Nutrigénomique appliquée aux gènes candidats modulant les profils lipidique, glycémique et inflammatoire suite à une supplémentation en acides gras polyinsaturés oméga-3

Thèse

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Sous la direction de :

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Résumé

La littérature suggère qu'une supplémentation en acides gras polyinsaturés (AGPI) oméga-3 (n-3) pourrait s'avérer bénéfique pour réduire les facteurs de risque métabolique liés aux maladies cardiovasculaires (MCV). Or, les effets rapportés par la supplémentation en AGPI n-3, notamment la diminution des concentrations de triglycérides (TG), peuvent varier d'un individu à l'autre, notamment en raison de variations génétiques. Il existe une grande variabilité interindividuelle dans la population, ce qui se répercute par des réponses différentes aux traitements nutritionnels. Dans le cadre de ce projet de doctorat, 210 participants ont été recrutés afin d'étudier la variabilité interindividuelle observée dans la réponse à une supplémentation en huile de poisson (5 grammes/jour) d'une durée de 6 semaines. Il a été démontré que 28,8% de l'échantillon ne répondait pas de la façon attendue à une supplémentation en AGPI n-3 où une augmentation des niveaux plasmatiques de TG était observée. Des polymorphismes nucléotidiques simples (SNPs) présents sur des gènes reliés à la voie de synthèse des AGPI n-3 et oméga-6 (n-6) (*FADS1-2-3* et *ELOVL2-5*) affectent seuls, ou en interaction avec la diète, la réponse des TG plasmatiques suite à la supplémentation en huile de poisson. Ces mêmes SNPs modulent les niveaux d'acides gras (AG) dans les phospholipides (PPLs) du plasma ainsi que l'insulinémie à jeun et l'indice HOMA de sensibilité à l'insuline (HOMA-IS). Outre l'effet sur les TG plasmatiques, il a été observé que la supplémentation en AGPI n-3 exerce un effet modeste sur l'expression des principaux gènes inflammatoires et abaisse les niveaux plasmatiques des principaux biomarqueurs de l'inflammation (CRP, TNFA et IL-6) sous l'influence de SNPs des gènes inflammatoires (*CRP*, *TNF-LTA, IL-1B* et *IL-6*). Cette thèse comprend aussi un volet d'application des connaissances où la perception de la nutrigénomique, le niveau d'intérêt envers cette science, la compréhension des principales limitations des tests de nutrigénétique, ainsi que l'acquisition de compétences ont été évalués par les diététistes membres de l'Ordre professionnel des diététistes du Québec (OPDQ). Globalement, ces résultats démontrent la présence d'une grande variabilité interindividuelle observée dans les niveaux plasmatiques de TG et que des SNPs présents sur des gènes impliqués dans le métabolisme des lipides et de l'inflammation modulent les réponses lipidique, glycémique et inflammatoire suite à une supplémentation en AGPI n-3.

Abstract

The literature suggests that an n-3 polyunsaturated fatty acid (PUFA) supplementation may be beneficial in reducing metabolic risk factors for cardiovascular diseases (CVD). However, the effects reported by an n-3 PUFA supplementation, including lower TG levels, vary from one individual to another due to genetic variations. There is a great interindividual variability in the population, which is reflected by different responses to a dietary intervention. As part of this PhD project, 210 participants were recruited. In order to study the interindividual variability observed in the response to a nutrient, a 6-week fish oil (5 g/day) supplementation was carried out in the participants. It was shown that 28.8% of the study participants did not respond as expected to an n-3 PUFA supplementation. Moreover, an increase in TG plasma levels was observed in these hypo-responders. SNPs on genes related to the biosynthetic pathway of n-3 and n-6 fatty acids (*FADS1-2-3* and *ELOVL2-5*) were associated alone or in an interaction effect with the fish oil supplementation with the plasma TG response following the 6-week n-3 PUFA supplementation. These same SNPs modulate fatty acid levels in plasma phospholipids as well as fasting insulin levels and homeostasis model assessment of insulin sensitivity (HOMA-IS) index. In addition to the effect on plasma TG, n-3 PUFA supplementation has been shown to exert a modest effect on the expression of inflammatory genes and to lower plasma levels of inflammatory biomarkers (CRP, TNFA and IL-6) under the influence of SNPs from inflammatory genes (*CRP, TNF-LTA, IL-1B* and *IL-6*). This thesis also includes a knowledge translation component where the perception of nutrigenomics, the level of interest in this science, the understanding of the main limitations of nutrigenetic testing, as well as the best ways to acquire knowledge in nutrigenomics were assessed by registered dietitians from the province of Quebec (Canada). Overall, these results demonstrate the presence of a large interindividual variability observed in the TG plasma levels and that SNPs on genes involved in lipid metabolism and inflammation may modulate the lipid, glycemic and inflammatory responses following an n-3 PUFA supplementation.

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Listes des abréviations *(et sigles)*

ADH/DHA :	Acide docosahexaénoïque
ADP/DPA :	Acide docosapentaénoïque
AEP/EPA :	Acide eicosapentaénoïque
AGPI :	Acide gras polyinsaturés
AHA :	*American Heart Association*
ALA :	Alpha-linolenic
ANOVA :	*Analyses of variance*
APOB :	Apolipoprotéine B
APOE :	Apolipoprotéine E
ARA :	Acide arachidonique
BMI :	*Body mass index*
C :	Cholestérol
CHD :	*Coronary heart disease*
CRP :	*C-reactive protein* (protéine C-réactive)
CVD :	*Cardiovascular disease*
D5D:	δ-*5 desaturase*
D6D:	δ-*6 desaturase*
DBP :	Diastolic blood pressure
DGLA:	Dihomo-γ-linolenic acid
DTC :	Direct-to-consumer (disponible directement au consommateur)
ELOVL2 :	*Fatty acid elongase 2*
ELOVL5 :	*Fatty acid elongase 5*
FAs:	Fatty acids
FADS1 :	*Fatty acid desaturase 1*
FADS2 :	*Fatty acid desaturase 2*
FADS3 :	*Fatty acid desaturase 3*
FAS :	*Fatty Acid Sensors Study*
FFQ :	*Food frequency questionnaire*
GLA:	*γ-linolenic acid*
GLM :	*General Linear Model*

GOLDN :	*Genetics of Lipid Lowering Drugs and Diet Network*
GWAS :	*Genome-wide association study*
HDL :	*High-density lipoprotein* (lipoprotéine de haute densité)
HOMA :	*Homeostasis model assessment*
HWE:	*Hardy-Weinberg equilibrium*
IL6 :	Interleukine 6
IMC :	Indice de masse corporelle
INAF :	Institut sur la nutrition et les aliments fonctionnels
IR :	*Insulin resistance*
IS :	*Insulin sensitivity*
LA:	*Linoleic acid*
LC:	*Long chain*
LD :	Linkage disequilibrium (déséquilibre de liaison)
LDL :	Low-densitylipoprotein (lipoprotéine de basse densité)
MAF :	*Minor allele frequency*
MCV :	Maladies cardiovasculaires
n-3 :	Oméga-3
n-6 :	Oméga-6
n-9:	Omega-9
NCBI :	*National Center for Biotechnology Information*
OGTT :	Test oral de tolérance au glucose
OMS :	Organisation mondiale de la Santé
OPDQ :	Ordre professionnel des diététistes du Québec
PBMCs :	*Peripheral blood mononuclear cells* (cellules mononuclées périphériques sanguines)
PCA :	*Principal component analysis* (analyse en composantes principales)
PPLs :	*Phospholipids* (phospholipides)
PPARA :	Peroxisome proliferator-activated receptor alpha
PUFA :	*Polyunsaturated fatty acid*
QFA :	Questionnaire de fréquence alimentaire

qRT-PCR :	*Real-time polymerase chain reaction* (réaction en chaîne de la polymérase à transcription quantitative inversée)
RD :	*Registered dietitian* (diététiste)
SAS :	*Statistical Analysis Software*
SBP :	*Systolic blood pressure* (pression systolique)
SD :	*Standard deviation* (déviation standard)
SFA :	*Saturated fatty acid* (acide gras saturé)
SMet :	Syndrome métabolique
SNP :	*Single-nucleotide polymorphism* (polymorphisme nucléotidique simple)
TC :	*Total cholesterol* (cholestérol total)
TG :	*Triglyceride* (triglycéride)
TNFA :	*Tumor necrosis factor alpha*
tSNP :	Tag SNP
UTR :	Untranslated region
VLDL :	Very-low-density lipoprotein (lipoprotéine de très basse densité)

Je dédie cette thèse à tous mes proches qui m'ont appuyé
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Avant-propos

Plusieurs personnes ont contribué aux travaux présentés dans cette thèse. Tout d'abord, ma directrice de recherche **Dr Marie-Claude Vohl**, professeure à l'École de nutrition de la Faculté des sciences de l'agriculture et de l'alimentation de l'Université Laval, a contribué à tous les articles présentés dans cette thèse de même qu'à chacune des étapes requises pour réaliser les différents projets. Plus précisément, le Dr Marie-Claude Vohl a participé à la planification du projet, à la révision des documents de demande de financement pour ce projet de doctorat, ainsi qu'à l'approbation et à la révision de chacun des articles. Le **Dr Simone Lemieux**, ma co-directrice de recherche est coauteure des travaux présentés aux chapitres 3, 4, 5, 6, et 7 de cette thèse. Le Dr Simone Lemieux a contribué à la planification du projet de recherche dans lequel s'insère ce projet de doctorat. Cette dernière détient également une expertise approfondie du métabolisme des lipides, ce qui lui a permis de réviser et de commenter les travaux des différents chapitres. Le **Dr Iwona Rudkowska** a contribué à la planification du projet de recherche dans lequel s'inscrivait ce projet de doctorat et a participé à la révision de même qu'à certaines analyses statistiques pour les articles présentés aux chapitres 3, 4, 5, 6, et 7. Le **Dr Patrick Couture** détient une expertise relative au DBT2 et aux MCV. Étant médecin, il a supervisé tout le volet médical de l'étude FAS. Ce dernier a également participé à l'élaboration et à la révision des travaux présentés aux chapitres 3, 4, 5, 6, et 7. Le **Dr Ann-Marie Paradis** a contribué aux articles présentés aux chapitres 6 et 8, de par sa grande implication à la coordination du volet clinique de l'étude FAS ainsi que sa participation au développement du questionnaire électronique envoyé aux diététistes membres de l'OPDQ. Le **Dr Pierre Julien**, de par sa grande expertise dans le domaine de la lipidologie, a contribué aux chapitres 3 et 5. Il a supervisé les étapes permettant le d'obtenir le dosage des AG dans les érythrocytes et dans les membranes des PPLs. Les **Drs Julie Robitaille** et **Sophie Desroches,** toutes deux aussi diététistes, ont contribué à l'article 8 de par leurs connaissances du domaine de la santé publique. Elles ont aussi contribué à la révision du questionnaire ainsi que de l'article. **Bénédicte L.-Tremblay** a contribué à l'analyse des résultats présentés au chapitre 8. **Élisabeth Thifault** a contribué aux articles 4 et 6 en prenant en charge le poste de coordinatrice du volet clinique de l'étude FAS. Finalement, **Véronique Garneau** a contribué aux chapitres 6 et 8 en analysant les données

brutes récoltées suite à la complétion de l'étude FAS et suite à l'obtention des résultats de la consultation en ligne sur la nutrigénétique auprès des diététistes membres de l'OPDQ.

Pour ma part, j'ai été responsable en collaboration avec ma directrice de recherche, Dr Marie-Claude Vohl, de la planification des projets (tous les chapitres), des manipulations de génotypage en laboratoire (chapitres 3, 4, 5, 6 et 7), de la révision du questionnaire (chapitre 8), de l'exécution et des analyses statistiques (tous les chapitres), de l'analyse des résultats (tous les chapitres) et de la rédaction entière des articles (tous les chapitres). Ensuite, j'ai été responsable de l'intégration des commentaires des coauteurs pour chacun des articles, de leur soumission à des revues scientifiques ainsi qu'à leur révision. Pour ces raisons, je suis le premier auteur sur tous les articles présentés dans le cadre de cette thèse.

Les articles scientifiques sont présentés sous la forme de manuscrits dans cette thèse. Les informations concernant l'état de publication et les références des articles sont présentées ici-bas.

Chapitre 3

Date de publication : 29 mai 2015

Cormier H, Rudkowska I, Lemieux S, Couture P, Julien P, Vohl MC. Changes in Plasma Phospholipid Fatty Acid Patterns and their Impact on Plasma Triglyceride Levels Following Fish Oil Supplementation. Int J Food Sci Nutr Diet 2015; *S*, *2*, 1-10.

Chapitre 4

Date de publication : 10 septembre 2013

Cormier H, Rudkowska I, Thifault E, Lemieux S, Couture P, Vohl MC. Polymorphisms in Fatty Acid Desaturase (*FADS*) Gene Cluster: Effects on Glycemic Controls Following an Omega-3 Polyunsaturated Fatty Acids (PUFA) Supplementation. Genes 2013; *4*(3), 485-498.

Chapitre 5

Date de publication : 4 novembre 2014

Cormier H, Rudkowska I, Lemieux S, Couture P, Julien P, Vohl MC. Effects of *FADS* and *ELOVL* polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation. Genes & nutrition 2014; *9*(6), 437.

Chapitre 6

Date de publication : 17 août 2012

Cormier H, Rudkowska I, Paradis AM, Thifault E, Garneau V, Lemieux S, Couture P, Vohl MC. 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), 1026-1041.

Chapitre 7

Date de publication : 15 mars 2016

Cormier H, Rudkowska I, Lemieux S, Couture P, Vohl MC. Expression and Sequence Variants of Inflammatory Genes; Effects on Plasma Inflammation Biomarkers Following a 6-Week Supplementation with Fish Oil. International journal of molecular sciences 2016;*17*(3), 375.

Chapitre 8

Date de publication : 6 janvier 2014

Cormier H, Tremblay BL, Paradis AM, Garneau V, Desroches S, Robitaille J, Vohl MC. Nutrigenomics- perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians. Journal of human nutrition and dietetics 2014; *27*(4), 391-400.

INTRODUCTION GÉNÉRALE

Selon la *Stratégie canadienne de santé cardiovasculaire (SCSC) – Facteurs de risque et répercussions sur les coûts futurs* du *Conference Board* du Canada, la prévention, la détection et la gestion des facteurs de risque associés aux MCV permettraient à l'État des économies relatives de l'ordre de 76,4 milliards de dollars pour la période 2005-2020 [1]. En 2020, on s'attend à ce que les coûts totaux des MCV atteignent 28,3 milliards de dollars et à ce que la part des coûts de la mortalité grimpe à 49%. Ces coûts comprennent les coûts directs (médicaments, hôpitaux, médecins, etc.) et les coûts indirects (pertes de revenus découlant de la mortalité, diminution de la productivité imputable à une incapacité à long terme ou à court terme, coûts fiscaux aux échelons fédéral et provincial) [1]. L'un des objectifs premiers de la *SCSC et Plan d'action – Pour un Canada au cœur qui bat* est de diminuer de 20% la prévalence de l'embonpoint ou d'obésité des adultes canadiens [2]. Tjepkema estimait que 36% des Canadiens souffraient d'embonpoint alors que 23% des Canadiens étaient obèses (indice de masse corporelle (IMC) ≥ 30 Kg/m^2) selon les données issues du *2004 Canadian Health Community Survey* [3].

La pandémie actuelle d'obésité accroît le développement de pathologies qui y sont reliées, dont les MCV et le diabète de type 2 (DBT2) en étant l'un des principaux facteurs de risque. L'*International Diabetes Federation* a établi un consensus quant à la définition du syndrome métabolique (SMet) où en plus d'un tour de taille élevé (supérieure à 94 centimètres pour les hommes et 80 centimètres pour les femmes), l'association avec au moins deux des quatre facteurs suivants est de mise pour établir le diagnostic : 1. Niveaux de TG plasmatiques ≥ à 1,7 mmol/L (ou l'administration d'un traitement hypotriglycéridémiant); 2. Valeurs de cholestérol-HDL < à 1,03 mmol/L; 3. Hypertension (systolique ≥ 130 et diastolique ≥ 85); et 4. Glycémie à jeun ≥ 5,6 mmol/L (ou un diagnostic de DBT2) [4]. L'étude du *National Health and Nutrition Examination Survey* (NHANES) a quant à elle évalué les associations entre les MCV et les cinq (5) facteurs entrant dans la composition du SMet et ont démontré que l'association la plus forte était avec les TG plasmatiques [5]. Les facteurs de risque des MCV : l'obésité, l'hypertension, la dyslipidémie, l'athérosclérose et le DBT2 sont causés par

une interaction complexe de facteurs génétiques, environnementaux (ex. : faible niveau de revenu ou d'instruction ou les deux, accès limité à des aliments sains ou à l'activité physique) et comportementaux (ex. : régime alimentaire malsain, inactivité physique, tabagisme, stress, dépression).

Partant de la prémisse que les concentrations de TG plasmatiques sont reconnues comme étant associées aux MCV et au DBT2, différents traitements hypotriglycéridémiants exercent des effets bénéfiques tels les fibrates [6] ou encore une supplémentation en AGPI n-3 [7]. La littérature suggère qu'une supplémentation en AGPI n-3 pourrait réduire les facteurs de risque métabolique liés aux MCV [8-17]. En effet, une supplémentation en AGPI n-3 réduit les taux plasmatiques de TG selon une courbe dose-réponse [16]. D'autres études rapportent également une réduction de l'ordre de 15 à 19% du taux de mortalité lié aux MCV de même qu'une amélioration du profil lipidique, une diminution de la pression artérielle ainsi qu'une diminution de l'inflammation [18]. Or, les effets rapportés de la supplémentation, notamment la diminution des concentrations de TG, varient d'un individu à l'autre notammen en raison de variations génétiques. Dans les études précédentes de notre groupe de recherche, il a été observé que la présence du SNP L162V du gène *peroxisome proliferator activated receptor alpha* (*PPARA*) peut affecter la réponse à une supplémentation en huile de poisson [19]. Cette variabilité dans la réponse à une supplémentation en huile de poisson a également été observée dans une cohorte finlandaise sur 312 sujets suite à la prise d'un supplément d'huile de poisson durant une période de 8 semaines. Dans cette étude, les auteurs ont observé que 31% des participants n'avaient pas diminué leurs concentrations de TG plasmatiques suite à la supplémentation [20]. Ces résultats ont par ailleurs été répliqués dans l'étude *Fatty Acid Sensors* (FAS) de notre groupe de recherche où 28,8% de la population était non-répondante (stabilité ou hausse significative des TG plasmatiques) à une supplémentation de 5g/jour d'huile de poisson fournissant 3-3,3g d'AGPI n-3/jour (1,9-2,2g d'acide eicosapentaénoique (AEP) + 1,1g d'acide docosahexaénoique (ADH)) [21]. Or, les recommandations nutritionnelles actuelles assument que chaque individu réagira de façon similaire à un traitement. Toutefois, il existe une grande variabilité interindividuelle dans la population, ce qui se répercute par des réponses différentes aux traitements nutritionnels [22]. La génétique permettrait d'expliquer une partie de cette grande variabilité interindividuelle observée dans

les résultats de certaines études en trouvant des variations génétiques associées aux pathologies étudiées [23-25]. Les résultats actuels de l'étude FAS montrent une diminution significative des niveaux de TG de l'ordre de 11,9 ± 25,9% (n = 210). Toutefois, lorsqu'on stratifie la cohorte en fonction de la réponse des niveaux de TG plasmatiques suite la supplémentation en AGPI n-3, les répondeurs (n = 148) ont alors une réduction significative des TG plasmatiques de l'ordre de 24,7 ± 15,0% alors que les non-répondeurs (n = 60) subissent une augmentation significative moyenne de l'ordre de 19,7 ± 19,4%, ce qui témoigne de la grande variabilité au sein d'une même population. L'*American Heart Association* (AHA) recommande la prise d'un supplément de 2 à 4 grammes d'AGPI n-3 (sous forme d'AEP et d'ADH) chez les individus hypertriglycéridémiques [26]. De ce fait, il serait pertinent de pouvoir cibler adéquatement les individus qui bénéficieront réellement des bienfaits qu'exercent les AGPI n-3 sur la santé cardiovasculaire. Le premier volet de cette thèse s'attarde aux principaux facteurs qui permettent d'expliquer une partie de la variabilité observée dans la réponse lipidique, glycémique et inflammatoire suite à une supplémentation en AGPI n-3.

Le deuxième volet de cette thèse porte sur la perception de la nutrigénomique et de la nutrigénétique par les diététistes membres de l'OPDQ. Actuellement, les tests génétiques sont utilisés dans la sphère publique pour dépister les maladies monogéniques telles que la phénylcétonurie et la tyrosinémie. Toutefois, on retrouve actuellement sur le marché une multitude de tests génétiques (i.e. *Nutrigenomix, 23andMe, Habit, DNAFit, ORIG3N*, etc.) que l'on peut commander en ligne ou via un professionnel de la santé, selon nos besoins. Une étude récente conclut que la population en général pourrait mal interpréter les résultats d'un test génétique *direct-to-consumer* (DTC) sans avoir recours à l'aide d'un professionnel habilité [27]. C'est pourquoi le *Genetic & Public Policy Center* (Johns Hopkins University) distingue deux catégories de tests génétiques : 1- *DTC genetic testings*, où les résultats sont envoyés directement aux clients ou encore à leur disposition via un portail web et 2- *DTC genetic testings - through physician*, où la compagnie exige que les résultats soient divulgués par un professionnel de la santé [28]. Le counseling génétique permettrait d'atténuer la détresse psychologique qui pourrait survenir suite aux résultats positifs à un test DTC [29]. Cependant, en ce moment, les professionnels de la santé sentent qu'ils manquent de

compétences afin de transmettre aisément l'information génétique aux clients/patients [30]. Des données tirées d'un sondage auprès de diététistes aux États-Unis montrent que 88 % d'entre eux croyaient ne pas avoir les connaissances nécessaires en matière de génomique nutritionnelle [31]. Comme les diététistes sont les professionnels de la santé les mieux placés pour parler de nutrition avec leurs clients/patients, il en va de soi quant à la divulgation d'informations génétiques issues de tests de nutrigénétique. La consultation panquébécoise en ligne sur la nutrigénomique auprès des diététistes membres de l'OPDQ avait pour but de dresser un portrait global de la situation actuelle de la nutrigénomique au Québec afin de pouvoir mieux cerner les besoins en matière de formation.

