acyltransferase and lipid transfer protein. *Atherosclerosis.* 1991;91(1-2):145-153.

36. Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br J Nutr.* 2004;92(3):477-483.

37. Hirayama S, Miida T. Small dense LDL: An emerging risk factor for cardiovascular disease. *Clin Chim Acta.* 2012;414:215-224.

38. Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab.* 2009;94(7):2537-2543.

39. Lee TC, Ivester P, Hester AG, Sergeant S, Case LD, Morgan T, Kouba EO, Chilton FH. The impact of polyunsaturated fatty acid-based dietary supplements on disease biomarkers in a metabolic syndrome/diabetes population. *Lipids Health Dis.* 2014;13:196.

40. Ooi EM, Ng TW, Watts GF, Barrett PHR. Dietary fatty acids and lipoprotein metabolism: new insights and updates. *Current opinion in lipidology.* 2013;24(3):192-197.

Tables

Table 6.1 Baseline characteristics of the kinetic sub-sample and the whole cohort

	Sub-sample (n=19)	Whole cohort (n=138)
Age, years	47 ± 16	52 ± 15
Female, n (%)	9 (47)	96 (70)
Weight, kg	84.0 ± 13.8	80.6 ± 14.5
BMI, kg/m^2	29.7 ± 4.8	29.4 ± 4.3
Waist circumference, cm	102.5 ± 9.9	100.7 ± 11.1
SBP, mmHg	115.2 ± 8.5	115.6 ± 12.8
DBP, mmHg	68.0 ± 8.1	69.7 ± 8.9
Total C, mmol/L *	5.08 ± 0.64	5.18 ± 0.93
LDL-C, mmol/L *	3.05 ± 0.55	3.03 ± 0.81
HDL-C, mmol/L *	1.43 ± 0.43	1.52 ± 0.42
TG, mmol/L *	1.30 ± 0.50	1.36 ± 0.58
CRP, mg/L	3.35 ± 2.71	3.32 ± 2.44
Glucose, mmol/L *	5.26 ± 0.37	5.28 ± 0.85
Insulin, pmol/L	93.4 ± 39.9	102.54 ± 58.61
HOMA-IR *	3.11 ± 1.27	3.55 ± 2.57

Data are presented as means ± SD. CRP values > 10 were excluded (n= 5 for the whole cohort). For the whole cohort, baseline characteristics of participants who completed at least one study phase are presented.

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure, C: cholesterol; TG: triglyceride; CRP: C-reactive protein; HOMA-IR: homeostatic model assessment of insulin resistance

*N = 137 for the whole cohort.

Table 6.2 LDL particle size features and PCSK9 concentration before control and after control, DHA and EPA phase in whole and kinetic sample

	Pre-control	Post-control	Post-DHA	% diff. DHA vs. control	P-value DHA vs. control*	Post-EPA	% diff. EPA vs. control	P-value EPA vs. control*	% diff DHA vs. △EPA	P-value DHA vs. EPA†
	All participants N=121a									
Total cholesterol, mmol/L ‡, §	5.29 ± 0.08	5.16 ± 0.08	5.33 ± 0.08	3.1	**0.001**	5.15 ± 0.08	-0.4	0.62	3.6	**<0.001**
LDL-C, mmol/L ‡, §	3.12 ± 0.07	2.99 ± 0.07	3.16 ± 0.08	5.4	**<0.001**	3.06 ± 0.07	2.5	0.046	3.2	**0.04**
Total apo B, g/L ‡, §	1.34 ± 0.04	1.31 ± 0.04	1.34 ± 0.04	2.8	**0.02**	1.32 ± 0.04	1.2	0.46	1.6	0.16
Triglycerides, mmol/L‡, §	1.34 ± 0.06	1.38 ± 0.06	1.14 ± 0.04	-21.3	**<0.001**	1.23 ± 0.05	-11.3	<0.001	-7.1	**0.005**
Mean LDL size, Å	252.10 ± 0.26	251.89 ± 0.21	252.15 ± 0.23	0.1	0.051	251.41 ± 0.20	-0.2	0.01	0.3	**<0.001**
LDL peak, Å	251.74 ± 0.28	251.56 ± 0.24	252.08 ± 0.25	0.2	**0.001**	251.19 ± 0.23	-0.1	0.059	0.4	**<0.001**
Proportion of large LDL, %	9.49 ± 0.72	9.00 ± 0.53	9.16 ± 0.62	1.8	0.85	8.42 ± 0.53	-6.4	0.20	8.8	0.19
Proportion of small LDL, %	69.29 ± 1.45	69.83 ± 1.20	68.88 ± 1.34	-1.4	0.25	72.00 ± 1.08	3.1	0.03	-4.3	**0.002**
PCSK9, ng/mL	NA	213.02 ± 5.65	189.12 ± 4.89	-12.6	**<0.001**	194.98 ± 5.40	-8.5	<0.001	-3.0	0.19
	Sub-study N=19b									
Total cholesterol, mmol/L	5.18 ± 0.16	4.70 ± 0.16	5.13 ± 0.14	9.1	**0.005**	5.01 ± 0.19	8.0	0.018	2.5	0.62
LDL-C, mmol/L	3.11 ± 0.14	2.70 ± 0.13	3.10 ± 0.15	14.7	**0.006**	3.05 ± 0.14	14.4	0.010	1.6	0.88
Total apo B, g/L	1.37 ± 0.09	1.26 ± 0.08	1.40 ± 0.12	11.0	**0.03**	1.35 ± 0.09	6.9	0.27	3.5	0.27
Triglycerides, mmol/L	1.29 ± 0.13	1.35 ± 0.17	1.20 ± 0.15	-11.1	0.10	1.39 ± 0.21	4.6	0.63	-13.6	0.06
Mean LDL size, Å	250.88 ± 0.75	250.95 ± 0.54	250.95 ± 0.63	0.0	0.90	250.42 ± 0.60	-0.2	0.29	0.2	0.33
LDL peak, Å	250.62 ± 0.76	250.68 ± 0.63	250.79 ± 0.71	0.0	0.89	250.20 ± 0.61	-0.2	0.12	0.2	0.17
Proportion of large LDL, %	7.52 ± 1.38	6.90 ± 1.07	7.01 ± 1.35	1.5	0.34	7.42 ± 1.12	4.2	0.12	-5.6	0.54
Proportion of small LDL, %	75.03 ± 3.16	76.13 ± 2.32	75.53 ± 2.88	-0.8	0.56	75.98 ± 2.55	0.0	0.72	-0.6	0.86
PCSK9, ng/mL	NA	196.92 ± 15.23	174.43 ± 13.53	-11.4	0.11	190.08 ± 15.33	1.2	0.75	-8.2	**0.04**

Values are presented as unadjusted means ± SEM. Bold indicates P<0.05. Because pre-DHA and -EPA values were essentially identical to the pre-control values, only pre-control values are presented.

a = Participants equaled 123 for DHA, 121 for EPA, 125 for control, 138 for baseline

b = Participants equaled 19 for DHA, 17 for EPA and 19 for control.

* P values for the EPA and DHA changes vs control, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models.

† This analysis compared the change with DHA or EPA compared with control based on post-treatment values. And the P values were taken from the main treatment effect in the mixed models.

‡ Log-transformed data were used in these analyses due to skewness of the distributions of values

§. Previously reported [4].

Apo: apolipoprotein; C: cholesterol; DHA:docosahexaenoic acid ; EPA: eicosapentaenoic acid ; LDL: low-density lipoprotein; NA: not available; PCSK9: proprotein convertase subtilisin/kexin type 9.

Table 6.3 Apo B100-containing lipoproteins and VLDL apo CIII kinetics after control, DHA and EPA phases

	Post-control	Post-DHA	% vs. Control	P-value △DHA *	Post-EPA	% vs. Control	P-value △EPA*	DHA vs. EPA (%)	P-value △DHA vs. △EPA†
VLDL apo B100									
PS, mg	336.07 ± 46.90	300.0 ± 47.4	-10.7	**0.03**	336.31 ± 74.52	0.1	0.70	-10.8	0.10
FCR, pools/d ‡	9.38 ± 0.94	11.3 ± 1.9	20.5	0.12	11.21 ± 1.87	19.4	0.20	0.9	0.73
PR, mg/kg/d ‡	30.42 ± 2.21	30.5 ± 2.6	0.1	1.00	32.91 ± 3.40	8.2	0.30	-7.4	0.22
Abs. conv. VLDL to IDL apoB100, mg/kg/d ‡	4.14 ± 1.29	4.0 ± 1.0	-3.2	0.34	2.37 ± 0.46	-42.7	0.31	69.0	**0.002**
Abs. conv. VLDL to LDL apoB100, mg/kg/d	9.40 ± 1.49	10.1 ± 1.5	7.5	0.29	10.08 ± 1.00	7.3	0.80	0.2	0.44
VLDL apoB100 direct catabolism, mg/kg/d ‡	16.06 ± 2.27	15.6 ± 2.6	-2.9	0.78	19.54 ± 3.22	21.7	0.25	-20.2	0.10
IDL apo B100									
PS, mg	16.06 ± 2.05	20.8 ± 2.7	29.7	0.11	15.24 ± 2.28	-5.2	0.58	36.7	0.05
FCR, pools/d ‡	11.54 ± 1.85	14.6 ± 2.3	26.4	**0.02**	13.46 ± 1.72	16.6	0.18	8.4	0.32
PR, mg/kg/d ‡	2.37 ± 0.64	3.4 ± 0.8	42.0	**0.03**	2.38 ± 0.46	0.6	0.78	41.1	**0.009**
Abs. conv. IDL to LDL apoB100, mg/kg/d ‡	3.77 ± 1.31	3.3 ± 0.8	-13.5	0.50	2.37 ± 0.46	-37.1	0.63	37.5	0.14
LDL apo B100									
PS, mg	2700.98 ± 159.05	2811.1 ± 192.1	4.1	0.67	2785.01 ± 155.81	3.1	0.54	0.9	0.84
FCR, pools/d ‡	0.43 ± 0.06	0.43 ± 0.06	-0.2	0.50	0.39 ± 0.04	-10.4	0.24	11.4	**0.008**
PR, mg/kg/d ‡	13.29 ± 1.45	13.5 ± 1.4	1.6	0.43	12.34 ± 1.04	-7.1	0.55	9.4	**0.03**
VLDL apo CIII									
PS, mg ‡	109.58 ± 15.77	100.41 ± 16.50	-8.4	0.10	115.30 ± 23.62	5.2	0.96	-12.9	0.11
FCR, pools/d	0.94 ± 0.06	0.83 ± 0.05	-11.3	**0.009**	0.88 ± 0.06	-6.9	0.04	-4.7	0.60
PR, mg/kg/d ‡	4.18 ± 0.54	3.67 ± 0.69	-12.2	**0.009**	4.24 ± 0.77	1.3	0.34	-13.3	0.092

Values are expressed as mean ± SEM. Bold indicates P<0.05.

Apo B100: Control: N = 17 (N = 18 for PS, FCR and PR VLDL); DHA: N = 16 (18 for PS VLDL, 17 for PS IDL and LDL, FCR and PR VLDL); EPA: N = 14 (16 for PS, FCR and PR VLDL, 15 for PS, FCR and PR LDL). Apo CIII: n = 18 for control, 18 for DHA, 16 for EPA.

Models were adjusted for values after the control treatment as well as for sex, weight, age, sequence and baseline value was considered only when these covariates were found to be significant at $P < 0.05$ in the models.

* P values for the EPA and DHA changes vs control, as determined by the LSMEANS statement and tested against the null hypothesis in the mixed models.

† This analysis compared the change with DHA or EPA compared with control based on post-treatment values. And the P values were taken from the main treatment effect in the mixed models.

‡ Log-transformed data were used in these analyses owing to skewness of the values.

Abs: absolute; Apo: apolipoprotein; PS: pool size; FCR: fractional catabolic rate; PR: production rate; Conv: conversion; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Table 6.4 Spearman correlation coefficient between changes in apoB-100-containing lipoproteins kinetics, PCSK9 levels and LDL-C after EPA and DHA vs. control

	DHA		EPA	
	LDL-C, mmol/L	**LDL apo B100, mg**	**LDL-C, mmol/L**	**LDL apo B100, mg**
VLDL apo B100				
FCR, pools/d	-0.203	-0.424	-0.303	-0.182
PR, mg/kg/d	0.159	0.229	0.135	-0.043
VLDL to IDL apoB100, mg/kg/d	0.165	-0.291	0.420	0.305
VLDL to LDL apoB100, mg/kg/d	0.238	0.276	-0.420	0.275
VLDL apoB100 direct catabolism, mg/kg/d	0.076	0.265	-0.055	-0.196
IDL apo B100				
FCR, pools/d	**0.503**	-0.129	0.301	0.270
PR, mg/kg/d	**0.674**	0.221	0.327	0.503
IDL to LDL apoB100, mg/kg/d	0.132	-0.226	**0.560**	0.380
LDL apo B100				
FCR, pools/d	-0.229	**-0.521**	-0.161	0.057
PR, mg/kg/d	0.044	-0.197	0.261	**0.625**
Mean LDL size, Å	0.103	-0.091	0.063	0.393
LDL peak, Å	0.148	0.324	0.062	0.254
Prop. large LDL, %	0.059	-0.181	0.133	0.046
Prop. small LDL, %	-0.111	0.028	-0.136	-0.196
PCSK9, ng/L	0.031	**0.642**	0.132	0.011

Abs: absolute; Apo: apolipoprotein; Conv: conversion; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; PCSK9: proprotein convertase subtilisin/kexin type 9; PR: production rate; Prop: proportion; PS: pool size; FCR: fractional catabolic rate; VLDL: very-low density lipoprotein.
Bold indicates P<0.05.

Supplemental Table 6.1 Apolipoproteins monitored into VLDL fractions for kinetic studies with the corresponding peptides selected for MRM analysis

Protein	Peptide sequence	Transition	Q1/Q3
apoB-100	**L**DVTTSIGR	$2y^{6}$	481.27/634.35
	L[+3]DVTTSIGR	$2y^{6}$	482.78/634.35
apoC-III	DA**L**SSVQESQVAQQAR	$2y^{10}$	858.93/1144.57
	DAL**[+3]**SSVQESQVAQQAR	$2y^{10}$	860.44/1144.57

Supplemental Material

Quantification and isolation of apoB-100 and apoC-III

The apoB-100 (Alerchek Inc., Portland, Maine, USA) and apoC-III (Assaypro LLC, St-Charles, MO, USA) concentrations in TRLs (VLDL) were determined using a non-competitive ELISA with immuno-purified polyclonal antibodies to calculate their respective pool sizes. We used three different time points during the kinetic study to estimate the average concentrations of the different apolipoproteins.

Protein in-solution digestion – ApoB-100 and ApoC-III

Proteins (20 µg) were solubilized in 25 µl digestion buffer (50 mM Ammonium bicarbonate – 1% Sodium Deoxycholate), heated to 95°C for 5 minutes and cooled down. Proteins were reduced with 1 µg of DTT at 37°C for 30 minutes and alkylated with 5 µg of iodoacetamide at 37°C for 20 minutes. Protein digestion was performed with trypsin (1 µg, Sequencing Grade Modified Trypsin, Promega, Madison, WI, USA) at 37°C o/n. Digestion was stopped and sodium deoxycholate was removed by acidification (3% acetonitrile, 1% trifluoroacetic acid, 0.5% acetic acid) at room temperature for 10 min. Sodium deoxycholate was pelleted at 16 000 g for 5 minutes. The supernatant was desalted on a C18 Empore filter (3M). Peptides were eluted in 80% acetonitrile – 0.1% trifluoroacetic acid and dried in a speed vac.

Liquid chromatography-multiple reaction monitoring (LC-MRM) analysis

Samples were analyzed on a ABSciex 5500QTRAP™ hybrid triple quadrupole/linear ion trap mass spectrometer equipped with an Eksigent nanoLC AS2 cHiPLC nanoflex controlled by Analyst 1.6™ and with a nanospray ionization source (ABSciex, Framingham, MA, USA). Mass spectrometry (MS) analysis was conducted in positive ion mode with an ion spray voltage of 2300V. Peptides were desalted on a 200um x 6 mm chip trap column packed with ChromXP C18, 3 um, (Eksigent) at 2 ul/min of Solvent A (formic 0.1%) then switched in line at a flow rate of 1000 nL/min on a 200um x 15 cm chip column packed with ChromXP C18, 3 um (Eksigent) with a 12 min linear gradient from 5 to 25% of solvent B (acetonitrile 0.1% FA), then a 2 min linear gradient from 25 to 80% B. In order to select tryptic peptides that are the most suitable for sensitive and selective leucine enrichment monitoring, MRM analysis were done on all the tryptic peptides unique to each proteins, containing one leucine with length ranging from 5 to 25 AA. Peptides containing Methionine and Cysteine were eliminated. Peptides were selected based on peak shape and intensity. The peptide selection process was done using Skyline Software v2.5 (MacCoss Lab, Washington University). For each lipoprotein, Table 1 (data supplement) shows the list of selected peptides as well as transition used for enrichment quantification. LC-MRM/MS analyses were performed using all the transitions from y and b ions with m/z greater than the precursor of the light (Leu)

and heavy (D3-Leu) form for each peptide [1]. Nebulizer gas was set to 8 (Gas1), curtain gas to 20 and heater to 150°C. Collision energy (CE) and declustering potential (DP) values for each peptide were predicted by the Skyline Software using a linear equation based on the precursor m/z. A blank solvent injection was run between each time course.

Isotopic Enrichment Calculation

The automatic integration of the peaks was done by MultiQuant 2.1 (ABSciex, Framingham, MA, USA) and was verify manually. For each protein, the isotopic enrichment was calculated as follow: area D3-Leu transition/(area D3-Leu transition + area Leu transition). Since the incorporation of D3-Leu leads to an addition of 3 Da, we investigated the isotope distribution of the light peptide in order to evaluate its contribution over the D3-Leu signal. For each protein, selected tryptic peptide have been synthesized (JPT, Germany) with isotopically coded amino acids ([(13)C(6)]Arg or [(13)C(6)]Lys) and reconstitute in 0.1% formic acid with peptide concentration equivalent to the corresponding endogenous peptide. The solution was injected into the mass spectrometer and the corresponding transition at [M + 2H]2+ and [M + 2H]2+ + 1.5 Da was monitored. The area of both peak was integrated and the percentage of contribution of the $[M + 2H]^{2+}$ over the $[M + 2H]^{2+}$ + 1.5 Da was calculated. This value was subtracted from the area of D3-Leu transition for each sample. In order to evaluate the linearity of the signal over the analysis range, the synthetic peptides have been introduced at variable concentration into the digested samples. Finally, measurement of isotopic enrichment using LC-MRM method has been validated against traditional gas chromatography-mass spectrometry method in four subjects for TRL apoB-48 (R^2=0.98, P<0.0001) and VLDL apoB-100 (R^2=0.99, P<0.0001) (data not shown).

Kinetic analysis

The kinetics of all apolipoproteins were derived using a multicompartmental model that has been previously described [2-4]. We assumed that the enrichment of the precursor pool was stable and used the VLDL apoB-100 plateau from the isotopic enrichment data as the forcing function to drive the appearance of the tracer into apoB-100 and apoC-III [5]. Under steady state conditions, the fractional catabolic rate (FCR) is equivalent to the fractional synthetic rate. The apoB production rates (PRs) were determined using the formula PR (mg · kg-1 · d-1)=[FCR (pools/d) X apoB concentration (mg/dL) X plasma volume (L)]/body wt (kg) [6]. The plasma volume was estimated at 4.5% body weight. The SAAM II program (SAAM Institute, Seattle, WA, USA) was used to fit the model to the observed tracer data.

References

1. Gillette MA, Carr SA. Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. *Nature methods.* 2013;10(1):28-34.

2. Tremblay AJ, Lamarche B, Hogue JC, Couture P. Effects of ezetimibe and simvastatin on apolipoprotein B metabolism in males with mixed hyperlipidemia. *J Lipid Res.* 2009;50(7):1463-1471.

3. Nguyen MN, Chan DC, Dwyer KP, Bolitho P, Watts GF, Barrett PH. Use of Intralipid for kinetic analysis of HDL apoC-III: evidence for a homogeneous kinetic pool of apoC-III in plasma. *J Lipid Res.* 2006;47(6):1274-1280.

4. Ooi EM, Ng TW, Watts GF, Chan DC, Barrett PH. Effect of fenofibrate and atorvastatin on VLDL apoE metabolism in men with the metabolic syndrome. *Journal of lipid research.* 2012;53(11):2443-2449.

5. Welty FK, Lichtenstein AH, Barrett PH, Dolnikowski GG, Schaefer EJ. Human apolipoprotein (Apo) B-48 and ApoB-100 kinetics with stable isotopes. *Arterioscler Thromb Vasc Biol.* 1999;19(12):2966-2974.

6. Cohn J, Wagner D, Cohn S, Millar J, Schaefer E. Measurement of very low density and low density lipoprotein apolipoprotein (Apo) B-100 and high density lipoprotein Apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *J Clin Invest.* 1990;85(3):804-811.

Chapitre 7 Comparaison de la réponse des triglycérides à une supplémentation en EPA et en DHA

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L'article présenté dans ce chapitre s'intitule: Comparing the serum triglyceride response to high-dose supplementation with either DHA or EPA among individuals with increased cardiovascular risk: The ComparED study.

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Résumé

Les études montrent que la diminution des triglycérides sériques en réponse à une supplémentation en acides gras polyinsaturés oméga-3 à longue chaîne est variable entre les individus. L'objectif de cette étude était de comparer la proportion d'individus qui présentent une diminution des triglycérides en réponse à une supplémentation en DHA et en EPA et d'identifier les prédicteurs de la réponse. Un total de 154 individus ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Chaque phase expérimentale était d'une durée de 10 semaines et les phases étaient séparées par une période libre de 9 semaines. La variation quotidienne moyenne des concentrations de triglycérides chez les participants a été calculée en utilisant l'écart type de la mesure au « screening » et avant chacune des trois phases de l'étude. La réponse des triglycérides à une supplémentation en DHA et en EPA est restée à l'intérieur de l'intervalle de la variation quotidienne moyenne des triglycérides (± 0,25 mmol/L) chez 47% et 57% des participants respectivement. Bien que le DHA a entraîné une réduction des triglycérides supérieure à 0,25 mmol/L chez une plus grande proportion de participants que l'EPA (45% vs 32%, P<0,001), la réduction moyenne des triglycérides était similaire en réponse au DHA et à l'EPA chez ce type de répondeurs (-0,59±0,04 et -0,57±0,05 mmol/L respectivement). Les participants qui ont présenté une réduction des triglycérides supérieure à 0,25 mmol/L en réponse à la supplémentation en DHA et en EPA avaient des concentrations de cholestérol non HDL, de triglycérides et d'insuline plus élevées que les autres types de répondeurs au début l'étude (P<0,05). En conclusion, une supplémentation de 2,7 g de DHA et d'EPA par jour a entraîné une réponse des triglycérides inférieure à la variation des triglycérides observée quotidiennement chez environ 50% des participants. Une supplémentation en DHA a entraîné une réduction des triglycérides supérieure à la variation observée quotidiennement chez une plus grande proportion d'individus que l'EPA. Toutefois, l'amplitude de la réduction des triglycérides sanguins entre le DHA et l'EPA était similaire parmi ces individus.