Cette thèse se distingue par la présentation de deux études. La première étude est issue d'un protocole d'intervention où 210 participants ont été supplémentés en huile de poisson (cohorte FAS). Celle-ci étudiait principalement la variabilité interindividuelle observée dans les réponses lipidique, glycémique et inflammatoire suite à une supplémentation en AGPI n-3 d'une durée de 6 semaines. Pour cette cohorte, plusieurs données ont été mesurées dont le dosage par chromatographie en phase gazeuse des AG dans les PPLs du plasma et des globules rouges. La deuxième étude a permis de recruter un échantillon de 373 diététistes ayant complété un questionnaire en ligne composé de 34 questions permettant d'évaluer la perception des diététistes de la nutrigénomique et des tests de nutrigénétique.

Cette thèse se subdivise en 9 chapitres. Le premier chapitre, chapitre 1, présente une revue de littérature portant sur les AGPI n-3 et leurs effets sur les facteurs de risque cardiovasculaire. Le chapitre 2 présente la problématique, les objectifs et les hypothèses de la présente thèse. Le chapitre 3 se constitue d'un article s'intitulant « *Changes in Plasma Phospholipid Fatty Acid Patterns and their Impact on Plasma Triglyceride Levels Following Fish Oil Supplementation* » qui a été publié dans la revue *International Journal of Food Sciences, Nutrition and Dietetics* en 2015. Le chapitre 4 se constitue d'un article s'intitulant « *Polymorphisms in Fatty Acid Desaturase (FADS) Gene Cluster: Effects on Glycemic Controls Following an Omega-3 Polyunsaturated Fatty Acids Supplementation* » qui a été publié dans la revue *Genes* en 2013. Le chapitre 5 se constitue d'un article s'intitulant « *Effects of FADS and ELOVL polymorphisms on indexes of desaturase and elongase*

activities: results from a pre-post fish oil supplementation » qui a été publié dans la revue *Genes & Nutrition* en 2014. Le chapitre 6 se constitue d'un article s'intitulant « *Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation* » qui a été publié dans la revue *Nutrients* en 2012. Le chapitre 7 se constitue d'un article s'intitulant « *Expression and Sequence Variants of Inflammatory Genes; Effects on Plasma Inflammation Biomarkers Following a 6-Week Supplementation with Fish Oil* » qui a été publié dans la revue *International Journal of Molecular Sciences* en 2016. Le chapitre 8 se constitue d'un article s'intitulant « *Nutrigenomics - perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians* » qui a été publié dans la revue *Journal of Human Nutrition and Dietetics* en 2014. Finalement, le chapitre 9 conclut cette thèse en intégrant les résultats présentés aux chapitres 3 à 8 et en présentant certaines voies à explorer afin de poursuivre ces recherches dans le futur.

Chapitre 1

Revue de littérature

1.1 Les acides gras polyinsaturés oméga-3

La composition de l'alimentation est l'un des piliers de la santé. Au pays, Santé Canada recommande une diète diversifiée telle que décrite dans le Guide alimentaire canadien dans sa dernière révision datant de 2007 [32]. Selon l'Enquête sur la santé dans les collectivités canadiennes, cycle 2.2, Nutrition (ESCC 2.2), 25 % des hommes et 23 % des femmes de 19 ans et plus ont outrepassé la limite supérieure de l'étendue des valeurs acceptables pour les macronutriments (ÉVAM) pour les lipides, qui est fixée à 35 % de l'apport énergétique total [33]. Bien que la quantité de lipides et de calories consommée revête une importance capitale pour enrayer l'actuelle pandémie d'obésité et toutes les complications qui y sont rattachées, la qualité y joue également un rôle prépondérant. En effet, la composition en lipides du régime alimentaire affecterait directement la santé cardiométabolique et l'apport en AGPI serait inversement associé au risque cardiovasculaire selon une étude observationnelle menée chez les femmes [34].

1.1.1 Les sources alimentaires

L'acide linoléique (LA) et l'acide alpha-linolénique (ALA) constituent à eux seuls (>95 %) la majorité des AGPI retrouvés dans la diète nord-américaine, où les quantités de LA sont près de dix fois plus importantes que celles de l'ALA [35]. La quantité de LA représente désormais entre 6 et 8% de l'apport énergétique total grâce à l'accroissement de la consommation de légumineuses, de produits transformés contenant des graisses végétales (huile de soja, de maïs, de palme et de canola entres autres), de margarine et de shortenings [36].

La consommation d'AGPI n-3 et n-6 a considérablement changé au cours du 20e siècle; l'ALA, l'AEP, l'acide docosapentaénoique (DPA) et l'ADH sont moins abondants dans le cadre de la diète occidentale. Ces changements ont ainsi affecté le ratio n-6:n-3 qui serait passé de 1:1 à plus de 30:1 [36, 37]. Le **Tableau 1.1** montre la teneur en AGPI n-3 de quelques aliments usuels. On remarque que les sources d'ALA proviennent généralement d'aliments du règne végétal ou encore d'aliments qui ont été enrichis, comme dans le cas de certaines marques d'œufs où les poules ont été nourries avec des graines de lin. Les sources

alimentaires d'ALA sont nombreuses : graines de chia, graines de lin, huile de lin, noix de Grenoble, huile de canola, etc. En ce qui concerne les aliments à riche teneur en AEP et ADH, ils proviennent essentiellement d'aliments d'origine marine dont certaines huiles de poisson, poissons gras, algues, fruits de mer ou encore d'aliments enrichis directement en ces deux AG.

1.1.2 Les recommandations nutritionnelles

Des apports nutritionnels de référence pour les AGPI n-3 ont été émis par Santé Canada pour l'ALA, mais pas pour l'AEP et l'ADH [38, 39]. Les Diététistes du Canada recommandent de consommer deux portions de 75 grammes de poisson par semaine, ce qui équivaut à des apports d'environ 0,3 à 0,45 grammes d'AEP et d'ADH par jour [38]. Pour tous les groupes d'âge, Santé Canada recommande également de consommer entre 0,6 à 1,2 % des apports énergétiques sous forme d'ALA, selon l'entendue l'ÉVAM [39]. Aucun apport maximal tolérable (AMT) n'a été établi pour la consommation d'ALA. Ailleurs dans le monde, le Conseil National Australien de la Santé et de la Recherche Médicale (NHMRC) définit un apport suffisant semblable à celui de l'Académie Nationale des Sciences (États-Unis) de 0,8 à 1,3 grammes d'ALA et 90 à 160 mg/jour pour les AGPI n-3 à longue chaîne.

À raison de deux repas de poisson par semaine, il est possible de combler les besoins en AGPI n-3 d'origine marine, mais l'AHA a conclu que des doses de l'ordre de 2 à 4 grammes par jour pourraient être bénéfiques pour les individus hypertriglycéridémiques [40]. Il est à noter qu'il est quasi impossible d'obtenir de telles concentrations d'AGPI n-3 d'origine marine par l'alimentation puisqu'il faudrait consommer environ cinq portions de saumon quotidiennement pour y arriver. De plus, des chercheurs ont démontré que l'apport moyen en AGPI n-3 d'origine marine est d'environ 150 mg par jour aux États-Unis et cette quantité équivaut à la consommation d'environ un repas de poisson tous les 10 jours [41].

Bien que les recommandations concernant la consommation d'AGPI n-3 soient basées sur une abondante littérature, dans la pratique, ces apports sont plutôt difficiles à atteindre parce que des changements dans les habitudes alimentaires sont toujours complexes et difficiles à implanter. Par conséquent, la plupart des gens hésitent à inclure régulièrement plusieurs

portions de poisson dans leur alimentation. De plus, des craintes sont soulevées quant à la présence de possibles contaminants environnementaux tels que les métaux lourds, le méthyl mercure et les organochlorés dans la chair des poissons [40]. La consommation de suppléments d'huile de poisson serait donc une façon efficace d'augmenter les apports en AGPI n-3 d'origine marine sans devoir changer les habitudes alimentaires.

L'effet d'une supplémentation pourrait aussi être important en prévention secondaire des MCV à des doses de 1 gramme/jour (AEP + ADH) selon les informations colligées par plusieurs experts [42]. Tel que mentionné précédemment, l'effet majeur d'une supplémentation en AGPI n-3 est la baisse impressionnante des taux de TG plasmatiques [43]. Cependant, à court terme, le cholestérol-LDL tend à monter légèrement puis à se stabiliser à long terme. Toutefois, la diminution des TG prévaut sur la légère augmentation du cholestérol-LDL, surtout que cette augmentation serait principalement due à une augmentation de la taille des particules plutôt qu'à leur nombre, ce qui serait moins athérogène [44].

Tableau 1.1 Teneur en acides gras n-3 d'origine marine ou végétale de quelques aliments usuels

Aliments	**Portion**	**ALA (g)**	**AEP + ADH (g)**
Édamames, cuit	125 ml (½ tasse)	0,29 – 0,34	0
Céréales de germe de blé, grillées	30 g	0,24	0
Lait, enrichi d'ADH	250 ml (1 tasse)	0	0,01
Boisson de soya	250 ml (1 tasse)	0,19	0
Œufs oméga-3, enrichis d'ADH	2 œufs	0,50 – 0,54	0,16 – 0,27
Anchois, en conserve dans l'huile	75 g (2 ½ oz)	0,01	1,54
Imitation de caviar (noir, rouge), granuleux	75 g (2 ½ oz)	0,01	1,96
Palourdes, cuites	75 g (2 ½ oz)	0,01	0,21
Flétan, cuit	75 g (2 ½ oz)	0,04 – 0,06	0,35 – 0,88
Hareng, cuit	75 g (2 ½ oz)	0,05 – 0,11	1,60
Maquereau, cuit	75 g (2 ½ oz)	0,03 – 0,08	0,90 – 1,39
Moules, cuites	75 g (2 ½ oz)	0,03	0,59
Huîtres, Pacifique, cuites	75 g (2 ½ oz)	0,05	1,04

Goberge, cuit	75 g (2 ½ oz)	0	0,40
Saumon d'élevage, Atlantique, cru ou cuit	75 g (2 ½ oz)	0,08 – 0,11	1,48 – 1,61
Saumon sauvage, Atlantique, cru ou cuit	75 g (2 ½ oz)	0,22 – 0,28	1,08 – 1,38
Sardines, en conserve	75 g (2 ½ oz)	0,17 – 0,37	0,74 – 1,05
Pétoncles, cuits	75 g (2 ½ oz)	0	0,27
Crevettes, cuites	75 g (2 ½ oz)	0,01	0,24
Sole ou plie, cuite	75 g (2 ½ oz)	0,01	0,37
Truite, cuite	75 g (2 ½ oz)	0,06 – 0,14	0,65 – 0,87
Thon, chair pâle, en conserve dans l'eau	75 g (2 ½ oz)	0	0,21
Haricots, cuits	175 ml (¾ tasse)	0,17 – 0,24	0
Tofu, cuit	150 g (¾ tasse)	0,27 – 0,48	0
Amandes, rôties dans l'huile, blanchies	60 ml (¼ tasse)	0,15	0
Graines de chia	15 ml (1 c. à table)	1,9	0
Graines de lin, moulues	15 ml (1 c. à table)	2,46	0
Graines de soya	60 ml (¼ tasse)	0,42	0
Noix de Grenoble	60 ml (¼ tasse)	2,30	0
Huile de canola	5 ml (1 c. à thé)	0,42	0
Huile de lin	5 ml (1 c. à thé)	2,58	0
Margarine oméga-3 faite à partir d'huile de canola	5 ml (1 c. à thé)	0,34	0
Supplément d'huile de poisson	5 ml (1 c. à thé)	0,04 – 0,06	0,48 – 1,44

Données tirées du Fichier canadien sur les éléments nutritifs, Santé Canada, www.santecanada.gc.ca/fcen

1.1.3 Formulation des suppléments d'AGPI n-3

Les études cliniques à répartition aléatoire menées sur les effets d'une supplémentation en AGPI n-3 utilisent souvent un supplément d'AGPI n-3 éthyl-esters 40/20. Les AG sont alors estérifiés avec une molécule d'éthanol plutôt que d'être pris sous la forme moléculaire naturelle liée à une molécule de glycérol (forme TG). L'utilisation d'AGPI n-3 éthyl-esters est souvent favorisée en raison de son coût moins élevé et de sa plus grande accessibilité. La forme éthyl-ester est hydrolysée de 10 à 50 fois plus lentement par la lipase pancréatique que la forme TG, ce qui retarde la resynthèse des TG. Selon une étude à répartition aléatoire en

chassé-croisé, menée par Beckermann *et al*., la teneur en AEP et en ADH dans le plasma était 50 % plus élevée pour les huiles de poisson sous la forme TG que pour les huiles de poisson sous la forme éthyl-esters [45], ce qui laisse entendre que non seulement le type d'acide gras, mais également la forme, influeraient sur les résultats.

1.1.4 Statut en acides gras polyinsaturés oméga-3

Plusieurs facteurs permettent de déterminer le statut en AG d'un individu. En effet, le profil des AG est la résultante de la consommation de lipides, de la digestion et de l'assimilation des AG et de la synthèse hépatique de ceux-ci [46]. Les apports alimentaires en AGPI n-3 et n-6 sont des déterminants critiques de la proportion d'AG bioactifs présents dans les membranes des PPLs [47]. Un phénomène dose-réponse est observé quant aux apports alimentaires en AGPI n-3 et le niveau d'incorporation dans les membranes. L'incorporation des AGPI n-3 dans les cellules est l'un des mécanismes d'action potentiel qui est responsable des bienfaits attribués aux n-3 [48]. Les PPLs composent la majeure partie de la membrane cytoplasmique et interviennent dans les propriétés physico-chimiques des membranes cellulaires. L'augmentation de la présence d'AGPI n-3 dans les membranes augmente ainsi la fluidité et la perméabilité membranaire en raison des queues hydrocarbonées insaturées avec inflexions qui peuvent aisément se rapprocher grâce aux forces de Van Der Waals. De plus, l'enrichissement des membranes en AGPI n-3 peut moduler les mécanismes de signalisation cellulaire, la fonction des protéines membranaires et l'expression de certains gènes [49].

La synthèse de ces AG peut être insuffisante en cas de diminution de l'activité enzymatique des désaturases ou en présence d'un déficit de l'un des cofacteurs des désaturases tel que le zinc, la pyridoxine (vitamine B6), la vitamine B3, l'acide ascorbique et la mélatonine [50-53]. Le profil d'AG peut être mesuré en utilisant deux matrices distinctes, soit les PPLs du plasma ou des érythrocytes. Ces mesures sont indépendantes de la concentration en TG et sont le reflet des apports nutritionnels des trois dernières semaines dans le cas des PPLs du plasma et des 4 derniers mois dans le cas des PPLs des globules rouges. La **Figure 1.1** montre bien les effets de l'incorporation des AGPI n-3 dans les membranes des cellules.

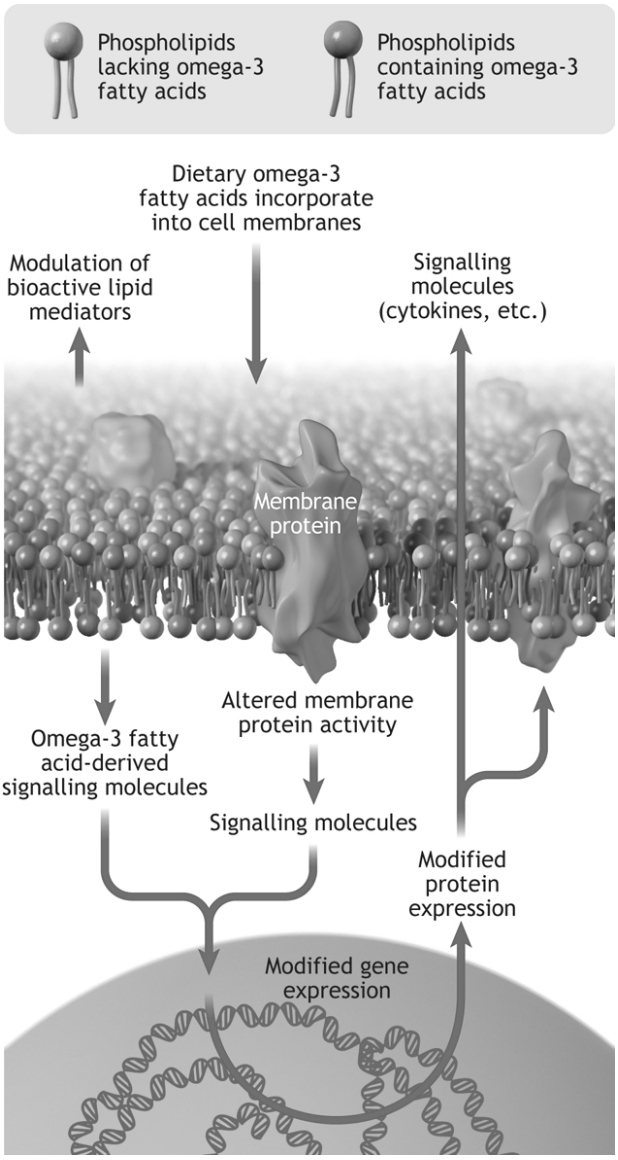


Figure 1.1 Incorporation des AGPI n-3 dans les membranes (Image par Lianne Friesen et Nicholas Woolridge)

1.1.5 Compétition entre les AGPI n-3 et n-6

Les AGPI n-3 provenant de la diète ou d'une supplémentation agissent directement sur le métabolisme de l'acide arachidonique (ARA), car ils déplacent ce dernier des membranes et entrent directement en compétition avec l'ARA pour les enzymes qui catalysent la biosynthèse des thromboxanes, des prostaglandines et des leucotriènes [54]. Un effet positif de la consommation d'aliments riches en AGPI n-3 est la diminution du potentiel des cellules comme les monocytes, les neutrophiles et les éosinophiles à synthétiser certains médiateurs

de l'inflammation dérivés de l'ARA en plus de diminuer la synthèse des thromboxanes A2 (TXA2). Cependant, la fonction des prostaglandines dérivées de l'ARA diffère de celles dérivées de l'AEP. La prostaglandines E2 (PGE2) et le TXA2 formés à partir de l'ARA sont produits dans les plaquettes et favorisent l'inflammation avec une chimio-activité et une agrégation plaquettaire puissantes. Ces métabolites possèdent également des propriétés vasoconstrictrices. Cependant, les métabolites dérivés de l'AEP agissent uniquement comme vasodilatateurs et antiagrégants [55]. Par conséquent, la source des eicosanoïdes joue un rôle majeur dans la fonction physiologique, et le déséquilibre pourrait mener à plusieurs conditions, y compris la thrombose, l'inflammation, l'asthme et la maladie inflammatoire de l'intestin [56].

1.1.5.1 Taux de conversion

Burdge & Wootton (2002) ont montré que chez les jeunes femmes en bonne santé, la conversion de l'ALA en AEP et ADH était respectivement de 21 % et 9 %, alors que chez les jeunes hommes, la conversion était de 8 % pour l'AEP et entre 0 et 4 % pour l'ADH [57]. En effet, les hormones pourraient influencer la synthèse des AGPI. Selon des études chez les animaux, la testostérone diminuerait l'activité de la désaturase delta-5 (D5D) et de la désaturase delta-6 (D6D). De plus, l'œstrogène diminuerait la biodisponibilité des *n*-6, notamment en raison de changements dans les propriétés physicochimiques des membranes microsomales. Une étude de Giltay et al. (2004) portant sur une cohorte de transsexuels a également permis de prouver le rôle prépondérant des hormones afin d'expliquer les différences perçues dans le taux de conversion des AGPI [58]. En effet, dans le groupe homme-à-femme traité avec de l'ethynil estradiol, les niveaux d'ADH ont augmenté de 40% alors que chez le groupe femme-à-homme traité avec de la testostérone, les concentrations d'ADH ont diminué de près de 20% [58]. Une autre étude a montré, en utilisant un isotope stable, que l'efficacité de conversion de l'AEP, de DPA et de l'ADH à partir de l'ALA était

respectivement de 0,2 %, 0,13 % et 0,05% [52]. Le taux de conversion est cependant très faible, voire insuffisant pour permettre de répondre aux besoins de l'organisme en AEP et en ADH, c'est pourquoi il est recommandé d'avoir des apports en AEP et ADH provenant directement de l'alimentation riche en produits d'origine marine ou via une supplémentation [48, 51, 59].

1.2 Acides gras polyinsaturés oméga-3 et santé

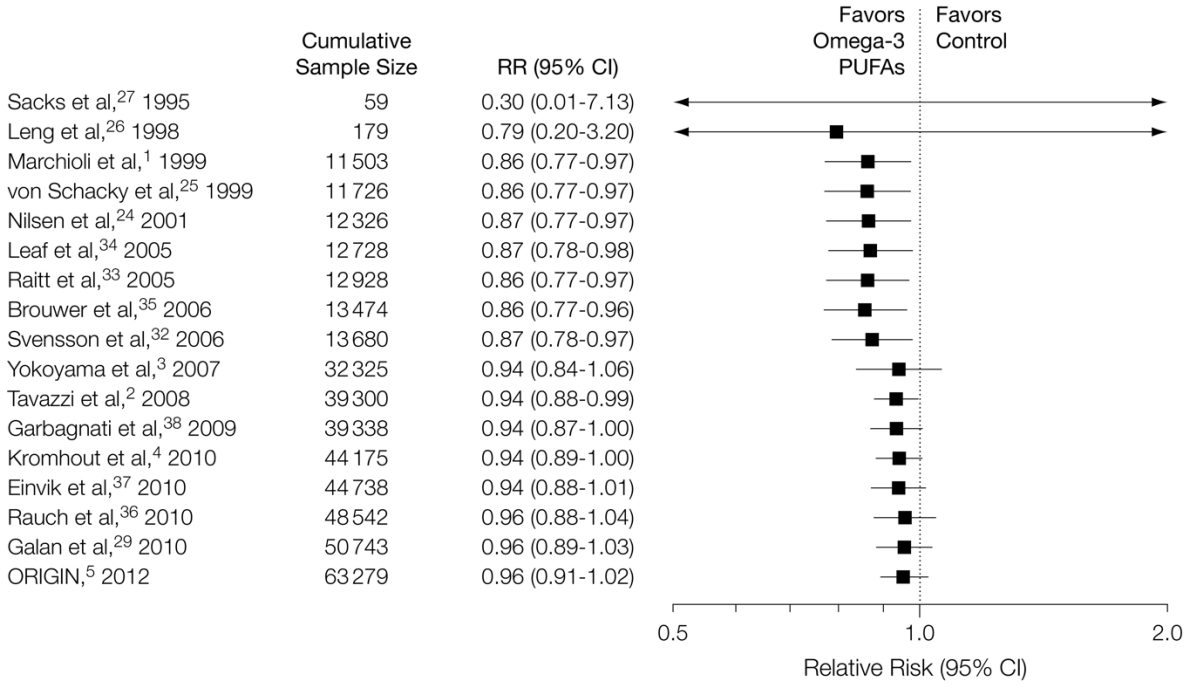
Dans les années 80, des chercheurs ont étudié la relation entre la consommation de poisson et la mortalité par maladies coronariennes. Ils se sont aperçus que, chez les Esquimaux du Groenland, le faible taux de mortalité par coronaropathies était notamment attribué à leur forte consommation de poisson [60]. Pour vérifier leur hypothèse, ils ont recueilli des données sur 20 ans auprès de 852 hommes de la ville de Zutphen aux Pays-Bas sans maladie coronarienne documentée. Une relation inverse suivant une courbe dose-réponse a été observée entre la consommation de poisson en 1960 et la mortalité par coronaropathies au cours des 20 années de suivi. De plus, les chercheurs ont noté que la mortalité y était 50% moins élevée chez les sujets qui consommaient au moins 30 grammes de poisson par jour que chez ceux qui ne mangeaient pas de poisson [60]. Cette étude a donné naissance aux recommandations actuelles concernant la consommation de poisson. En effet, l'AHA recommande aux personnes n'ayant pas d'antécédent de MCV de manger une variété de poisson, de préférence des poissons gras (saumon, thon, maquereau, hareng et truite), au minimum deux fois par semaine [40]. Quant aux individus ayant des antécédents de MCV, des apports de 1 gramme/jour d'AEP et d'ADH sont recommandés, préférablement provenant de poissons gras et la prise de suppléments d'AEP et d'ADH devraient être considérée, sous recommandation médicale [12]. Finalement, les individus ayant des taux élevés de TG devraient prendre de 2 à 4 g/jour d'AEP et d'ADH sous supervision médicale [40].

Par la suite, plusieurs autres études ont établi des liens entre des concentrations plus élevées d'AGPI n-3 dans les globules rouges ou les PPLs du plasma – ou des apports alimentaires plus élevés en AGPI n-3 – sur la longévité et le risque cardiovasculaire. En effet, dans l'étude *Cardiovascular Health Study*, les concentrations d'AGPI n-3 dans les PPLs du plasma étaient inversement associées aux taux de mortalité totaux [61]. Les études *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico* (GISSI-Prevenzione) et *Japan EPA Lipid Intervention Study* (JELIS), ont montré des effets bénéfiques d'une supplémentation en AGPI n-3 en prévention secondaire des MCV sur des cohortes de plus de 11 000 et 18 000 sujets respectivement [62, 63]. Les chercheurs ont ainsi observé des diminutions au niveau de la

morbidité et de la mortalité cardiovasculaires suite à un premier événement cardiovasculaire [62, 63]. Des résultats similaires ont été obtenus dans la *Heart and Soul Study* où les niveaux sanguins d'AEP et d'ADH étaient inversement associés aux taux de mortalité totaux et ce, même après des ajustements statistiques pour l'âge, le sexe, l'ethnicité, le statut socioéconomique, les facteurs de risque cardiovasculaire traditionnels et les marqueurs de l'inflammation [64, 65].

Toutefois, ces résultats demeurent controversés et des études plus récentes n'arrivent plus à confirmer l'effet protecteur des AGPI n-3 [66-70]. Plusieurs raisons ont été avancées pour expliquer ces disparités dont l'utilisation plus commune de statines [71], les courtes périodes de suivi, les faibles doses d'AGPI n-3 et l'amélioration des soins de santé [72]. Selon von Schacky (2014), les récentes contradictions observées dans les résultats des récentes études sur les bienfaits des AGPI n-3 pourraient être expliquées par des niveaux de départ différents en AEP et ADH, d'importants prédicteurs d'événements cardiovasculaires [73]. Les niveaux d'AEP et d'ADH suivent une distribution normale qui est compromise en raison de la grande variabilité interindividuelle de la réponse suite à l'augmentation des apports en AGPI n-3. Cela crée un chevauchement important des niveaux d'AEP et d'ADH entre les groupes d'intervention et de contrôle [73]. De surcroît, le moment de la journée où les capsules d'huile de poisson ont été consommées peut grandement influencer l'incorporation de l'AEP et de l'ADH puisque les apports en lipides lors d'un repas ont un impact sur la biodisponibilité des AGPI n-3. Il a été démontré que la prise de suppléments d'AGPI n-3 lors du déjeuner était associée à une plus faible biodisponibilité de l'AEP et de l'ADH, puisque le déjeuner est reconnu comme un repas qui contient souvent de faibles quantités de matières grasses [73].

Bref, certaines études publiées avant les années 2010 observent généralement une diminution d'environ 10% de la mortalité par coronaropathies et par mort subite d'origine cardiaque alors que les études plus récentes ne montrent pas les mêmes effets ou des effets moindres tel que le montre la **Figure 1.2** [70].



Error bars indicate 95% CIs of the relative risk (RR) estimates. The size of the squares corresponds to the study weight in the random-effects meta-analysis. Diamonds represent the meta-analysis summary effect estimate. ICD indicates implantable cardioverter-defibrillator; PUFAs, polyunsaturated fatty acids.

Figure 1.2 Méta-analyses sur les suppléments d'AGPI n-3 et la mortalité totale (tirée de [70])

Un rapport récent de l'AHA suggérait que la supplémentation en AGPI n-3 n'apportait aucun bénéfice pour la prévention des MCV chez les patients atteints de DBT2/pré-diabète, pour la prévention des maladies coronariennes chez les individus à haut risque de MCV, pour la prévention primaire pour les infarctus du myocarde ni pour la prévention secondaire de fibrillation atriale ou fibrillation atriale post-chirurgie [74]. Toutefois, une supplémentation en AGPI n-3 pourrait réduire le taux de mortalité par maladie coronarienne chez les patients ayant une histoire de maladie coronarienne connue, possiblement en réduisant la mort subite d'origine cardiaque induite par ischémie. Par contre, la supplémentation en AGPI n-3 n'a pas réduit l'incidence des récidives d'infarctus du myocarde non mortel [74]. Malgré ces résultats mitigés, la relation entre les effets des AGPI n-3 et la mortalité par coronaropathies n'est pas linéaire [75].

Toutefois, bien que les effets des AGPI n-3 sur la mortalité semblent neutres ou modestes, il n'en demeure pas moins que d'importants effets sur la santé leur sont attribués. En effet, la prise d'AGPI n-3 semble être bénéfique en prévention des dyslipidémies, pour abaisser la tension artérielle, pour améliorer la fonction vasculaire, pour inhiber certaines réponses inflammatoires, et pour diminuer l'agrégation plaquettaire [11, 59, 76, 77]. De plus, les AGPI n-3 auraient des effets bénéfiques en prévention secondaire des arythmies cardiaques [78], de la variabilité de la fréquence cardiaque [79] et de l'inflammation causée par les plaques athéroscléreuses avancées [80, 81]. Dans le cadre de ce doctorat, nous nous sommes intéressés aux effets hypotriglycéridémiants des AGPI n-3, à leur impact sur les contrôles glycémiques et sur l'inflammation.

1.2.1 Effets hypotriglycéridémiant des acides gras oméga-3

Les AGPI n-3, à des doses élevées de l'ordre de 2 à 4 grammes/jour d'AEP et d'ADH, diminueraient les concentrations de TG sériques chez les sujets normo- et hyperlipidémiques et à de plus faibles doses lorsque les acides gras saturés (AGS) sont substitués par les AGPI n-3 [82]. Le phénomène suit une courbe dose-réponse et l'ampleur des effets obtenus dépendrait également des concentrations de départ de TG [83]. Selon Harris (1997), la prise d'AGPI n-3 n'affecte pas le cholestérol total, tend à augmenter de 5 à 10% les niveaux de cholestérol-LDL et de 1 à 3% les niveaux de cholestérol-HDL tout en diminuant les concentrations de TG d'environ 25 à 30% [83]. Des résultats similaires ont également été confirmés par Balk *et al*. (2006) dans une revue systématique rassemblant 21 études cliniques évaluant les effets des AGPI n-3 sur les marqueurs sériques du risque cardiovasculaire [84]. La diminution des concentrations de TG est également plus marquée chez les sujets atteints d'hypertriglycéridémie où la baisse se chiffrerait en moyenne à 35 % [83]. De plus, une méta-analyse publiée par Wei *et al*. (2011), a montré que l'AEP et l'ADH ont tous deux permis de réduire les concentrations de TG, mais avec une plus grande réduction pour l'ADH [85].

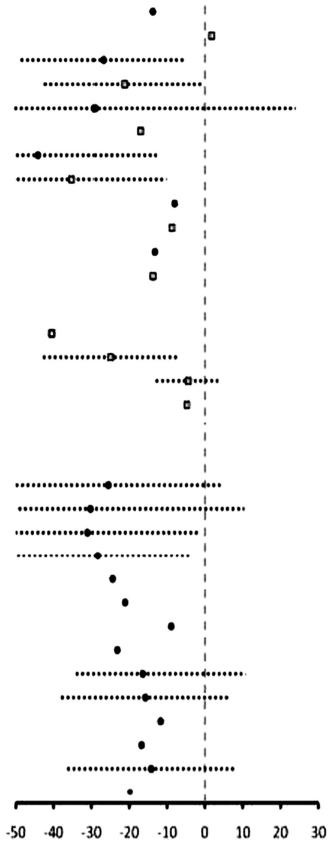
Les procédés par lesquels les AGPI n-3 provoquent un effet hypotriglycéridémiant ont fait l'objet de nombreuses études [86]. Globalement, les AGPI n-3 suppriment de manière coordonnée la lipogenèse en réduisant les niveaux du facteur de transcription *sterol regulatory element binding protein-1c* (SREBP-1c) [87]. Les facteurs de transcription de la

famille (SREBP) sont des acteurs majeurs de la régulation du métabolisme lipidique puisqu'ils contrôlent l'expression des gènes du métabolisme des acides gras, des TG et du cholestérol [88]. De plus, les AGPI n-3 activent PPARA, ce qui diminue la sécrétion de TG et de lipoprotéines de faible densité (VLDL), en plus d'augmenter l'oxydation des AG dans le foie. En effet, *PPARA* exercerait son effet sur les concentrations de TG entre autres via une augmentation de la bêta-oxydation des AG dans les mitochondries et les peroxysomes ce qui diminuerait la disponibilité des AG pour la synthèse des TG [7, 86, 89]. De plus, les effets hypotriglycéridémiant seraient la résultante d'une diminution de l'activité des enzymes responsables de la synthèse des TG dont la diacylglycerol acyltransférase ou la *phosphatidic acid phosphohydrolase* et d'une augmentation de la synthèse des PPLs [7].

Le **Tableau 1.2** montre un recensement des principaux résultats des essais cliniques avec différents protocoles de supplémentation pour l'AEP seul, l'ADH seul ou encore pour l'AEP vs. l'ADH. Les niveaux plasmatiques de TG ont diminué dans 5 études sur 6 pour l'AEP (de +1,8% à -34,9 %), dans toutes les études pour le ADH (de -8,0 % à -43,7 %) et ont également diminué dans les études AEP vs. ADH (moyenne : -22,4 ± 13,3 % [ADH] vs. -15,6 ± 12,3% [AEP]). Une diminution des niveaux plasmatiques de TG a été observée par pratiquement toutes les études incluses dans l'article de revue de Jacobson *et al*. (2012), à l'exception de l'étude d'Egert *et al*. (2009) qui conclut qu'une supplémentation avec 2,2 grammes/jour d'AEP pendant 6 semaines hausserait légèrement les niveaux de TG. Les changements rapportés ne corrélaient pas significativement avec la durée de la supplémentation en AGPI n-3 [43].

Tableau 1.2 Comparaisons des pourcentages de changement des niveaux plasmatiques de TG ajustées pour les niveaux de départ suite à une supplémentation en AEP vs. ADH, AEP seul ou ADH seul. (adapté de [43])

Études	Dose (g/jour)	Durée	N EXP (Placebo)	Acide gras	Pourcentage moyen de changement à partir des valeurs de départ (intervalle de confiance de 95%) Triglycérides plasmatiques	Niveaux de départs (mg/dL)	Niveaux de départ (mmol/L)
EPA vs. DHA							
Egert et al, 2009	2.3	6 semaines	25 (24)	DHA		86.8	0.981
	2.2		25 (24)	EPA		82.4	0.931
Grimsgaard et al, 1997	4	7 semaines	72 (77)	DHA		109.8	1.241
	4		75 (77)	EPA		108.9	1.231
Mori et al, 2000	4	6 semaines	17 (20)	DHA		199.3	2.252
	4		19 (20)	EPA		178.0	2.011
Nestel et al, 2002	3	7 semaines	12 (14)	DHA		175.4	1.982
	3		12 (14)	EPA		139.1	1.572
Park & Harris, 2003	4	4 semaines	11 (11)	DHA		90.0	1.017
	4		11 (11)	EPA		74.0	0.836
Woodman et al, 2002	4	6 semaines	18 (16)	DHA		143.5	1.622
	4		17 (16)	EPA		118.7	1.341
EPA seul							
Ando et al, 1999	1.8	12 semaines	19 (19)	EPA		258.6	2.922
Kurabayashi et al, 2000	1.8	48 semaines	69 (72)	EPA		135.6	1.532
Satoh et al, 2007	1.8	12 semaines	22 (22)	EPA		170.9	1.931
Tanaka et al, 2008	1.8	5 ans	9326 (9319)	EPA		153.2	1.731
DHA seul							
Agreen et al, 1996	1.68	15 semaines	14 (14)	DHA		103.6	1.171
Conquer & Holub, 1996	1.62	6 semaines	12 (12)	DHA		85.0	0.961
Conquer & Holub, 1998	0.75	6 semaines	6 (6)	DHA		124.9	1.411
	1.5	6 semaines	7 (6)	DHA		92.1	1.041
Davidson et al, 1997	1.25	6 semaines	9 (8)	DHA		250.0	2.825
	2.5	6 semaines	9 (8)	DHA		296.0	3.345
Engler et al, 2004	1.2	6 semaines	20*	DHA		139.0	1.571
Geppert et al, 2006	0.94	8 semaines	53 (53)	DHA		95.7	1.081
Kelley et al, 2007	3	90 jours	17 (17)	DHA		247.1	2.792
Maki et al, 2005	1.52	6 semaines	27 (30)	DHA		178.8	2.020
Sanders et al, 2006	1.5	4 semaines	40 (39)	DHA		95.7	1.081
Stark & Holub, 2004	2.8	4 semaines	32 (32)	DHA		142.6	1.611
Theobald et al, 2004	0.7	12 semaines	38*	DHA		91.2	1.031
Wu et al, 2006	2.14	6 semaines	14 (13)	DHA		124.0	1.401



1.2.2 Acides gras polyinsaturés oméga-3 et contrôles glycémiques

Une méta-analyse récente publiée par Yanai *et al*. (2018), a conclu qu'il y a une grande hétérogénéité statistique dans les associations entre la consommation de poisson et d'AGPI n-3 et le risque de DBT2. Cette hétérogénéité serait principalement expliquée par des différences géographiques où certaines populations seraient plus répondantes, comme la population asiatique [90]. Aussi, plusieurs études épidémiologiques ont rapporté une prévalence plus faible d'intolérance au glucose et de DBT2 chez les populations consommant de grandes quantités de poisson [60, 91-94]. La **Figure 1.3** montre que plusieurs facteurs peuvent accroître le risque de DBT2 dont les profils d'alimentation, les variations génétiques ainsi que la nutrition paternelle, maternelle, *in utero* et périnatale.

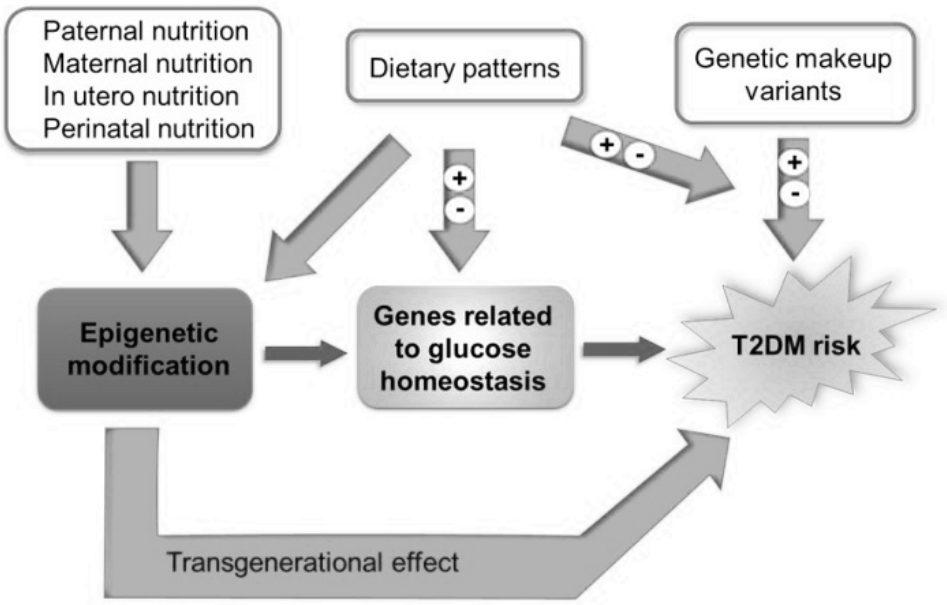


Figure 1.3 Schéma du rôle de la nutrition, des facteurs génétique et épigénétique, dans le développement du DBT2 (tirée de [95])

Une revue systématique publiée dans le *British Journal of Nutrition* en 2012 s'est penchée sur l'impact des AGPI n-3 sur les patients atteints du SMet, une accumulation de plusieurs facteurs de risque des MCV [96]. Outre l'effet bien documenté des AGPI n-3 sur la réduction des concentrations de TG, les chercheurs n'ont pas répertorié d'autres effets significatifs sur

les autres marqueurs du SMet, dont la glycémie [96]. Malgré une légère hausse des concentrations de glucose suite à une supplémentation en AGPI n-3, trois méta-analyses différentes ont conclu que l'utilisation de quantités modérées à élevées d'huile de poisson chez les diabétiques n'avait aucun effet délétère sur le contrôle de la glycémie [84, 97, 98].

Toutefois, les AGPI n-3 amélioreraient la sensibilité à l'insuline grâce à un certain nombre de mécanismes, notamment par la diminution des TG en circulation, augmentant ainsi la fluidité de la membrane et la transduction du signal. Les AGPI n-3 amélioreraient la sensibilité à l'insuline en stimulant GPR120, un récepteur couplé à la protéine G activé par ces derniers [90, 99]. En outre, l'activation de GPR120 exercerait des effets anti-inflammatoires et des effets bénéfiques sur l'homéostasie glucidique via un rôle dans la sécrétion et la sensibilité à l'insuline [99]. La sensibilité à l'insuline serait aussi influencée par le statut en AGPI n-3 et n-6 où un plus grand ratio d'AGPI n-6 diminuerait cette dernière [46, 100]. La sensibilité à l'insuline serait donc positivement associée à la proportion d'AGPI n-3 dans les membranes [101-103].

De plus, la substitution des graisses saturées par des graisses insaturées, telles que les AGPI n-3, pourrait avoir des effets bénéfiques sur la sensibilité à l'insuline [104]. Une revue de littérature sur les effets des AGPI n-3 sur le risque de DBT2 publiée par Nettleton et Katz (2005) a montré qu'une réduction des apports en graisses saturées pourrait réduire le risque de conversion d'intolérance au glucose vers le DBT2 chez une population présentant un embonpoint. Ces changements observés dans la qualité des lipides exerceraient aussi une myriade d'effets bénéfiques dont l'amélioration de l'hémostase, de l'albuminurie, de l'inflammation, du stress oxydatif et le ralentissement de la progression de l'artériosclérose [105].