Abstract

Studies show that the reduction in serum triglyceride concentrations with long-chain omega-3 fatty acid supplementation is highly variable among individuals. The objectives of this study were to compare the proportions of individuals whose triglyceride concentrations are lowered after high-dose docosahexaenoic (DHA) and eicosapentaenoic acid (EPA), and to identify predictors of the response to both modalities. In a double-blind controlled crossover study, 154 men and women were randomized to three supplemented phases of 10 weeks each: 1) 2.7g/d of DHA, 2) 2.7g/d of EPA and 3) 3g/d of corn oil, separated by nine-week washouts. As secondary analyses, the mean intra-individual variation in triglyceride was calculated using the standard deviation from the mean of four off-treatment samples. The response remained within the intra-individual variation (±0.25 mmol/L) in 47% and 57% of participants after DHA and EPA respectively. Although there was a greater proportion of participants with a reduction greater than 0.25 mmol/L after DHA than after EPA (45% vs. 32%, $P<0.001$), the mean triglyceride reduction was comparable between groups (-0.59±0.04 vs. -0.57±0.05 mmol/L). Participants with a reduction greater than 0.25 mmol/L after both DHA and EPA had higher non-HDL-cholesterol, triglyceride and insulin concentrations compared with other responders at baseline (all $P<0.05$). In conclusion, supplementation with 2.7g/d of DHA or EPA has no meaningful effect on triglyceride concentrations in a large proportion of individuals with normal mean triglyceride concentrations at baseline. Although DHA lowers triglyceride in a greater proportion of individuals than EPA, the magnitude of the triglyceride lowering among them is similar.

Title page

Comparing the serum triglyceride response to high-dose supplementation with either DHA or EPA among individuals with increased cardiovascular risk: The ComparED study

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Abbreviations: Apo B: total apolipoprotein B100; Apo E: apolipoprotein E; CRP: C-reactive protein; CVD: cardiovascular disease; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FADS1: fatty acid desaturase 2 or delta-5 fatty acid desaturase; FADS2: fatty acid desaturase 2 or delta-6 fatty acid desaturase; GAPDH : gene glyceraldehyde 3-phosphate dehydrogenase; HDL-C: high-density lipoprotein cholesterol; HMG-CoA reductase: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase ; IL: interleukin; LCn3-PUFA : long-chain omega-3 polyunsaturated fatty acid; LDL-C: low-density lipoprotein cholesterol; LDL-R: LDL receptor; PCSK9: proprotein convertase subtilisin/kexin type 9; SREBP: sterol regulatory element-binding protein; TG: triglyceride; TNF-α : tumor-necrosis factor alpha.

Introduction

High fasting serum triglyceride (TG) concentration is associated with high risk of cardiovascular disease (CVD)[1; 2; 3] and, when very high, of pancreatitis[4]. Long-chain omega-3 polyunsaturated fatty acids (LCn3-PUFAs), including docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3), are known to decrease TG concentrations[5]. However, results from clinical studies have shown that there is a large inter-individual variability in the TG response to LCn3-PUFA supplementation[6; 7; 8; 9; 10]. The heterogeneous TG response to LCn3-PUFA may be attributed to many factors including measurement errors in the laboratory, day-to-day biological variations, genetic factors, and their interaction with other extrinsic factors such as the background diet and concurrent medications[11].

A number of studies have examined the variability in the TG response to LCn3-PUFA supplementation[6; 7; 8; 9; 10]. In the Fish Oil Intervention and Genotype Study, 8-week supplementation of fish oil supplementation (1.8 g EPA+DHA per day) reduced mean TG concentrations by 11.2% compared with baseline values among healthy subjects[6; 7; 8; 9; 10]. However, fewer than 70% were defined as responders, i.e. with a reduction in TG greater than 0 mmol/L[6; 10]. A similar proportion of participants of the Fatty Acid Sensor study presented a reduction in TG concentrations after 6-week supplementation with LCn3-PUFAs (1.9 to 2.2 g EPA + 1.1 g DHA per day)[12]. One may argue that using a threshold of 0 mmol/L to identify responders (any reduction in TG concentrations) and non-responders (any increase in TG concentrations) provides a limited perspective on the true variability of the response to LCn3-PUFAs. Indeed, TG concentrations are known to vary intra-individually by about 20%[13]. Thus, any individual change in TG concentrations that remains within the boundaries of usual intra-individual variation should not be considered as a truly meaningful change. Intra-individual variation, which includes biological variation and analytical variation, may be calculated as the average within-subject standard deviation based on two or more measurements taken on different days when no true change is expected. Any change smaller than this intra-individual variation should be considered within the variability of the measurement and thus, as a non-response. To our knowledge, no study has yet documented the proportion of “true” responders to LCn3-PUFA supplementation.

Furthermore, almost all previous clinical trials on LCn3-PUFA supplements have tested the combined impact of DHA and EPA in various forms and proportions. After years of negative results in studies investigating the association between LCn3-PUFA supplements and cardiovascular risk, the REDUCE-IT study has recently shown that supplementation with 4 g/d of EPA reduces the risk of cardiovascular events by 25% compared with a mineral oil placebo in individuals with high baseline TG concentrations[14]. These observations highlight the importance of investigating further the individual effects of DHA and EPA on cardiometabolic health. To date, data indicate that DHA may be more potent than EPA in modulating lipids and inflammation risk factors[15; 16], including TG concentrations[16], but very few studies have undertaken a head-to-head comparison of the

differential effect of high-dose DHA and EPA supplementation. The inter- and intra-individual variabilities in the TG response to DHA and EPA taken separately remain unknown.

The first objective of this study was to compare the proportions of individuals whose serum TG concentrations are meaningfully lowered after high-dose supplementation with either DHA and EPA among men and women with abdominal obesity and a subclinical inflammation state. The second objective was to identify factors that predict the serum TG response to DHA and EPA supplementation. We hypothesized that high-dose DHA meaningfully reduces serum TG concentrations in more individuals than a similar dose of EPA. We also hypothesized that individuals with the highest serum TG concentrations at baseline experience the greatest TG reductions after both DHA and EPA.

Material and Methods

Study design: Details of the study design have been published previously[16]. This study used a double-blind randomized, controlled crossover design with three treatment phases: 3 x 1 g capsule of 90% purified LCn3-PUFAs per day providing 1) 2.7 g/d of DHA, 2) 2.7 g/d of EPA, 3) 0 g/d of DHA+EPA (corn oil control). Each treatment phase had a median duration of 10 weeks and were separated by nine-week washouts. The original and primary objective of this study was to compare the effect of DHA and EPA on plasma C-reactive protein (CRP) concentrations, and thus, CRP was used as the primary outcome measure for the sample size calculation[16]. *A priori* sample size calculations indicated that N=150 would be needed to detect a minimal difference of 10% in plasma CRP concentrations when comparing any two of the three treatments with a power of 81% and P<0.01 (two-tailed)[16]. The sample size of N=150 also provide 92% power to detect a 10% difference in serum TG between treatments at P=0.01 (two-tailed, not shown). This is a secondary analysis of this trial. A total of 154 participants were randomized using an in-house computer program and all participants signed an informed consent document approved by local Ethics Committees at the beginning of the study. The study protocol was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003) and the study was conducted according to the Declaration of Helsinki. Allocation to treatments were concealed to participants as well as to study coordinators throughout the study.

Study population: Primary eligibility criteria were to have abdominal obesity (waist circumference ≥80 cm for women, ≥94 cm for men)[17] with a subclinical inflammation state reflected by plasma CRP concentrations >1 mg/L but <10 mg/L.

Compliance: Compliance to supplementation was assessed by counting supplements that were returned by participants. As previously reported, subjects with a compliance <80% were considered as non-compliant and therefore excluded from analyses (N=2)[16].

Anthropometric and cardiometabolic markers assessment: Methods of this study have been published previously[18; 19]. Briefly, anthropometric measures including weight and waist circumference were obtained according to standardized procedures[20] at screening, beginning and end of each treatment phase. Blood samples were collected after a 12-h overnight fasting at screening, beginning of each treatment phase and at the end of each treatment phases. Total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TG, total apolipoprotein B100 (apo B), CRP, adiponectin, interleukin-6 (IL-6), LDL peak particle size, mean LDL particles size, proportion of LDL in the various size categories (small and large), insulin and glucose were measured before and after each study phase. Tumor necrosis factor alpha (TNF-α), proprotein convertase subtilisin/kexin type 9 (PCSK9) and interleukin-18 (IL-18) were measured after

each treatment phase. Serum Non-HDL-C concentration was calculated as the difference between total serum cholesterol concentrations and HDL-C concentrations.

Fatty acid composition of red blood cell membranes was analyzed by OmegaQuant Analytics, LLC, (Sioux Falls, SD) according to the Omega-3 Index® methodology[21]. Each fatty acid is expressed as a weight percent of total identified fatty acids after a response factor correction (based on calibration curves) was applied to each fatty acid.

Gene expression of lipid metabolism: Fasting fresh blood was collected in PAXgene Blood RNA tubes (Becton Dickinson, Canada) after each treatment in a subsample of 44 randomly selected participants for gene expression analyses. RNA was isolated using a PAXgene RNA-kit according to the manufacturer's instructions (Qiagen, Canada). Quantity of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and total RNA quality was assayed on an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Reverse transcription was performed on 1.5-2 µg total RNA. cDNA corresponding to 20 ng of total RNA was used to perform fluorescent-based Realtime PCR quantification using the LightCycler 480 (Roche Diagnostics, Mannheim, DE). The genes targeted were 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), LDL-receptor (LDL-R) and sterol regulatory element-binding protein 1c and 2 (SREBP1c and SREBP2). Values were normalized to the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

All personnel involved in the intervention and the measurements of the study outcomes were blinded to treatments.

Statistical analyses: As per our a priori define statistical plan[16], and since the corn oil control treatment had no significant impact on serum TG concentrations compared with baseline value (-0.04 ± 0.04 mmol/L, P=0.31, data not shown), the change in serum TG concentrations after DHA and EPA supplementation was calculated as the difference between post-treatment serum TG concentrations (EPA or DHA) minus the serum TG concentrations after the control phase.

Mean intra-individual variation in serum TG concentrations, reflecting the combination of usual biological as well as the analytical variation, was calculated as the standard deviation of the mean TG values from four off-treatment samples, i.e. one sample taken at screening and samples taken before each of the three treatment phases, which were preceded by a median washout period of nine weeks from the previous treatment. The mean intra-individual variation in TG concentrations in our sample was ± 0.25 mmol/L. The median intra-individual variation was ± 0.20 mmol/L (min ± 0.04 mmol/L; max ± 0.91 mmol/L). Responders to either DHA or EPA were defined as those among whom serum TG were reduced by more than 0.25 mmol/L compared with

the control phase. Non-responders were those among whom TG variation was within the ± 0.25 mmol/L variation. Concordant responders were those among whom both DHA and EPA supplementation reduced serum TG concentrations by more than 0.25 mmol/L compared with the control treatment.

Pearson correlation analysis was used to assess the association between the TG response to DHA and the TG response to EPA. Anthropometric measurement and cardiometabolic marker values and changes in anthropometric measurements and cardiometabolic marker values among the subgroups of responders were compared using generalized linear models (proc MIXED with a VC or a CS covariance matrix for unrepeated and repeated measurements respectively) for continuous variables and Fisher's exact tests for categorical variables. Models with the VC covariance matrix were adjusted for age, sex and waist circumference at screening and models with the CS covariance matrix were adjusted for age, sex, waist circumference at screening and value of the variable of interest after the control phase. EPA- and DHA-induced changes in cardiometabolic outcomes compared with values after the control phase were determined with the LSMEANS statement and were tested against the null hypothesis in the mixed models. Difference in cardiometabolic outcomes between EPA and DHA were determined by the main treatment effect (no post hoc test needed)[16]. The skewness of the distribution of the variables and model residuals was assessed and data were transformed when required. All analyses were computed using SAS (v9.3, Cary, NC).

Results

Subjects' characteristics

As shown previously, of the 154 individuals randomized to the treatment sequences, a total of 121 subjects completed all three study phases and were included in the analyses[16]. Screening characteristics of these subjects are presented in Table 1. All subjects had a relatively high waist circumference and serum CRP concentrations as per the main entry criteria. The mean age was 58 ± 10 years for men (N=36) and 52 ± 16 years for women (N=85). The mean screening TG concentration was within the normal range and similar among men and women (1.4 ± 0.8 mmol/L vs. 1.4 ± 0.7 mmol/L respectively).

Triglyceride response to DHA and EPA

As previously shown, the overall mean reduction in serum TG concentrations compared with control was greater with DHA than with EPA (-13.3% vs. -11.9%; P<0.01)[16]. The individual TG response to DHA and EPA supplementation is presented in Figure 1. The proportion of responders (those among whom serum TG concentrations were reduced by more than 0.25 mmol/L) was greater with DHA than with EPA (45% and 32%, respectively; P<0.001). However, the TG reduction was similar in magnitude between those two groups (-0.59 mmol/L for DHA and -0.57 mmol/L for EPA). Inversely, the variation in TG concentrations remained within the ± 0.25 mmol/L intra-individual variation range in fewer participants after DHA than after EPA (47% and 57% respectively, P<0.01). The proportion of participants among whom serum TG concentrations increased by more than 0.25 mmol/L was similar after DHA and EPA (8% and 11% respectively, P=0.29).

The percentage of shared variance in the TG response to DHA and EPA was 39.0% (r=0.62; P<0.001, data not shown). A total of 26% of participants were concordant responders to both DHA and EPA, 34% presented no change in TG after both DHA and EPA supplementation and 2% presented an increase in TG after both supplements (data not shown). A total of 24% of participants presented a fully discordant TG response to DHA and EPA (reduction greater than 0.25 mmol/L in TG with DHA and increase greater than 0.25 mmol/L with EPA, or *vice versa*, data not shown).

Concordant responders to DHA and EPA

Table 2 presents the characteristics after the control phase of the participants with a concordant reduction (N=32) in TG concentrations after DHA and EPA supplementation compared with all other participants. Responders to both DHA and EPA had higher serum non-HDL-C (4.01 vs. 3.50 mmol/L; P=0.02), TG (1.90 vs. 1.21 mmol/L; P<0.001), PCSK9 (241.35 vs. 203.00 ng/mL; P=0.03) and insulin (110. 34 vs. 98.40 pmol/L; P=0.03) concentrations and a greater proportion of large LDL particles (11.50 vs. 8.35 %; P=0.03) after the control phase

compared to the other participants (i.e. non-responders and those with a discordant response to EPA and DHA). The reduction in TG concentrations among concordant responders was similar after DHA and EPA supplementation (-0.67 vs. -0.61 mmol/L respectively; P=0.22, Supplemental Table 1). However, the increase in HDL-C concentrations among concordant responders was greater after DHA than after EPA supplementation (+0.14 vs. -0.01 mmol/L respectively; P<0.001) and the reduction in IL-18 concentrations was greater after DHA than after EPA supplementation (-36.79 vs. -10.60 pg/mL respectively; P=0.023, Supplemental Table 1).

Correlates of the TG response to DHA

Compared with non-responders to DHA, responders to DHA had a greater concentration of TG (P<0.001), PCSK9 (P<0.01) and insulin (P=0.02) after the control phase (Table 3). As shown in Table 4, the reduction in TG concentrations among responders to DHA supplementation was observed along with a concomitant reduction in body mass index (-0.3 kg/m^2; P=0.03), diastolic blood pressure (-2.4 mmHG; P=0.04), PCSK9 (-40.1 ng/mL; P<0.001), CRP (-0.33 mg/L, P=0.02) and IL-18 (-35.1 pg/mL; P=0.003) concentrations, and with an increase in HDL-C (+0.13 mmol/L; P=0.001). The changes in body mass index, total cholesterol and non-HDL-C concentrations with DHA were significantly different among TG responders and non-responders to DHA (all P<0.05, Table 4).

Correlates of the TG response to EPA

Similarly, responders to EPA had greater TG (P<0.001) and PCSK9 (P=0.03) concentrations after the control phase than non-responders (Table 5). The reduction in TG concentrations among responders to EPA was observed along with a reduction in PCSK9 (-39.9 ng/mL, P<0.001) and IL-6 (-0.69 pg/mL; P=0.03) concentrations after EPA supplementation (Table 6). None of these changes among responders to EPA were different from those seen in non-responders (Table 6).

Participants with a low intra-individual TG variability (i.e. variability <0.20 mmol/L based on four off-treatment measurements, which is the median variability of the sample) were more likely to be responders to both DHA and EPA (OR 3.35, 95% CI 1.12 to 10.08) than participants with an individual TG variability greater than 0.20 mmol/L (data not shown).

Gene expression of the lipid metabolism

Neither DHA nor EPA supplementation modified expression of HMG-CoA reductase, LDL-R, SREBP1c and SREBP2 in whole blood cells compared with the control treatment among the 44 participants (Supplemental Table 2). Moreover, neither DHA nor EPA modified the gene expression of HMG-CoA reductase, LDL-R, SREBP1c and SREBP2 among TG responders or non-responders to DHA and EPA (data not shown).

Discussion

To the best of our knowledge, this is the first study accounting for the intra-individual variability in serum TG concentrations to identify and characterize TG responders to DHA and EPA supplementation among individuals at increased risk of CVD[22]. This randomized controlled crossover study revealed important inter- and intra-variability in the serum TG response to similar doses of DHA and EPA. First, the greater reduction in mean TG concentrations with high-dose DHA compared with high-dose EPA[16] is largely due to the fact that there are more "true" responders to DHA than to EPA supplementation, i.e. among whom the TG reduction is greater than the intra-individual variation of 0.25 mmol/L. Indeed, the magnitude of the reduction in TG concentrations after DHA and EPA was similar among responders. Second, only a small proportion of the participants (26%) are "true" responders to both DHA and EPA. Finally, individuals with elevated non-HDL-C, TG and insulin concentrations and with a lower TG variability are more likely to present a reduction in TG concentrations after high-dose DHA and EPA.

Variability in the response to pharmacological treatments has been widely reported in the literature[23], and thus variability in the response to LCn3-PUFA supplementation is not surprising. Clinical studies have consistently shown a mean TG reduction after LCn3-PUFA supplementation, but a substantial inter-individual variability in TG response to LCn3-PUFA supplementation has also been observed[6; 8; 9; 12]. Previous studies on this topic have assessed the change in TG concentrations using a reference of 0 mmol/L to identify responders and non-responders to LCn3-PUFAs. We argue that a change in serum TG concentrations that remains within the boundaries of intra-individual variability, i.e. accounting for biological as well measurement variability, should not be considered "meaningful". Hence, the proportion of individuals with a "meaningful" change in TG (positive or negative) may be smaller than what previous studies have suggested. Indeed, the present study suggests that the proportion of individuals with a "meaningful" TG reduction is approximately 30% for EPA and 45% for DHA rather than the 70% reported previously[6; 8; 9; 12]. This suggests that less than 50% of individuals (at least those with baseline TG within the normal range) may benefit from LCn3-PUFA supplementation in terms of TG-lowering.

This study has shown that the greater reduction in mean TG concentration observed after DHA compared with EPA[16] seems largely due to this greater proportion of responders to DHA compared to EPA because the magnitude of the TG lowering was similar between responders to DHA and EPA supplementation. LCn3-PUFAs are known to reduce TG concentrations primarily by reducing the production of VLDL-apo B100 and by increasing the conversion of VLDL- to LDL-apo B100[24]. However, we have previously shown that DHA and EPA exert similar effects on the rate of VLDL-apo B100 production and catabolism and hence, on VLDL-apoB pool size[19]. These observations support the hypothesis that the larger reduction in TG concentrations after DHA compared with EPA is rather due to a greater proportion of responders to DHA compared with EPA than to a

greater hypotriglyceridemic effect of DHA compared with EPA. Gender, body mass index, baseline TG concentrations and a number of gene polymorphisms have been identified as potential contributors to the TG response to LCn3-PUFA supplementation[10; 25; 26]. These covariates may explain, at least partly, why DHA exerts an hypotriglyceridemic effect in a greater proportion of individuals than EPA.

EPA may be converted into docosapentaenoic acid (DPA, 22:5n-3) and DHA through a metabolic pathway involving *in vivo* elongation, desaturation and peroxisomal beta-oxidation in humans[27]. We observed that the changes in LCn3-PUFA content of red blood cells after DHA and EPA supplementation was similar between responders and non-responders compared with control (Tables 4 and 6), suggesting that the TG response appears to be independent of the rate of incorporation of DHA and EPA into red blood cells. Polymorphisms in genes involved in endogenous LCn3-PUFAs biosynthesis (e.g. fatty acid desaturase 1 and 2, *FADS1* and *FADS2*), metabolism (e.g. peroxisome proliferator-activated receptors family, *PPAR*s, ATP citrate lyase, *ACYL*, and acetyl-CoA carboxylase, *ACACA*) and transport (e.g. apolipoprotein E*, apo E*) have been identified as modulators of the TG response to LCn3-PUFA supplementation[12; 28; 29; 30]. To our knowledge, only one randomized controlled trial has assessed how the apo E genotype modulates the TG response to DHA and EPA-rich oils[31]. Authors found no effect of the apo E genotype on the TG response to a 4-week supplementation with either 3.3 g/d of EPA or 3.7 g/d of DHA in a sample of 38 healthy normolipidaemic males[31]. Importantly, the TG response was modeled as a continuous variable and thus, the intra-individual variability in the TG response was not considered. Moreover, the association between the apo E genotype and the consistency in the TG response to DHA and EPA has not been documented. The present study revealed that supplementation with either DHA or EPA exerts no effect on the gene expression of *HMG-CoA reductase*, *LDL-R*, *SREBP1c* and *SREBP2* (Supplemental Table 2) measured in whole blood cells. We have previously observed that supplementation with either DHA or EPA also had similar effects on *FADS1, FADS2, ELOVL fatty acid elongase 2 (ELOVL2)* and *ELOVL fatty acid elongase 5 (ELOVL5)*[21]. Of note, the change in the expression of these genes after DHA and EPA supplementation was similar among the subgroups of TG responders to DHA and EPA (data not shown). These observations remain to be validated in future studies.

Our data showed that the TG responses to DHA and EPA were inconsistent, with only 26% of participants showing meaningful TG reductions after both DHA and EPA supplementation. However, responders to a given LCn3-PUFA (i.e. DHA or EPA) were more likely to be responders to the other fatty acid, and so were those with a smaller intra-individual variability in their TG concentrations. Participants with a concordant reduction in TG concentrations after DHA and EPA supplementation also had higher non-HDL-C, TG and insulin concentrations at baseline compared with non-responders. Consistent with these observations, participants with higher TG concentrations at baseline usually achieve the greatest TG reduction after LCn3-PUFA supplementation[25]. In a small clinical study conducted in hypertriglyderidemic individuals, men with a lower body mass index were more

likely to present a reduction in TG concentrations greater than 30% in response to a LCn3-PUFAs and metformin combined supplementation[26]. Also consistent with our observations, results of the Fatty Acids Sensor Study conducted among 254 healthy subjects have shown that neither age, sex nor body mass index explain the variability in the TG response to LCn3-PUFA supplementation in a multivariate model[7]. However, responders to LCn3-PUFA supplementation were those with the highest TG and insulin concentrations, and the lowest HDL-C concentrations at baseline[32]. Thus, a small number of studies have shown that individuals with high TG concentrations, low HDL-C concentrations and alterations in glucose/insulin homeostasis at baseline are those most likely to be responders to LCn3-PUFA supplementation, at least in terms of TG lowering. Because DHA and EPA are nearly always consumed and studied in combination, further research is needed to determine the impact of gender, body weight, and alterations of the glucose, insulin and lipid metabolism on the consistency in the TG response to individual fatty acids.