1.2.3 Acides gras polyinsaturés oméga-3 et inflammation

Selon Calder (2018), une inflammation inappropriée, excessive ou incontrôlée contribue à un éventail de maladies, dont les MCV [106]. De ce fait, une multitude de types de cellules, de médiateurs chimiques et d'effets d'interactions sont impliqués. En outre, l'AEP et l'ADH sont capables d'inhiber en partie de nombreux aspects de l'inflammation, y compris la

chimiotaxie leucocytaire, l'adhésion des leucocytes aux cellules endothéliales, la production d'eicosanoïdes dérivés de l'acide arachidonique (ARA) (i.e. les prostaglandines (PG) de série 2 et les leucotriènes (LT) de série 4) et la production de cytokines inflammatoires, dont TNFA et IL-1β [106]. L'AEP et l'ADH agissent sur l'inflammation par l'intermédiaire de divers mécanismes présentés à la **Figure 1.4**.

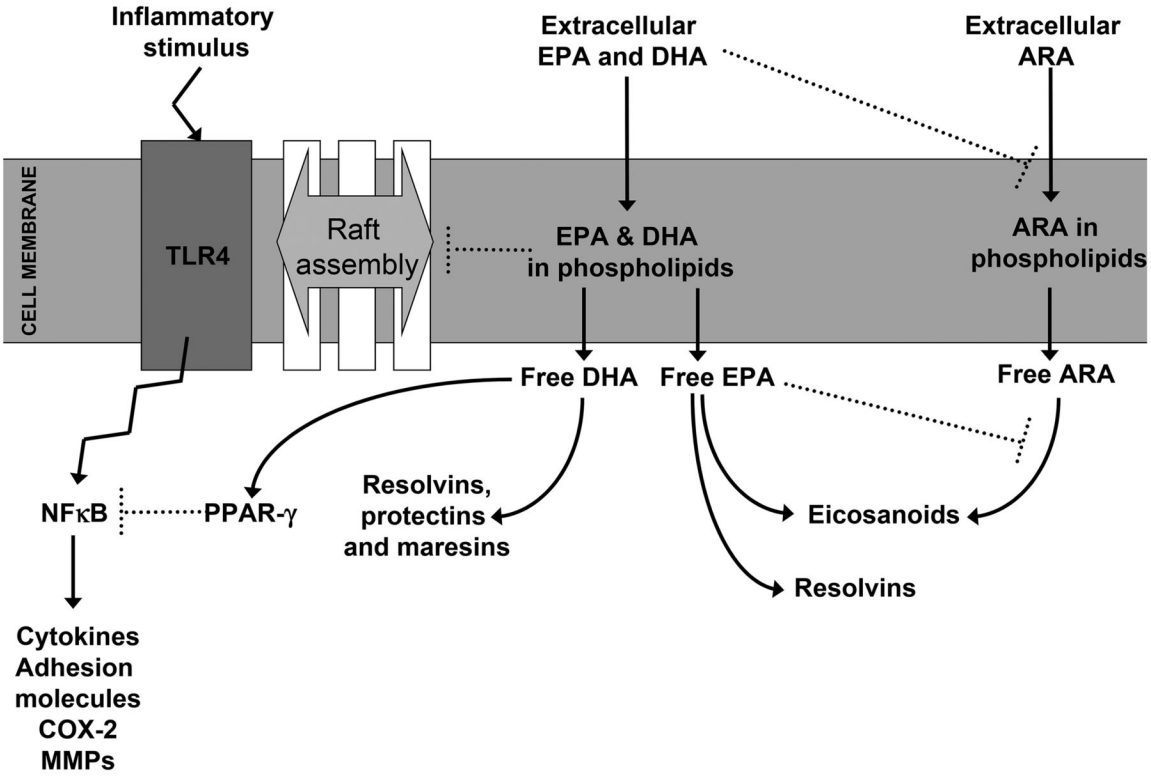


Figure 1.4 Schéma des principales actions anti-inflammatoires des AGPI n-3 (Tirée de Calder 2018 [106])

La composition en AGPI n-3 et n-6 des membranes cellulaires intervient dans l'inflammation [106]. En outre, la consommation d'AGPI n-3, via l'alimentation ou par une supplémentation en huile de poisson, augmente les proportions de ces AG dans les PPLs des cellules de l'inflammation [107-113]. L'incorporation de l'AEP et de l'ADH dans les cellules de l'inflammation se produit selon une relation dose-réponse et se fait principalement au détriment de l'ARA, tel que démontré sur la **Figure 1.4**. Comme moins de substrat est disponible pour la synthèse des eicosanoïdes dérivés de l'ARA, la production de PGE2 [109,

112, 114, 115], de thromboxanes B2 [112], LTB_4 [108, 110], d'acide 5-hydroxyeicosatétraénoïque [108, 110] et LTE_4 [116] est diminuée dans les cellules de l'inflammation. De surcroit, des changements dans la composition en AG des membranes cellulaires peuvent modifier la formation des radeaux lipidiques, le profil des médiateurs lipidiques produits ainsi que la signalisation cellulaire, entraînant une modification de l'expression de certains gènes.

Les évidences récentes indiquent que les AGPI n-3 et n-6 ainsi que tous leurs métabolites respectifs n'ont pas seulement des effets différents, mais parfois complètement opposés sur l'immunité et l'inflammation [47, 117, 118]. Globalement, les métabolites obtenus à partir des AGPI n-6 et plus particulièrement à partir de l'ARA, ont des effets délétères sur l'inflammation aigue et chronique alors que les médiateurs produits du côté des AGPI n-3 interviennent plutôt dans la résolution de l'inflammation [119, 120]. Toutefois, les lipoxines, des métabolites dérivés à partir de l'ARA, semblent exercer des effets anti-inflammatoires [121].

1.3 Génétique et acides gras oméga-3

L'émergence de la nutrition de précision, qui tient compte de la variabilité génétique individuelle et populationnelle, dans le contexte actuel des recherches en nutrition, promet de fournir des recommandations nutritionnelles plus spécifiques et individualisées permettant de prévenir l'apparition de plusieurs pathologies [122]. Selon Chilton *et al*. (2017), une meilleure compréhension de la contribution des variations génétiques des gènes impliqués dans la biosynthèse des AG à longue chaîne et de leurs métabolites aiderait au développement de protocoles de supplémentation en AGPI n-3 individualisés, tel qu'illustré à la **Figure 1.5** [122].

Jusqu'à tout récemment, on supposait que la capacité métabolique de la voie de biosynthèse des AGPI à longue chaîne était limitée et relativement uniforme dans la population. Cette prémisse était soutenue par des études dans les populations d'ascendance européenne, qui suggéraient que seulement une petite proportion d'AGPI, de l'ordre de 2 à 3 % des apports énergétiques totaux, était convertie en AGPI à longue chaîne [123-126]. Cependant, de nouvelles études ont démontré que plusieurs variations génétiques et épigénétiques dans les gènes *FADS1, FADS2, ELOVL5* et *ELOVL2* étaient fortement associées aux niveaux plasmatiques d'AGPI à longue chaîne dans les PPLs du plasma ou des globules rouges [127-139]. Une revue de littérature récente sur l'impact du génotype du groupe de gènes *FADS* sur le statut en AG et la santé cardiovasculaire a suggéré que les allèles mineurs de *FADS* sont associés à une diminution de l'inflammation et du risque cardiovasculaire et que les apports en gras totaux ou en certains AG pourraient modifier la relation entre plusieurs SNPs présents dans le groupe de gènes FADS et les niveaux d'AG circulants [140]. Toutefois, à ce jour, ni la taille d'effet ni le SNP fonctionnel dans *FADS1* ou *FADS2* n'ont été identifiés [140]. Ces informations pourraient contribuer à affiner et personnaliser les recommandations nutritionnelles en AEP et ADH où des apports plus élevés en AGPI n-3 pourraient être recommandés chez les porteurs de certains allèles mineurs de *FADS* [141].

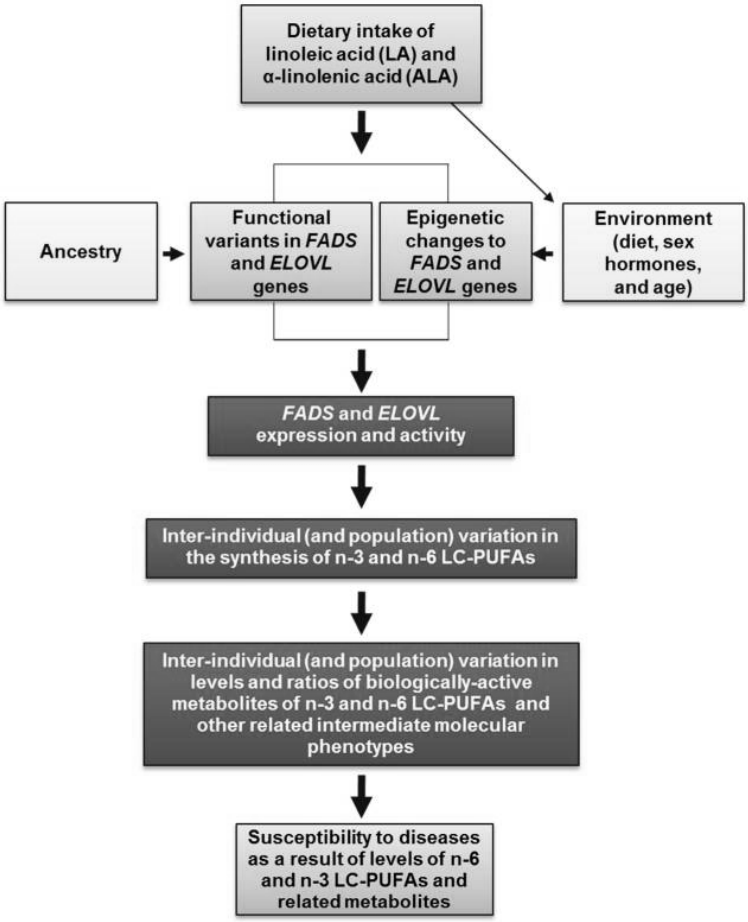


Figure 1.5. Effets d'interactions gène-diète dans la biosynthèse des AGPI à longue chaîne (tirée de [122])

1.3.1 Variabilité interindividuelle

La variabilité interindividuelle est un concept qui permet d'expliquer l'obtention de résultats inconstants concernant la relation entre des composantes spécifiques de l'alimentation et le risque de souffrir d'une maladie. Bien que l'effet de modifications alimentaires sur le profil lipidique semble bénéfique en général, l'amplitude des changements varie d'un individu à l'autre. Ainsi, certaines personnes retirent un bénéfice plus important que d'autres lorsqu'elles modifient leur alimentation. Tel que le montre la **Figure 1.6**, certains individus seront considérés comme étant hyper-répondeurs ou hypo-répondeurs à la supplémentation en AGPI n-3 sur la base de la variation relative des concentrations de TG plasmatiques dans le temps où une hyper-réponse représente une diminution des concentrations de TG plasmatiques suite à la supplémentation.

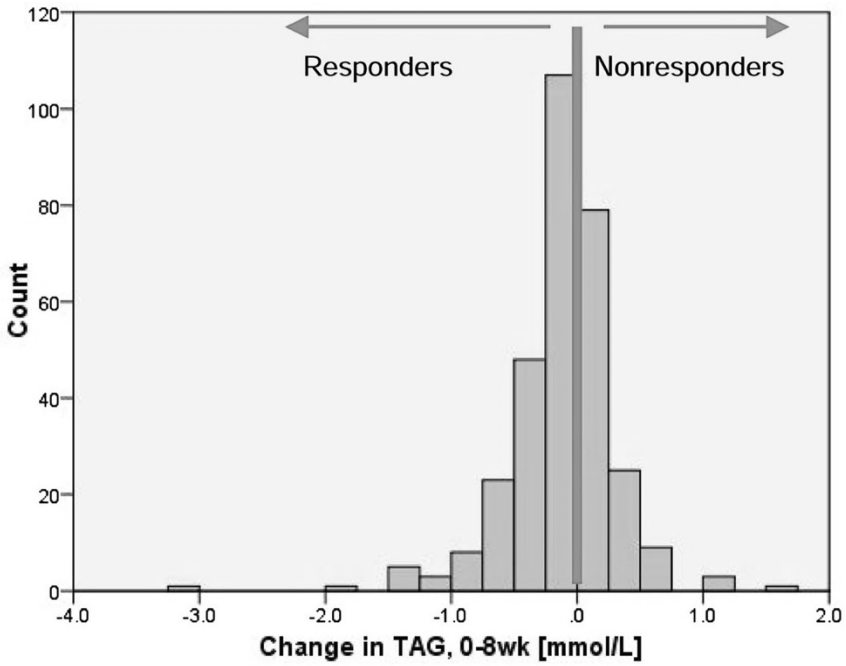


Figure 1.6 Réponse interindividuelle des niveaux de TG suite à une supplémentation de 1,8g d'AEP + ADH chez 312 individus sains pendant 8 semaines (Tirée de [142])

Cette variabilité interindividuelle pourrait avoir un impact significatif sur le succès des politiques de santé publique et des interventions nutritionnelles [143]. Il est reconnu que la consommation d'AGPI n-3 abaisse les concentrations plasmatiques de TG tel que décrit précédemment. Toutefois, certains individus ne répondent pas tous physiologiquement de manière identique à une supplémentation en AGPI n-3, ce qui peut être, en partie, expliqué par la présence de facteurs génétiques. Des travaux de notre laboratoire ont montré qu'un score de risque génétique construit selon un modèle additif incluant 13 allèles à risque avec des fréquences alléliques significativement différentes entre les hypo- et les hyper-répondeurs à la supplémentation permettait d'expliquer 21,5% de la variation observée dans la réponse plasmatique des TG suite à une supplémentation en AGPI n-3 après des ajustements pour plusieurs facteurs confondants tels que le sexe, l'âge et l'IMC [144].

Plusieurs gènes tels que *FADS1-2-3* ont été étudiés afin de mieux comprendre les causes génétiques impliquées dans le métabolisme et le statut en AGPI n-3 [20, 141].

1.3.1.1 Le groupe de gènes *FADS*

L'ensemble de gènes *FADS* comprend 3 gènes (*fatty acid desaturase 1* (*FADS1*); *fatty acid desaturase 2* (*FADS2*)*;* et *fatty acid desaturase 3* (*FADS3*)) situés sur le chromosome 11q12.2. Ces trois gènes sont souvent étudiés comme étant une seule entité en raison du fort déséquilibre de liaison (LD) entre les SNPs présents sur ces gènes, sur leurs régions promotrices et même dans les régions situées entre les gènes [137]. Ces gènes codent pour deux désaturases, la D5D et la D6D, deux enzymes qui introduisent une double liaison carbone-carbone par déshydrogénation dans la chaîne carbone d'un AG. Ces enzymes permettent alors la biosynthèse des AGPI n-3, n-6 et oméga-9 (n-9) en association avec des élongases qui permettent l'allongement de la chaîne carbonée.

Dans deux voies parallèles et concurrentes, les produits enzymatiques de ces gènes convertissent les AGPI n-6 ou n-3 à 18 carbones (abondants dans la diète occidentale) en AGPI à longue chaîne. Du côté de la voie des AGPI n-6, l'ARA est synthétisé à partir du LA en utilisant trois étapes enzymatiques : deux étapes de désaturation et une étape d'élongation

[53]. Les AGPI n-3 primaires, dont l'AEP, l'ADH et le DPA peuvent être synthétisés à partir de l'ALA. Cependant, il faut compter sept étapes enzymatiques : trois étapes de désaturation, trois étapes d'élongation et une étape de β-oxydation pour arriver à synthétiser l'ADH. Il est important de noter que les substrats des AGPI n-6 et n-3 se disputent les premières étapes enzymatiques de la voie. Les étapes de désaturation ont longtemps été reconnues comme étant les étapes limitant la vitesse dans cette voie. Comme le montre la **Figure 1.7**, l'étape de désaturation initiale convertit le LA et l'ALA en acide γ-linolénique (GLA) et en acide stéaridonique, respectivement, via une désaturase codée par le gène *FADS2* situé sur le chromosome 11 (11q12.2) [53, 145, 146]. La seconde étape de désaturation est catalysée par une enzyme codée par une autre désaturase encodée par le gène *FADS1*, sur le chromosome 11q12.2-q13.1) [146]. Cette étape convertit l'acide dihomo-γ-linolénique (DGLA) et l'acide eicosatétraénoïque en ARA et en AEP, respectivement [147].

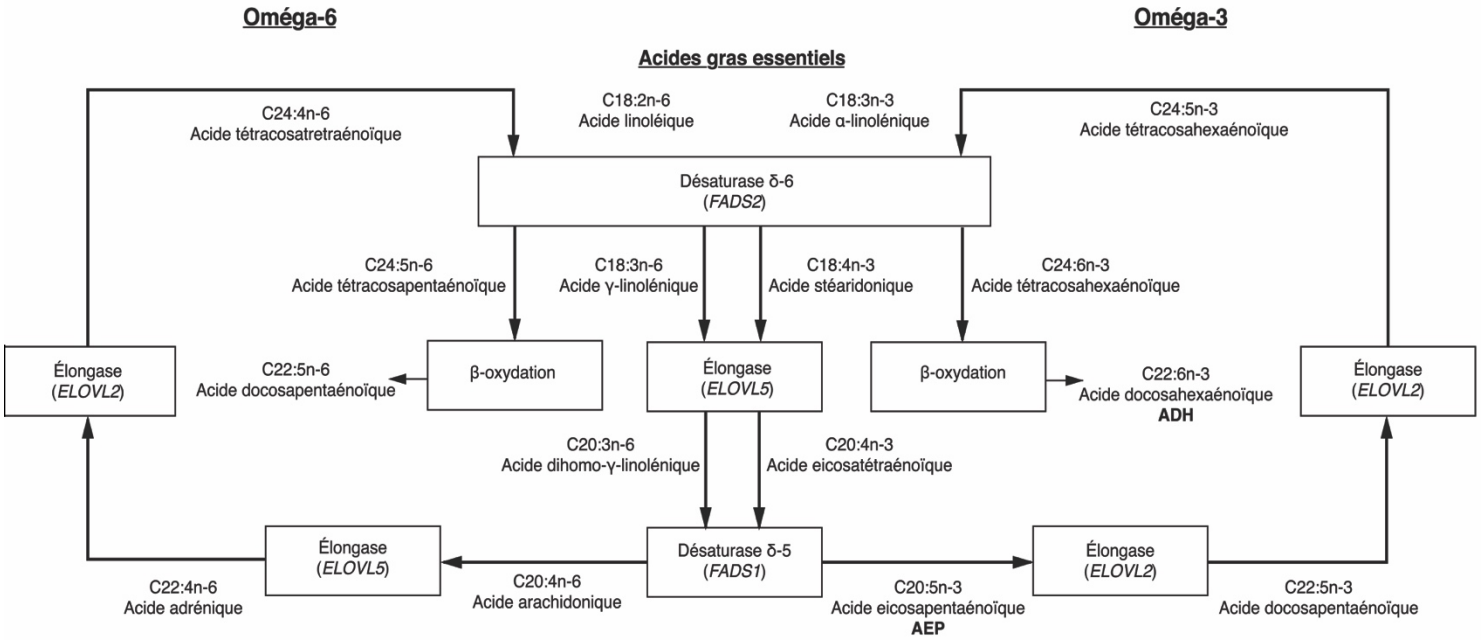


Figure 1.7 Synthèse des AGPI n-3 et n-6 chez les humains.

Suite à la première étude sur l'ensemble de gènes *FADS* publiée par Schaeffer et al., (2006) qui a montré des associations entre les SNPs de *FADS1* et *FADS2* et la composition en AG des PPLs [127], plusieurs autres études ont confirmé ces associations non pas seulement sur les niveaux d'AG seuls, mais également avec des rapports produits sur précurseurs tel que

ARA:DGLA permettant ainsi de mesurer l'indice d'activité enzymatique des désaturases [128, 130, 132-135, 137, 139, 148-151]. De plus, des études pangénomiques (GWAS) ont permis d'identifier des SNPs associés au risque de MCV, de DBT2 et de cancer dont certains impliquent des SNPs présents dans le groupe de gènes *FADS* [152, 153]. D'autres GWAS ont observé des associations entre plusieurs SNPs du groupe de gènes *FADS* et une grande variété de phénotypes moléculaires et cliniques. Par exemple, les SNPs rs174537 et rs174547 étaient associées aux niveaux d'ARA et d'AEP dans plusieurs populations, dont les populations italienne, européenne et chinoise [131, 138, 154, 155].

Les SNPs du groupe de gènes *FADS* sont également associés à de nombreux autres phénotypes incluant les MCV, les taux de lipides sanguins (y compris les taux de cholestérol-LDL, cholestérol total et de TG), les maladies coronariennes, la résistance à l'insuline, la dépression périnatale, les maladies atopiques, les troubles d'attention/d'hyperactivité ainsi qu'à l'intelligence et la mémoire chez les enfants [128, 129, 133, 136, 149, 151, 156-163].

1.3.1.2 Le groupe de gènes *ELOVL*

Fatty acid elongase 2 (*ELOVL2*) et *fatty acid elongase 5* (*ELOVL5*) sont des gènes codant pour des élongases impliquées dans la biosynthèse des AG à longue chaîne. Les élongases catalysent la première réaction et la réaction limitante des quatre qui constituent le cycle d'élongation des AG à longue chaîne. Ce processus enzymatique permet l'ajout de deux molécules de carbone par cycle d'élongation à la chaîne des AG à longue et très longue chaîne. Les AG ainsi produits sont impliqués dans plusieurs processus biologiques en tant que précurseurs de lipides membranaires et de médiateurs. Une étude a montré que l'élongase-5 encodée par *ELOVL5* jouait un rôle dans la régulation des lipides et dans le métabolisme des glucides [164].