Interestingly, we observed that responders to DHA or to EPA had greater serum PCSK9 concentrations at "baseline" (i.e. after the control phase) than non-responders. PCSK9 regulates cholesterol metabolism by degrading cellular LDL-receptors, hence blunting the clearance of LDL from the circulation, and then leading to an increase in LDL concentrations[33]. We have previously shown that DHA and EPA reduce PCSK9 concentrations in a similar manner[19]. Accordingly, responders presented a reduction in PCSK9 concentrations after either DHA or EPA, which did not differ, however, from changes seen in groups of non-responders. While the role of PCSK9 in regulating cholesterol homeostasis is well understood, its impact on TG metabolism is less known. The relationship between LCn3-PUFAs, PCSK9 and TG metabolism remains to be investigated in the future.

It has been consistently reported that lowering LDL-C concentrations reduces the incidence of major cardiovascular events[34]. However, LCn3-PUFA supplementation tends to slightly increase LDL-C concentrations, and DHA may do more so than EPA[16]. Using supplements of LCn3-PUFAs in order to prevent cardiovascular events may appear counterintuitive in this context. In the present study, supplementation with DHA and EPA had neutral effect on LDL-C concentrations among TG responders to DHA and EPA supplementation. Moreover, the reduction in TG concentrations after DHA among responders was also associated with favorable changes in diastolic blood pressure, HDL-C, CRP and IL-18 concentrations. The reduction in TG after EPA among responders was associated with reduced IL-6 concentrations. These observations support the previous assumption that DHA may be more potent than EPA in modulating cardiometabolic risk factors[16], especially among those with a meaningful reduction in TG concentrations after LCn3-PUFA supplementation. Because a high proportion of individuals are non-responders to LCn3-PUFAs and that TG concentrations may even increase in a small proportion of them, it is not surprising that most previous clinical trials have failed to observe reductions in CVD risk after LCn3-PUFA supplementation[35; 36; 37]. This does

not exclude the possibility that LCn3-PUFA supplementation may exert beneficial effects on cardiovascular risk, through the modulation of TG concentrations as well as of other cardiovascular risk factors, in some individuals. More recently, data from the REDUCE-IT study have shown that supplementation with EPA alone (4 g/d) reduces the risk of cardiovascular events by 25% in individuals at high cardiovascular risk and with high baseline TG concentrations[14]. Considering that DHA may be more a more potent modulator of cardiometabolic health than EPA[15; 16], it will be most interesting to further investigate the potential cardiovascular benefits of DHA alone in future hard endpoint studies.

This study has several strengths but also some limitations. To our knowledge, this is the first crossover trial to assess the variability in TG response to DHA and EPA supplementation taken individually and using the intra-individual TG variation to assess the response. The results of the present study are specific to a population with high waist circumference and elevated CRP concentrations only. Also, the sample size was calculated *a priori* to compare the effect of DHA and EPA on CRP concentrations and not to characterize the responders to LCn3-PUFA supplementation. While sufficient to characterize the TG response to DHA and EPA supplementation, the number of subjects in the study (N=121) may have been insufficient to identify predictors of the TG response. Participants were asked to maintain their usual dietary habits and were also counselled on how to exclude fatty fish meals, fish-oil supplements, flax products, walnuts, and LCn3-PUFA-enriched products during the study. Since the diet was not assessed before the study, we cannot exclude the fact that the background diet may have influenced the TG variability as well as its response to DHA and/or EPA supplementation. Variability in the response to interventions in clinical trials may be attributed to non-compliance to treatments. However, since the mean compliance to supplementation based on returned capsules was higher than 95% for all three phases of the present study and the changes in DHA and EPA content of red blood cells consistent with the given treatment (i.e. DHA and EPA respectively)[16], the intra- and inter-individual variability in the TG response observed in this study seems more likely attributable to other factors rather than a consequence of the non-compliance.

In sum, data from this randomized controlled crossover trial have shown that a small proportion of individuals present a meaningful reduction in TG concentration after supplementation with either high-dose DHA or EPA. The greater reduction in TG concentrations after DHA compared with EPA is mainly explained by a greater proportion of responders to DHA than to EPA. In this population at risk of CVD, participants with high TG, non-HDL-C and insulin concentrations at baseline and those with TG levels that appear to be less variable were more likely to present a concordant reduction in TG concentrations after both DHA and EPA supplementation. In light of the present data and of data from the REDUCE-IT study, further studies must be conducted to better identify individuals who may benefit from DHA and EPA supplementation and to compare the effect of DHA and EPA on the risk of cardiovascular outcomes.

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Authorship

BL, PC and AT have designed and obtained funding for this study. PC was responsible of the medical supervision of the study. WSH and KHJ conducted the laboratory analyses. CV provided significant help with the analysis of the results. JA performed statistical analyses and wrote de manuscript, which was reviewed critically by all authors. BL had primary responsibility for final content. All authors critically revised the manuscript and contributed intellectually to its development, provided final approval of the submitted manuscript, had full access to all of the data in the study, take responsibility for the integrity of the data and the accuracy of the data in the analysis, affirm that the article is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

References

1. Austin MA (1998) Plasma triglyceride as a risk factor for cardiovascular disease. *Can J Cardiol* **14 Suppl B**, 14b-17b.
2. Hokanson JE, Austin MA (1996) Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk* **3**, 213-219.
3. Liu J, Zeng FF, Liu ZM *et al.* (2013) Effects of blood triglycerides on cardiovascular and all-cause mortality: a systematic review and meta-analysis of 61 prospective studies. *Lipids Health Dis* **12**, 159.
4. Valdivielso P, Ramirez-Bueno A, Ewald N (2014) Current knowledge of hypertriglyceridemic pancreatitis. *Eur J Intern Med* **25**, 689-694.
5. Kris-Etherton PM, Harris WS, Appel LJ *et al.* (2003) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology* **23**, e20-30.
6. Caslake MJ, Miles EA, Kofler BM *et al.* (2008) Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. *Am J Clin Nutr* **88**, 618-629.
7. Thifault E, Cormier H, Bouchard-Mercier A *et al.* (2013) Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. *J Nutrigenet Nutrigenomics* **6**, 73-82.
8. Minihane AM, Khan S, Leigh-Firbank EC *et al.* (2000) ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arteriosclerosis, thrombosis, and vascular biology* **20**, 1990-1997.
9. Minihane AM (2010) Fatty acid-genotype interactions and cardiovascular risk. *Prostaglandins, leukotrienes, and essential fatty acids* **82**, 259-264.
10. Madden J, Williams CM, Calder PC *et al.* (2011) The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. *Annual review of nutrition* **31**, 203-234.
11. Meisel C, Gerloff T, Kirchheiner J *et al.* (2003) Implications of pharmacogenetics for individualizing drug treatment and for study design. *J Mol Med (Berl)* **81**, 154-167.
12. Cormier H, Rudkowska I, Paradis AM *et al.* (2012) Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation. *Nutrients* **4**, 1026-1041.
13. Bookstein L, Gidding SS, Donovan M *et al.* (1990) Day-to-day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. *Arch Intern Med* **150**, 1653-1657.
14. Bhatt DL, Steg PG, Miller M *et al.* (2019) Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. *The New England journal of medicine* **380**, 11-22.
15. Wei MY, Jacobson TA (2011) Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. *Current atherosclerosis reports* **13**, 474-483.
16. Allaire J, Couture P, Leclerc M *et al.* (2016) A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study. *Am J Clin Nutr* **104**, 280-287.
17. Alberti KG, Zimmet P, Shaw J *et al.* (2005) The metabolic syndrome--a new worldwide definition. *Lancet* **366**, 1059-1062.
18. Pirro M, Bergeron J, Dagenais GR *et al.* (2001) Age and duration of follow-up as modulators of the risk for ischemic heart disease associated with high plasma C-reactive protein levels in men. *Arch Intern Med* **161**, 2474-2480.
19. Allaire J, Vors C, Tremblay AJ *et al.* (2018) High-Dose DHA Has More Profound Effects on LDL-Related Features Than High-Dose EPA: The ComparED Study. *J Clin Endocrinol Metab* **103**, 2909-2917.
20. Airlie, Lohman T, Roche A *et al.* (1988) Standardization of anthropometric measurements. In *The Airlie (VA) Concensus Conference*, pp. 39-80. Champaign, Ill: Human Kinetics.

21. Allaire J, Harris WS, Vors C *et al.* (2017) Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)* **120**, 8-14.
22. Jellinger PS, Handelsman Y, Rosenblit PD *et al.* (2017) AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND AMERICAN COLLEGE OF ENDOCRINOLOGY GUIDELINES FOR MANAGEMENT OF DYSLIPIDEMIA AND PREVENTION OF CARDIOVASCULAR DISEASE. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* **23**, 1-87.
23. Nicolas JM, Espie P, Molimard M (2009) Gender and interindividual variability in pharmacokinetics. *Drug Metab Rev* **41**, 408-421.
24. Lamarche B, Couture P (2015) Dietary fatty acids, dietary patterns, and lipoprotein metabolism. *Curr Opin Lipidol* **26**, 42-47.
25. Skulas-Ray AC, Kris-Etherton PM, Harris WS *et al.* (2011) Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr* **93**, 243-252.
26. Sirtori CR, Tremoli E, Sirtori M *et al.* (1977) Treatment of hypertriglyceridemia with metformin. Effectiveness and analysis of results. *Atherosclerosis* **26**, 583-592.
27. Cottin SC, Sanders TA, Hall WL (2011) The differential effects of EPA and DHA on cardiovascular risk factors. *Proc Nutr Soc* **70**, 215-231.
28. Bouchard-Mercier A, Rudkowska I, Lemieux S *et al.* (2013) Polymorphisms, de novo lipogenesis, and plasma triglyceride response following fish oil supplementation. *Journal of lipid research* **54**, 2866-2873.
29. Tremblay BL, Cormier H, Rudkowska I *et al.* (2015) Association between polymorphisms in phospholipase A2 genes and the plasma triglyceride response to an n-3 PUFA supplementation: a clinical trial. *Lipids Health Dis* **14**, 12.
30. Lindi V, Schwab U, Louheranta A *et al.* (2003) Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to n-3 fatty acid supplementation. *Mol Genet Metab* **79**, 52-60.
31. Olano-Martin E, Anil E, Caslake MJ *et al.* (2010) Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. *Atherosclerosis* **209**, 104-110.
32. Rudkowska I, Guenard F, Julien P *et al.* (2014) Genome-wide association study of the plasma triglyceride response to an n-3 polyunsaturated fatty acid supplementation. *Journal of lipid research* **55**, 1245-1253.
33. Tavori H, Rashid S, Fazio S (2015) On the function and homeostasis of PCSK9: reciprocal interaction with LDLR and additional lipid effects. *Atherosclerosis* **238**, 264-270.
34. Cholesterol Treatment Trialists C, Mihaylova B, Emberson J *et al.* (2012) The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* **380**, 581-590.
35. Aung T, Halsey J, Kromhout D *et al.* (2018) Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77917 Individuals. *JAMA Cardiol* **3**, 225-234.
36. Abdelhamid AS, Brown TJ, Brainard JS *et al.* (2018) Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *The Cochrane database of systematic reviews* **7**, CD003177.
37. Manson JE, Cook NR, Lee IM *et al.* (2018) Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. *The New England journal of medicine*.

Tables

Table 7.1 Characteristics at screening of the 121 subjects included in the analyses

	Men N=36	Women N=85
Age, years	58(10)	52(16)
Body mass index, kg/m^2	29.5(3.6)	28.9(4.2)
Waist circumference, cm	106.2(8.3)	97.4(9.5)
Systolic blood pressure, mm HG	118.3(12.7)	110.5(12.2)
Diastolic blood pressure, mm HG	77.5(8.9)	70.5(7.4)
Total cholesterol, mmol/L	4.9(0.8)	5.5(0.9)
LDL-C, mmol/L	3.0(0.7)	3.2(0.8)
HDL-C, mmol/L	1.3(0.3)	1.7(0.4)
Triglycerides, mmol/L	1.4(0.8)	1.4(0.7)
C-reactive protein, mg/L	2.8(1.8)	3.5(2.4)
Glucose, mmol/L	5.5(1.0)	5.2(0.8)
% with metabolic syndrome (n)	22(8)	16(14)

Values are expressed as means (SD) unless stated otherwise.

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 7.2 Characteristics after the control phase of participants with a concordant reduction in triglyceride concentrations after both DHA and EPA supplementation and of participants among other categories of responders to DHA and EPA

	Participants with a concordant TG reduction N = 32	Other participants N = 89	P-value §
Age, years	56(16)	53(14)	0.19
Female, % (n)	22(26)	49(59)	0.12
Body mass index, kg/m² *	28.4(3.9)	29.6(4.3)	0.26
Waist cirumference, cm	99.3(10.5)	100.9(11.0)	0.63
Systolic blood pressure, mm HG	117.1(12.6)	115.6(13.3)	0.36
Diastolic blood pressure, mm HG	71.2(8.6)	69.6(10.2)	0.19
Total cholesterol, mmol/L	5.54(0.84)	5.04(0.92)	0.06
LDL-C mmol/L	3.14(0.77)	2.95(0.80)	0.58
Total apolipoprotein B100, g/L *	1.46(0.52)	1.26(0.36)	0.06
HDL-C, mmol/L	1.52(0.40)	1.54(0.42)	0.28
Non-HDL-C, mmol/L	4.01(0.94)	3.50(0.83)	0.02
Triglycerides, mmol/L *	1.90(0.70)	1.21(0.53)	<0.001
Mean LDL size, Å	252.19(2.53)	251.85(2.42)	0.91
LDL peak size, Å	251.80(2.71)	251.55(2.73)	0.94
% small LDL	65.0(14.5)	71.0(13.0)	0.09
% large LDL*	11.5(6.6)	8.4(5.7)	0.03
PCSK9, ng/mL	241.35(56.81)	203.00(63.46)	0.02
% with metabolic syndrome (n)	10(12)	15(18)	0.06
C-reactive protein, mg/L *, †	2.72(1.93)	3.15(2.21)	0.67
Adiponectin, mg/L *	6.88(4.72)	7.14(5.34)	0.50
Interleukin-6, pg/mL*	1.60(1.12)	1.64(1.99)	0.91
Interleukin-18, pg/mL*	278.62(153.26)	274.27(135.68)	0.70
TNF-α, pg/mL*	1.16(0.34)	1.41(1.83)	0.69
Glucose, mmol/L *	5.31(0.69)	5.41(1.01)	0.76
Insulin, pmol/L *	110.34(47.10)	98.40(50.01)	0.03
% N3 in red blood cells	9.44(1.08)	9.36(0.12)	0.75
% EPA in red blood cells	0.91(0.26)	0.88(0.28)	0.97
% DPA in red blood cells	3.01(0.10)	2.92(0.05)	0.59
% DHA in red blood cells	5.31(0.84)	5.38(1.11)	0.48
EPA intakes, mg/d *,‡	66.0(53.2)	77.4(73.2)	0.38
DHA intakes, mg/d *,‡	94.3(64.8)	114.3(102.8)	0.43

Values are expressed as unadjusted means (SD) unless stated otherwise. Concordant reduction group are individuals with a reduction in TG concentrations greater than 0.25 mmol/L after both DHA and EPA. Other responders group includes all other individuals.

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-tranformed values due to the skewness of the distribution.

† N = 29 for the concordant reduction and 84 for other responders due to exclusions for CRP > 10 mg/L.

‡ N = 27 for concordant reduction and 77 for other responders.

§ P-values were obtained with generalized linear models for continuous values or Fisher's exact tests for proportions. Models were adjusted for age, sex and waist circumference at screening.

Table 7.3 Anthropometric measures and cardiometabolic risk factors of the different groups of responders to DHA supplementation after the control phase

	Responders (reduction in TG) N = 54	Non-responders N = 57	Increase in TG N = 10	P-value ‡
Age, years	55 (15)	53 (14)	48 (14)	0.08
% Female (n)	78 (42)	67 (38)	50 (5)	0.15
Body mass index, kg/m²	28.48 (3.69)	29.13 (3.52)	34.59 (6.52)	0.11
Waist cirumference, cm	98.83 (9.87) [a]	99.81 (10.22) [a]	112.64 (12.33) [b]	<0.01
Systolic BP, mmHG	116.50 (11.15)	114.51 (13.72)	121.87 (17.88)	0.51
Diastolic BP, mmHG	70.56 (9.29)	68.98 (10.05)	72.87 (11.15)	0.45
Total cholesterol, mmol/L	5.44 (0.88) [a]	5.09 (0.88) [a,b]	4.21 (0.69) [b]	0.02
LDL-C, mmol/L	3.07 (0.80)	3.04 (0.79)	2.38 (0.57)	0.23
Total apo B, g/L	1.41 (0.48)	1.26 (0.34)	1.12 (0.40)	0.12
HDL-C, mmol/L	1.55 (0.43)	1.56 (0.39)	1.33 (0.40)	0.69
Non HDL-C, mmol/L	3.88 (0.93) [a]	3.54 (0.81) [a,b]	2.88 (0.47) [b]	0.03
Triglyceride, mmol/L*	1.77 (0.64) [a]	1.08 (0.52) [b]	1.09 (0.34) [b]	<0.001
Mean LDL size, Å	252.10 (2.44)	251.95 (2.41)	251.01 (2.68)	0.85
LDL peak size, Å	251.78 (2.54)	251.61 (2.87)	250.73 (2.85)	0.85
% small LDL	67.03 (14.08)	70.52 (13.08)	76.16 (12.29)	0.33
% large LDL*	10.44 (6.35)	8.54 (5.91)	6.05 (3.67)	0.32
% with metabolic syndrome (n)	26 (14)	14 (8)	0 (0)	0.08
PCSK9, ng/mL	239.43 (54.9) [a]	194.22 (62.88) [b]	179.04 (65.47) [a,b]	<0.01
C-reactive protein, mg/L *, †	2.82 (1.95)	2.91 (2.07)	4.85 (2.73)	0.30
Adiponectin, mg/L *	6.84 (4.85)	7.38 (5.41)	6.57 (5.83)	0.54
IL-6, pg/mL *	1.66 (1.12)	1.59 (2.38)	1.69 (0.71)	0.47
IL-18, pg/mL *	280.98 (151.03)	268.52 (125.78)	284.72 (166.16)	0.69
TNF-α, pg/mL *	1.52 (2.26)	1.21 (0.67)	1.21 (0.27)	0.53
Glucose, mmol/L *	5.33 (0.69)	5.44 (1.18)	5.32 (0.46)	0.55
Insulin, pmol/L *	107.11 (51.62) [a]	91.16 (42.57) [b]	130.90 (60.61) [a,b]	0.03
% N3 in red blood cells	9.54 (1.05)	9.54 (1.29)	9.30 (1.26)	0.55
% EPA in red blood cells	0.91 (0.26)	0.91 (0.29)	0.92 (0.25)	0.54
% DPA in red blood cells	2.97 (0.56)	2.97 (0.43)	2.94 (0.36)	0.80
% DHA in red blood cells	5.46 (0.92)	5.46 (1.16)	5.26 (1.05)	0.77

Values are expressed as unadjusted means (SD) unless stated otherwise.

The responders group includes individuals with a reduction in TG concentrations greater than 0.25 mmol/L after DHA (vs. control). The non-responders group includes individuals with a change in TG concentrations between -0.25 and +0.25 mmol/L after DHA (vs. control). The increase group includes individuals with an increase in TG concentrations greater than 0.25 mmol/L after DHA (vs. control).

P values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) and are adjusted for age, sex, and waist circumference after the control phase for continuous values and using Fisher's exact tests for proportions.

Values with different superscript letters are different from each other, $P < 0.05$.

Apo: apolipoprotein; BP: blood pressure; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; IL: interleukin; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-tranformed values due to the skewness of the distribution.

† N = 48 for the responders (reduction) and 55 for non-responders due to exclusions for CRP > 10 mg/L.

‡ Main treatment P-values for the comparison between groups were determined by the main effect.

Table 7.4 Change in anthropometric variables and in cardiometabolic risk factors among the different groups of responders to DHA supplementation (vs. control)

	Responders (TG reduction) N = 54	P-value vs. control ‡,§	Non-responders N = 57	P-value vs. control ‡,§	Increase in TG N = 10	P-value vs. control ‡,§
Body mass index, kg/m^2 *	-0.26 (0.10) [a]	0.03	+0.18 (0.10) [b,c]	0.04	+0.62 (0.26) [b,c]	<0.01
Waist cirumference, cm	-0.86 (0.53)	0.16	+0.17 (0.53)	0.56	+1.01 (1.24)	0.09
Systolic BP, mmHG	-0.40 (1.19)	0.92	-1.80 (1.09)	0.10	+1.97 (2.33)	0.28
Diastolic BP, mmHG	-2.36 (0.84)	0.04	-1.25 (0.90)	0.08	+1.47 (1.98)	0.46
Total cholesterol, mmol/L	-0.07 (0.08) [a]	0.69	+0.28 (0.07) [b,c]	<0.01	+0.61 (0.20) [b,c]	<0.01
LDL-C, mmol/L	+0.07 (0.07)	0.12	+0.22 (0.06)	<0.01	+0.33 (0.21)	0.06
Total apo B, g/L	-0.03 (0.03)	0.57	+0.07 (0.03)	0.05	+0.19 (0.07)	0.05
HDL-C, mmol/L	+0.13 (0.03)	0.001	+0.08 (0.03)	0.01	+0.09 (0.05)	0.05
Non HDL-C, mmol/L	-0.12 (0.06) [a]	0.06	+0.20 (0.06) [b,c]	<0.01	+0.53 (0.21) [b,c]	<0.01
Triglyceride, mmol/L	-0.59 (0.04) [a]	<0.001	-0.05 (0.02) [b]	<0.001	+0.42 (0.06) [c]	<0.001
Mean LDL size, Å	+0.04 (0.27)	0.99	+0.55 (0.26)	0.04	+0.68 (0.74)	0.44
LDL peak size, Å	+0.44 (0.29)	0.14	+0.77 (0.27)	<0.01	+1.04 (0.76)	0.19
% small LDL	+0.94 (1.70)	0.49	-3.17 (1.35)	0.06	-2.02 (2.96)	0.62
% large LDL	-0.94 (0.75)	0.17	+1.10 (0.60)	0.16	+0.01 (1.23)	0.90
PCSK9, ng/mL	-40.12 (6.77)	<0.001	-12.34 (5.97)	<0.001	-11.53 (11.42)	0.01
C-reactive protein, mg/L *, †	-0.33 (0.20)	0.02	-0.14 (0.20)	0.04	-0.47 (0.45)	0.65
Adiponectin, mg/L *	+0.02 (0.19)	0.74	+0.37 (0.22)	0.04	+0.54 (0.19)	0.09
IL-6, pg/mL *	-0.18 (0.24)	0.04	-0.27 (0.31)	0.04	-0.10 (0.06)	0.22
IL-18, pg/mL *	-35.07 (10.00)	0.003	-1.14 (11.5)	0.75	-28.96 (18.46)	0.47
TNF-α, pg/mL *	-0.40 (0.31)	0.07	-0.06 (0.10)	0.06	+0.23 (0.17)	0.86
Glucose, mmol/L *	+0.05 (0.04)	0.15	+0.15 (0.06)	<0.01	+0.26 (0.07)	0.02
Insulin, pmol/L *	-10.30 (4.74)	0.10	+1.82 (3.11)	0.83	+18.10 (16.07)	0.30
% N3 in red blood cells	+4.87 (0.20)	<0.001	+4.66 (0.16)	<0.001	+4.24 (0.38)	<0.001
% EPA in red blood cells	+0.63 (0.05)	<0.001	+0.60 (0.03)	<0.001	+0.47 (0.11)	<0.001
% DPA in red blood cells	-0.85 (0.07)	<0.001	-0.78 (0.05)	<0.001	-0.79 (0.16)	<0.001
% DHA in red blood cells	+5.11 (0.20)	<0.001	+4.84 (0.16)	<0.001	+4.56 (0.40)	<0.001

Values are unadjusted means (SEM).