1.3.1.3 Les gènes de l'inflammation

Les AGPI n-3 exercent des effets bénéfiques sur plusieurs maladies inflammatoires telles que l'arthrite rhumatoïde, le syndrome du côlon irritable et l'asthme [106, 165]. Des études ont montré que les AGPI n-3 d'origine marine permettent de diminuer les niveaux d'expression de certains gènes de l'inflammation en plus de diminuer les concentrations de plusieurs cytokines pro-inflammatoires et de la protéine C-réactive (CRP) [109, 112, 114, 166-170]. Dans le cadre du présent doctorat, quatre gènes de l'inflammation ont été étudiés. *Lymphotoxin Alpha* (*LTA*), aussi connu sous le nom *Tumor Necrosis Factor Ligand Superfamily Member 1*, code pour une protéine membre de la famille des facteurs de nécrose tumorale qui forme un hétérotrimère avec la lymphotoxine-bêta permettant d'ancrer la lymphotoxine-alpha à la surface des cellules. La protéine intervient dans une grande variété de réponses inflammatoires et les SNPs présents sur le gène *LTA* sont notamment associés au risque d'infarctus du myocarde [171].

Tumor necrosis factor (*TNF*) code pour une cytokine pro-inflammatoire (TNFA) principalement sécrétée par les macrophages. Cette cytokine est impliquée dans un large spectre de processus biologiques comprenant notamment la prolifération cellulaire, l'apoptose, le métabolisme des lipides et la coagulation en plus d'être impliquée dans diverses maladies y compris certaines maladies auto-immunes, la résistance à l'insuline et le cancer. Selon Grimble *et al*. (2002), la capacité de l'huile de poisson à diminuer la production de TNFA par les cellules mononucléées du sang périphérique (PBMCs) est influencée par la production inhérente de TNFA et par des SNPs dans les gènes *TNF* et *LTA* [172].

Interleukin 6 (*IL6*) code pour une cytokine inflammatoire principalement produite sur les sites d'inflammation aigue et chronique. *IL6* serait impliqué dans plusieurs pathologies associées à l'inflammation, dont l'arthrite rhumatoïde et le DBT2 [106].

Interleukin 1 beta (*Il1B*) code pour une cytokine qui est un important médiateur de la réponse inflammatoire en contribuant à l'hypersensibilité à la douleur [106].

1.4 Perceptions de la génomique nutritionnelle par les professionnels de la santé

Avec les avancées technologiques récentes des sciences omiques (nutrigénomique, métabolomique, protéomique, transcriptomique, etc.) et suite à l'accessibilité et à la baisse des prix des tests génétiques, la popularité des tests de nutrigénétique est appelée à croître au cours de la prochaine décennie. Selon une étude longitudinale publiée en 2016 auprès de 1026 répondants ayant discuté des résultats issus de tests génétiques avec un professionnel de la santé, 22% des participants ont noté un manque d'engagement ou d'intérêt de la part du professionnel suite à la divulgation de leurs résultats génétiques en raison d'une remise en question de l'efficacité de tels tests [94]. À mesure que les tests génétiques deviendront plus largement accessibles, les professionnels de la santé devront apprendre à s'engager plus activement auprès des clients/patients à propos de leurs résultats [94]. De plus, 61% des répondants à un sondage portant sur les tests génétiques estimaient que les professionnels de la santé, dont les médecins, ont l'obligation professionnelle d'aider à interpréter les résultats [173]. Bien que les professionnels de la santé soient intéressés à incorporer le profil génomique dans les soins dispensés aux clients/patients [174], plusieurs croient qu'ils ne sont pas suffisamment préparés à y faire face [175]. De plus, selon Weir *et al*., les professionnels de la santé au Canada sentent qu'ils manquent de compétences afin de transmettre ce savoir à leurs clients/patients en plus d'être sceptiques envers l'applicabilité immédiate de la génomique nutritionnelle dans l'état des connaissances actuelles [30].

1.4.1 La place des diététistes dans l'implémentation de la nutrigénomique dans la pratique professionnelle

L'application de la génomique nutritionnelle dans la pratique professionnelle des diététistes est récente. L'*Academy of Nutrition and Dietetics*, la plus grande organisation regroupant des professionnels de la nutrition au monde, ne considère pas encore cette science comme étant prête à être intégrée dans le cadre de la pratique courante des diététistes [176]. Cette position freine l'application de la nutrigénomique qui tend à être faible auprès de ses membres [177, 178]. Pourtant, l'intérêt des consommateurs pour la nutrigénétique est élevée et la demande pour des professionnels de la santé qualifiés est appelée à croître [179]. Au Québec, l'OPDQ et le Collège des médecins du Québec (CMQ) ont publié en mai 2016 leurs prises de position

sur l'utilisation de la nutrigénomique et de ses tests par les professionnels de la santé. Dans ce document, on stipule qu'à ce stade-ci de l'évolution de cette science, le recours à la nutrigénomique est plus utile et pertinent dans le cadre de la recherche, car l'application de cette science dans un contexte clinique ne peut conduire à la détermination d'un plan de traitement à valeur ajoutée, faute de données probantes et de lignes directrices [180].

La théorie de la diffusion postule que l'attitude face à l'acceptation des nouvelles technologies est le résultat d'un compromis entre les avantages et les risques perçus [181]. Lorsqu'un risque est perçu, particulièrement lorsque les connaissances sont limitées, des incertitudes quant aux avantages de l'utilisation d'une nouvelle technologie telle que la nutrigénomique peuvent être générées [181]. Toutefois, la pré-adoption d'une nouvelle technologie chez les professionnels de la santé est augmentée si l'innovation est perçue comme à faible risque [182, 183]. La nutrigénomique est perçue comme une science à faible risque puisqu'elle est entièrement basée sur des évidences scientifiques et cible la promotion de la santé plutôt que la réduction du risque de maladies.

Actuellement, les diététistes sentent que la nutrigénomique n'est pas reconnue par les communautés scientifique et médicale, qui demeurent plus conservatrices, les rendant ainsi insensibles à l'émergence des avancées scientifiques en nutrition [184]. De plus, des différences d'opinion et de tolérance du risque sont rapportées entre les diététistes pratiquant dans le secteur public et le secteur privé, ce qui limite l'application de la nutrigénomique. Cela implique une certaine dissociation entre les adopteurs précoces de la nutrigénomique de celle de l'ensemble de la profession [184].

Les diététistes seront les professionnels de la santé les plus pressentis pour mettre en pratique la science de la nutrigénomique [184-187]. Selon une étude récente, la réticence à intégrer la nutrigénétique dans la pratique professionnelle des diététistes est associée à une faible connaissance de cette science, à un manque de confiance en la science elle-même et au scepticisme envers les tests de nutrigénétique [184]. Les facteurs favorables à une application efficace et réussie de la nutrigénétique dans le champ de pratique des diététistes découlent d'une meilleure connaissance de la nutrigénomique, d'une confiance et d'une attitude

positive en cette science, d'une accessibilité aux produits tels que les tests de nutrigénétique, d'un milieu de travail positif et stimulant et de travailler dans le milieu clinique plutôt que dans le milieu de la santé publique [184]. Les diététistes du milieu de la pratique privée sont plus enclins à adopter la nutrigénomique dans leur pratique professionnelle que les diététistes qui travaillent dans le secteur public [184]. Les diététistes du secteur privé démontrent un intérêt plus élevé envers la nutrigénétique de par leur intérêt financier afin d'offrir un nouveau service à leur clientèle, leur intérêt face à l'implémentation de nouvelles technologies, leur amour pour cette science et leur désir d'ajouter une plus-value à leur pratique actuelle [184, 188, 189]. Une étude qualitative auprès de 12 diététistes qui proposent déjà le service à leur clientèle a montré que l'application de la nutrigénétique dans leur pratique professionnelle est empreint de positivisme et que les clients sont motivés, excités et ont une observance plus élevées quant aux recommandations nutritionnelles personnalisées qui découlent de ces tests [190].

Selon Wright (2014), l'enseignement de la génomique nutritionnelle par des professionnels qualifiés dans le cadre des études de premier cycle en nutrition ainsi que pendant les stages cliniques est décisif [191]. Les stratégies d'enseignement optimales comprendraient des méthodes d'exposition répétées à la nutrigénomique, la résolution de problèmes, l'apprentissage collaboratif et l'apprentissage basé sur des études de cas [191].

1.4.2 Les tests de nutrigénétique

Depuis plusieurs années, le public peut se procurer des tests de nutrigénétique en ligne appelés *DTC genetic testing* (tests offerts directement aux consommateurs) ou via un professionnel de la santé. Les tests de nutrigénétique peuvent fournir de l'information reliant la diète à la santé, aux habitudes de vie, aux facteurs anthropométriques ou encore à certaines données en lien avec l'activité physique [192, 193]. Des changements au niveau de l'alimentation, aussi mineurs soient-ils, peuvent exercer un impact sur l'état de santé général. Ainsi, des recommandations nutritionnelles sur mesure tenant compte du génotype et non seulement du phénotype, ouvrent la voie à une amélioration des habitudes alimentaires. En effet, 87 % des participants d'un sondage ont admis qu'ils tireraient profit d'une meilleure compréhension de la façon dont leur patrimoine génétique interagit avec leur alimentation

[194]. En accord avec l'énoncé précédent, 75 % affirment que le fait d'en connaître plus sur leur génétique pourrait avoir des répercussions sur leurs choix alimentaires [194]. Ces tests pavent la voie vers l'arrivée de plus en plus probable de la génétique dans le secteur public afin de dépister non seulement les maladies monogéniques, mais également les maladies polygéniques complexes (où plusieurs gènes seraient en cause) telles que l'obésité, le cancer ou les MCV.

Pour le moment, les tests de nutrigénétique présentent une faible valeur prédictive du risque [195]. Selon Janssens *et al*., les preuves scientifiques pour la plupart des associations entre les SNPs et le risque de maladie sont insuffisantes pour supporter le développement d'applications utiles [196]. En dépit de leur faible valeur prédictive, les individus sont quand même prêts à faire des changements dans leur alimentation comme le montrent les statistiques susmentionnées. Une étude publiée par Nielsen et El-Sohemy a démontré que les participants qui recevaient les résultats personnalisés suite à l'analyse de leur génome (groupe intervention) considéraient que les recommandations alimentaires leur seraient utiles dans une plus grande proportion que le groupe ayant reçu les recommandations nutritionnelles générales (88 % contre 72 % respectivement, $p = 0,02$) et que seulement 9 % des individus dans le groupe intervention se sentaient mal à l'aise avec l'information révélée à l'issu de leur test génétique [194]. Ces résultats suggèrent que les recommandations nutritionnelles personnalisées basées sur le génotype pourraient possiblement être mieux comprises et plus utiles que les recommandations nutritionnelles générales [194]. Par ailleurs, une autre étude démontre que les individus à risque de contracter une maladie X durant leur vie étaient plus sensibles à se renseigner sur cette dernière, à discuter des résultats avec un professionnel de la santé, à changer leur diète et à exercer une activité physique [197].

Toutefois, les résultats de ces tests peuvent alors engendrer de la confusion auprès de leurs utilisateurs. Hesketh a démontré que le public aurait de la difficulté à comprendre la complexité de l'influence de la diète et de la génétique sur son état de santé lorsqu'il est confronté aux résultats de tests personnalisés en nutrigénétique [198]. En fait, il va de l'exemple suivant : La compagnie X m'a dit que mon gène « A » augmenterait mes chances

de souffrir d'une maladie du cœur à moins de prendre un supplément de folate. Cependant, trop de folate pourrait alors affecter mes chances de développer un cancer. D'un autre côté, mon gène « B » me protègerait contre les MCV, à l'unique condition que je mange moins de gras polyinsaturés.

Le modèle actuel de transmission des données qui implique les diététistes est celui du *DTC-genetic testing through healthcare providers* (tests génétiques offerts directement au consommateur via un professionnel de la santé tel un nutritionniste ou un médecin) où le diététiste explique les résultats génétiques en plus de fournir des recommandations nutritionnelles. Il s'agit donc d'une forme améliorée des tests génétiques offerts directement au consommateur puisque les résultats sont acheminés directement à un professionnel de la santé qui, par la suite, rencontrera le client dans le but de faire du counseling nutritionnel. Or, selon Abrahams *et al*., le modèle pourrait être appelé à changer afin d'impliquer une équipe pluridisciplinaire où la première étape consisterait en un counseling génétique en utilisant une approche de nutrition de précision suivie d'un counseling en nutrition principalement axé sur les changements de comportement alimentaire [184].

Selon la position actuelle de l'OPDQ, la population québécoise doit être mieux informée afin que le public ayant accès à cette technologie puisse donner un consentement libre et éclairé [180].

Chapitre 2
Hypothèses et objectifs

Les études en nutrition sont complexes puisque plusieurs facteurs peuvent intervenir et ainsi influencer les résultats. D'une part, des facteurs environnementaux – dont l'environnement alimentaire, les habitudes de vie, la pratique d'activité physique, les profils d'alimentation, etc. – peuvent moduler les résultats attendus. D'autre part, la génétique d'un individu est également à considérer puisque la présence de variations génétiques peut exercer un effet seul ou en interaction avec l'environnement affectant la réponse métabolique à un traitement nutritionnel donné. Conséquemment, des études en nutrition observant l'effet de telles variations génétiques sur plusieurs biomarqueurs du risque métabolique pourraient permettre de mieux comprendre la place de la génomique nutritionnelle dans l'enchevêtrement des facteurs – environnementaux et non environnementaux – qui permettent d'expliquer une partie de la grande variabilité interindividuelle de la réponse observée. La recherche en génomique nutritionnelle permet de mieux comprendre la relation complexe entre la santé d'un individu, son génome et l'alimentation avec le potentiel d'implémenter des recommandations pour la prévention des MCV via une meilleure nutrition.

Le principal effet documenté d'une supplémentation en huile de poisson concerne la baisse significative des niveaux de TG. Cet effet hypotriglycéridémiant n'est toutefois pas le même chez tous les individus et varie considérablement d'un individu à l'autre. Des effets d'interaction complexes entre des facteurs génétiques impliqués dans la voie de synthèse des n-3 pourraient expliquer une partie de la variabilité interindividuelle dans la réponse des niveaux de TG suite à une supplémentation en AGPI n-3. Les gènes candidats ont été sélectionnés pour leur implication dans cette voie métabolique où ils codent pour des enzymes régulatrices – des désaturases et des élongases – qui sont considérées comme les enzymes limitantes de la voie de synthèse des AGPI n-3 et n-6. De plus, comme l'AEP et l'ADH sont reconnus pour leurs effets anti-inflammatoires et pour leur action concertée dans la résolution de l'inflammation, des gènes de l'inflammation ont également été étudiés dans le cadre de ce projet de doctorat.

La recherche scientifique en génomique nutritionnelle est importante et ne cesse de croître d'année en année poussant les consommateurs à s'y intéresser via la présence sur le marché de tests de nutrigénétique. Les diététistes sont les professionnels de la santé les mieux

qualifiés pour fournir de l'information sur la nutrition à leurs patients/clients. Or, la demande croissante de la part des consommateurs pour des tests de nutrigénétique place les diététistes dans une position délicate où ces professionnels de la santé seront de plus en plus sollicités et consultés pour accompagner leurs patients/clients dans leurs démarches. De surcroit, les diététistes ne détiennent pas, pour la plupart, les bases afin d'intégrer plusieurs aspects de cette science à leur pratique professionnelle.

Conséquemment, les objectifs généraux de ce projet de doctorat sont de vérifier si la variabilité de la réponse des facteurs du risque métabolique induite par une supplémentation en AGPI n-3 est influencée par des variations génétiques et de dresser un portrait global de la situation de la nutrigénomique au Québec du point de vue des diététistes membres de l'OPDQ.

Afin d'atteindre ces objectifs, plusieurs objectifs spécifiques ont été déterminés et poursuivis à l'aide de deux études différentes. Premièrement, l'étude FAS au cours de laquelle j'ai participé autant au volet clinique en rencontrant les participants et en administrant les questionnaires de fréquence alimentaire en tant que diététiste, qu'aux analyses de laboratoires et à l'analyse de données. Les résultats présentés aux chapitres 3, 4, 5, 6 et 7 sont issus de cette étude. Deuxièmement, les résultats issus de l'étude effectuée auprès d'un échantillon de diététistes membres de l'OPDQ et pour laquelle j'ai participé à la majorité des étapes allant de la révision du sondage électronique, à l'analyse des résultats ainsi qu'à l'écriture de l'article, sont présentés au chapitre 8.

Les objectifs spécifiques étaient les suivants :

Objectif spécifique #1 : Étudier les associations entre les SNPs présents dans les gènes *FADS1-2-3* et *ELOVL2-5* et l'indice d'activité des désaturases et des élongases.

Hypothèse :

1. L'indice d'activité des désaturases et des élongases est modulé par les SNPs présents dans les gènes *FADS1-2-3* et *ELOVL2-5* suite à une supplémentation en AGPI n-3 d'une durée de 6 semaines.

Objectif spécifique #2 : Étudier les associations entre les SNPs présents dans les gènes *FADS1-2-3* et les niveaux d'AG et les profils d'AG dérivés d'une analyse factorielle avant et après une supplémentation en AGPI n-3 d'une durée de 6 semaines.

Hypothèses :

1. Les niveaux plasmatiques de TG sont modulés par les niveaux plasmatiques d'AG seuls et par les profils d'AG dérivés d'une analyse factorielle suite à une supplémentation en AGPI n-3 d'une durée de 6 semaines.
2. La variabilité génétique des gènes *FADS1-2* explique une partie de la variabilité interindividuelle observée dans les niveaux plasmatiques d'AG seuls et les profils d'AG dérivés d'une analyse factorielle à l'étude avant et après une supplémentation en AGPI n-3 d'une durée de 6 semaines.

Objectif spécifique #3 : Étudier les associations entre les SNPs présents dans les gènes *FADS1-2-3* et *ELOVL2-5* encodant pour des enzymes impliqués dans la voie de synthèse des AGPI n-3 et la réponse des contrôles glycémiques suite à une supplémentation en huile de poisson.

Hypothèse :

1. La variabilité génétique des gènes *FADS1-2-3* et *ELOVL2-5* explique une partie de la variabilité interindividuelle observée dans les concentrations des contrôles glycémiques à l'étude suite à une supplémentation en huile de poisson.

Objectif spécifique #4 : Étudier les associations entre les SNPs présents dans les gènes de l'inflammation *LTA-TNF, IL6, IL-1B et CRP* et la réponse des niveaux plasmatiques des

principaux biomarqueurs de l'inflammation (i.e. CRP, TNFA et IL6) suite à une supplémentation en huile de poisson d'une durée de 6 semaines.

Hypothèses :

1. L'expression des gènes *LTA-TNF* et *IL6* est diminuée suite à une supplémentation en huile de poisson d'une durée de 6 semaines.
2. La variabilité génétique des gènes *LTA-TNF, IL6, IL-1B et CRP* explique une partie de la variabilité interindividuelle observée dans les niveaux plasmatiques des principaux biomarqueurs de l'inflammation (i.e. CRP, TNFA et IL6) suite à une supplémentation en huile de poisson d'une durée de 6 semaines.

Objectif spécifique #5 : Étudier les associations entre les SNPs du groupe de gènes *FADS1-2-3* et *ELOVL2-5* et l'indice d'activité des désaturases et des élongases suite à une supplémentation en AGPI n-3 d'une durée de 6 semaines.

Hypothèse :

1. La variabilité génétique des gènes *FADS1-2-3* et *ELOVL2-5* explique une partie de la variabilité interindividuelle observée dans les indices d'activité des désaturases et des élongases suite à une supplémentation en AGPI n-3 d'une durée de 6 semaines.

Objectif spécifique #6 : Étudier l'état des connaissances actuelles en nutrigénomique auprès d'une population de diététistes membres de l'OPDQ

Hypothèses :

1. Les diététistes membres de l'OPDQ ne sont pas convenablement outillés en matière de nutrigénomique.
2. Les diététistes membres de l'OPDQ sont capables d'identifier les limites perçues des tests de nutrigénétique.

Chapitre 3

Changements dans les profils des acides gras des phospholipides du plasma et leur impact sur les niveaux de triglycérides plasmatiques suite à une supplémentation en huile de poisson

Hubert Cormier, Iwona Rudkowska, Simone Lemieux, Patrick Couture, Pierre Julien et Marie-Claude Vohl

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Changes in Plasma Phospholipid Fatty Acid Patterns and their Impact on Plasma Triglyceride Levels Following Fish Oil Supplementation

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Résumé

L'objectif de la présente étude était de tester les associations entre les changements dans les profils des AG des PPLs du plasma et les changements dans les niveaux de TG plasmatiques suite à une supplémentation en AGPI n-3 et de vérifier si les SNPs du groupe de gènes *FADS1-2-3* étaient associés aux niveaux plasmatiques d'AG (seuls ou combinés en facteurs dérivés par analyse en composantes principales). Un total de 210 participants a complété une période de pré-inclusion de 2 semaines suivie d'une période de supplémentation de 6 semaines avec 5 g/jour d'huile de poisson. Des profils d'AG des PPLs ont été obtenus et 19 SNPs du groupe de gènes *FADS1-2-3* ont été génotypés. Une analyse en composantes principales a été effectuée et les scores ont été calculés. Une augmentation des niveaux d'AEP, d'ADP et d'ADH dans les PPLs du plasma a été observée, ainsi qu'une diminution des niveaux d'ALA et de tous les niveaux d'AGPI n-6 suite à la supplémentation. Quatre profils d'AG des PPLs du plasma ont été identifiés post-supplémentation. Les changements dans les quantités absolues d'ARA, d'ALA, de DGLA, ainsi que les changements dans les quantités absolues des n-3 totaux et des n-6 totaux étaient tous associés à un changement des niveaux de TG tandis que la corrélation ne restait significative seulement que pour l'ARA et le DGLA lorsque les AG étaient exprimés en pourcentage du total des AG. Plusieurs SNPs du groupe de gènes *FADS1-2-3* ont été associés à des niveaux d'AG post-supplémentation. Ces résultats suggèrent que les AG seuls ou regroupés en facteurs pourraient moduler les niveaux plasmatiques de TG après la supplémentation en huile de poisson. De plus, les SNPs du groupe de gènes *FADS1-2-3* interagissent avec les AG et/ou les facteurs pour moduler les niveaux de TG.