The responders group includes individuals with a reduction in TG concentrations greater than 0.25 mmol/L after DHA (vs. control). The non-responders group includes individuals with a change in TG concentrations between -0.25 and +0.25 mmol/L after DHA (vs. control). The increase group includes individuals with an increase in TG concentrations greater than 0.25 mmol/L after DHA (vs. control).

P-values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer).

Values with different superscript letters are different from each other, $P < 0.05$.

Apo: apolipoprotein; BP: blood pressure; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; IL: interleukin; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-transformed values due to the skewness of the distribution.

† N = 46 for the responders (reduction) and 54 for non-responders due to exclusions for CRP > 10 mg/L.

‡ Adjusted for age, sex, waist circumference at screening and value of the variable of interest after the control phase.

§ P-values for DHA and EPA changes compared with control values in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.

Table 7.5 Anthropometric variables and in cardiometabolic risk factors among the different groups of responders to EPA supplementation after the control phase

	Responders (reduction in TG) N = 39	Non-responders N = 69	Increase in TG N = 13	P value ‡
Age, years	54 (16)	54 (14)	51 (12)	0.58
% Female (n)	77 (30)	71 (49)	46 (6)	0.11
Body mass index, kg/m² *	28.41 (4.02)	29.86 (4.48)	28.93 (2.69)	0.58
Waist cirumference, cm	98.43 (11.07)	101.51 (11.21)	100.74 (7.09)	0.35
Systolic BP, mmHG	116.58 (13.16)	116.56 (12.88)	111.36 (13.9)	0.17
Diastolic BP, mmHG	70.45 (9.70)	70.52 (9.80)	65.92 (9.83)	0.13
Total cholesterol, mmol/L	5.38 (0.92) [a]	5.19 (0.93) [a,b]	4.46 (0.46) [b]	0.04
LDL-C, mmol/L	3.07 (0.79)	3.04 (0.82)	2.57 (0.52)	0.23
Total apo B, g/L *	1.42 (0.49)	1.28 (0.38)	1.19 (0.34)	0.18
HDL-C, mmol/L	1.49 (0.41)	1.60 (0.42)	1.36 (0.28)	0.06
Non HDL-C, mmol/L	3.90 (0.95) [a]	3.59 (0.84) [a,b]	3.10 (0.65) [b]	0.04
Triglyceride, mmol/L *	1.79 (0.68) [a]	1.21 (0.48) [b]	1.15 (0.88) [b]	<0.001
Mean LDL size, Å	252.03 (2.72)	252.02 (2.31)	251.22 (2.33)	0.81
LDL peak size, Å	251.62 (2.90)	251.72 (2.62)	251.06 (2.79)	0.90
% small LDL *	66.20 (14.43)	70.15 (13.51)	75.25 (9.43)	0.20
% large LDL*	10.72 (6.39)	8.64 (5.96)	7.43 (4.99)	0.17
% with metabolic syndrome (n)	31 (12)	10 (7)	23 (3)	0.02
PCSK9, ng/mL	237.70 (66.88) [a]	204.38 (61.85) [b]	185.91 (41.33) [a,b]	0.03
C-reactive protein, mg/L *, †	2.59 (1.82)	3.32 (2.38)	2.88 (1.53)	0.64
Adiponectin, mg/L *	6.68 (4.62)	7.56 (5.73)	5.68 (3.11)	0.46
IL-6, pg/mL *	2.01 (2.92)	1.48 (0.87)	1.27 (0.58)	0.44
IL-18, pg/mL *	269.87 (148.51)	276.81 (129.57)	284.69 (174.75)	0.90
TNF-α, pg/mL *	1.24 (0.51)	1.43 (2.05)	1.20 (0.52)	0.95
Glucose, mmol/L *	5.29 (0.64)	5.45 (1.13)	5.30 (0.42)	0.90
Insulin, pmol/L *	109.13 (46.64) [a]	102.01 (52.48) [a,b]	76.46 (31.36) [b]	<0.01
% N3 in red blood cells	9.38 (1.15)	9.47 (1.24)	8.89 (0.93)	0.45
% EPA in red blood cells	0.90 (0.26)	0.90 (0.28)	0.82 (0.23)	0.90
% DPA in red blood cells	2.96 (0.54)	2.95 (0.47)	2.90 (0.44)	0.99
% DHA in red blood cells	5.33 (0.95)	5.44 (1.15)	5.00 (0.68)	0.45

Values are expressed as unadjusted means (SD) unless stated otherwise.

The responders group includes individuals with a reduction in TG concentrations greater than 0.25 mmol/L after EPA (vs. control). The non-responders group includes individuals with a change in TG concentrations between -0.25 and +0.25 mmol/L after EPA (vs. control). The increase group includes individuals with an increase in TG concentrations greater than 0.25 mmol/L after EPA (vs. control).

P values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) and are adjusted for age, sex, and waist circumference after the control phase for continuous values and using Fisher's exact tests for proportions.

Values with different superscript letters are different from each other, $P < 0.05$.

Apo: apolipoprotein; BP: blood pressure; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; IL: interleukin; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-transformed values due to the skewness of the distribution.

† N = 34 for the responders (reduction) and 65 for non-responders due to exclusions for CRP > 10 mg/L.

‡ Main treatment P-values for the comparison between groups were determined by the main effect.

Table 7.6 Change in anthropometric variables and in cardiometabolic risk factors among the different groups of responders to EPA supplementation (vs. control)

	Responders (reduction in TG) N = 39	P-value vs. control ‡,§	Non-responders N = 69	P-value vs. control ‡,§	Increase in TG N = 13	P-value vs. control ‡,§
Body mass index, kg/m² *	-0.21 (0.11) [a]	0.10	+0.06 (0.09) [a,b]	0.28	+0.40 (0.20) [b]	0.05
Waist cirumference, cm	-0.33 (0.70)	0.46	+0.22 (0.37)	0.35	+0.49 (0.70)	0.53
Systolic BP, mmHG	-2.35 (1.43)	0.39	-0.30 (0.98)	0.52	+4.82 (2.30)	0.12
Diastolic BP, mmHG	-2.15 (1.00) [a]	0.15	-0.27 (0.76) [a,b]	0.72	+4.74 (2.04) [b]	0.04
Total cholesterol, mmol/L	-0.14 (0.10) [a]	0.11	-0.05 (0.05) [a]	0.35	+0.43 (0.14) [b]	0.03
LDL-C, mmol/L	+0.11 (0.08)	0.14	-0.01 (0.04)	0.90	+0.33 (0.14)	0.03
Total apo B, g/L	-0.05 (0.03) [a]	0.12	+0.02 (0.01) [a]	0.75	+0.15 (0.05) [b]	<0.01
HDL-C, mmol/L	+0.02 (0.03)	0.48	-0.02 (0.02)	0.26	-0.01 (0.04)	0.43
Non HDL-C, mmol/L	-0.16 (0.09) [a]	0.09	-0.02 (0.05) [a]	0.64	+0.44 (0.12) [b]	<0.01
Triglyceride, mmol/L	-0.57 (0.05) [a]	<0.001	-0.04 (0.01) [b]	0.05	+0.40 (0.05) [c]	<0.001
Mean LDL size, Å	-0.20 (0.30)	0.21	-0.39 (0.17)	0.02	-1.12 (0.46)	<0.01
LDL peak size, Å	+0.08 (0.31) [a]	0.84	-0.28 (0.18) [a,b]	0.09	-1.27 (0.57) [b]	0.00
% small LDL *	+2.01 (2.10)	0.19	+2.01 (1.07)	0.01	+2.99 (1.98)	0.05
% large LDL *	-0.91 (0.88)	0.25	-0.31 (0.53)	0.03	-0.78 (0.73)	0.62
PCSK9, ng/mL	-39.90 (8.10)	<0.001	-10.72 (6.09)	<0.01	+7.61 (10.63)	0.90
C-reactive protein, mg/L *, †	+0.29 (0.29)	0.98	-0.25 (0.19)	0.12	-0.03 (0.54)	0.52
Adiponectin, mg/L *	-0.06 (0.26)	0.24	-0.18 (0.21)	0.02	+0.34 (0.30)	0.20
IL-6, pg/mL *	-0.69 (0.44)	0.03	-0.03 (0.12)	0.04	+0.23 (0.47)	0.83
IL-18, pg/mL *	-5.18 (10.57) [a]	0.62	-13.97 (8.78) [a]	0.55	+77.66 (41.53) [b]	0.00
TNF-α, pg/mL *	-0.17 (0.08)	0.06	-0.18 (0.28)	0.77	+0.48 (0.46)	0.27
Glucose, mmol/L *	+0.03 (0.05)	0.08	+0.01 (0.05)	0.09	+0.02 (0.06)	0.89
Insulin, pmol/L *	-5.59 (4.92)	0.89	+5.12 (4.66)	0.22	+25.08 (8.41)	0.02
% N3 in red blood cells	+6.34 (0.24)	<0.001	+5.59 (0.18)	<0.001	+5.22 (0.32)	<0.001
% EPA in red blood cells	+4.27 (0.15)	<0.001	+3.82 (0.12)	<0.001	+3.50 (0.19)	<0.001
% DPA in red blood cells	+2.61 (0.10)	<0.001	+2.47 (0.07)	<0.001	+2.36 (0.22)	<0.001
% DHA in red blood cells	-0.51 (0.17)	<0.01	-0.68 (0.12)	<0.001	-0.66 (0.17)	0.06

Values are unadjusted means (SEM).

The responders group includes individuals with a reduction in TG concentrations greater than 0.25 mmol/L after EPA (vs. control). The non-responders group includes individuals with a change in TG concentrations between -0.25 and +0.25 mmol/L after EPA (vs. control). The increase group includes individuals with an increase in TG concentrations greater than 0.25 mmol/L after EPA (vs. control).

P-values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer).

Values with different superscript letters are different from each other, $P < 0.05$.

Apo: apolipoprotein; BP: blood pressure; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; IL: interleukin; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-transformed values due to the skewness of the distribution.

† N = 36 for the responders (reduction) and 64 for non-responders due to exclusions for CRP > 10 mg/L.

‡ Adjusted for age, sex, waist circumference at screening and value of the variable of interest after the control phase.

§ P-values for DHA and EPA changes compared with control values in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.

Supplemental Table 7.1 Change in anthropometric variables and cardiometabolic risk factors among TG responders to both DHA and EPA supplementation

	DHA vs. control N=32	P-value vs. control ‡,§	EPA vs. control N=32	P-value vs. control ‡,§	P-value DHA vs. EPA ‡,¶
Body mass index, kg/m^2	-0.27(0.11)	0.49	-0.20(0.13)	0.80	0.67
Waist circumference, cm	-0.94(0.70)	0.60	-0.59(0.83)	0.92	0.59
Systolic blood pressure, mmHg	-2.89(1.53)	0.36	-2.95(1.62)	0.34	0.96
Diastolic blood pressure, mmHg	-4.19(1.08)	0.02	-2.59(0.99)	0.20	0.13
Total cholesterol, mmol/L	-0.11(0.08)	0.35	-0.20(0.09)	0.08	0.35
LDL-C, mmol/L	+0.06(0.07)	0.84	0.08(0.08)	0.64	0.72
Total apolipoprotein B100, g/L	-0.06(0.03)	0.24	-0.06(0.03)	0.35	0.78
HDL-C, mmol/L	+0.14(0.02)	<0.001	-0.01(0.03)	0.89	<0.001
Non-HDL-C, mmol/L	-0.11(0.09)	0.02	+0.09(0.09)	0.07	0.54
Triglycerides, mmol/L *	-0.67(0.06)	<0.001	-0.61(0.06)	<0.001	0.22
Mean LDL size, Å	+0.01(0.37)	0.37	-0.44(0.33)	0.05	0.21
LDL peak size, Å	+0.53(0.42)	0.50	-0.17(0.34)	0.34	0.07
% small LDL	+2.20(2.55)	0.16	+2.88(2.29)	0.11	0.77
% large LDL *	-1.63(1.03)	0.002	-1.12(1.02)	0.06	0.08
PCSK9, ng/mL	-37.31(10.15)	<0.001	-39.14(8.73)	<0.001	0.83
C-reactive protein, mg/L *,†	0.00(0.20)	0.44	+0.30(0.32)	0.81	0.37
Adiponectin, mg/L	+0.11(0.28)	0.91	+0.01(0.24)	0.67	0.63
Interleukin-6, pg/mL*	-0.25(0.18)	0.16	-0.31(0.19)	0.08	0.54
Interleukin-18, pg/mL*	-36.79(9.83)	<0.001	-10.60(10.56)	0.04	0.02
TNF-α, pg/mL*	-0.04(0.13)	0.77	-0.15(0.07)	0.32	0.19
Glucose, mmol/L *	+0.05(0.04)	0.39	+0.02(0.05)	0.76	0.54
Insulin, pmol/L *	-10.06(6.51)	0.07	-4.63(5.89)	0.58	0.29

Values are expressed as unadjusted means (SEM).

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; N3: long-chain omega-3 fatty acids; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.

* Analyses were performed on log-tranformed values due to the skewness of the distribution.

† n = 28 for DHA and 29 for EPA due to exclusions for CRP > 10 mg/L.

‡ P-values were obtained with generalized linear models for repeated measurements for continuous values or Fisher's exact tests for proportions. Models were adjusted for age, sex and waist circumference at screening and the respective value after the control phase.

§ P-values for EPA and DHA changes compared with control values in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.

¶ Main treatment P-values for the comparison between EPA and DHA changes compared with control values in the outcome were determined by the main treatment effect.

Supplemental Table 7.2 Gene expression of lipid metabolism after DHA and EPA, vs. control (N=44)

Gene	Control	DHA	% vs. control	P-value vs. control *,†	EPA	% vs. control	P-value vs. control *,†	P-value DHA vs. EPA*,‡
HMG CoA reductase	1884 (396)	1971 (449)	+5%	0.25	1911 (498)	+1%	0.72	0.46
LDL receptor	387 (146)	367 (95)	-5%	0.27	374 (104)	-3%	0.45	0.68
SREBP1c	1995 (518)	2015 (478)	+1%	0.83	2003 (473)	0%	0.96	0.83
SREBP2	2485 (661)	2456 (526)	-1%	0.72	2436 (415)	-2%	0.57	0.80

Values are expressed as unadjusted means (SD). For each gene, expression is presented as no. of copies of mRNA normalized for the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GADPH).

HMG-CoA reductase: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; LDL: low-density lipoprotein; SREBP: sterol regulatory element-binding protein.

* P-values were obtained with generalized linear models for repeated measurements for continuous values or Fisher's exact tests for proportions. Models were adjusted for age, sex and waist circumference at screening.

† P-values for DHA and EPA changes compared with control values in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.

‡ Main treatment P-values for the comparison between DHA and EPA changes compared with control values in the outcome were determined by the main treatment effect.

Figure

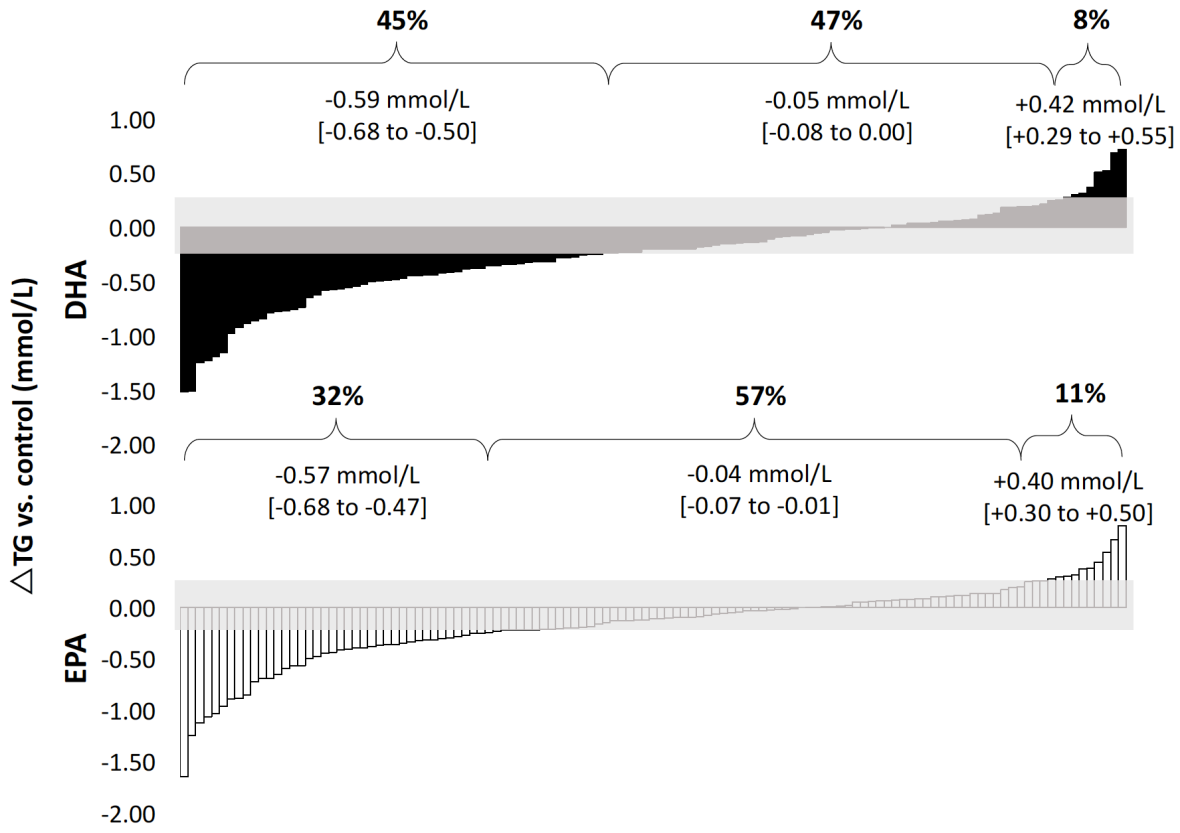

Figure 7.1 Individual change in TG (vs. control) after DHA and EPA supplementation

One column represents one subject. Data are sorted to show the range of variation in the TG response after both DHA (black) and EPA (white). The grey zone represents the intra-individual variation range of ± 0.25 mmol/L. Values in brackets are 95% confidence intervals. After DHA: 45% (N=54) were responders, 47% (N=57) were non-responders and 8% (N=10) had an increase in TG greater than 0.25 mmol/L. After EPA: 32% (N=39) were responders 57% (N=69) were non-responders and 11% (N=13) had an increase in TG greater than 0.25 mmol/L.

Chapitre 8 Comparaison de la réponse du cholestérol des LDL à une supplémentation en EPA et en DHA

Janie Allaire, Cécile Vors, William S Harris, Kristina Harris Jackson, André Tchernof, Patrick Couture, Benoît Lamarche

L'article présenté dans ce chapitre s'intitule: Is the increase in LDL-C after high-dose supplementation with either DHA or EPA consistent, and should we worry about it? The ComparED study.

Cet article a été soumis pour publication dans *The Journal of Nutrition* le 7 mai 2019.

Résumé

La supplémentation en acide docosahexaénoïque (DHA) augmente les concentrations sériques de C-LDL de façon plus importante qu'une supplémentation en acide eicosapentaénoïque (EPA). Or, il semble y avoir une importante variabilité intra- et inter- individuelle en réponse à chacun de ces deux acides gras. L'objectif de cette étude était de caractériser la variabilité intra- et inter- individuelle dans la réponse du C-LDL à une supplémentation en DHA et en EPA. Un total de 154 individus ont été assignés de façon aléatoire à une séquence de trois phases expérimentales de 2,7 g par jour d'EPA, 2,7 g par jour de DHA et 3 g par jour d'huile de maïs à titre de témoin. Chaque phase expérimentale était d'une durée de 10 semaines et les phases étaient séparées par une période libre de 9 semaines. La variation quotidienne moyenne des concentrations de C-LDL a été calculée en utilisant l'écart type de la moyenne des valeurs de triglycérides au « screening » et avant chacune des trois phases de l'étude. La réponse du C-LDL à une supplémentation en DHA et en EPA est restée à l'intérieur de la variation quotidienne (± 0,30 mmol/L) chez environ la moitié des participants (51% et 54% respectivement). Des proportions similaires de participants ont présenté une augmentation de la concentration de C-LDL supérieure à 0,30 mmol/L après le DHA (28%) et l'EPA (24%). L'augmentation absolue était plus grande après le DHA qu'après l'EPA (DHA : +0,70 mmol/L [IC à 95% +0,60 à +0,80 mmol/L] ; EPA : +0,56 mmol/L [IC à 95% +0,46 à +0,66 mmol/L]) chez ce groupe de répondeurs. Des proportions similaires de participants ont présenté une diminution de la concentration de C-LDL supérieure à 0,30 mmol/L après le DHA et l'EPA (21% vs 23% respectivement). La diminution du C-LDL était similaire après le DHA et l'EPA (DHA : -0,55 mmol/L [IC à 95% -0,63 à -0,48 mmol/L] ; EPA : -0,62 mmol/L [IC à 95% -0,71 à -0,54 mmol/L]) chez ce groupe de répondeurs. Bien qu'en moyenne, l'augmentation des concentrations de C-LDL est plus importante suite à une supplémentation en DHA qu'en EPA, plus de 70% des individus ont présenté une réponse à l'intérieur de la variabilité observée quotidiennement ou ont présenté une diminution des concentrations de C-LDL après le DHA ou l'EPA. Des études supplémentaires sont nécessaires pour documenter comment ces différences dans la réponse influencent l'effet de l'EPA et du DHA sur le risque cardiovasculaire.