Abstract

The objective of the present study was to test for associations between changes in fatty acids (FAs) and changes in plasma TG levels after an n-3 FAs supplementation and to test whether SNPs from the *FADS* gene cluster were associated with plasma FA levels or with specific FA patterns. A total of 210 subjects completed a 2-wk run-in period followed by 6-wk supplementation with 5g/d of fish oil. FA profiles of plasma PPLs were obtained and 19 SNPs from the *FADS* gene cluster were genotyped. Principal component analysis was conducted and scores were calculated. There was an increase in EPA, DPA and DHA levels in PPLs as well as a decrease in ALA and all n-6 FA levels after the supplementation. Factor analysis suggested 4 post-n-3 FA supplementation patterns. Changes in ARA, ALA, DGLA, as well as changes in total n-3 and omega-6 FAs in absolute quantities of FAs were all associated with a change in TG levels whereas the correlation remained significant only for ARA and DGLA when FAs were expressed as percentage of total FAs. Several SNPs from the *FADS* gene cluster were associated with post-supplementation FA levels. These results suggest that FAs alone or regrouped in factors could play a role in modulating plasma TG levels after fish oil supplementation. SNPs from the *FADS* gene cluster interact with both FAs and/or factors to modulate TG levels.

Introduction

Low levels of long-chain (LC) omega-3 (n-3) fatty acids (FA) in tissues are a marker of increased risk for coronary heart disease [1]. Though, results from a recent meta-analysis of the role of n-3 FA supplementation on major cardiovascular outcomes did not support a causal link between n-3 FA intakes and low risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke based on relative and absolute measures of association [2]. Results from this meta-analysis remain controversial, as the mean doses of n-3 FAs used in several randomized clinical trials included in the analysis were lower than the doses of 2-4 grams/day of n-3 FAs recommended by the AHA for patients with hypertriglyceridemia [3].

Incorporation of n-3 FAs into transport, functional and storage pools occur in a dose- and time-dependent manner [4]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) influence the physical nature of cell membranes, thus directly impacting on membrane protein-mediated responses, lipid mediators, cell signaling, and gene expression [5]. Moreover, an increased intake of polyunsaturated FAs of the n-3 series, in particular EPA and DHA, may have a favorable effect on serum lipids. These n-3 FAs act both directly by substituting ARA in cell membranes and by inhibiting ARA metabolism [6]. The inhibition of ARA metabolism favors the incorporation of n-3 FAs into cell membranes.

Any modifications in dietary fat intake affect the composition of cell membranes. Thus, principal component analysis (PCA) may be useful to show which variables are correlated with one another, possibly measuring the same construct. PCA allows reducing the number of observed variables, in the present study plasma phospholipid (PPL) FAs, into a smaller number of components that will account for most of the variance in comparison to one measured by FA itself. In the present study, PCA were made prior to- and after the n-3 FA supplementation to see if the supplementation itself may affect correlations between FAs.

Recent studies suggested that plasma n−6 and n−3 FA levels are associated with several common single nucleotide polymorphisms (SNPs) of the *fatty acid desaturase (FADS)* gene

cluster [7-9]. Genome- wide association studies (GWAS) have shown that SNPs of genes encoding enzymes involved in LC polyunsaturated FA metabolism affect plasma FA levels [10-12]. Genetic susceptibility may lead to a defect in the activity of specific enzymes named desaturases and elongases and could possibly lead to the development of low-grade inflammation, insulin resistance, type 2 diabetes mellitus, metabolic syndrome, hypertension, atherosclerosis and ischemic heart disease [13].

We have previously reported that SNPs within the *FADS* gene cluster are associated with plasma desaturase activities after n-3 FA supplementation [14]. *FADS*1 and *FADS*2 genes encode respectively for two desaturases: δ-5 desaturase (D5D) and δ-6 desaturase (D6D) [7]. The D5D and D6D, responsible for double bonds formation in the n-3 FA pathways, have been associated with differences in FA composition of plasma [15], erythrocyte membranes [9] and adipose tissue [15].

The objectives of the present study were to test for associations between changes in percentage of FAs alone or regrouped as factors with changes in plasma TG levels after the n-3 FAs supplementation and to test whether SNPs from the *FADS* gene cluster were associated with plasma FA levels or with specific FA patterns derived from PCA after the supplementation.

Methods

Study Population

A total of 254 subjects from the greater Quebec City metropolitan area were recruited between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees. Subjects had to be aged between 18 and 50 years old with a body mass index (BMI) between 25 and 40 kg/m^2. They had to be nonsmokers and free of any thyroid or metabolic disorders requiring treatment. Participants were excluded if they had taken n-3 FA supplements for at least 6 months prior to the beginning of the study. However, only 210 subjects completed the intervention protocol and blood lipid profile pre-and/or post-supplementation are missing for two participants. Statistical analyses were then performed on 208 individuals. The ethics committees of Laval University Hospital Research Center and Laval University approved the experimental protocol. This trial was registered at clinicaltrials.gov as NCT01343342.

Study Design and Diets

The complete study design has been previously reported [16]. Briefly, subjects followed a run-in period of two weeks where a trained registered dietitian gave individual dietary instructions. Recommendations were drawn from the *Canada's Food Guide to Healthy Eating* [17]. After a 2-week run-in period, each participant received a bottle containing capsules of n-3 FAs (Ocean Nutrition, Nova Scotia, Canada) covering the following six-week period. They had to take 5 capsules per day, which gave them a total of 3-3.3g of n-3 FAs (1.9-2.2g EPA and 1.1g DHA) per day. Subjects had to report any deviations that may have occurred during the protocol. They also had to write their alcohol and fish consumption on a log sheet. Before each phase of the study, subjects received written and oral dietary instructions by a registered dietitian.

SNPs Selection and Genotyping

SNPs in *FADS*1, *FADS*2, and *FADS*3 were identified using the International Hap Map Project SNP database, based on the National Center for Biotechnology Information (NCBI) B36 assembly Data Rel 28. phase II + III, build 126. The *FADS* gene cluster is made of three

genes that are located very close to each other on chromosome 11. Because of the head-to-head orientation of *FADS*1 and *FADS*2 and the tail-to-tail orientation of *FADS*2 and *FADS*3, we added 500 kilo-base pairs (kbp) downstream of *FADS*1 and 2500 kbp upstream of *FADS*3 to cover promoter regions. Intergenic areas were also covered. Gene Tagger procedure in Haploview V4.2 was used to determine tag SNPs (tSNPs) using a minor allele frequency (MAF) ≥ 3% and pairwise tagging (r2≥0.8). Subsequently, we examined linkage disequilibrium (LD) out of the nineteen SNPs of the *FADS* gene cluster area using the LD Plot procedure in Haploview V4.2. The SIGMA Gen- Elute Gel Extraction Kit (Sigma-Aldrich Co. St.Louis. Missouri. USA) has been used to extract genomic DNA. Selected SNPs of the *FADS* gene cluster (rs174456, rs174627, rs482548, rs2072114, rs12807005, rs174448, rs2845573, rs7394871, rs7942717, rs74823126, rs174602, rs498793, rs7935946, rs174546, rs174570, rs174579, rs174611, rs174616 and rs968567) have been genotyped using validated primers and TaqMan probes (Thermo Fisher Scientific, Waltham, MA, USA). DNA was mixed with TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), with a gene-specific primer and with probe mixture (predeveloped TaqMan SNP Genotyping Assays; Thermo Fisher Scientific, Waltham, MA, USA)) in a final volume of 10µl. Genotypes were determined using a 7500 RT-PCR System and analyzed using ABI Prism SDS version 2.0.5 (Thermo Fisher Scientific, Waltham, MA, USA).

Biochemical Parameters measurements

Fasting insulin concentrations were measured by radioimmunoassay with polyethylene glycol separation [18]. Fasting glucose concentrations were enzymatically measured [19]. Plasma C-reactive protein (CRP) was measured by nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously [20]. Plasma total cholesterol and TG concentrations were measured using enzymatic assays [21]. The high-density lipoprotein cholesterol fraction was obtained after precipitation of very low-density lipoprotein and low-density lipoprotein particles in the infranatant with heparin manganese chloride [22]. Low-density lipoprotein cholesterol was calculated with the Friedewald formula [23].

Measurement of FA Composition in Plasma Phospholipids

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after 12 hours overnight fast and 48 hours alcohol abstinence. Plasma was separated by centrifugation at 500 g for 6 min and stored at –80°C for subsequent analyses. Plasma lipids were extracted with chloroform:methanol (2:1,by volume) according to a modified Folch method [24]. FA composition of PPLs was determined by gas chromatography. Total phospholipids were isolated with isopropyl ether:acetic acid (96:4) by thin layer chromatography [25]. Isolated PPLs were then methylated [26]. FA profiles were obtained after methylation in methanol/ benzene 4:1 (v/v) [26] and capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, Canada) equipped with a HP-88 capillary column (100m x 0.25 mm i.d. x 0.20μm film thickness; Agilent Technologies, Palo Atto, CA) coupled with a flame ionization detector (FID). Helium was used as carrier gas (split ratio 1:80). FA were identified according to their retention time, using the following standard mixtures as a basis for comparison: the FAME 37 mix (Supelco Inc., Bellefonte, PA) and the GLC-411 FA mix (NuChek Prep Inc, Elysian, MN), as well as the following methylated FAs C22:5n-6 (Larodan AB, Malmö, Sweden) and C22:5n-3 (Supelco Inc., Bellefonte, PA).

Principal Component Analysis (PCA)

Variables with abnormal distribution where logarithmically transformed before further analyses. Principal factor analysis was conducted in SAS v9.3 (SAS Institute Inc., Cary, North Carolina, USA) using the FACTOR procedure. Measured FAs (all-cis-8,11,14-eicosatrienoic acid (C20:3n-6, DGLA), docosatetraenoic acid (C22:4n-6), 4,7,10,13,16-docosapentaenoic acid (C22:5n-6), docosanoic acid (C22:0), tetracosanoic acid (C24:0), eicosanoic acid (C20:0), cis-9-octadecenoic acid (C18:1n-9), docosahexaenoic acid (C22:6n-3, DHA), 7,10,13,16,19-docosapentaenoic acid (C22:5n-3), eicosapentaenoic acid (C20:5n-3, EPA), octadecadienoic acid (C18:2n-6, LA), trans-11-octadecenoic acid (C18:1 trans-11), cis-6-octadecenoic acid (C18:1), trans-9-hexadecenoic acid (C16:1 trans-9), hexadecanoic acid (C16:0), hexadecenoic acid (C16:1), octadecanoic acid (C18:0), tetradecanoic acid (C14:0), octadecatrienoic acid (C18:3n-3, ALA), octadecenoic acid (C18:1n-7), all-cis-5, 8, 1, 14-eicosatetraenoic acid (C20:4n-6, ARA), cis-11-eicosenoic acid (C20: 1n-9),

eicosadienoic acid (C20:2n-6), cis-9-tetradecenoic acid (C14:1), cis-15-tetracosenoic acid (C24:1n-9), trans-9-octadecenoic acid (C18:1 trans-9) and eicosatrienoic acid (C20:3)) pre- and post-supplementation were analyzed separately in order to develop components. To determine the number of factors to retain, components with Eigenvalue ≥ 2 at Screen test were considered. FAs with positive loadings contribute to the pattern; FAs with negative loadings are inversely associated with the pattern. In post-supplementation, 4 factors were considered (Figure 1). Looking at the rotated solution (rotation method: Varimax), FAs with absolute factor loadings ≥ 0.5 were regarded as significant contributors to the pattern. Each participant was given a score for the 4 post-supplementation factors. These scores were calculated from the sum of phospholipid FA concentrations multiplied by their matching scoring coefficients. These scores reflect the degree of each participant PPL FAs conforming to a FA pattern.

Statistical Analysis

All genotype distributions were tested for any deviation from Hardy-Weinberg equilibrium (HWE) using the ALLELE procedure in S.A.S Genetics v9.3 (S.A.S Institute Inc., Cary, North Carolina, USA). Significance testing for linkage disequilibrium (LD) coefficient D was obtained using a chi-square test, likelihood ratio and Fisher exact test ($p \leq 0.01$). All other statistical analyses were carried out using S.A.S statistical software v9.3 (S.A.S Institute Inc., Cary, North Carolina, USA). Normal distribution was evaluated looking at the box-plot as well as skewness and kurtosis ranges for normal distribution. When needed, variables non-normally distributed were log10-transformed. A general linear model (GLM) was used to test for associations between PPL FAs and SNPs from the *FADS* gene cluster after an n-3 FA supplementation. GLM was adjusted for baseline PPL FAs levels for analyses involving post-supplementation data. For some SNPs, heterozygotes and homozygotes for the minor allele were grouped if the genotype frequency of the homozygotes for the minor allele was under 5%. Statistical significance was defined as $p \leq 0.05$.

Results

SNPs within the *FADS* gene cluster are shown in Table 1. All SNPs were in HWE except rs7935946, located in an intron of *FADS*2. Thus, this SNP was not considered for further analyses. Daily energy intakes were calculated by a food frequency questionnaire validated for healthy French-Canadians [27] and are presented in Table 2. After the supplementation, carbohydrates, saturated fats, proteins and PUFA intakes were significantly different from the pre-n-3 FA period (p=0.0005; p=0.0008; p=0.02; and p=0.003, respectively). Subjects have significantly decreased their fasting plasma TG levels (-11.9%, p<0.0001) and have slightly increased their fasting glucose levels (+2.4%, p=0.02) (Table 2).

Changes in plasma phospholipid FAs following an n-3 FA supplementation

Changes in PPL FAs (in percentage of total FAs) following a 6-week n-3 FA supplementation are reported in Table 3. Briefly, there was an increase in EPA, DPA and DHA levels in PPLs as well as a decrease in ALA and all n-6 FA levels after the supplementation ($p \leq 0.0002$, for all). A significant decrease was observed for all MUFA levels in PPLs except for cis-9-tetradecenoic acid (C14: 1) and for cis-15-tetracosenoic acid (C24: 1n-9) for which non-significant increases were observed. For SFA levels, small, but significant increases were observed in PPLs with> 18-carbon atoms FAs ($p < 0.007$ for all). Factor analysis suggested 4 post-n3 FA supplementation patterns. Figure 1 shows the composition of the 4 main post-supplementation factors. A factor loading ≥ 0.5 indicates a strong positive association with the FAs profile whereas a factor loading ≤ -0.5 indicates a strong inverse association with the FAs profile. Factor 1 included all-cis-8,11,14-eicosatrienoic acid (C20:3n-6, DGLA), docosatetraenoic acid (C22:4n-6), 4, 7, 10, 13, 16-docosapentaenoic acid (C22:5n-6) and eicosapentaenoic acid (C20:5n-3, EPA). Factor 2 included docosanoic acid (C22:0), tetracosanoic acid (C24:0), eicosanoic acid (C20:0) and cis-9-octadecenoic acid (C18:1n-9). Factor 3 included docosahexaenoic acid (C22:6n-3, DHA), 7, 10, 13, 16, 19-docosapentaenoic acid (C22:5n-3), eicosapentaenoic acid (C20:5n-3, EPA) and octadecadienoic acid (C18:2n-6, LA). Factor 4 included trans-11-octadecenoic acid (C18:1trans-11), cis-6-octadecenoic acid (C18:1) and trans-9-hexadecenoic acid (C16:1 trans-9).

Table 4 shows the differences in post-supplementation FA pattern scores between positive (delta TG < 0) and negative (delta TG ≥ 0) responders based on their plasma TG levels after a 6-wk fish oil supplementation. Overall, there appears to be a tendency to show FA pattern scores in the opposite direction between positive and negative responders and this difference is significant for factor 2 (p=0.02).

In a general linear model adjusted for the effects of age, sex, BMI and baseline FA levels, several SNPs from the *FADS* gene cluster were associated with post-supplementation FA levels, especially with ALA levels (7 SNPs), ARA levels (2 SNPs) and EPA levels (2 SNPs) as shown in Table 6. Associations between SNPs from the *FADS* gene cluster and post-supplementation factors derived from PCA (1 SNP associated with Factor 1 and 2 SNPs associated with Factors 2, 3 and 4) were also observed using the same statistical model, but with post-supplementation factors as the dependent variable (Table 6).

Discussion

In this study, we tested whether PPL FAs individually or regrouped as FA patterns were associated with a change in TG levels following an n-3 FA supplementation and whether SNPs from the *FADS* gene cluster influence changes in PPL FAs and FA patterns. To our knowledge, this is the first study to investigate the effects of *FADS* SNPs on FA profiles derived from factor analysis after a supplementation with high doses of n-3 FAs.

Despite Rizos et al. (2012) have had difficulty demonstrating the causality link between n-3 FA intakes and lower risk of all-cause mortality, Hartweg et al. have conducted a systematic review including 23 randomized clinical trials and have shown that n-3 FAs exert beneficial effects on CVD risk factors such as plasma TG and VLDL- cholesterol levels with no adverse effects of the intervention [2, 28]. There is a growing body of evidence underlining the importance of the FA composition of membranes in health and disease. For instance, higher plasma levels of DHA are associated with a more favorable CVD risk profile [29] and higher plasma levels of EPA are inversely associated with the risk of major coronary events [30]. A recent review by Kaur et al. (2011) suggested that n-3 DPA may also have potential beneficial properties on metabolic health [31].

FA patterns can be altered by factors known to modulate lipid metabolism such as visceral obesity, insulin resistance and inflammation [32]. In the present study, there were no significant changes in BMI, insulin resistance and inflammation-related traits (data not shown). However, only results with PCA post-supplementation are presented in this paper to better reflect the changes in FA patterns after the intake of n-3 FA supplements, because FA patterns depend largely on dietary fat intake [33, 34]. The lipid profile of individuals undertaking a supplementation with n-3 FAs is altered as shown by variations in individual FA levels after the supplementation (Table 3). This metabolic signature could provide new insights on the role of groups of FAs strongly correlated and their ability to act as better biomarkers than FA levels alone measured in PPLs after a supplementation with high doses of n-3 FAs.

There was a difference in post-supplementation FA pattern scores between positive and negative responders with regard to Factor 2 after a 6-wk fish oil supplementation. Although this difference did not remain significant for the other factors, we observed a trend with scores going in the opposite direction between positive and negative responders, meaning that FA composition of PPLs is probably different among these individuals. The composition of Factor 2 indicates a strong positive correlation between long chain and very long chain saturated FAs in addition to a negative correlation with oleic acid. In our study, there was a small but significant increase of saturated FAs in PPLs (expressed as % of total FAs, Table 3), but not when looking at absolute quantities (data not shown). This change observed in plasma TG levels does not seem to be related to the activation of PPAR-α by saturated FAs because saturated FAs with ≥20 carbon units failed to bind well with any of the PPARs [35]. The mechanism by which Factor 2 may be associated with a decrease in plasma TG levels could rely on the substrate specificity of the lipoprotein lipase (LPL) depending of the FA chain length and the degree of FA saturation. Even if LPL has a lower affinity for monoacylglycerol that contains a saturated acyl group, it has a higher affinity for LC TGs vs. medium-chain TGs [36, 37].

Post-supplementation Factor 1 included long-chain n-6 FAs with positive factor loadings as well as EPA with negative factor loadings. Moreover, Factor 1 correlates significantly with the change in plasma TG levels in positive responders as shown in Table 5 ($r=-0.25$, $p=0.003$). According to these correlation patterns, individuals with a high positive score (characterized by ↑ DGLA, ↑ 4,7,10,13,16-Docosapentaenoic acid, ↑ docosatetraenoic acid, ↓ EPA) are more likely to be characterized by an increase in TG concentrations while individuals having a high negative score (characterized by ↓ DGLA, ↓ 4,7,10,13,16-Docosapentaenoic acid, ↓ docosatetraenoic acid, ↑EPA) were more likely to display a decrease in TG after the 6-week n-3 FA supplementation. Factor 1 is similar to the product-to-precursor ratio of EPA to ALA often used as a surrogate measure of desaturase activity [38, 39]. In this study, SNP rs12807005 was associated with post-supplementation Factor 1. We have previously shown that carriers of the minor allele of rs12807005 had significantly higher plasma TG levels after the supplementation [16]. Moreover, we have shown that this SNP could also modulate estimate of D5D activity following fish oil supplementation

whereas carriers of the minor allele had a lower D5D activity post-supplementation, often associated with adverse profiles of several metabolic risk factors [14, 40, 41]. Altogether, these results suggest that genetic predispositions may lead to more or less long-chain FAs conversion depending on the genotype and may affect plasma TG response. Interestingly, Lemaitre et al. have shown that minor alleles of SNPs in *FADS1* and *FADS2* were associated with higher levels of ALA and lower levels of EPA and DPA, which is in accordance with our results showing that SNPs from the FADS gene cluster could also modulate FAs levels regrouped as a unique pattern [12]. A previous GWAS on our study population has highlighted new loci that could possibly explain the difference observed in plasma TG response after an n-3 FA supplementation between positive and negative responders characterized by delta TG levels < 0 or ≥ 0, respectively [42].