Abstract

Background: Although supplementation with docosahexaenoic acid (DHA) increases serum LDL-cholesterol (LDL-C) concentrations more than eicosapentaenoic acid (EPA), there seems to be important intra- and inter-individual variations in the response to either treatment.

Objective: The objective of this study was to better understand the magnitude of the intra- and inter-individual LDL-C response to DHA and EPA. In a randomized, double-blind, crossover study, 154 individuals with abdominal obesity and subclinical inflammation were randomized to a sequence of three 10-week supplementation phases: 2.7 g/d DHA, 2.7 g/d EPA and 3 g/d corn oil (0 g DHA+EPA), separated by nine-week washouts.

Methods: As secondary analyses, intra-individual variation in LDL-C concentrations (±0.30 mmol/L), which accounts for the measurement error as well as biological variability, was calculated as the standard deviation from the mean of four samples taken at screening and before each phase.

Results: Variation in LDL-C was within the ±0.30 mmol/L range in half of participants after DHA (51%) and EPA (54%). The increase in LDL-C among those with an increase > 0.30 mmol/L (28% of participants after DHA and 24% after EPA) was greater after DHA than after EPA (DHA: +0.70 mmol/L [95%CI +0.60 to +0.80 mmol/L] vs. EPA: +0.56 mmol/L [95%CI +0.46 to +0.66 mmol/L]). Similar proportions of individuals had their LDL-C reduced > 0.30 mmol/L after DHA and EPA (21% vs. 23% respectively). The mean reduction in LDL-C in both groups was also of comparable magnitude (DHA: -0.55 mmol/L [95%CI -0.63 to -0.48 mmol/L]; EPA: -0.62 mmol/L [95%CI -0.71 to -0.54 mmol/L]).

Conclusions: Although on average the increase in LDL-C was greater after DHA than after EPA, more than 70% of individuals showed no change or a reduction in LDL-C after either supplement. Futures studies should document how some but not all individuals may benefit from DHA and EPA supplementation.

Title page

Is the increase in LDL-C after high-dose supplementation with either DHA or EPA consistent, and should we worry about it? The ComparED study

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Running title: Variability in LDL-C response after EPA and DHA

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Clinical Trial Registry number and website: http://www.clinicaltrials.gov (NCT01810003).

Abbreviations: Apo : apolipoprotein ; CRP : C-reactive protein; DHA : docosahexaenoic acid; EPA : eicosapentaenoic acid; HDL-C : high-density lipoprotein cholesterol; HMG-CoA reductase : 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HOMA-IR : homeostatic model assessment of insulin resistance; IL : interleukin; LCn3-PUFA : long-chain omega-3 polyunsaturated fatty acid; LDL-C : low-density lipoprotein cholesterol; LPL : lipoprotein lipase; PCSK9 : proprotein convertase subtilisin/kexin type 9; REDUCE-IT : Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; SD : standard deviation; SREBP : sterol regulatory element-binding protein; TG : triglyceride; TNF-α : tumor necrosis factor alpha; VLDL : very-low density lipoprotein.

Introduction

Recent evidence from large randomized controlled trials has generated conflicting results regarding the protective effect of long-chain omega-3 polyunsaturated fatty acid (LCn3-PUFA) supplementation on fatal and non-fatal vascular events (1). The majority of available studies have used supplements containing mixtures of docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in various forms and proportions. DHA and EPA have generally been recommended indiscriminately in the management of cardiovascular risk (2), but there are important differences in the response to either LCn3-PUFA, including a greater increase in low-density lipoprotein cholesterol (LDL-C) concentration after DHA than after EPA supplementation (3, 4). While DHA may exert beneficial effects on a number of other cardiovascular risk factors such as high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations compared with EPA (3, 4), there is now evidence that supplementation with 4 g/d of EPA alone reduces the risk of cardiovascular events by 25% among individuals at risk of cardiovascular events (5). The extent to which DHA alone influences the risk of cardiovascular diseases remains unknown.

The inter-individual variability in the plasma TG response to LCn3-PUFA supplementation has been fairly well documented in several clinical studies (6-8). This is not the case for LDL-C. One study reported that the LDL-C response to supplementation with 3 g/d of EPA + DHA varied from -49% to +87% among 55 hyperlipidemic individuals (9). Such variability accounts for day-to-day fluctuations in LDL-C concentration, which may be as high as 20% (10-12). Intra-individual variation in LDL-C, which includes biological variation as well as analytical variation, can be estimated as the average within-subject standard deviation based on two or more measurements taken on different days, when no change in LDL-C is expected. To our knowledge, no study has yet assessed the inter- and intra- variability in the LDL-C response to high doses of DHA and EPA. This is not a trivial issue considering that LDL-C is a key risk factor for cardiovascular disease and that the LCn3-PUFA supplement is one of the most consumed supplements in United-States (13).

The objectives of this study were to: 1- examine the magnitude of the intra- and the inter-individual LDL-C response to both DHA and EPA supplementation; 2- examine the change in other risk factors associated with the LDL-C response to DHA and EPA; and 3- identify characteristics associated with the LDL-C response to DHA and EPA. Because previous studies have shown that DHA increases LDL-C more than EPA, we hypothesized that DHA increases LDL-C in a greater proportion of individuals than EPA. We also hypothesized that individuals with higher LDL-C concentrations at baseline are more likely to present an increase in LDL-C after DHA and EPA supplementation.

Material and Methods

Study design: Details of the study design have been published previously (14). Briefly, we used a double-blind randomized, controlled crossover design with 3 phases of 3 identical capsules per day providing: 1- 2.7 g/d of DHA; 2- 2.7 g/d of EPA; and 3- 0 g/d of DHA+EPA (corn oil control). DHA and EPA were provided as 90% purified re-esterified LCn3-PUFAs. Phases had a median duration of 10 weeks and were separated by nine-week washouts. Participants were recruited at the Institute of Nutrition and Functional Foods in Quebec City and were randomized to one of the six sequences of treatments stratified by sex using an in-house computer program. Allocations to sequences of treatments were concealed to participants as well as to study coordinators throughout the study. The original and primary objective of this study was to compare the effect of DHA and EPA on plasma C-reactive protein (CRP) concentrations, and thus, CRP was used as the primary outcome measure for the sample size calculation (14). *A priori* sample size calculations indicated that N=150 would be needed to detect a minimal difference of 10% in plasma CRP concentrations when comparing any two of the three treatments with a power of 81% and $P<0.01$ (two-tailed) (14). A total of 154 randomized participants signed an informed consent document approved by local Ethics Committees at the beginning of the study. The study protocol was registered March 4, 2013 at ClinicalTrials.gov (NCT01810003). This is a secondary analysis of this trial.

Study population: Eligibility criteria were to have a high waist circumference ($\geq$80 cm for women and $\geq$94 cm for men (15)) and subclinical inflammation (plasma CRP concentration between 1 and 10 mg/L).

Compliance: Compliance to supplementation was high based on accounts of returned supplements (14) as well as on measurements of DHA and EPA incorporation into red blood cells (16).

Characteristics and risk factors assessment*:* As indicated previously (14), blood samples were collected after a 12-h overnight fast at screening, beginning of each treatment phase and on two consecutive days at the end of each treatment phase. The mean of the two measurements after each phase was used in the analyses for total cholesterol, LDL-C, HDL-C, TG, total apolipoprotein B (apo B), CRP, adiponectin and interleukin-6 (IL-6) (14). Tumor necrosis factor alpha (TNF-α) and interleukin-18 (IL-18) were measured once. Methods to assess cardiometabolic outcomes have been published elsewhere (14). Serum non-HDL-C concentration was calculated as the difference between total serum cholesterol concentrations and HDL-C concentrations. Serum LDL-C concentrations were calculated using the Friedewald Equation. Non-denaturing 2%-16% polyacrylamide gradient gel electrophoresis was used to characterize various features of the LDL particle size phenotype (17), including LDL peak particle size and mean LDL particle size as well as the proportion of LDL in the various size categories (small and large). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the formula developed by Matthews *et al* (18).

All personnel involved in the intervention and the measurements of the study outcomes were blinded to treatments.

***Statistical analyses**:* The primary objective of this study was to compare the effect of DHA and EPA on inflammation markers (14). Because polyunsaturated omega-6 fatty acids exert neutral effect on inflammation markers (19), corn oil was selected as the control treatment for this randomized controlled trial. However, 10-week corn oil supplementation decreased LDL-C compared to baseline (-0.15 mmol/L, $P<0.01$, not shown). Thus, we used data from the DHA and EPA phases only for the specific purpose of this study. The effects of DHA and EPA supplementation on serum LDL-C concentrations were calculated as the difference between the post-treatment minus pre-treatment values for both DHA and EPA treatments.

Intra-individual variation in serum LDL-C concentrations, reflecting usual biological fluctuations as well as analytical variation, was calculated as the standard deviation (±SD mmol/L) of the mean of four samples taken at screening and before the three study phases. The mean intra-individual variation in LDL-C concentrations was ±0.30 mmol/L. Participants were separated in three different groups according to their LDL-C response to DHA and EPA supplementation: those among whom the reduction in serum LDL-C was greater than 0.30 mmol/L (favorable response, i.e. LDL-C reduction), those among whom LDL-C variation was within ±0.30 mmol/L (non-responders, i.e. no change in LDL-C) and those among whom the increase in serum LDL-C was greater than 0.30 mmol/L (unfavorable response, i.e. increase in LDL-C).

The differences in baseline characteristics and risk factors among the various LDL-C responder groups after each treatment (DHA and EPA) were assessed using generalized linear models (proc MIXED with a Variance Components covariance matrix). Categorical variables were compared using Fisher's exact test. Changes in anthropometric measurements and risk factors after DHA and EPA supplementation among the LDL-C responder groups were compared using generalized linear models (proc MIXED with a Variance Components covariance matrix). Models were adjusted for age, sex, waist circumference at screening and the baseline value of the variable of interest before the specific treatment. The skewness of the distribution of the model residuals was assessed and data were transformed when required. All analyses were computed using SAS (v9.3, Cary, NC).

Results

Participants' characteristics

Of the 154 individuals randomized to the treatment sequences, 121 participants completed all three study phases between April 3, 2013 and June 19, 2015. Screening, pre-treatment and post-treatment LDL-C data were available for 119 participants for both the EPA and DHA phases. Characteristics of the 119 participants at screening are presented in Table 1. As per our recruitment criteria, men and women had a high waist circumference (mean (SD) 106 $\pm$ 8 and 97 $\pm$ 10 cm respectively) and plasma CRP concentrations of 2.8 $\pm$ 1.9 and 3.5 $\pm$ 2.4 mg/L respectively but were otherwise healthy. Participants had serum TG and LDL-C concentrations within the normal range.

LDL-C response to DHA and EPA

The LDL-C response to DHA and EPA supplementation compared with the treatment-specific baseline value is presented in Figure 1. Change in LDL-C concentrations was within the ±0.30 mmol/L range in approximately half of participants after DHA (51%) and EPA (54%). LDL-C increased by more than 0.30 mmol/L in 28% of participants after DHA (mean increase: +0.70 mmol/L [95% CI +0.60 to +0.80 mmol/L]) and in 24% of participants after EPA (+0.56 mmol/L [95% CI +0.46 to +0.66 mmol/L]). Similar proportions of individuals had a reduction in LDL-C greater than 0.30 mmol/L after DHA and EPA (21% vs. 23%), also with similar mean absolute reduction in LDL-C (DHA: -0.55 mmol/L [95% CI -0.63 to -0.48 mmol/L]; EPA: -0.62 mmol/L [95% CI -0.71 to -0.54 mmol/L]). A total of 8% of participants presented a concordant increase in LDL-C after both DHA and EPA, 29% were non-responders to both treatments and 6% had a reduction in LDL-C after both DHA and EPA (data not shown). Thus, 57% had a discordant response to DHA and EPA (data not shown).

Determinants of the LDL-C response to DHA and EPA

None of the baseline characteristics of participants were associated with their LDL-C response to DHA (Table 2). On the other hand, individuals among whom LDL-C concentrations were increased after EPA had a lower baseline body mass index (BMI), systolic blood pressure and CRP concentrations compared with non-responders to EPA (P=0.003, 0.01 and 0.03 respectively, Table 3). They also had lower total cholesterol concentrations and LDL-C concentrations at baseline than participants with a reduction in LDL-C after EPA (P=0.03 and 0.02 respectively).

Cardiometabolic changes associated with the LDL-C response to DHA and EPA

Individuals among whom LDL-C concentrations increased after DHA also presented an increase in total cholesterol (+14.4%), total apo B (+17.8%) and non-HDL-C (+16.8%) compared to non-responders and to those with a reduction in LDL-C (Table 4, all P<0.0001). They also presented increases in HDL-C (+10.6%) and IL-6 (+10.5%) concentrations that were greater than among participants with a reduction in LDL-C (Table 4, all P<0.05). Individuals with an increase in LDL-C after EPA supplementation showed increases in total cholesterol (+11.4%) and in non-HDL-C (+15.5%) concentrations compared to individuals with a reduction in LDL-C or to non-responders (Table 5, all P<0.0001). Individuals with an increase in LDL-C after EPA supplementation also presented an increase in blood glucose (+3.7%) that was greater than that of individuals with a reduction in LDL-C after EPA (P=0.03). The reduction in TG concentration was similar among the various groups of responders after both DHA and EPA supplementation (Tables 4 and 5) and the change in LDL-C concentrations was not correlated with the change in TG concentrations (data not shown).

Discussion

Most previous clinical studies have reported neutral effects of LCn3-PUFA supplementation on the risk of cardiovascular events (1). However, recent evidence from The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) indicated that supplementation with 4 g/d of EPA alone reduces the risk of cardiovascular events by 25% in high-risk individuals (5). The impact of DHA supplementation alone in studies with hard endpoints has not been investigated yet. LCn3-PUFAs are known to increase LDL-C concentrations, with greater effects of DHA compared with EPA, thus emphasizing the importance to better understand the impact of such differences from a cardiovascular disease prevention perspective. To the best of our knowledge, this is the first study to characterize the variability and to identify predictors of the LDL-C response to high doses of DHA and EPA. In this double-blind randomized crossover study, supplementation with 2.7 g/d of DHA and 2.7 g/d of EPA produced an increase in LDL-C in a similar proportion of participants, approximately 25%. However, the absolute increase in LDL-C was greater after DHA than after EPA among those with such an unfavorable response. The increase in LDL-C concentrations was also associated with an increase in other cholesterol-related variables after both DHA and EPA supplementation. Importantly, almost 50% of individuals experienced no meaningful change in LDL-C concentrations after DHA or EPA supplementation, while both meaningfully reduced LDL-C concentrations in more than 20% of individuals.

Increased LDL-C concentration is an independent and strong predictor of cardiovascular risk (20, 21), despite the fact that visit-to-visit LDL-C concentration may vary by more than 20% (12) and a large inter-individual variations in the LDL-C response to statin regimen (22, 23). Here, we used the standard deviation of four untreated LDL-C measurements to determine its coefficient of variation in our sample of participants. This metric comprises both analytical and biological variations. We have previously shown that, compared with the corn oil control, the mean increase in LDL-C concentrations was greater after DHA than after EPA supplementation (+0.16 mmol/L vs. +0.07 mmol/L respectively, P=0.038) (14). The present study reveals that even if DHA and EPA increase LDL-C concentrations in a similar proportion of individuals, this increase remains more important after DHA than after EPA among those in whom LDL-C is increased. The proportions of individuals with an increased in LDL-C after DHA (28%) or EPA (24%) were, of course, lower than proportions seen if we had used a threshold of zero mmol/L as a cutoff to mark a “meaningful” LDL-C increase (55% and 49% for DHA and EPA respectively, not shown). Thus, almost half of individuals with an increase in LDL-C > 0 mmol/L after DHA and EPA are in fact non-responders to DHA and EPA supplementation. These observations first emphasize the importance of not considering only the mean change in LDL-C after supplementation with LCn3-PUFA, but also to consider the “usual” variation of a biomarker to assess the effect of a diet or a treatment on key cardiometabolic risk factors.

Common polymorphisms related to the production and catabolism of LDL have been identified as determinants of the LDL-C response to statin regimen (24). But to our knowledge, data on the effect of various gene polymorphisms on the LDL-C response to DHA and EPA are still missing. One study reported the impact of the apo E genotype on the response of various cardiovascular risk factors to four weeks of supplementation with EPA- and DHA- rich oils among 38 normolipidaemic men (25). There was an increase in LDL-C concentrations in the apo E3/E4 genotype group (P=0.029), but not in the apo E3/E3 genotype group after the DHA treatment compared with baseline. Authors found no association between the LDL-C response and the apo E genotype after the EPA treatment. We analyzed the expression of genes involved in lipid metabolism among a subgroup of participants (N=44) in this study and found that neither DHA nor EPA modified the expression of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*HMG-CoA reductase)*, *LDL-receptor*, and sterol regulatory element-binding protein 1c and 2 (*SREBP1* and *SREBP2)* in whole blood cells compared with the control treatment (26). Interestingly, the change in LDL-C concentrations was correlated with the expression of the *LDL-receptor* and *SREBP1c* after DHA, but not after EPA (data not shown).

We and others have previously observed that DHA reduces apo CIII production compared with EPA (27, 28). Apo CIII inhibits the activity of lipoprotein lipase (LPL), an enzyme responsible for lipoprotein-bound and hydrolysis of very-low density lipoprotein (VLDL) (29). In this study, DHA and EPA had no effect on the expression of the LPL gene in whole blood cells (30). It has been suggested that the LDL-C raising effect of DHA is partly due to a reduction in whole body LDL uptake (25) and to an increase in the production of smaller VLDL particles, which are the precursors of LDL (31). Interestingly, the change in proprotein convertase subtilisin/kexin type 9 (PCSK9) concentrations was not associated with the change in *LDL-receptor* gene expression in whole blood cells after either DHA or EPA compared with the control in this study (26). PCSK9 regulates cholesterol metabolism by degrading cellular LDL-receptors, thus inhibiting the clearance of LDL from the circulation (32). In the present study, the different types of responders presented similar PCSK9 concentrations after both DHA and EPA supplementation (data not shown). We have previously shown that DHA increases the size of LDL as well as the production and the clearance rate of LDL-apo B100 compared with EPA (27), but that DHA and EPA equally reduce PCSK9 concentrations (27). Taken together, these observations suggest that DHA and EPA exert differential effects on LDL metabolism, more specifically on LDL production but perhaps also on LDL clearance, however to an extent that may be independent of PCSK9. How each of these pathways contributes to explaining the variability in the LDL-C response to EPA and DHA needs to be further investigated.

The LCn3-PUFA content of red blood cells after DHA and EPA supplementation was similar between the different groups of responders, suggesting that DHA and EPA absorption and rate of incorporation into cells has no influence in determining the LDL-C response to DHA and EPA (data not shown). Baseline characteristics such as age, sex, BMI and baseline LDL-C response have been identified as modulators of the statin-induced

LDL-C response (33, 34), but none of these characteristics were associated with the increase in LDL-C concentrations after DHA supplementation in the present study. Participants with an unfavorable LDL-C response after EPA supplementation had lower BMI, systolic blood pressure, total cholesterol and LDL-C concentrations at baseline. On the other hand, participants with a favorable LDL-C response after EPA supplementation were those who had elevated LDL-C concentrations at baseline. These observations suggest that high-risk participants (i.e. those with the highest LDL-C concentrations at baseline) are those who may benefit more from EPA supplementation. Importantly, we observed that the reduction in LDL-C concentrations was associated with a concomitant improvement in other cardiovascular risk factors after supplementation with either DHA or EPA. The LDL-C response showed no parallel association with the TG response to DHA and EPA supplementation, with all groups having similar TG reductions after both treatments. Because our study participants had normal TG concentrations at baseline, LCn3-PUFA supplements are not necessarily indicated, and our current observations must be validated in individuals with high TG concentrations, who are a more traditional target population for LCn3-PUFA supplementation.

Key strengths of this randomized trial include the crossover design, the large number of participants and the high compliance to treatments. Non-adherence to treatments is probably one of the most important factors influencing the variability in the cardiometabolic response to interventions in clinical trials. However, the mean compliance measured both by counting returned supplements and incorporation of DHA and EPA in red blood cells was high in all arms of this study (14). Thus, the variability in the LDL-C response seen in this analysis is likely attributable to factors other than variability in the compliance to treatments. The number of participants in the study (N=119) may have been sufficient to characterize the LDL-C response to DHA and EPA supplementation, but insufficient to identify predictors of the LDL-C response to either supplement. The primary objective of this randomized controlled trial was to compare the effect of DHA and EPA supplementation on inflammation markers. Because the control treatment with corn oil decreased LDL-C concentration by -0.15 mmol/L compared with baseline value (P=0.001, not shown), only data from the EPA and the DHA phase were used in our analysis. However, the baseline value of DHA and EPA was similar (3.08 ± 0.07 mmol/L for DHA and 3.08 ± 0.07 mmol/L for EPA).

In conclusion, data from this randomized crossover trial have demonstrated that approximately 25% of individuals show an unfavorable serum LDL-C response to supplementation with either DHA and EPA, while more than 50% have no meaningful change in LDL-C. We could not identify predictors of an increase in LDL-C after DHA supplementation, whereas individuals with an increase in LDL-C after EPA supplementation were those with a favorable baseline lipid profile. A reduction in LDL-C after either DHA or EPA supplementation was seen in approximately 25% of individuals and was accompanied with an improvement in several other cardiovascular risk factors. Previous clinical studies have consistently shown neutral effects of LCn3-PUFA

supplementation combining EPA and DHA in various proportions on cardiovascular events (1). Data from REDUCE-IT have challenged the field by demonstrating cardiovascular benefits after supplementation with high-dose EPA (5). Futures studies are needed to identify individuals most likely to benefit from DHA and EPA supplementation taken individually in terms of cardiovascular prevention.

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Other authors have no disclosure.

Conflicts of interests

Authors report no conflict of interest.

Authorship

BL, PC and AT have designed and obtained funding for this study. PC was responsible of the medical supervision of the study. CV provided significant help with the analysis of the results. JA performed statistical analyses and wrote de manuscript, which was reviewed critically by all authors. BL had primary responsibility for final content. All authors critically revised the manuscript and contributed intellectually to its development, provided final approval of the submitted manuscript, had full access to all of the data in the study, take responsibility for the integrity of the data and the accuracy of the data in the analysis, affirm that the article is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted.