Interestingly, factor 3 post-supplementation included DHA (factor loading: 0.85), DPA (factor loading: 0.76), EPA (factor loading: 0.58) and LA (factor loading: -0.80) meaning that the increase of plasma FAs levels of DHA, DPA and EPA after the supplementation is highly correlated to a strong decrease in LA levels as measured in PPLs. However, this factor was not associated with a change in plasma TG levels in this study.

According to Lemaitre et al., it is estimated that SNPs could account for 40 to 70% of the inter-individual variability observed in red blood cell (RBC) FAs and that there are shared genetic effects that could either be positive or negative between several FAs [43]. Thereby, we believed that changes in FA patterns could be partly attributable to genetic factors such as SNPs of the three studied genes. This study shows that SNPs from the *FADS* gene cluster may affect the FA composition of PPLs after the supplementation. Some SNPs are even associated to a specific FA pattern, herein named Factors 1, 2, 3 or 4. Previous studies from our group have shown that *FADS*2 gene expression was changed after the 6-week n-3 FA supplementation, possibly leading to alterations in the metabolic pathways of n-6 and n-3 FAs because of the involvement of the D6D [44].

SNPs in *FADS*1, which catalyzes the biosynthesis of highly unsaturated FAs from precursor essential PUFAs, and two other members of the same gene family, *FADS*2 and *FADS*3, have

been previously associated with FA levels (measured in PPLs or in RBCs) or with cardiometabolic traits, even at genome-wide significance levels [12, 45, 46]. By directing FAs down this metabolic pathway, increased activity of these enzymes may lower circulating TG concentrations [47]. Moreover, the use of D6D twice in the conversion of ALA to DHA in the n-3 FAs pathway and in the conversion of LA to ARA implies that this enzyme may play a key regulatory role in the PUFA metabolism [48]. Previous data from our research group have shown that gene-diet interactions with several SNPs from the *FADS* gene cluster could potentially modulate the enzyme activities of desaturases and elongases involved in the FA metabolism post-supplementation, possibly leading to different FA levels in PPLs [14]. According to Hong et al., SNPs from the *FADS* gene cluster can affect age-associated changes in serum phospholipid LC FAs in addition to D5D activity, and oxidative stress in middle-aged non-obese men [49]. A recent GWAS of PUFAs in the InCHIANTI Study have shown that the mutated allele of rs174537 was associated with higher ALA and LA levels and lower ARA, EPA, DPA and DHA levels [50]. Results from that GWAS have been replicated in the GOLDN study, where there were significant associations of rs174537 (in high LD with rs174546, r2=0.99) with ALA, LA, ARA, EPA and DHA ($p<0.001$) and marginal association with DPA ($p=0.068$). In the present study, rs174546 (in high LD with rs174537, r2=0.99), has been associated with ALA, LA, ARA, DGLA levels post-supplementation, but not with LC n-3 FAs, as shown by others. However, this SNP was associated with post-supplementation Factor 2 that regroups several saturated FAs as one component.

Strengths and Limitations

This is a well-designed study to look at associations with genetic factors after a 6-week n-3 FA supplementation with sufficient statistical power to show significant associations. High doses of n-3 FAs were given to study participants and the compliance was really high as calculated by the remaining capsules from bottles returning and as shown by increased levels of n-3 FAs into PPLs. Recent evidence suggests that PPL FA pool may be more affected by recent fat consumption and that it would be more effective to look at FAs in RBCs especially in the context of genetic studies [51]. FAs were also measured in RBCs in the present study, but only in 30 individuals. However, the combined levels of EPA and DHA in PPLs

correlated with levels from RBCs after the supplementation ($r=0.47$, $p=0.02$) [52]. Thus, PCA were done using FAs measured in PPLs.

Conclusion

It is clear that each FA has its own particular effect on lipid metabolism. This study shows that not only polyunsaturated FAs alone could play a role in modulating plasma TG levels in response to a supplementation with n-3 FAs, but also that several FAs strongly correlated and regrouped as factors could be considered. These factors could potentially act as better biomarkers than FA levels alone measured in PPLs. Dissemination of negative or positive responders to the n-3 FA supplementation may be more effective when looking at individuals scoring high to a specific FA pattern rather than looking at FAs alone. Moreover, SNPs from the *FADS* gene cluster have the ability to interact with both FAs and/ or factors to possibly modulate TG levels.

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Tableau 3.1 Characteristics of SNPs within the FADS gene cluster

dbSNP No.[1]	Sequence[2]	Position	Alleles	AA	CC	CA	CG	CT	GA	GG	GT	TT	MAF
				n[3] (%)									
FADS1													
rs174546	TGC[**C/T**]TTG	3′ UTR	C/T		103 (49.8)			86 (41.2)				10 (9.1)	T=33.0
FADS2													
rs482548	CAC[**C/T**]GTG	3′ UTR	C/T		161 (77.8)			40 (19.3)				6 (2.9)	T=3.9
rs2072114	TTC[**A/G**]GGT	Intron	A/G	167 (79.9)					38 (18.2)	4 (1.9)			G=20.0
rs2845573	TCA[**C/T**]GTT	Intron	A/G	177 (84.7)					30 (14.4)	2 (1.0)			G=15.3
rs174602	CCC[**A/G**]TCC	Intron	T/C		9 (4.3)			59 (28.2)				141 (67.5)	C=39.2
rs498793	AAC[**A/G**]CAG	Intron	C/T		62 (9.8)			99 (71.7)				43 (18.6)	T=31.3
rs174570	TGA[**C/T**]GTA	Intron	C/T		159 (76.4)			46 (22.1)				3 (1.4)	T=23.5
rs174579	TTT[**C/T**]CAG	Intron	C/T		127 (61.1)			78 (37.5)				3 (1.4)	T=16.3
rs174611	GGA[**C/T**]CCT	Intron	T/C		12 (5.7)			84 (40.2)				113 (54.1)	C=14.3
rs174616	TCA[**C/T**]GTT	Intron	A/G	51 (24.4)					158 (51.7)	50 (23.9)			A=45.4
rs968567	CGG[**A/G**]AGC	5′ UTR	G/A	2 (1.0)					63 (30.1)	144 (68.9)			T=7.9
FADS3													
rs174456	TAC[**A/C**]TGG	Intron	A/C	102 (48.8)	18 (8.6)	89 (42.6)							C=44.3
rs7394871	GAC[**A/C**]CCT	Intron	C/A	2 (1.0)	181 (86.6)	26 (12.4)							A=16.1
rs7942717	ACG[**A/G**]GTG	Intron	A/G	161 (77.0)					47 (22.5)	1 (0.5)			G=8.8
Intergenic regions within the *FADS* gene cluster													
rs174627	CTG[**C/T**]GTA	Intergenic	G/A	2 (1.0)					48 (23.0)	159 (76.1)			A=7.1
rs12807005	ATG[**A/G**]ATC	Intergenic	G/A	0 (0)					5 (2.4)	204 (97.6)			A=1.0
rs174448	TGA[**C/T**]TTC	Intergenic	A/G	78 (37.5)					109 (52.4)	21 (10.1)			G=37.6
rs7482316	CAA[**A/G**]CTG	Intergenic	A/G	168 (80.4)					39 (18.7)	2 (1.0)			G=6.7

[1] **dbSNP No. from HapMap Data Rel 28 Phase II + III, August 10 on NCBI b36 Assembly dbSNP b126 database.**
[2] **Genes sequences from dbSNP short genetics variations NCBI reference assembly.**
[3] **Number of subjects for each genotype.**

Tableau 3.2 Pre- and post-supplementation descriptive characteristics (n = 208)

	Pre-Supplementation	Post-Supplementation (including capsules)	p-values[3]
Weight (kg)[4]	81.3 ± 13.9	81.7 ± 14.3	0.83
BMI (kg/m²)[2,4]	27.8 ± 3.7	27.9 ± 3.9	0.81
Waist circumference (cm)[4]	93.3 ± 10.5	93.4 ± 10.8	0.93
Daily energy intake			
Energy (Kcal)	2272 ± 590	2143 ± 566	0.08
Total lipids, (g)	86.5 ± 29.2	86.6 ± 29.8	0.48
MUFA (g)	30.8 ± 11.8	29.6 ± 12.4	0.13
PUFA (g)	15.2 ± 6.6	17.1 ± 6.9	0.003
SFA (g)	29.0 ± 12.0	25.5 ± 10.4	0.0008
Cholesterol (mg)	303.7 ± 147.4	297.3 ± 169.4	0.41
Carbohydrates (g)	286.7 ± 78.9	263.4 ± 77.7	0.0005
Proteins (g)	97.8 ± 30.2	92.6 ± 29.6	0.02
Alcohol (g)	3.2 ± 6.0	3.2 ± 6.1	0.81
Cardiometabolic risk factors			
Total cholesterol (mmol/L)	4.75 ± 0.90	4.72 ± 0.94	0.74
HDL-cholesterol (mmol/L)	1.44 ± 0.36	1.47 ± 0.40	0.28
LDL-cholesterol (mmol/L)	2.76 ± 0.81	2.78 ± 0.85	0.77
Triacylglycerols (mmol/L)[2]	1.21 ± 0.63	1.02 ± 0.52	<0.0001
CRP (mg/L)[2]	1.82 ± 2.17	1.85 ± 2.12	0.98
Glucose (mmol/L)	4.95 ± 0.46	5.06 ± 0.49	0.02
Insulin (pmol/L)[2]	87.1 ± 75.7	83.6 ± 40.8	0.91

[1] **Values are means ± SD;**
[2] **Data were $\log_{10}$-transformed;**
[3] **p-values are derived from a repeated measures ANOVA adjusted for the effects of age, sex and BMI;**
[4] **p-values are derived from a repeated measures ANOVA adjusted for the effects of age only.**

Tableau 3.3 Changes in plasma phospholipid fatty acids prior to and after an n-3 FA supplementation.

	Pre-suppl.	Post-suppl.	Change	p-value[1]
Plasma phospholipids fatty acids			**(%)**	
SFA (% of total fatty acids)				
Tetradecanoic acid (C14:0)	0.38±0.10	0.37±0.10	-2.3	0.18
Hexadecanoic acid (C16:0)	27.73±1.50	27.65±1.41	-0.3	0.21
Octadecanoic acid (C18:0)	13.53±1.29	13.97±1.36	3.2	<0.0001
Eicosanoic acid (C20:0)	0.62±0.11	0.64±0.12	3.4	0.007
Docosanoic acid (C22:0)	1.79±0.28	1.85±0.31	2.9	0.0006
Tetracosanoic acid (C24:0)	1.43±0.25	1.50±0.27	4.5	<0.0001
MUFA (% of total fatty acids)				
***cis*-9-tetradecenoic acid (C14:1)**	0.11±0.10	0.12±0.10	5.3	0.42
Hexadecenoic acid (C16:1)	0.52±0.19	0.45±0.16	-14.3	<0.0001
***trans*-9-Hexadecenoic acid (C16:1 *trans*-9)**	0.16±0.10	0.13±0.10	-15.5	0.002
***cis*-6-Octadecenoic acid (C18:1)**	0.07±0.10	0.05±0.09	-30.4	0.003
***cis*-9-Octadecenoic acid (C18:1n-9)**	8.58±1.00	7.99±1.18	-6.8	<0.0001
Octadecenoic acid (C18:1n-7)	1.33±0.21	1.29±0.17	-3.2	0.006
***trans*-9-Octadecenoic acid (C18:1 *trans*-9)**	0.02±0.06	0.02±0.05	-34.4	0.07
***trans*-11-Octadecenoic acid (C18:1 *trans*-11)**	0.12±0.11	0.10±0.11	-13.6	0.06
***cis*-11-eicosenoic acid (C20:1n-9)**	0.10±0.12	0.08±0.09	-24.0	0.004
***cis*-15-tetracosenoic acid (C24:1n-9)**	2.57±0.56	2.67±0.62	3.9	0.08
PUFA (% of total fatty acids)				
Total n-6				
Octadecadienoic acid (C18:2n-6, LA)	19.71±2.08	17.69±2.22	-10.2	<0.0001
Eicosadienoic acid (C20:2n-6)	0.32±0.07	0.27±0.06	-15.4	<0.0001
***all-cis*-8,11,14-eicosatrienoic acid (C20:3n-6, DGLA)**	3.29±0.74	2.38±0.60	-27.6	<0.0001
***all-cis*-5,8,1,14-Eicosatetraenoic acid (C20:4n-6, ARA)**	11.05±1.74	9.74±1.44	-11.9	<0.0001
Docosatetraenoic acid (C22:4n-6)	0.35±0.07	0.19±0.09	-46.2	<0.0001
4,7,10,13,16-Docosapentaenoic acid (C22:5n-6)	0.28±0.12	0.10±0.10	-65.1	<0.0001
Total n-3				
Octadecatrienoic acid (C18:3n-3, ALA)	0.18±0.15	0.14±0.12	-22.3	0.0002
Eicosapentaenoic acid (C20:5n-3, EPA)	1.12±0.52	4.10±1.28	266.9	<0.0001
7,10,13,16,19-Docosapentaenoic acid (C22:5n-3, DPA)	0.96±0.23	1.39±0.31	44.0	<0.0001
Docosahexaenoic acid (C22:6n-3, DHA)	3.53±0.77	5.03±0.86	42.6	<0.0001

Values are means ± SD expressed as percentage of total fatty acids.
[1] ***p*-values are from a paired Student's T-Test.**

Tableau 3.4 Differences in post-supplementation FA pattern scores between positive and negative responders after a 6-wk fish oil supplementation.

	Post-supplementation factor scores (n=208)		***P*[1]**
	ΔTG (n=60, negative responders) Mean ± SD	ΔTG (n=148, positive responders) Mean ± SD	
Factor 1	0.13 ± 0.96	-0.05 ± 1.02	0.21
Factor 2	-0.24 ± 0.91	0.10 ± 1.02	0.02
Factor 3	0.04 ± 1.20	-0.02 ± 0.86	0.67
Factor 4	-0.05 ± 1.04	0.02 ± 0.99	0.63

[1]ANOVA adjusted for age, sex and BMI;
Negative responders have a ΔTG ≥ 0;
Positive responders have a ΔTG < 0.

Tableau 3.5 Pearson correlations between relative change in plasma TG levels and relative changes in FAs alone or regrouped as factors after a 6-week fish oil supplementation.

	ΔTG (n = 60, negative responders)		**ΔTG (n = 148, positive responders)**		**ΔTG (n = 208)**	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Factor 1		r = 0.19 p = 0.14		**r = -0.25** **p = 0.003**		r = -0.09 p = 0.22
Factor 2		r = -0.21 p = 0.11		r = 0.10 p = 0.22		r = -0.07 p = 0.32
Factor 3		r = 0.008 p = 0.95		r = -0.15 p = 0.07		r = -0.06 p = 0.37
Factor 4		r = 0.13 p = 0.34		r = 0.06 p = 0.50		r = 0.04 p = 0.58
ΔEPA	r = -0.01 p = 0.94	r = -0.06 p = 0.68	r = 0.13 p = 0.12	**r = 0.17** **p = 0.04**	r = 0.11 p = 0.11	r = 0.06 p = 0.43
ΔDHA	r = 0.09 p = 0.49	r = 0.02 p = 0.89	r = -0.05 p = 0.53	**r = -0.18** **p=0.03**	r = 0.12 p = 0.09	r = -0.09 p = 0.20
ΔARA	r = 0.04 p = 0.76	r = -0.02 p = 0.89	**r = 0.24** **p = 0.004**	r = -0.04 p = 0.63	**r = 0.24** **p = 0.0006**	**r = -0.17** **p = 0.01**
ΔALA	r = -0.02 p = 0.90	r = -0.03 p = 0.82	**r = 0.17** **p = 0.04**	r = 0.12 p = 0.14	**r = 0.15** **p = 0.04**	r = 0.07 p = 0.28
ΔDGLA	**r = 0.32** **p = 0.02**	**r = 0.32** **p = 0.01**	**r = 0.27** **p = 0.001**	r = 0.08 p = 0.31	**r = 0.41** **p < 0.0001**	**r = 0.24** **p = 0.0006**
Δ total n-3 FAs	r = 0.04 p = 0.75	r = -0.02 p = 0.88	r = 0.09 p = 0.30	r = 0.08 p = 0.34	**r = 0.16** **p = 0.02**	r = 0.03 p = 0.62
Δ total n-6 FAs	r = 0.15 p = 0.27	r = 0.06 p = 0.63	**r = 0.24** **p = 0.04**	r = -0.13 p = 0.12	**r = 0.33** **p < 0.0001**	r = -0.12 p = 0.08

Pearson correlations with partials for age, sex and BMI.
Model 1 -> Correlations with changes in FA concentrations.
Model 2 -> Correlations with changes in FAs expressed as % of total FAs.
Negative responders have a ΔTG ≥ 0;
Positive responders have a ΔTG < 0;

Tableau 3.6 Associations of SNPs within the *FADS* gene cluster with plasma phospholipid fatty acids AFTER a 6-week n-3 FAs supplementation.

	Fatty acids										Post-supplementation factors derived from PCA**			
	Omega-6 fatty acids*						Omega-3 fatty acids*							
	18:2n-6 (LA)	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	22:4n-6	22:5n-6	18:3n-3 (ALA)	20:5n-3 (EPA)	22:5n-3 (DPA)	22:6n-3 (DHA)	Factor 1	Factor 2	Factor 3	Factor 4
FADS1														
rs174546	--	0.04	--	0.06	--	--	0.02	--	--	--	--	0.03	--	--
FADS2														
rs482548	--	--	--	--	--	--	--	--	--	--	--	--	--	--
rs2072114	--	--	--	0.06	--	--	0.04	--	--	--	--	--	--	--
rs2845573	--	--	--	0.03	--	--	0.007	--	--	--	--	--	--	--
rs174602	--	--	--	--	--	--	--	0.05	0.01	--	--	--	0.01	--
rs498793	--	--	--	0.007	--	--	--	0.02	--	--	--	--	--	--
rs174570	--	--	--	0.09	--	--	--	--	--	--	--	--	--	--
rs174579	--	--	--	--	--	--	--	--	--	--	--	--	--	--
rs174611	--	--	--	--	--	--	0.003	--	--	--	--	--	--	--
rs174616	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--
rs968567	--	--	--	--	--	--	0.03	--	--	--	--	--	0.02	--
FADS3														
rs174456	--	--	--	--	--	--	--	--	--	--	--	--	--	--
rs7394871	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006
rs7942717	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Intergenic regions within the *FADS* gene cluster														
rs174627	--	--	--	--	--	--	0.006	--	--	--	--	0.05	--	--
rs12807005	--	--	0.04	--	--	--	--	--	--	--	0.03	--	--	--
rs174448	--	--	--	--	--	--	--	--	--	--	--	--	--	--
rs7482316	--	--	--	--	0.003	--	--	--	--	--	--	--	--	0.01

***p*-values are from a general linear model adjusted for the effects of age, sex, BMI and baseline FA levels.**
****p*-values are from a general linear model adjusted for the effects of age, sex, BMI.**

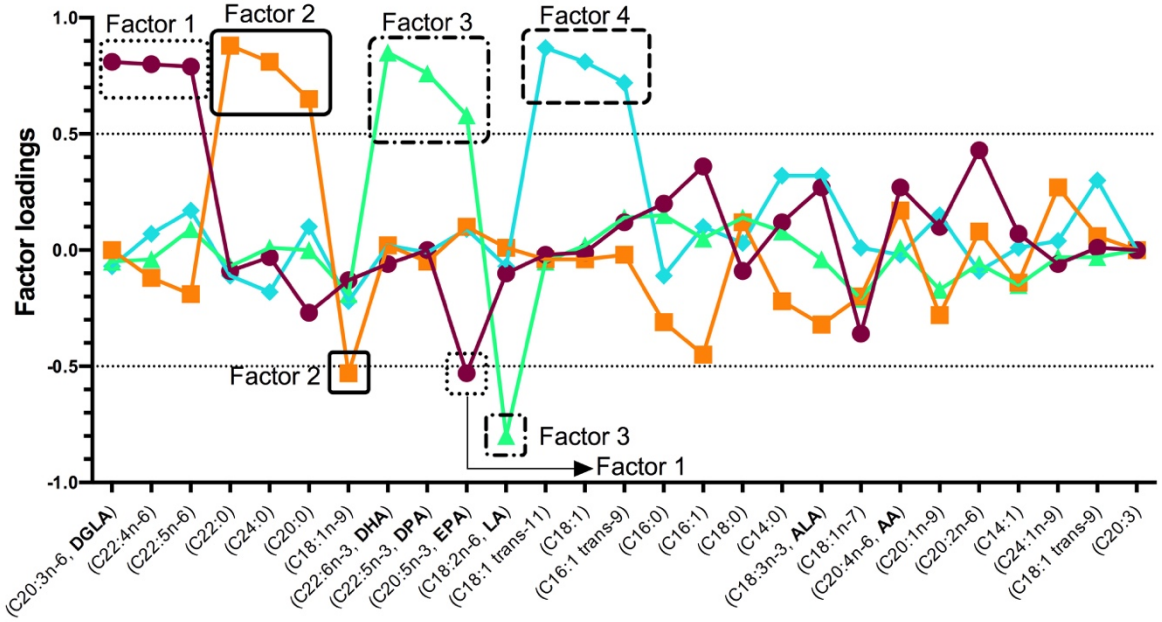

Figure 3.1 Post-n-3 FA supplementation patterns derived from PCA.