References

1. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, et al. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77917 Individuals. JAMA Cardiol. 2018;3(3):225-34.
2. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, et al. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory From the American Heart Association. Circulation. 2017;135(15):e867-e84.
3. Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. Current atherosclerosis reports. 2011;13(6):474-83.
4. Innes JK, Calder PC. The Differential Effects of Eicosapentaenoic Acid and Docosahexaenoic Acid on Cardiometabolic Risk Factors: A Systematic Review. International journal of molecular sciences. 2018;19(2).
5. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. The New England journal of medicine. 2019;380(1):11-22.
6. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, et al. Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr. 2008;88(3):618-29.
7. Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, et al. Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. J Nutrigenet Nutrigenomics. 2013;6(2):73-82.
8. Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, et al. ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(8):1990-7.
9. Minihane AM. Fatty acid-genotype interactions and cardiovascular risk. Prostaglandins, leukotrienes, and essential fatty acids. 2010;82(4-6):259-64.
10. Alexander KS, Kazmierczak SC, Snyder CK, Oberdorf JA, Farrell DH. Prognostic utility of biochemical markers of cardiovascular risk: impact of biological variability. Clin Chem Lab Med. 2013;51(9):1875-82.
11. Bookstein L, Gidding SS, Donovan M, Smith FA. Day-to-day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. Arch Intern Med. 1990;150(8):1653-7.
12. Boey E, Gay GM, Poh KK, Yeo TC, Tan HC, Lee CH. Visit-to-visit variability in LDL- and HDL-cholesterol is associated with adverse events after ST-segment elevation myocardial infarction: A 5-year follow-up study. Atherosclerosis. 2016;244:86-92.
13. Clarke TC BL, Stussman BJ, Barnes PM, Nahin RL. Trends in the use of complementary health approaches among adults: United States, 2002–2012. National health statistics reports. Hyattsville, MD: National Center for Health Statistics.2015.
14. Allaire J, Couture P, Leclerc M, Charest A, Marin J, Lepine MC, et al. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study. Am J Clin Nutr. 2016;104(2):280-7.
15. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366(9491):1059-62.
16. Allaire J, Harris WS, Vors C, Charest A, Marin J, Jackson KH, et al. Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA). 2017;120:8-14.
17. St-Pierre A, Ruel I, Cantin B, Dagenais G, Bernard P-M, Després J-P, et al. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. Circulation. 2001;104(19):2295-9.
18. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

19. Lee TC, Ivester P, Hester AG, Sergeant S, Case LD, Morgan T, et al. The impact of polyunsaturated fatty acid-based dietary supplements on disease biomarkers in a metabolic syndrome/diabetes population. Lipids Health Dis. 2014;13:196.
20. Cholesterol Treatment Trialists C, Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 2012;380(9841):581-90.
21. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association Between Lowering LDL-C and Cardiovascular Risk Reduction Among Different Therapeutic Interventions: A Systematic Review and Meta-analysis. Jama. 2016;316(12):1289-97.
22. Boekholdt SM, Hovingh GK, Mora S, Arsenault BJ, Amarenco P, Pedersen TR, et al. Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta-analysis of statin trials. Journal of the American College of Cardiology. 2014;64(5):485-94.
23. Karlson BW, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. A VOYAGER Meta-Analysis of the Impact of Statin Therapy on Low-Density Lipoprotein Cholesterol and Triglyceride Levels in Patients With Hypertriglyceridemia. Am J Cardiol. 2016;117(9):1444-8.
24. Chasman DI, Giulianini F, MacFadyen J, Barratt BJ, Nyberg F, Ridker PM. Genetic determinants of statin-induced low-density lipoprotein cholesterol reduction: the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. Circ Cardiovasc Genet. 2012;5(2):257-64.
25. Olano-Martin E, Anil E, Caslake MJ, Packard CJ, Bedford D, Stewart G, et al. Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. Atherosclerosis. 2010;209(1):104-10.
26. Allaire J, Vors C, Harris WS, Jackson KH, Tchernof A, Couture P, et al. Comparing the serum triglyceride response to high-dose supplementation with either DHA or EPA among individuals with increased cardiovascular risk: The ComparED study. Br J Nutr. 2019:1-32.
27. Allaire J, Vors C, Tremblay AJ, Marin J, Charest A, Tchernof A, et al. High-Dose DHA Has More Profound Effects on LDL-Related Features Than High-Dose EPA: The ComparED Study. J Clin Endocrinol Metab. 2018;103(8):2909-17.
28. Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. Br J Nutr. 2004;92(3):477-83.
29. Norata GD, Tsimikas S, Pirillo A, Catapano AL. Apolipoprotein C-III: From Pathophysiology to Pharmacology. Trends Pharmacol Sci. 2015;36(10):675-87.
30. Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. Journal of lipid research. 2003;44(3):455-63.
31. Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, et al. The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annual review of nutrition. 2011;31:203-34.
32. Tavori H, Rashid S, Fazio S. On the function and homeostasis of PCSK9: reciprocal interaction with LDLR and additional lipid effects. Atherosclerosis. 2015;238(2):264-70.
33. Simon JA, Lin F, Hulley SB, Blanche PJ, Waters D, Shiboski S, et al. Phenotypic predictors of response to simvastatin therapy among African-Americans and Caucasians: the Cholesterol and Pharmacogenetics (CAP) Study. Am J Cardiol. 2006;97(6):843-50.
34. Shear CL, Franklin FA, Stinnett S, Hurley DP, Bradford RH, Chremos AN, et al. Expanded Clinical Evaluation of Lovastatin (EXCEL) study results. Effect of patient characteristics on lovastatin-induced changes in plasma concentrations of lipids and lipoproteins. Circulation. 1992;85(4):1293-303.

Tables

Table 8.1 Characteristics at screening of the 119 subjects included in the analyses

	Men (N=36)	Women (N=83)
Age, years	58 ± 10	51 ± 16
Body mass index, kg/m^2	29.45 ± 3.59	28.93 ± 4.25
Waist circumference, cm	106.17 ± 8.32	97.31 ± 9.60
Systolic blood pressure, mm HG	118.25 ± 12.74	110.30 ± 12.24
Diastolic blood pressure, mm HG	77.51 ± 8.86	70.32 ± 7.44
Total cholesterol, mmol/L	4.87 ± 0.81	5.48 ± 0.87
LDL-C, mmol/L *	2.95 ± 0.70	3.17 ± 0.81
HDL-C, mmol/L	1.28 ± 0.29	1.68 ± 0.38
Triglycerides, mmol/L	1.41 ± 0.77	1.36 ± 0.62
C-reactive protein, mg/L	2.78 ± 1.85	3.43 ± 2.39
Glucose, mmol/L	5.52 ± 1.02	5.20 ± 0.75
% with metabolic syndrome (n)	56 (20)	37 (31)

Values are expressed as means ± SD unless stated otherwise.

*N = 35 for men.

HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol

Table 8.2 Baseline characteristics of groups with a different LDL-C response to DHA supplementation

	LDL-C response			
	Favorable response (N = 25)	**Non-responders (N = 61)**	**Unfavorable response (N = 33)**	**P-value**
Age, years	51.8 ± 15.5	53.1 ± 15.3	54.7 ± 13.2	0.83
Female, % (n)	84 (21)	66 (40)	67 (22)	0.25
Post-menopausic, % (n)	16 (13)	30 (25)	19 (16)	0.73
Body mass index, kg/m² *	29.4 ± 4.1	29.3 ± 4.6	28.7 ± 3.5	0.74
Waist cirumference, cm	99.4 ± 11.3	100.2 ± 11.7	99.6 ± 10.3	0.68
Systolic blood pressure, mm HG	112.5 ± 11.1	116.2 ± 13.7	117.9 ± 13.5	0.43
Diastolic blood pressure, mm HG	68.5 ± 8.6	70.4 ± 9.2	70.4 ± 11.5	0.75
Total cholesterol, mmol/L	5.5 ± 0.8	5.2 ± 0.9	5.2 ± 0.8	0.16
LDL-C, mmol/L	3.3 ± 0.7	3.0 ± 0.8	3.0 ± 0.7	0.26
Total apo B, g/L	1.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.5	0.93
Mean LDL size, Å	252.1 ± 3.1	252.2 ± 2.9	251.7 ± 2.8	0.64
LDL peak, Å	252.0 ± 3.6	251.9 ± 2.9	251.3 ± 3.1	0.60
Prop. large LDL, % *	9.5 ± 9.3	9.9 ± 9.7	8.9 ± 6.9	0.88
Prop. small LDL, % *	70.2 ± 16.9	68.8 ± 18.0	70.7 ± 15.4	0.75
HDL-C, mmol/L	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.18
Non-HDL-C, mmol/L	3.9 ± 0.8	3.6 ± 0.8	3.7 ± 0.8	0.45
Triglycerides, mmol/L *	1.3 ± 0.5	1.3 ± 0.6	1.5 ± 0.6	0.52
% with metabolic syndrome (n)	8 (2)	15 (9)	27 (9)	0.15
C-reactive protein, mg/L *, †	2.8 ± 2.6	3.1 ± 2.3	3.2 ± 1.9	0.07
Adiponectin, mg/L *	8.7 ± 7.6	7.1 ± 4.6	6.3 ± 4.5	0.41
IL-6, pmol/L *	1.6 ± 1.9	1.6 ± 1.1	1.7 ± 1.1	0.34
IL-18, pmol/L *	243.7 ± 175.5	278.9 ± 143.6	267.2 ± 87.8	0.43
TNF-alpha, pmol/L *	1.1 ± 0.4	1.3 ± 1.4	1.3 ± 0.4	0.29
Glucose, mmol/L *	5.2 ± 0.4	5.4 ± 0.9	5.4 ± 1.0	0.55
Insulin, pmol/L *	86.8 ± 39.1	105.6 ± 48.1	103.7 ± 60.3	0.07
HOMA-IR *	2.9 ± 1.3	3.8 ± 2.6	3.8 ± 3.0	0.07

Values are unadjusted means ± SD.

The favorable response group includes individuals with a reduction in LDL-C greater than 0.30 mmol/L after DHA. The non-responders group includes individuals with a change in LDL-C within the ±0.30 mmol/L range after DHA. The unfavorable response group includes individuals with an increase in LDL-C concentrations greater than 0.30 mmol/L after DHA.

P-values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) for continuous values or Fisher exact test for proportions. Generalized linear models were adjusted for age, sex and waist circumference at screening.

* on transformed values, due to the skewness of the distribution.

† N = 22 for the reduction group, 60 for the non-responders and 30 for the increase group due to exclusions for CRP > 10.

Apo: apolipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; IL: interleukin; TNF-α: tumor necrosis factor alpha.

Table 8.3 Baseline characteristics of groups with a different LDL-C response to EPA supplementation

	LDL-C response			
	Favorable response (N = 27)	**Non-responders (N = 64)**	**Unfavorable response (N = 28)**	**P-value**
Age, years	54.93 ± 12.85	52.83 ± 15.22	52.57 ± 15.47	0.81
Female, % (n)	15 (18)	35 (42)	19 (23)	0.27
Post-menopausic, % (n)	16 (13)	33 (27)	17 (14)	0.75
Body mass index, kg/m^2	28.69 ± 3.92[a,b]	29.62 ± 4.27[a]	28.50 ± 3.98[b]	**<0.01**
Waist circumference, cm	99.79 ± 12.07	100.14 ± 10.24	99.99 ± 9.44	0.25
Systolic blood pressure, mm HG	116.77 ± 14.45[a,b]	117.38 ± 12.44[a]	109.88 ± 14.64[b]	**0.01**
Diastolic blood pressure, mm HG	71.38 ± 8.78	69.90 ± 7.80	67.40 ± 9.46	0.20
Total cholesterol, mmol/L	5.58 ± 0.89[a]	5.21 ± 0.88[a,b]	5.01 ± 0.87[b]	**0.04**
LDL-C, mmol/L	3.40 ± 0.79[a]	3.05 ± 0.76[a,b]	2.82 ± 0.75[b]	**0.03**
Total apo B, g/L *	1.41 ± 0.45	1.34 ± 0.37	1.33 ± 0.39	0.84
Mean LDL size, Å	252.73 ± 2.84	252.18 ± 2.96	251.91 ± 3.37	0.47
LDL peak, Å	252.11 ± 3.13	251.67 ± 2.94	251.64 ± 3.78	0.71
Prop. large LDL, % *	10.36 ± 7.29	9.18 ± 6.55	9.15 ± 6.84	0.63
Prop. small LDL, %	66.23 ± 17.58	69.45 ± 14.89	68.91 ± 15.74	0.71
HDL-C, mmol/L	1.57 ± 0.40	1.54 ± 0.46	1.51 ± 0.36	0.65
Non-HDL-C, mmol/L	4.01 ± 0.91	3.67 ± 0.88	3.50 ± 0.83	0.12
Triglycerides, mmol/L *	1.32 ± 0.63	1.35 ± 0.56	1.48 ± 0.70	0.70
% with metabolic syndrome (n)	4 (5)	8 (10)	4 (5)	0.90
C-reactive protein, mg/L *, †	2.31 ± 2.17[a,b]	3.79 ± 2.88[a]	2.60 ± 1.75[b]	**0.01**
Adiponectin, mg/L *	8.94 ± 8.35	6.93 ± 4.70	5.99 ± 3.68	0.18
IL-6, pmol/L *	1.41 ± 1.10	1.80 ± 2.02	1.75 ± 1.63	0.46
IL-18, pmol/L *	278.61 ± 158.44	271.94 ± 127.72	264.98 ± 178.23	0.89
TNF-alpha, pmol/L *	2.29 ± 5.38	1.29 ± 0.80	1.12 ± 0.42	0.42
Glucose, mmol/L *	5.44 ± 0.52	5.38 ± 1.01	5.20 ± 0.76	0.44
Insulin, pmol/L *	90.93 ± 34.76	98.11 ± 43.70	99.86 ± 49.62	0.92
HOMA-IR *	3.21 ± 1.44	3.53 ± 2.56	3.38 ± 1.94	0.88

Values are unadjusted means ± SD.

The favorable response group includes individuals with a reduction in LDL-C greater than 0.30 mmol/L after EPA. The non-responders group includes individuals with a change in LDL-C within the ±0.30 mmol/L range after EPA. The unfavorable response group includes individuals with an increase in LDL-C concentrations greater than 0.30 mmol/L after EPA.

Bold indicates P<0.05. Values with different superscript letters are different from each other, P<0.05.

P-values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) for continuous values or Fisher exact test for proportions. Generalized linear models were adjusted for age, sex and waist circumference at screening.

* on transformed value, due to the skewness of the distribution.

† N = 63 for the non-responders and 26 for the increase group due to exclusions for CRP > 10.

Apo: apolipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; IL: interleukin; TNF-α: tumor necrosis factor alpha.

Table 8.4 Change in cardiometabolic risk factors according to the LDL-C response to DHA supplementation (post-value minus pre-value)

	LDL-C response						
	Favorable response (N = 25)	**P-value‡**	**Non-responders (N = 61)**	**P-value‡**	**Unfavorable response (N = 33)**	**P-value‡**	**P-value§**
BMI, kg/m^2	+0.20 ± 0.09	**0.04**	+0.16 ± 0.08	**0.02**	+0.17 ± 0.09	0.06	0.88
Waist cir., cm	-0.01 ± 0.42	0.76	+0.02 ± 0.36	0.78	+1.05 ± 0.50	**0.03**	0.22
Systolic BP, mm HG	+1.01 ± 1.66	0.67	-1.56 ± 0.94	0.15	-0.47 ± 1.28	0.91	0.45
Diastolic BP, mm HG	-1.65 ± 1.04	0.15	-1.91 ± 0.77	**0.03**	-0.44 ± 1.28	0.91	0.41
Total C, mmol/L	-0.63 ± 0.05[a]	**<0.001**	+0.01 ± 0.04[b]	0.50	+0.73 ± 0.06[c]	**<0.001**	**<0.001**
LDL-C, mmol/L	-0.55 ± 0.04[a]	**<0.001**	+0.02 ± 0.02[b]	0.91	+0.70 ± 0.05[c]	**<0.001**	**<0.001**
Total apo B, g/L	-0.15 ± 0.03[a]	**<0.001**	-0.06 ± 0.02[b]	**0.01**	+0.19 ± 0.05[c]	**<0.001**	**<0.001**
Mean LDL size, Å	-0.14 ± 0.62	0.80	+0.14 ± 0.32	0.54	+0.71 ± 0.51	0.30	0.67
LDL peak, Å	-0.25 ± 0.75	0.79	+0.48 ± 0.32	0.10	+1.08 ± 0.49	0.09	0.37
Prop. large LDL, %	+0.01 ± 1.87	0.96	-1.04 ± 1.01	0.33	+0.89 ± 1.48	0.80	0.68
Prop. small LDL, %	-0.73 ± 3.74	0.99	-0.20 ± 2.12	0.81	-3.95 ± 3.07	0.30	0.71
HDL-C, mmol/L	+0.03 ± 0.04[a]	0.97	+0.11 ± 0.02[a,b]	**<0.001**	+0.14 ± 0.03[b]	**<0.01**	**0.04**
Non HDL-C, mmol/L	-0.66 ± 0.05[a]	**<0.001**	-0.09 ± 0.03[b]	**0.001**	+0.58 ± 0.05[c]	**<0.001**	**<0.001**
Triglycerides, mmol/L *	-0.22 ± 0.07	**<0.001**	-0.25 ± 0.04	**<0.001**	-0.26 ± 0.05	**<0.001**	0.54
CRP, mg/L *, †	-0.48 ± 0.30	0.07	-0.43 ± 0.21	0.06	-0.03 ± 0.37	0.75	0.43
Adiponectin, mg/L *	+0.50 ± 0.25	0.07	-0.17 ± 0.18	0.23	+0.43 ± 0.29	0.40	0.06
IL-6, pmol/L *	-0.61 ± 0.40[a]	**0.001**	-0.26 ± 0.13[a,b]	**0.01**	+0.11 ± 0.27[b]	0.95	**0.02**
IL-18, pmol/L *	+4.86 ± 14.02	0.63	-24.34 ± 8.90	**0.02**	-0.12 ± 17.62	0.93	0.17
TNF-a, pmol/L *	-0.09 ± 0.07	**0.02**	-0.22 ± 0.18	0.11	+0.12 ± 0.16	0.93	0.17
Glucose, mmol/L *	+0.01 ± 0.07	0.92	+0.10 ± 0.07	0.23	+0.15 ± 0.06	**0.03**	0.31
Insulin, pmol/L *	-3.80 ± 4.04	0.07	+0.31 ± 3.66	0.91	-5.58 ± 5.98	0.21	0.21
HOMA-IR *	-0.12 ± 0.15	0.12	+0.10 ± 0.13	0.71	-0.17 ± 0.30	0.50	0.27

Values are unadjusted means ± SD.

The favorable response group includes individuals with a reduction in LDL-C greater than 0.30 mmol/L after DHA. The non-responders group includes individuals with a change in LDL-C within the ±0.30 mmol/L range after DHA. The unfavorable response group includes individuals with an increase in LDL-C concentrations greater than 0.30 mmol/L after DHA.

P values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) and are adjusted for age, sex, and waist circumference at screening and pre-value of the variable of interest for continuous values and using Fisher's exact tests for proportions.
Bold indicates P<0.05. Values with different superscript letters are different from each other, P<0.05.
Apo: apolipoprotein; BMI: body mass index; BP: blood pressure; C: cholesterol; CRP: C-reactive protein; HOMA-IR: homeostatic model assessment of insulin resistance; IL: interleukin; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.
* Analyses were performed on log-transformed values due to the skewness of the distribution.
† N = 21 for the favorable response group and 59 for non-responders due to exclusions for CRP > 10 mg/L.
‡ § P-values for DHA changes compared with the pre-value in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.
§ Main treatment P-values for the comparison between groups were determined by the main effect.

Table 8.5 Change in cardiometabolic risk factors according to the LDL-C response to DHA supplementation (post-value minus pre-value)

	LDL-C response						
	Favorable response (N = 27)	**P-value‡**	**Non-responders (N = 64)**	**P-value‡**	**Unfavorable response (N = 28)**	**P-value‡**	**P-value§**
BMI, kg/m^2	+0.04 ± 0.11	0.61	+0.16 ± 0.06	**0.04**	+0.26 ± 0.12	**0.02**	0.31
Waist circumference, cm	-0.14 ± 0.46	0.56	+0.79 ± 0.36	0.06	+0.31 ± 0.59	0.87	0.27
Systolic BP, mm HG	-2.62 ± 1.57	0.30	+1.01 ± 1.11	**0.02**	+0.39 ± 1.39	0.78	0.09
Diastolic BP, mm HG	-3.02 ± 1.05[a]	0.06	+1.21 ± 0.71[b]	0.05	-0.11 ± 1.49[a,b]	0.94	**0.03**
Total C, mmol/L	-0.75 ± 0.07[a]	**<0.001**	-0.13 ± 0.04[b]	**<0.01**	+0.54 ± 0.07[c]	**<0.001**	**<0.001**
LDL-C, mmol/L	-0.62 ± 0.04[a]	**<0.001**	-0.02 ± 0.02[b]	0.33	+0.56 ± 0.05[c]	**<0.001**	**<0.001**
Total apo B, g/L	-0.21 ± 0.04[a]	**<0.001**	-0.02 ± 0.02[b]	0.23	+0.07 ± 0.04[b]	0.14	**<0.001**
Mean LDL size, Å	-1.48 ± 0.56	**<0.01**	-0.63 ± 0.31	**<0.01**	-0.17 ± 0.50	0.14	0.26
LDL peak, Å	-1.05 ± 0.64	**0.01**	-0.31 ± 0.30	0.07	-0.12 ± 0.53	0.33	0.42
Prop. large LDL, %	-2.27 ± 1.24	**0.02**	-0.79 ± 0.67	0.05	-0.01 ± 1.33	0.42	0.51
Prop. small LDL, %	+6.88 ± 3.11	**<0.01**	+2.51 ± 1.62	**0.01**	+1.12 ± 2.91	0.22	0.34
HDL-C, mmol/L	-0.06 ± 0.03	0.07	-0.01 ± 0.02	0.35	+0.04 ± 0.03	0.68	0.28
Non-HDL-C, mmol/L	-0.69 ± 0.06[a]	**<0.001**	-0.12 ± 0.03[b]	**<0.01**	+0.49 ± 0.06[c]	**<0.001**	**<0.001**
Triglycerides, mmol/L *	-0.15 ± 0.09	**0.02**	-0.18 ± 0.04	**<0.001**	-0.14 ± 0.07	0.57	0.12
CRP, mg/L *, †	+0.52 ± 0.37	0.28	-0.48 ± 0.27	0.31	+0.29 ± 0.29	0.99	0.34
Adiponectin, mg/L *	-0.39 ± 0.23	0.13	-0.24 ± 0.16	0.72	+0.38 ± 0.31	0.28	0.17
IL-6, pmol/L *	-0.15 ± 0.20	0.19	-0.27 ± 0.25	0.24	-0.44 ± 0.24	**0.01**	0.29
IL-18, pmol/L *	-27.60 ± 23.15	0.71	+13.51 ± 8.83	0.29	+6.49 ± 9.10	0.48	0.63
TNF-a, pmol/L *	-1.22 ± 1.02	0.15	-0.02 ± 0.13	0.45	+0.21 ± 0.32	0.61	0.70
Glucose, mmol/L *	-0.10 ± 0.08[a]	0.38	+0.03 ± 0.04[a,b]	0.28	+0.18 ± 0.07[b]	**0.01**	**0.04**
Insulin, pmol/L *	+9.59 ± 6.39	0.26	+9.03 ± 3.74	**0.01**	+5.04 ± 4.34	0.36	0.78
HOMA-IR *	+0.25 ± 0.23	0.41	+0.41 ± 0.18	**0.01**	+0.30 ± 0.19	0.16	0.78

Values are unadjusted means ± SD.

The favorable response group includes individuals with a reduction in LDL-C greater than 0.30 mmol/L after EPA. The non-responders group includes individuals with a change in LDL-C within the ±0.30 mmol/L range after EPA. The unfavorable response group includes individuals with an increase in LDL-C concentrations greater than 0.30 mmol/L after EPA.

P values were obtained with generalized linear models (multiple comparisons between groups adjusted with Tukey-Kramer) and are adjusted for age, sex, and waist circumference at screening and pre-value of the variable of interest for continuous values and using Fisher's exact tests for proportions.
Bold indicates P<0.05. Values with different superscript letters are different from each other, P<0.05.
Apo: apolipoprotein; BMI: body mass index; BP: blood pressure; C: cholesterol; CRP: C-reactive protein; HOMA-IR: homeostatic model assessment of insulin resistance; IL: interleukin; PCSK9: proprotein convertase subtilisin/kexin type 9; TNF-α: tumor necrosis factor alpha.
* Analyses were performed on log-transformed values due to the skewness of the distribution.
† N = 59 for non-responders and 26 for the unfavorable response due to exclusions for CRP > 10 mg/L.
‡ § P-values for EPA changes compared with the pre-value in the outcome were determined with the LSMEANS statement and were tested against the null hypothesis.
§ Main treatment P-values for the comparison between groups were determined by the main effect.

Figure

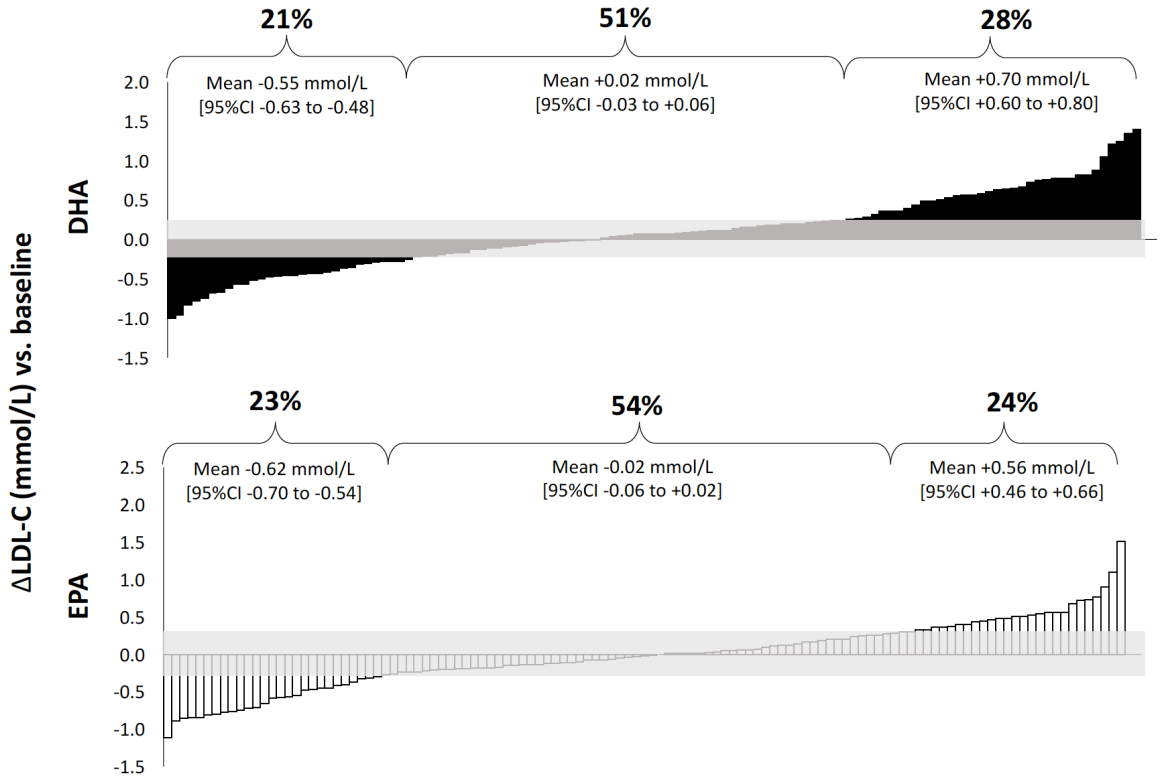

Figure 8.1 Change in LDL-C (post vs. pre-value) after DHA and EPA supplementation

One column represents one subject (N=119). Data are sorted to show the range of variation in the TG response after both DHA (black) and EPA (white). The grey zone represents the intra-individual variation range of ± 0.30 mmol/L. Values in brackets are 95% confidence intervals. After DHA: 21% (N=25) presented a favorable response, 51% (N=61) were non-responders and 28% (N=33) presented and unfavorable response. After EPA: 23% (N=27) presented a favorable response, 54% (N=64) were non-responders and 24% (N=28) presented an unfavorable response.

Discussion et conclusion

Après des années de résultats neutres quant à l'effet de la supplémentation en AGn3-LC combinant l'EPA et le DHA sur le risque d'évènements cardiovasculaires (6, 7), l'étude REDUCE-IT a montré que lorsqu'administré seul, l'EPA (4 g par jour) permet de diminuer de façon importante le risque d'évènements cardiovasculaires comparativement à un groupe témoin (13). Les résultats de cette étude clinique de grande envergure amènent une nouvelle perspective au débat sur le lien entre la supplémentation en AGn3-LC et le risque d'évènements cardiovasculaires. Les résultats suggèrent entre autres que la proportion d'EPA et de DHA pourrait avoir influencé l'effet de la supplémentation en AGn3-LC (EPA+DHA) observé dans les études cliniques menées jusqu'à maintenant. Les résultats de ce projet de doctorat vont également en ce sens. Toutefois, de par ses effets sur les facteurs de risque cardiométabolique, le DHA pourrait avoir des effets plus importants que l'EPA sur la prévention d'évènements cardiovasculaires.

Effets de l'EPA et du DHA sur les concentrations de triglycérides et les mécanismes sous-jacents

Nous avons d'abord observé qu'une supplémentation de 2,7 g par jour de DHA diminuait en moyenne les concentrations de triglycérides de façon plus importante qu'une dose similaire d'EPA. Il a précédemment été montré que la diminution des triglycérides suite à une supplémentation en AGn3-LC était en grande partie due à la diminution de la production des triglycérides et des VLDL au foie, mais aussi à une augmentation de la conversion de l'apo B100 des VLDL en LDL (128, 129). Selon nos observations, la différence entre l'effet du DHA et de l'EPA sur les concentrations de triglycérides semble en grande partie due à une plus grande augmentation du FCR de l'apo B100 des VLDL avec le DHA plutôt qu'à la modulation du PR. Ces observations peuvent être expliquées par l'effet de l'EPA et du DHA sur l'expression des gènes impliqués dans la lipogenèse. En effet, nous avons observé que l'EPA et le DHA exercent des effets similaires sur l'expression de *l'HMG-CoA réductase*, *SREBP1c* et *SREBP2* mesurée dans les cellules sanguines totales. Le DHA et l'EPA semblent exercer des effets différents sur le PR de l'apo CIII des VLDL et donc potentiellement sur le type de VLDL produit par le foie. Par exemple, le DHA pourrait diminuer la taille ou moduler le ratio apoB/E/C sur la surface des VLDL, mais des études supplémentaires sont nécessaires pour vérifier cette hypothèse. Dans l'ensemble, ces changements permettraient en grande partie d'expliquer la diminution des concentrations de triglycérides, l'augmentation des concentrations de C-LDL et l'augmentation dans la taille des LDL suite à une supplémentation en DHA comparativement à l'EPA. Jusqu'à maintenant, peu d'évidences permettent de comprendre les mécanismes sous-jacents aux différences entre les effets de l'EPA et du DHA sur les concentrations sanguines de triglycérides et de C-LDL.

Les résultats de ce projet de doctorat ont aussi montré que la différence entre l'effet de l'EPA et du DHA sur les triglycérides sanguins était en grande partie due au fait que le DHA diminue les concentrations de triglycérides chez une plus grande proportion d'individus que l'EPA. Nous avons caractérisé la réponse des triglycérides à l'EPA et en DHA en utilisant l'écart type de la moyenne de quatre mesures répétées chez un même individu pour identifier les différents types de répondeurs dans notre échantillon. Cette approche innovatrice permet selon nous de différencier les effets qui sont dus à une intervention de ceux qui sont dus à la variation quotidienne ou normale du biomarqueur en question. Environ 50% des individus ont présenté une réponse des triglycérides sanguins à la supplémentation en DHA et à l'EPA inférieure à la variation des concentrations de triglycérides observée quotidiennement. Nous avons observé qu'une diminution des concentrations de triglycérides était accompagnée de changements bénéfiques dans les autres facteurs de risque cardiométabolique tant avec l'EPA qu'avec le DHA. Nous avons également observé qu'une augmentation des triglycérides après une supplémentation en EPA et en DHA était associée à des changements indésirables dans les autres facteurs de risque cardiométabolique. Enfin, environ le tiers des individus ont présenté une réponse des triglycérides discordante à une supplémentation en EPA et en DHA. Au meilleur de nos connaissances, bien que la variabilité dans la réponse aux AGn3-LC ait été rapportée précédemment dans la littérature, l'impact de cette variabilité sur le risque d'évènements cardiovasculaires n'a pas encore été étudié. Les méta-analyses d'études cliniques menées jusqu'à maintenant ont montré un effet neutre de la supplémentation en AGn3-LC sur le risque d'évènements cardiovasculaires en général (6, 7). Bien qu'il soit possible que les AGn3-LC exercent réellement un effet neutre sur le risque d'évènements cardiovasculaires, il est aussi possible que cet effet soit en partie dû au fait qu'il existe différents types de répondeurs à l'EPA et au DHA. Il serait intéressant d'observer l'impact des différents types de réponses à une supplémentation en AGn3-LC de façon rétrospective avec les données des études cliniques qui ont été menées précédemment. Si des études futures le permettent, il serait intéressant d'observer l'effet des différents types de réponses à l'EPA et au DHA sur le risque d'évènements cardiovasculaires. Advenant le cas où le type de réponses à l'EPA et au DHA influencerait leurs effets sur le risque d'évènements cardiovasculaires, il serait important d'identifier les individus qui peuvent bénéficier ou non de cette supplémentation.

Un score de risque génétique développé à partir de l'étude FAS a montré que l'âge, le sexe et l'indice de masse corporelle permettaient d'expliquer moins de 1% de la variabilité dans la réponse des triglycérides à une supplémentation en AGn3-LC (1,9 à 2,2 g d'EPA + 1,1 g de DHA par jour), alors que les variations génétiques permettaient d'expliquer presque 50% de la variabilité (6, 7). Les résultats de ce projet de doctorat ont montré que les individus qui ont présenté une diminution des concentrations de triglycérides supérieure à la variation quotidienne après l'EPA et le DHA avaient des concentrations sanguines de cholestérol total, de cholestérol non-HDL, de triglycérides et de PCSK9 plus élevées au départ que les individus qui ont présenté une augmentation des concentrations de triglycérides. Toutefois, cette étude n'a pas été réalisée dans l'objectif

d'identifier les prédicteurs de la réponse à l'EPA et au DHA. De plus, puisque la variabilité génétique semble avoir un rôle important sur la réponse des triglycérides à une supplémentation en AGn3-LC, il serait intéressant de conduire des analyses afin d'identifier des déterminants génétiques de la réponse à l'EPA et au DHA lorsqu'administrés séparément. Dans le but d'optimiser les programmes de prévention des maladies cardiovasculaires par la supplémentation en EPA ou en DHA, des études supplémentaires sont nécessaires pour caractériser les individus qui pourraient bénéficier ou non d'une telle supplémentation.

Effets de l'EPA et du DHA sur les concentrations de C-LDL et les mécanismes sous-jacents

Nous avons observé que l'augmentation dans les concentrations de C-LDL était environ deux fois plus importante que l'augmentation des concentrations d'apo B totale après une supplémentation de 2,7 g par jour de DHA en comparaison avec le témoin. Puisque ≥90% de l'apo B totale mesurée à jeun dans le sang se trouve sur les LDL (51), nous avions émis l'hypothèse que le DHA modulait la taille des particules LDL. Nous avons effectivement observé que le DHA augmentait la taille moyenne et la taille maximale des particules LDL, et diminuait la proportion de LDL petites et denses comparativement à l'EPA. L'étude du métabolisme des lipoprotéines nous a permis d'observer que cette augmentation dans la concentration du C-LDL suite à une supplémentation en DHA comparativement à l'EPA était en partie due à l'effet net de l'augmentation combinée du PR et du FCR de l'apo B100 des LDL. Comme mentionné précédemment, l'augmentation du PR et de la taille des LDL avec le DHA pourrait en partie être due à la diminution de la taille des VLDL et à la modulation du ratio apoB/E/C sur leur surface. De plus, puisque le DHA accélère le métabolisme de l'apo B100 des LDL en augmentant à la fois le PR et le FCR, il est possible que le DHA puisse diminuer le temps de résidence de la particule LDL dans le sang. Il a précédemment été rapporté qu'une particule LDL qui réside plus longtemps dans la circulation sanguine est plus athérogène qu'une particule qui est métabolisée rapidement (160). Ainsi, l'augmentation de la concentration du C-LDL par le DHA pourrait être compensée, du moins en partie, par des changements bénéfiques sur le métabolisme et le phénotype de la particule LDL. Des études cliniques de grande envergure sont nécessaires pour déterminer comment ces changements dans le métabolisme du LDL après une supplémentation en DHA influencent le risque d'évènements cardiovasculaires.

Nous avons observé que les changements dans les concentrations de C-LDL n'étaient pas corrélés avec les changements dans le PR et le FCR de l'apo B100 des LDL, ni avec les changements dans les concentrations de PCSK9 après une supplémentation en DHA et en EPA. Toutefois, le changement dans les concentrations de PCSK9 était corrélé avec le changement dans les concentrations d'apo B100 des LDL en réponse au DHA seulement. Ces observations suggèrent que PCSK9 pourrait être impliqué dans l'effet du DHA sur le FCR des LDL. Néanmoins, nous avons observé que la supplémentation en EPA et en DHA n'a pas influencé l'expression du gène du *récepteur LDL* ni de *SREBP2* qui régule la synthèse de PCSK9 et du récepteur LDL au foie (161).

L'augmentation du FCR de l'apo B100 des LDL après le DHA comparativement à l'EPA pourrait donc être en partie modulée par des mécanismes indépendants de l'apo B100. Puisque la supplémentation en DHA, mais pas en EPA, influence les concentrations sanguines de C-HDL, il serait intéressant de comparer leurs effets sur l'expression des gènes et l'activité des protéines impliquées dans le métabolisme du transport inverse du cholestérol comme la *CETP*, *l'ABCA1* et le *SRB1*. De plus, des investigations supplémentaires sont nécessaires pour mieux comprendre les mécanismes sous-jacents aux changements dans les concentrations sanguines de PCSK9 et de C-LDL suite à une supplémentation en EPA et en DHA. En résumé, les résultats de ce projet de doctorat ont montré qu'une supplémentation en DHA augmentait les concentrations de C-LDL de façon plus importante qu'une supplémentation en EPA. Cependant, le DHA pourrait aussi avoir des effets favorables sur le métabolisme des LDL en accélérant le FCR de l'apo B100 des LDL et par la production de LDL de plus grande taille comparativement à l'EPA. L'impact de telles différences sur le risque d'évènements cardiovasculaires en soi mérite des études plus approfondies.

L'étude de la variabilité dans la réponse du C-LDL a permis d'observer que l'EPA et le DHA ont entraîné une augmentation des concentrations de C-LDL chez une proportion similaire de participants, mais que cette augmentation était plus importante suite au DHA qu'à l'EPA chez ce groupe de répondeurs. L'augmentation des concentrations de C-LDL au-delà de la variation quotidienne en réponse une supplémentation en EPA ou en DHA était aussi accompagnée d'une augmentation du cholestérol total. Les individus qui ont présenté une augmentation dans les concentrations de C-LDL au-delà de la variation quotidienne en réponse au DHA avaient tendance à avoir des concentrations de CRP et d'insuline plus élevées au départ que les individus qui ont présenté une diminution du C-LDL. En ce qui concerne la réponse à l'EPA, les individus qui ont présenté une augmentation des concentrations de C-LDL au-delà de la variation quotidienne avaient des concentrations de C-LDL plus élevées au départ que les individus qui ont présenté une diminution du C-LDL. Les résultats de ce doctorat n'ont pas permis d'identifier les individus qui sont le plus susceptibles de présenter une augmentation des concentrations de C-LDL suite à une supplémentation en EPA et en DHA. Des études supplémentaires sont nécessaires pour identifier les déterminants de la réponse du C-LDL à une supplémentation en EPA et en DHA et les individus qui sont le plus susceptibles de bénéficier ou pas de cette supplémentation d'un point de vue cardiovasculaire.

Bref, les résultats de ce projet de doctorat ont montré qu'une supplémentation en DHA avait des effets bénéfiques plus importants sur les facteurs de risque cardiométabolique qu'une supplémentation en EPA, à l'exception de son effet sur les concentrations de C-LDL. Néanmoins, le DHA semble accélérer le métabolisme des LDL comparativement à l'EPA, ce qui serait bénéfique sur le risque cardiovasculaire, mais aucune étude clinique ne permet de vérifier cette hypothèse actuellement.

Effets de l'EPA et du DHA sur l'Indice Oméga-3

Nous avons ensuite comparé les effets de l'EPA et du DHA sur l'Indice Oméga-3 qui est un marqueur du risque d'évènements coronariens de plus en plus reconnu et utilisé (116). Rappelons que l'Indice Oméga-3 permet de déterminer le statut en AGn3-LC des individus. Il est calculé en effectuant la somme de la composition des globules rouges en EPA et en DHA (en % des acides gras totaux). Nous avons observé qu'une supplémentation de 2,7 g par jour de DHA augmentait l'Indice Oméga-3 de façon plus importante qu'une dose similaire d'EPA. Encore une fois, ces observations pointent vers l'hypothèse que le DHA aurait des effets bénéfiques plus importants que l'EPA sur le risque d'évènements cardiovasculaires.

Effets de l'EPA et du DHA sur les marqueurs de l'inflammation

Nous avons observé qu'à une dose de 2,7 g par jour, le DHA diminuait les concentrations d'IL-18 et augmentait les concentrations d'adiponectine de façon plus importante qu'une dose similaire d'EPA. Il y a toutefois quelques limites à l'utilisation des marqueurs de l'inflammation comme facteurs de risque cardiométabolique. D'abord, bien que ces marqueurs aient précédemment été associés au risque cardiovasculaire, il s'agit pour la majorité de marqueurs non spécifiques du risque cardiovasculaire (86). De plus, comme nous pouvons le constater par les écarts types et les erreurs types dans le tableau 2 de l'article présenté au chapitre 4, ces marqueurs sont associés à une très grande variabilité et ont une distribution qui ne suit pas une loi normale. Ce sont donc des marqueurs qui sont assez difficiles à modéliser et à analyser. Néanmoins, les effets bénéfiques du DHA comparativement à l'EPA sur les marqueurs de l'inflammation concordent avec les constats généraux provenant des travaux de cette thèse, soit que le DHA pourrait exercer des effets plus importants que l'EPA sur la prévention du risque d'évènements cardiovasculaires.

Conclusion générale

Plusieurs aspects de ce doctorat sont novateurs. Au meilleur de nos connaissances, il s'agit des premières données publiées sur la comparaison de l'effet de l'EPA et du DHA sur le métabolisme lipidique, l'expression des gènes et sur la variabilité dans la réponse des triglycérides et du C-LDL. L'étude des prédicteurs de la réponse est plutôt de nature exploratoire, mais ces travaux permettent d'établir les bases pour l'élaboration de futurs projets de recherche de plus grande envergure.

À la lumière des résultats de ce projet de doctorat et de la revue de la littérature effectuée dans le chapitre 1 et 2 de cette thèse, il est intéressant de mettre perspective ce qui a été fait jusqu'à maintenant et ce qu'il serait pertinent de faire dans le futur. Il a précédemment été observé que l'effet protecteur des AGn3-LC sur le risque d'évènements coronariens serait plus important en prévention secondaire qu'en prévention primaire (7). C'est

également ce que suggèrent les résultats des études JELIS et REDUCE-IT (13, 147) . Néanmoins, l'étude VITAL et ASCEND ont montré qu'à faible dose, une supplémentation en AGn3-LC avait aussi des effets protecteurs sur le risque d'évènements coronariens en prévention primaire (145, 146). Selon moi, ces données supportent les recommandations actuelles disant que l'adoption de saines habitudes de vie, qui inclut la consommation régulière de poisson, permettrait de prévenir le risque de maladies cardiovasculaires chez une population en santé. La consommation d'environ deux repas de poisson gras par semaine permettrait d'atteindre un effet cardioprotecteur maximal selon l'effet sur l'Indice Oméga-3 (116). Cependant, selon les plus récentes données sur l'alimentation des Canadiens, 0% des Canadiens consomment habituellement deux repas de poisson gras par semaine (données non publiées de l'Enquête sur la santé dans les collectivités canadiennes de 2015). En ce qui concerne l'effet d'une supplémentation en AGn3-LC chez la population à risque d'évènements cardiovasculaires, elle ne semble pas avoir d'effet négatif en général, mais pourrait avoir des effets protecteurs. En ce sens, la recommandation actuelle de l'AHA qui est de prendre une supplémentation en AGn3-LC en prévention secondaire est supportée par les évidences scientifiques. Advenant le cas où des études cliniques de grande envergure permettraient de montrer un effet différentiel de l'EPA et du DHA sur le risque d'évènements cardiovasculaires, il ne serait pas impossible de voir s'ajouter une précision concernant la dose d'EPA et de DHA dans les recommandations futures.

Il y a d'autres facteurs que ceux qui ont été discutés précédemment qui pourrait expliquer en partie l'effet neutre des AGn3-LC sur le risque cardiovasculaire qui a été observé dans les études cliniques. Par exemple, il est généralement observé que les individus qui consomment peu de poisson, comparativement à ceux qui en consomment plus, sont ceux qui bénéficient le plus d'une supplémentation en AGn3-LC sur le risque cardiovasculaire (146). Il est donc important de considérer les habitudes de vie de la population à l'étude. En raison de leurs concentrations sanguines de C-LDL et de triglycérides dans les limites des valeurs normales, les participants de ce projet de doctorat ne représentaient pas nécessairement la population cible pour une supplémentation en AGn3-LC. De plus, les participants avaient déjà un statut élevé en AGn3-LC et un risque cardiovasculaire modéré selon leur Indice Oméga-3 moyen (116). On peut donc penser que les effets du DHA auraient été encore plus importants chez une population dont le risque cardiovasculaire aurait été plus élevé. Dans le cadre de cette thèse, nous avons comparé les effets de l'EPA et du DHA sur le risque cardiométabolique. Évidemment, les conclusions de cette thèse ne sont donc pas généralisables aux effets potentiels de l'EPA et du DHA sur la prévention ou le traitement d'autres problèmes de santé comme les maladies inflammatoires auto-immunes, la maladie d'Alzheimer, la dépression ou le cancer pour ne nommer que quelques exemples (121).

À la lumière des résultats de ce doctorat, il me semble évident que la prochaine étape serait de mener une étude clinique de grande envergure afin de comparer les effets d'une dose élevée d'EPA et de DHA (2 à 4 g par jour)

sur le risque d'évènements cardiovasculaires. Il serait également important de déterminer comment la variabilité dans la réponse à l'EPA et au DHA influence le risque d'évènements cardiovasculaires et d'identifier les individus qui pourraient bénéficier ou non de cette supplémentation, toujours d'un point de vue cardiovasculaire.

Bibliographie

1. Bang H, Dyerberg J, Hjørne N. The composition of food consumed by Greenland Eskimos. Acta Medica Scandinavica. 1976;200(1-6):69-73.
2. Fodor JG, Helis E, Yazdekhasti N, Vohnout B. "Fishing" for the origins of the "Eskimos and heart disease" story: facts or wishful thinking? Can J Cardiol. 2014;30(8):864-8.
3. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med. 2014;160(6):398-406.
4. Del Gobbo LC, Imamura F, Aslibekyan S, Marklund M, Virtanen JK, Wennberg M, et al. omega-3 Polyunsaturated Fatty Acid Biomarkers and Coronary Heart Disease: Pooling Project of 19 Cohort Studies. JAMA internal medicine. 2016;176(8):1155-66.
5. Harris WS, Del Gobbo L, Tintle NL. The Omega-3 Index and relative risk for coronary heart disease mortality: Estimation from 10 cohort studies. Atherosclerosis. 2017;262:51-4.
6. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, et al. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77917 Individuals. JAMA Cardiol. 2018;3(3):225-34.
7. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. The Cochrane database of systematic reviews. 2018;11:CD003177.
8. Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. Prostaglandins, leukotrienes, and essential fatty acids. 2013;89(1):1-8.
9. Wei MY, Jacobson TA. Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. Current atherosclerosis reports. 2011;13(6):474-83.
10. Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. J Clin Lipidol. 2012;6(1):5-18.
11. Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, Beilin LJ. Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. Free Radic Biol Med. 2003;35(7):772-81.
12. Tsunoda F, Lamon-Fava S, Asztalos BF, Iyer LK, Richardson K, Schaefer EJ. Effects of oral eicosapentaenoic acid versus docosahexaenoic acid on human peripheral blood mononuclear cell gene expression. Atherosclerosis. 2015;241(2):400-8.
13. Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. The New England journal of medicine. 2019;380(1):11-22.
14. Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, et al. The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annual review of nutrition. 2011;31:203-34.
15. Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, et al. Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr. 2008;88(3):618-29.
16. Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S, et al. Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation. Nutrients. 2012;4(8):1026-41.
17. Thifault E, Cormier H, Bouchard-Mercier A, Rudkowska I, Paradis AM, Garneau V, et al. Effects of age, sex, body mass index and APOE genotype on cardiovascular biomarker response to an n-3 polyunsaturated fatty acid supplementation. J Nutrigenet Nutrigenomics. 2013;6(2):73-82.
18. Agence de la santé publique du Canada. Rapport du système canadien de surveillance des maladies chriniques: Les maladies du coeur au Canada, 2018. 2018.
19. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41.

20. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1459-544.
21. Institut national de santé publique du Québec. Portrait de l'ensemble des maladies vasculaires au Québec : prévalence, incidence et mortalité. 2018.
22. Canada Adlspd. Le fardeau économique de la maladie au Canada, 2005-2008. 2014.
23. Husain K, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. World J Biol Chem. 2015;6(3):209-17.
24. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. Nat Rev Genet. 2009;10(2):109-21.
25. Morita SY. Metabolism and Modification of Apolipoprotein B-Containing Lipoproteins Involved in Dyslipidemia and Atherosclerosis. Biol Pharm Bull. 2016;39(1):1-24.
26. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340(2):115-26.
27. Cox RA, Garcia-Palmieri MR. Cholesterol, Triglycerides, and Associated Lipoproteins. In: rd, Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. Boston1990.
28. Ramasamy I. Recent advances in physiological lipoprotein metabolism. Clin Chem Lab Med. 2014;52(12):1695-727.
29. Chan DC, Barrett PH, Watts GF. Lipoprotein transport in the metabolic syndrome: methodological aspects of stable isotope kinetic studies. Clinical science (London, England : 1979). 2004;107(3):221-32.
30. Larsson SL, Skogsberg J, Bjorkegren J. The low density lipoprotein receptor prevents secretion of dense apoB100-containing lipoproteins from the liver. J Biol Chem. 2004;279(2):831-6.
31. Blasiole DA, Oler AT, Attie AD. Regulation of ApoB secretion by the low density lipoprotein receptor requires exit from the endoplasmic reticulum and interaction with ApoE or ApoB. J Biol Chem. 2008;283(17):11374-81.
32. Wolska A, Dunbar RL, Freeman LA, Ueda M, Amar MJ, Sviridov DO, et al. Apolipoprotein C-II: New findings related to genetics, biochemistry, and role in triglyceride metabolism. Atherosclerosis. 2017;267:49-60.
33. Norata GD, Tsimikas S, Pirillo A, Catapano AL. Apolipoprotein C-III: From Pathophysiology to Pharmacology. Trends Pharmacol Sci. 2015;36(10):675-87.
34. Hogue J-C. Étude des mécanismes athérogènes associés à l'insulino-résistance et l'hypercholestérolémie familiale: Citeseer; 2008.
35. Chistiakov DA, Bobryshev YV, Orekhov AN. Macrophage-mediated cholesterol handling in atherosclerosis. J Cell Mol Med. 2016;20(1):17-28.
36. Tremblay AJ, Lamarche B, Kelly I, Charest A, Lepine MC, Droit A, et al. Effect of sitagliptin therapy on triglyceride-rich lipoprotein kinetics in patients with type 2 diabetes. Diabetes Obes Metab. 2014;16(12):1223-9.
37. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-52.
38. Sytkowski PA, Kannel WB, D'Agostino RB. Changes in risk factors and the decline in mortality from cardiovascular disease. The Framingham Heart Study. The New England journal of medicine. 1990;322(23):1635-41.
39. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuniga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovascular diabetology. 2018;17(1):122.
40. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. The New England journal of medicine. 1998;339(4):229-34.
41. Fox CS, Coady S, Sorlie PD, D'Agostino RB, Sr., Pencina MJ, Vasan RS, et al. Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. Circulation. 2007;115(12):1544-50.

42. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). Jama. 1986;256(20):2823-8.
43. Stamler J, Daviglus ML, Garside DB, Dyer AR, Greenland P, Neaton JD. Relationship of baseline serum cholesterol levels in 3 large cohorts of younger men to long-term coronary, cardiovascular, and all-cause mortality and to longevity. JAMA. 2000;284(3):311-8.
44. Cholesterol Treatment Trialists C, Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 2012;380(9841):581-90.
45. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2001;104(10):1108-13.
46. Anderson TJ, Gregoire J, Pearson GJ, Barry AR, Couture P, Dawes M, et al. 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult. Can J Cardiol. 2016;32(11):1263-82.
47. Unni SK, Quek RG, Biskupiak J, Lee VC, Ye X, Gandra SR. Assessment of statin therapy, LDL-C levels, and cardiovascular events among high-risk patients in the United States. J Clin Lipidol. 2016;10(1):63-71 e1-3.
48. Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. Lancet. 2016;388(10059):2532-61.
49. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ. 2003;326(7404):1423.
50. Yusuf HR, Giles WH, Croft JB, Anda RF, Casper ML. Impact of multiple risk factor profiles on determining cardiovascular disease risk. Prev Med. 1998;27(1):1-9.
51. Sniderman AD, Marcovina SM. Apolipoprotein A1 and B. Clin Lab Med. 2006;26(4):733-50.
52. Thanassoulis G, Williams K, Ye K, Brook R, Couture P, Lawler PR, et al. Relations of change in plasma levels of LDL-C, non-HDL-C and apoB with risk reduction from statin therapy: a meta-analysis of randomized trials. J Am Heart Assoc. 2014;3(2):e000759.
53. Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, et al. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. Circulation. 1994;90(1):42-9.
54. Dittrich J, Beutner F, Teren A, Thiery J, Burkhardt R, Scholz M, et al. Plasma levels of apolipoproteins C-III, A-IV, and E are independently associated with stable atherosclerotic cardiovascular disease. Atherosclerosis. 2019;281:17-24.
55. Luc G, Fievet C, Arveiler D, Evans AE, Bard JM, Cambien F, et al. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. Journal of lipid research. 1996;37(3):508-17.
56. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. Circulation. 2000;102(16):1886-92.
57. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, et al. Very-Low-Density Lipoprotein-Associated Apolipoproteins Predict Cardiovascular Events and Are Lowered by Inhibition of APOC-III. J Am Coll Cardiol. 2017;69(7):789-800.
58. Onat A, Hergenc G, Sansoy V, Fobker M, Ceyhan K, Toprak S, et al. Apolipoprotein C-III, a strong discriminant of coronary risk in men and a determinant of the metabolic syndrome in both genders. Atherosclerosis. 2003;168(1):81-9.
59. Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. Circulation. 2010;121(15):1722-34.
60. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA. 1988;260(13):1917-21.

61. Griffin BA. Lipoprotein atherogenicity: an overview of current mechanisms. Proc Nutr Soc. 1999;58(1):163-9.
62. Campos H, Blijlevens E, McNamara JR, Ordovas JM, Posner BM, Wilson PW, et al. LDL particle size distribution. Results from the Framingham Offspring Study. Arterioscler Thromb. 1992;12(12):1410-9.
63. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. The New England journal of medicine. 2012;367(22):2089-99.
64. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, et al. Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. The New England journal of medicine. 2017;376(20):1933-42.
65. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. The New England journal of medicine. 2007;357(21):2109-22.
66. Group HTRC, Bowman L, Hopewell JC, Chen F, Wallendszus K, Stevens W, et al. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. The New England journal of medicine. 2017;377(13):1217-27.
67. Emerging Risk Factors C, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009;302(18):1993-2000.
68. Liu J, Zeng FF, Liu ZM, Zhang CX, Ling WH, Chen YM. Effects of blood triglycerides on cardiovascular and all-cause mortality: a systematic review and meta-analysis of 61 prospective studies. Lipids Health Dis. 2013;12:159.
69. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA. 2007;298(3):309-16.
70. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007;298(3):299-308.
71. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469-80.
72. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143-421.
73. McPherson R, Frohlich J, Fodor G, Genest J, Canadian Cardiovascular S. Canadian Cardiovascular Society position statement--recommendations for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease. Can J Cardiol. 2006;22(11):913-27.
74. Nichols GA, Philip S, Reynolds K, Granowitz CB, Fazio S. Increased Cardiovascular Risk in Hypertriglyceridemic Patients With Statin-Controlled LDL Cholesterol. J Clin Endocrinol Metab. 2018;103(8):3019-27.
75. Valdivielso P, Ramirez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. Eur J Intern Med. 2014;25(8):689-94.
76. de Nijs T, Sniderman A, de Graaf J. ApoB versus non-HDL-cholesterol: diagnosis and cardiovascular risk management. Critical reviews in clinical laboratory sciences. 2013;50(6):163-71.
77. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, et al. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. Jama. 2012;307(12):1302-9.
78. Poirier S, Mayer G, Poupon V, McPherson PS, Desjardins R, Ly K, et al. Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation: evidence for an intracellular route. J Biol Chem. 2009;284(42):28856-64.
79. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003;34(2):154-6.
80. Abifadel M, Rabes JP, Devillers M, Munnich A, Erlich D, Junien C, et al. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum Mutat. 2009;30(4):520-9.
81. Hess CN, Low Wang CC, Hiatt WR. PCSK9 Inhibitors: Mechanisms of Action, Metabolic Effects, and Clinical Outcomes. Annu Rev Med. 2018;69:133-45.

82. Turgeon RD, Tsuyuki RT, Gyenes GT, Pearson GJ. Cardiovascular Efficacy and Safety of PCSK9 Inhibitors: Systematic Review and Meta-analysis Including the ODYSSEY OUTCOMES Trial. Can J Cardiol. 2018;34(12):1600-5.
83. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England journal of medicine. 2005;352(16):1685-95.
84. Kalupahana NS, Moustaid-Moussa N, Claycombe KJ. Immunity as a link between obesity and insulin resistance. Molecular aspects of medicine. 2012;33(1):26-34.
85. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107(3):499-511.
86. Calder PC, Ahluwalia N, Albers R, Bosco N, Bourdet-Sicard R, Haller D, et al. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. Br J Nutr. 2013;109 Suppl 1:S1-34.
87. Swarbrick MM, Havel PJ. Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord. 2008;6(2):87-102.
88. Ablij H, Meinders A. C-reactive protein: history and revival. Eur J Intern Med. 2002;13(7):412.
89. Krishnan SM, Sobey CG, Latz E, Mansell A, Drummond GR. IL-1beta and IL-18: inflammatory markers or mediators of hypertension? Br J Pharmacol. 2014;171(24):5589-602.
90. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, et al. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. J Biol Chem. 2010;285(9):6153-60.
91. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation. 2001;103(8):1057-63.
92. Kim DH, Kim C, Ding EL, Townsend MK, Lipsitz LA. Adiponectin levels and the risk of hypertension: a systematic review and meta-analysis. Hypertension. 2013;62(1):27-32.
93. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286(3):327-34.
94. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. Diabetes. 2005;54(10):2932-8.
95. Liu C, Feng X, Li Q, Wang Y, Li Q, Hua M. Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. Cytokine. 2016;86:100-9.
96. Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diabetes Care. 2013;36(1):166-75.
97. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. Jama. 2009;302(2):179-88.
98. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. The New England journal of medicine. 2000;342(12):836-43.
99. Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Circulation. 2003;108(20):2453-9.
100. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004;291(14):1730-7.
101. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101(15):1767-72.
102. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation. 2000;101(18):2149-53.

103. Ridker PM, Libby P, MacFadyen JG, Thuren T, Ballantyne C, Fonseca F, et al. Modulation of the interleukin-6 signalling pathway and incidence rates of atherosclerotic events and all-cause mortality: analyses from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). Eur Heart J. 2018;39(38):3499-507.
104. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet. 2018;391(10118):319-28.
105. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63(25 Pt B):2960-84.
106. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77(5):1146-55.
107. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr. 2006;83(6 Suppl):1467S-76S.
108. Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men*. Br J Nutr. 2002;88(4):355-63.
109. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr. 2002;88(4):411-20.
110. Tang C, Cho HP, Nakamura MT, Clarke SD. Regulation of human delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. Journal of lipid research. 2003;44(4):686-95.
111. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohdrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Journal of the Academy of Nutrition and Dietetics. 2002;102(11):1621.
112. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, et al. Polyunsaturated fatty acids in the food chain in the United States. The American journal of clinical nutrition. 2000;71(1):179S-88S.
113. Serra-Majem L, Nissensohn M, Overby NC, Fekete K. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. Br J Nutr. 2012;107 Suppl 2:S64-76.
114. Drouin G, Rioux V, Legrand P. The n-3 docosapentaenoic acid (DPA): A new player in the n-3 long chain polyunsaturated fatty acid family. Biochimie. 2019.
115. Statistique Canada. Niveaux d'acides gras oméga-3 chez les adultes, 2012 et 2013. 2015.
116. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med. 2004;39(1):212-20.
117. Harris WS, Tintle NL, Etherton MR, Vasan RS. Erythrocyte long-chain omega-3 fatty acid levels are inversely associated with mortality and with incident cardiovascular disease: The Framingham Heart Study. J Clin Lipidol. 2018;12(3):718-27 e6.
118. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, et al. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory From the American Heart Association. Circulation. 2017;135(15):e867-e84.
119. Ghasemi Fard S, Wang F, Sinclair AJ, Elliott G, Turchini GM. How does high DHA fish oil affect health? A systematic review of evidence. Critical reviews in food science and nutrition. 2018:1-44.
120. Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial. J Am Heart Assoc. 2013;2(6):e000513.
121. Saini RK, Keum YS. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance - A review. Life Sci. 2018;203:255-67.
122. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol. 2011;58(20):2047-67.
123. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA. 2006;296(15):1885-99.

124. Zibaeenezhad MJ, Ghavipisheh M, Attar A, Aslani A. Comparison of the effect of omega-3 supplements and fresh fish on lipid profile: a randomized, open-labeled trial. Nutr Diabetes. 2017;7(12):1.
125. Bays HE, Tighe AP, Sadovsky R, Davidson MH. Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications. Expert Rev Cardiovasc Ther. 2008;6(3):391-409.
126. Filippatos TD, Kei A, Rizos CV, Elisaf MS. Effects of PCSK9 Inhibitors on Other than Low-Density Lipoprotein Cholesterol Lipid Variables. J Cardiovasc Pharmacol Ther. 2018;23(1):3-12.
127. Toth PP, Worthy G, Gandra SR, Sattar N, Bray S, Cheng LI, et al. Systematic Review and Network Meta-Analysis on the Efficacy of Evolocumab and Other Therapies for the Management of Lipid Levels in Hyperlipidemia. J Am Heart Assoc. 2017;6(10).
128. Lamarche B, Couture P. Dietary fatty acids, dietary patterns, and lipoprotein metabolism. Curr Opin Lipidol. 2015;26(1):42-7.
129. Ooi EM, Watts GF, Ng TW, Barrett PH. Effect of dietary Fatty acids on human lipoprotein metabolism: a comprehensive update. Nutrients. 2015;7(6):4416-25.
130. Blake WL, Clarke SD. Suppression of rat hepatic fatty acid synthase and S14 gene transcription by dietary polyunsaturated fat. J Nutr. 1990;120(12):1727-9.
131. Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis. 2008;197(1):12-24.
132. Khan S, Minihane AM, Talmud PJ, Wright JW, Murphy MC, Williams CM, et al. Dietary long-chain n-3 PUFAs increase LPL gene expression in adipose tissue of subjects with an atherogenic lipoprotein phenotype. Journal of lipid research. 2002;43(6):979-85.
133. Parks JS, Johnson FL, Wilson MD, Rudel LL. Effect of fish oil diet on hepatic lipid metabolism in nonhuman primates: lowering of secretion of hepatic triglyceride but not apoB. Journal of lipid research. 1990;31(3):455-66.
134. Backes J, Anzalone D, Hilleman D, Catini J. The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids Health Dis. 2016;15(1):118.
135. Buckley R, Shewring B, Turner R, Yaqoob P, Minihane AM. Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. Br J Nutr. 2004;92(3):477-83.
136. Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, et al. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. Am J Clin Nutr. 2000;71(5):1085-94.
137. Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. Journal of lipid research. 2003;44(3):455-63.
138. Ishida T, Ohta M, Nakakuki M, Kami H, Uchiyama R, Kawano H, et al. Distinct regulation of plasma LDL cholesterol by eicosapentaenoic acid and docosahexaenoic acid in high fat diet-fed hamsters: participation of cholesterol ester transfer protein and LDL receptor. Prostaglandins, leukotrienes, and essential fatty acids. 2013;88(4):281-8.
139. Graversen CB, Lundbye-Christensen S, Thomsen B, Christensen JH, Schmidt EB. Marine n-3 polyunsaturated fatty acids lower plasma proprotein convertase subtilisin kexin type 9 levels in pre- and postmenopausal women: A randomised study. Vascul Pharmacol. 2016;76:37-41.
140. Rodriguez-Perez C, Ramprasath VR, Pu S, Sabra A, Quirantes-Pine R, Segura-Carretero A, et al. Docosahexaenoic Acid Attenuates Cardiovascular Risk Factors via a Decline in Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Plasma Levels. Lipids. 2016;51(1):75-83.
141. Bennett M, Gilroy DW. Lipid Mediators in Inflammation. Microbiol Spectr. 2016;4(6).
142. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. PLoS One. 2014;9(2):e88103.
143. Tortosa-Caparros E, Navas-Carrillo D, Marin F, Orenes-Pinero E. Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. Critical reviews in food science and nutrition. 2017;57(16):3421-9.

144. Wan Y, Zheng J, Wang F, Li D. Fish, long chain omega-3 polyunsaturated fatty acids consumption, and risk of all-cause mortality: a systematic review and dose-response meta-analysis from 23 independent prospective cohort studies. Asia Pacific journal of clinical nutrition. 2017;26(5):939-56.
145. Group ASC, Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. The New England journal of medicine. 2018;379(16):1540-50.
146. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. The New England journal of medicine. 2019;380(1):23-32.
147. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet. 2007;369(9567):1090-8.
148. Sekikawa A, Curb JD, Ueshima H, El-Saed A, Kadowaki T, Abbott RD, et al. Marine-derived n-3 fatty acids and atherosclerosis in Japanese, Japanese-American, and white men: a cross-sectional study. J Am Coll Cardiol. 2008;52(6):417-24.
149. Nicolas JM, Espie P, Molimard M. Gender and interindividual variability in pharmacokinetics. Drug Metab Rev. 2009;41(3):408-21.
150. Ross R, Goodpaster BH, Koch LG, Sarzynski MA, Kohrt WM, Johannsen NM, et al. Precision exercise medicine: understanding exercise response variability. Br J Sports Med. 2019.
151. Minihane AM, Khan S, Leigh-Firbank EC, Talmud P, Wright JW, Murphy MC, et al. ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(8):1990-7.
152. Minihane AM. Fatty acid-genotype interactions and cardiovascular risk. Prostaglandins, leukotrienes, and essential fatty acids. 2010;82(4-6):259-64.
153. Rudkowska I, Caron-Dorval D, Verreault M, Couture P, Deshaies Y, Barbier O, et al. PPARalpha L162V polymorphism alters the potential of n-3 fatty acids to increase lipoprotein lipase activity. Mol Nutr Food Res. 2010;54(4):543-50.
154. Rudkowska I, Garenc C, Couture P, Vohl MC. Omega-3 fatty acids regulate gene expression levels differently in subjects carrying the PPARalpha L162V polymorphism. Genes Nutr. 2009;4(3):199-205.
155. Ouellette C, Cormier H, Rudkowska I, Guenard F, Lemieux S, Couture P, et al. Polymorphisms in genes involved in the triglyceride synthesis pathway and marine omega-3 polyunsaturated fatty acid supplementation modulate plasma triglyceride levels. J Nutrigenet Nutrigenomics. 2013;6(4-5):268-80.
156. Tremblay BL, Cormier H, Rudkowska I, Lemieux S, Couture P, Vohl MC. Association between polymorphisms in phospholipase A2 genes and the plasma triglyceride response to an n-3 PUFA supplementation: a clinical trial. Lipids Health Dis. 2015;14:12.
157. Vallee Marcotte B, Guenard F, Lemieux S, Couture P, Rudkowska I, Calder PC, et al. Fine mapping of genome-wide association study signals to identify genetic markers of the plasma triglyceride response to an omega-3 fatty acid supplementation. Am J Clin Nutr. 2019;109(1):176-85.
158. Olano-Martin E, Anil E, Caslake MJ, Packard CJ, Bedford D, Stewart G, et al. Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. Atherosclerosis. 2010;209(1):104-10.
159. Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. Am J Clin Nutr. 2011;93(2):243-52.
160. Sacks FM, Campos H. Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab. 2003;88(10):4525-32.
161. Nozue T. Lipid Lowering Therapy and Circulating PCSK9 Concentration. J Atheroscler Thromb. 2017;24(9):895-907.