PCA revealed four post-supplementation phospholipid fatty acid factors. Factor 1 included *all-cis*-8,11,14-eicosatrienoic acid (C20:3n-6, DGLA), docosatetraenoic acid (C22:4n-6), 4,7,10,13,16-docosapentaenoic acid (C22:5n-6) and eicosapentaenoic acid (C20:5n-3, EPA). Factor 2 included docosanoic acid (C22:0), tetracosanoic acid (C24:0), eicosanoic acid (C20:0) and *cis*-9-octadecenoic acid (C18:1n-9). Factor 3 included docosahexaenoic acid (C22:6n-3, DHA), docosapentaenoic acid (C22:5n-3), eicosapentaenoic acid (C20:5n-3, EPA) and octadecadienoic acid (C18:2n-6, LA). Factor 4 included *trans*-11-octadecenoic acid (C18:1 *trans*-11), *cis*-6-octadecenoic acid (C18:1) and *trans*-9-hexadecenoic acid (C16:1 *trans*-9).

Chapitre 4

Polymorphismes dans le groupe de gènes *FADS* : Effets sur les contrôles glycémiques suite à une supplémentation en acides gras polyinsaturés oméga-3

Hubert Cormier, Iwona Rudkowska, Élisabeth Thifault, Simone Lemieux, Patrick Couture et Marie-Claude Vohl

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Polymorphisms in Fatty Acid Desaturase (*FADS*) Gene Cluster: Effects on Glycemic Controls Following an Omega-3 Polyunsaturated Fatty Acids (PUFA) Supplementation

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Résumé

Des changements dans l'indice d'activité des désaturases sont associés à la sensibilité à l'insuline et peuvent être associés au DBT2. Certains SNPs du groupe de gènes *FADS* ont été associés à l'indice HOMA-IS et à la composition en AG du sérum. **Objectif :** Déterminer si des variations génétiques dans le groupe de gènes *FADS* modulent les réponses de la glycémie à jeun (FG) et de l'insulinémie à jeun (FI) après une supplémentation en AGPI n-3 de 6 semaines. **Méthodes :** 210 sujets ont complété une période de pré-inclusion de 2 semaines suivie d'une supplémentation de 6 semaines avec 5 g/j d'huile de poisson fournissant 1,9 g-2,2 g d'AEP + 1,1 g d'ADH. Le génotypage de 18 SNPs du groupe de gènes *FADS* (couvrant 90% de toutes les variations génétiques communes; MAF ≥ 0,03) a été réalisé. **Résultats :** Les porteurs de l'allèle mineur pour rs482548 (*FADS2*) ont augmenté leurs taux de glucose plasmatique à jeun suite à la supplémentation en AGPI n-3 dans un modèle statistique ajusté pour les niveaux de glucose de départ, l'âge, le sexe et l'IMC. Un effet d'interaction génotype × supplémentation modulant les niveaux de glucose plasmatique à jeun a été observé pour rs482548 ($p = 0,008$). Pour les niveaux d'insuline à jeun, un effet du génotype a été observé avec un SNP (rs174456). Pour l'indice HOMA-IS, plusieurs effets d'interaction génotype × supplémentation ont été observés pour rs7394871, rs174602, rs174570, rs7482316 et rs482548 ($p = 0,03$; $p = 0,01$; $p = 0,03$; $p = 0,05$; et $p = 0,07$, respectivement). **Conclusion :** Les résultats suggèrent que les SNPs présents dans le groupe de gènes *FADS* peuvent moduler les niveaux plasmatiques de glucose et d'insuline à jeun en plus de moduler l'indice HOMA-IS en réponse à la supplémentation en AGPI n-3.

Abstract

Changes in desaturase activity are associated with insulin sensitivity and may be associated with type 2 diabetes mellitus (T2DM). SNPs in the *FADS* gene cluster have been associated with the homeostasis model assessment of insulin sensitivity (HOMA-IS) and serum fatty acid composition. **Objective:** To investigate whether common genetic variations in the *FADS* gene cluster influence fasting glucose (FG) and fasting insulin (FI) responses following a 6-week *n*-3 PUFA supplementation. **Methods:** 210 subjects completed a 2-week run-in period followed by a 6-week supplementation with 5 g/d of fish oil (providing 1.9 g–2.2 g of EPA + 1.1 g of DHA). Genotyping of 18 SNPs of the *FADS* gene cluster covering 90% of all common genetic variations (minor allele frequency ≥ 0.03) was performed. **Results:** Carriers of the minor allele for rs482548 (*FADS2*) had increased plasma FG levels after the *n*-3 PUFA supplementation in a model adjusted for FG levels at baseline, age, sex, and BMI. A significant genotype × supplementation interaction effect on FG levels was observed for rs482548 (p = 0.008). For FI levels, a genotype effect was observed with one SNP (rs174456). For HOMA-IS, several genotype × supplementation interaction effects were observed for rs7394871, rs174602, rs174570, rs7482316 and rs482548 (p = 0.03, p = 0.01, p = 0.03, p = 0.05 and p = 0.07; respectively). **Conclusion:** Results suggest that SNPs in the *FADS* gene cluster may modulate plasma FG, FI and HOMA-IS levels in response to *n*-3 PUFA supplementation.

Introduction

Type 2 diabetes mellitus (T2DM) and CVD are major public health concerns worldwide and especially in North America where 40 million people are going to be affected by 2030 [1]. Adults with diabetes have heart disease death rates about 2 to 4 times higher than adults without diabetes [2].

It is therefore important to study T2DM risk factors. The degree of obesity, the presence of the metabolic syndrome, family history of diabetes, impaired glucose tolerance, a low physical activity level, high blood levels of TG, high-density lipoprotein cholesterol (HDL-c) levels under 0.91 mmol/L as well as certain ethnic groups are well-known T2DM risk factors [2]. Fish oil and omega-3 (*n*-3) polyunsaturated fatty acids (PUFA) are of particular interest because of their roles in improving the plasma lipid profile, especially by reducing plasma TG [3]. *N*-3 PUFAs represent a reasonable therapeutic strategy to improve the dyslipidemic profile in individuals with T2DM [4]. On the other hand, *n*-3 PUFA supplementation is often associated with a significant but marginal increase in fasting plasma glucose (FG) concentrations [5]. A review of 18 trials with 823 subjects on the effects of fish oil supplementation on plasma lipid levels and glycemic controls showed that the pooled weight mean difference for FG was an increase of 0.26 mmol/L after an *n*-3 PUFA supplementation with doses ranging from 3 to 18 g/d of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6].

Changes in desaturase activity are associated with insulin sensitivity, which can be estimated using the homeostasis model assessment (HOMA-IS) [7] and might be part of T2DM development. HOMA-IS and serum fatty acid composition have been associated with single-nucleotide polymorphisms (SNPs) in the fatty acid desaturase (*FADS*) gene cluster [8]. The fatty acid desaturase-1 (*FADS1*) and fatty acid desaturase-2 (*FADS2*) genes encode respectively for two desaturases: δ-5 desaturase (D5D) and δ-6 desaturase (D6D) [9]. The D5D and D6D, responsible for double bonds formation in the *n*-3 PUFA and omega-6 (*n*-6) PUFA pathways, have been associated with differences in fatty acid composition of plasma, adipose tissue, and membrane fluidity [10]. In general, D5D

activity is inversely related to obesity and insulin resistance, whereas D6D activity shows positive associations [10].

Prospective studies have shown a strong inverse relationship between D5D and T2DM risk and a strong positive relation with D6D, using the desaturase indexes that estimate aggregate desaturase activity (ADA) using products to precursors ratios [11,12]. However, these studies cannot predict with certainty how confounding factors such as diet, lifestyle habits and heredity or reverse causality could explain part of the variance. It is thus relevant to study the effects of SNPs within the *FADS* gene cluster, mostly *FADS1* and *FADS2* that encode the desaturases in response to *n*-3 PUFA. By giving high doses of *n*-3 PUFA above 3 g/d, the *n*-3 PUFA pathway is promoted at the expense of the *n*-6 pathway which would potentially reduce the *n*-6:*n*-3 ratio by increasing D5D and D6D ADA and generate improvements in the metabolic profile of individuals.

The objective of the present study was to investigate whether the FG, the fasting insulin (FI), and the HOMA-IS responses to a 6-week *n*-3 PUFA supplementation were influenced by common genetic variations in the *FADS* gene cluster. We hypothesized that SNPs within the *FADS* gene cluster are associated with glycemic controls parameters after an *n*-3 PUFA supplementation.

Methods

Study Population

A total of 254 subjects from the greater Quebec City metropolitan area were recruited between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees. Subjects had to be aged between 18 and 50 years with a BMI between 25 and 40. Subjects were excluded from the study if they had taken *n*-3 PUFA supplements for at least 6 months prior to the study. However, only 210 subjects completed the intervention protocol and glycemic control parameters pre- and/or post-supplementation are missing for two participants [13]. Statistical analyses were then performed on 208 individuals. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This trial was registered at clinicaltrials.gov as NCT01343342 [14].

Study Design and Diets

Subjects followed a run-in period of two weeks during which a trained registered dietitian gave individual dietary instructions. Recommendations were drawn from the *Eating Well with Canada's Food Guide* [15]. All subjects were asked to apply these dietary recommendations and to maintain their body weight stable throughout the protocol. Among these recommendations, some specifications have been imposed to ensure the success of this study such as not to exceed two portions of fish or seafood per week (max. 150 g) and to choose, preferably, fish with white flesh and to avoid products fortified with *n*-3 PUFA during the study period. Subjects were also asked to limit their alcohol intakes to no more than two drinks per week. Subjects were not allowed to take *n*-3 PUFA supplements, including those of vegetable sources and to take vitamins or natural health products during the protocol.

After the run-in period, each participant received a bottle containing *n*-3 PUFA capsules (Ocean Nutrition, Nova Scotia, Canada) covering the following six weeks period. They had to take five capsules of fish oil per day, which gave them a total of 3–3.3 g of *n*-3 PUFA (1.9–2.2 g EPA and 1.1 g DHA) per day. Compliance was measured by bottles returning and

by calculating the number of remaining capsules in the bottles at the end of the supplementation. Subjects had to report any deviations that may have occurred during the protocol. They also had to write their alcohol and fish consumption on a log sheet. Before each phase of the study, subjects received written and verbal dietary instructions by a registered dietitian. Detailed methods were previously presented in Cormier *et al*. [13].

Anthropometric Measurements

Body weight, height, and waist girth were measured according to the procedures recommended by the Airlie Conference and were taken before the run-in period, as well as pre- and post-*n*-3 PUFA supplementation [16]. BMI was calculated as weight in kilograms divided by height in meters-squared (kg/m2).

Biochemical Parameters

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after 12 h overnight fast and 48 h alcohol abstinence. Blood samples were taken to identify and exclude individuals with metabolic disorders such as diabetes (FG > 7.0 mmol/L), hypertension (>140 mmHG/>90 mmHG), hypo/hyperthyroidism (based on thyroid-stimulating hormone (TSH) levels) or severe dyslipidemias [17]. Afterwards, selected participants had blood samples taken at prior and after the *n*-3 PUFA supplementation period. Plasma was separated by centrifugation (2,500 × g for 10 min at 4 °C) and samples were aliquoted and frozen for subsequent analyses. FI was measured by radioimmunoassay with polyethylene glycol separation [18]. FG concentrations were enzymatically measured [19]. HOMA-IS was calculated using the following formula: $\frac{22.5}{FGxFI}$ [7]. Plasma C-reactive protein (CRP) was measured by nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously [20]. Plasma total cholesterol (TC) and TG concentrations were measured using enzymatic assays [21]. The high-density lipoprotein cholesterol (HDL-C) fraction was obtained after precipitation of very low-density lipoprotein and low-density lipoprotein particles in the infranatant with heparin manganese chloride [22]. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula [23]. Apolipoprotein B-100 (ApoB100) concentrations were measured

in plasma by the rocket immunoelectrophoretic method of Laurell, as previously described [24].

SNP Selection and Genotyping

SNPs in *FADS1*, *FADS2* and *FADS3* were identified using the International HapMap Project SNP database, based on the National Center for Biotechnology Information (NCBI) B36 assembly Data Rel 28. phase II + III, build 126. The *FADS* gene cluster is made of three genes that are located very close to each other among chromosome 11. Because of the head-to head orientation of *FADS1* and *FADS2* and the tail-to-tail orientation of *FADS2* and *FADS3*, we added 500 kilo-base pairs (kbp) downstream of *FADS1* and 2,500 kbp upstream of *FADS3* to cover the promoter region. Intergenic areas were also covered. Tagger procedure in Haploview V4.2 was used to determine tag SNPs (tSNPs) using a minor allele frequency (MAF) ≥3% and pairwise tagging (r2 ≥ 0.8). Subsequently, we examined linkage disequilibrium (LD) out of the 19 SNPs covering all common variations in the *FADS* gene cluster area, using the LD Plot procedure in Haploview V4.2. Since the majority of the SNPs within the *FADS* gene cluster are in high LD (r2 ≥ 0.8) with each other, 19 SNPs were sufficient to cover 96% of the entire area. The SIGMA GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis, MO, USA) has been used to extract genomic DNA. Selected SNPs of the *FADS* gene cluster (rs174456, rs174627, rs482548, rs2072114, rs12807005, rs174448, rs2845573, rs7394871, rs7942717, rs74823126, rs174602, rs498793, rs7935946, rs174546, rs174570, rs174579, rs174611, rs174616 and rs968567) have been genotyped using validated primers and TaqMan probes (Life Technologies Corporation, Carlsbad, CA, USA). DNA was mixed with TaqMan Universal PCR Master Mix (Life Technologies Corporation, Carlsbad, CA, USA), with a gene-specific primer and with probe mixture (predeveloped TaqMan SNP Genotyping Assays; Life Technologies Corporation, Carlsbad, CA, USA) in a final volume of 10 μL. Genotypes were determined using a 7500 RT-PCR System and analyzed using ABI Prism SDS version 2.0.5 (Life Technologies Corporation, Carlsbad, CA, USA). The Exonic Splicing Enhancer (ESE) finder Webbased program was used to determine the potential effect of variants of the *FADS* gene cluster on pre-mRNA splicing. All tests were run under default threshold values [25].

Statistical Analyses

All genotype distributions were tested for any deviation from Hardy-Weinberg equilibrium (HWE) using the ALLELE procedure in SAS Genetics v9.3 (SAS Institute Inc., Cary, NC, USA). All SNPs were in HWE except one: rs7935946. Significance testing for LD coefficient D was obtained using a chi-square test, likelihood ratio and Fisher exact test ($p \leq 0.01$).

All other statistical analyses were carried out using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Normal distribution of outcome variables was evaluated looking at the box-plot, and also skewness and kurtosis ranges for normal distribution. When needed, outcome variables non-normally distributed were log10-transformed. A linear regression using the stepwise bidirectional elimination approach was used to assess which SNPs could explain part of the glycemic control parameters' variance where the effects of the 18 SNPs that were in HWE and the effects of age, sex, and BMI were included in the statistical model. The MIXED procedure was used to test for the effects of the genotype, the supplementation and the genotype by supplementation interaction for each SNP on FG, FI and HOMA-IS levels when age, sex and BMI were included in the model. The repeated statement was used to indicate the within subjects (repeated) variables. Genotypic groups were assessed as three groups expressed as Major Allele Homozygotes (11), Heterozygotes (12) and Minor Allele Homozygotes (22). For some SNPs, Heterozygotes and Minor Allele Homozygotes were grouped if the genotypic frequency of the Minor Allele Homozygotes was under 5%. Since polymorphisms tested in complex diseases rarely account for a large amount of variance, characterized by very low *p*-values ($p< 0.001$), we decided to present the results without correction for multiple testing and using a *p*-value ≤ 0.05. Statistical significance was defined as $p \leq 0.05$.

Results and Discussion

Baseline characteristics of study participants are presented in Table 1. Since we recruited overweight participants, the mean BMI is above 25 kg/m^2 in both men and women. Gender differences are evident with respect to weight, TC/HDL-C ratio, HDL-C, TG and CRP levels. In both men and women, the *n*-3 PUFA supplementation was associated with a decrease of plasma TG levels as well as with an increase of FG levels [5].

A previous paper from our group reported that *n*-3 PUFA supplementation slightly increased FG in the present study (mean ± SD, pre-*n*-3 PUFA: 4.95 ± 0.46; post-*n*-3 PUFA: 5.06 ± 0.49, $p = 0.02$) [5]. We then wanted to test whether SNPs of the *FADS* gene cluster may modulate glycemic control parameters' response after the 6-week supplementation period.

All SNPs were in HWE except one: rs7935946. This SNP was not considered for further analysis. Therefore, associations with 18 SNPs were tested in statistical analyses. The percentage of gene coverage with these 18 SNPs was of 90%.

To validate the presence of associations between glycemic control parameters and SNPs of the *FADS* gene cluster, all SNPs were included in a linear regression model with values post-*n*-3 PUFA supplementation as the dependent variable, adjusted for baseline values (FG, FI or HOMA-IS levels), age, sex and BMI. With the use of the stepwise bidirectional selection method, 2 SNPs were associated with FG levels: rs482548 (2.05%, $p = 0.01$) and rs498793 (1.75%, $p = 0.02$); 1 SNP was associated with FI levels: rs174602 (0.89%, $p = 0.04$); and 2 SNPs were associated with HOMA-IS: rs174602 (1.97%, $p= 0.005$) and rs482548 (0.90% $p = 0.05$) (Data not shown).

To test the potential interaction between the *FADS* gene polymorphisms and the *n*-3 PUFA supplementation on plasma FG, FI and HOMA-IS levels, a general linear model adjusted for sex, age and BMI was used in order to verify whether the genotype, the supplementation or the interaction (genotype by supplementation) were associated with

plasma levels of glycemic control parameters. As shown in Table 2, independently of the genotype, the supplementation was associated with FG concentrations (significant associations with 16 SNPs of the *FADS* gene cluster) meaning that the supplementation had an independent effect on plasma FG levels, as expected. One SNP (rs482548) was associated with plasma FG levels meaning that the genotype for that particular polymorphism may modulate the plasma FG during the intervention. A significant genotype by supplementation interaction effect on FG levels was observed for rs482548 ($p = 0.008$). For FI levels, a genotype effect was observed with one SNP (rs174456). For HOMA-IS, several genotype by supplementation interaction effects were observed for rs7394871, rs174602, rs174570, rs7482316 and rs482548 ($p = 0.03$, $p= 0.01$, $p = 0.03$, $p = 0.05$ and $p = 0.07$ respectively) (Table 2). Table 3 shows the genotype by supplementation interaction effect on HOMA-IS and FG levels by genotypic groups and the β-values for the four significant SNPs after the 6-week *n*-3 PUFA supplementation.

Insulin resistance is a condition in which glucose intakes by sensitive tissues decreased. Therefore, insulin resistance and insulin sensitivity are strong predictors of T2DM [26]. Individuals affected with T2DM also have higher cardiovascular risk. An *n*-3 PUFA supplementation may exert beneficial effects on cardiovascular risk on that specific population. Connor *et al*. showed that *n*-3 PUFA intake (6 g/d of EPA + DHA for 6 months), along with oral therapy for T2DM, can lower TG concentrations, with no adverse effects on glycemic control [27]. In addition to decrease TG levels, an *n*-3 PUFA supplementation may increase slightly FG levels. As previously reported by our research group, FG increased by 2.44% ± 49.55% ($p = 0.0004$) after the 6-week *n*-3 PUFA supplementation [5]. The high standard deviation associated with changes in FG levels shows that there is a large inter-individual variability in FG response following an *n*-3 PUFA supplementation. Because FG is part of the HOMA-IS formula and correlates negatively with this model, it is believed that the high inter-individual variability may also be seen in HOMA-IS values. As shown in Figure 1, 99 individuals decreased their HOMA-IS (mean ± SD; –23.2 ± 14.3%) while 107 individuals increased their HOMA-IS (mean ± SD; 30.4 ± 48.4%) after the *n*-3 PUFA supplementation.

The fatty acid profile of membrane phospholipids and the activity of desaturases are strongly linked to the incidence of T2DM [11]. The large inter-individual variability in HOMA-IS response after an *n*-3 PUFA supplementation could be due to different dietary factors such as a diet rich in saturated and trans fats, a reduced enzymatic activity of D5D and D6D, a low dietary contribution of C20-C22 PUFAs or high amount of 18:2 *n*-6 (linoleic acid) in the diet [28]. Increasing the content of longer chain PUFAs increases membrane fluidity, the number of insulin receptors and the action of insulin [29,30]. Remarkably, it has been demonstrated that an increase in cell membrane fluidity increases both the number of insulin receptors on the membrane and their affinity to insulin, thus improving insulin sensitivity [31,32]. Defects in D5D and D6D could induce decreases in C20-C22 PUFAs in skeletal muscle membrane phospholipids that lead to less unsaturation of membrane phospholipids. Less unsaturation of membrane phospholipids could possibly affect glucose metabolism.

The mechanism by which insulin resistance increases cardiovascular risk is unclear. However, the emerging literature demonstrates that genetic factors may be involved [33,34]. D5D and D6D, key enzymes in the PUFAs metabolism and encoded by genes *FADS1* and *FADS2*, may influence glucose metabolism [35]. The D6D activity may be strongly and positively related to the incidence of T2DM, whereas the literature reports an inverse relationship with the activity of D5D [36]. These desaturases have a concomitant activity in the *n*-3 and *n*-6 PUFA metabolism, creating a competition where the *n*-6 PUFA pathway is often favored [37]. An unbalanced ratio of *n*-3:*n*-6 due to the enzymatic activity of desaturases involved in PUFA metabolism might alter glucose metabolism [34].

A recent study published by Manning *et al*. has identified many loci implicated in T2DM, including SNPs in *FADS1*, which were associated with FG [38]. In the present study, the majority of SNPs that showed associations with glycemic control parameters were from the *FADS2* gene, but as the study covered 90% of all common genetic variations of the *FADS* gene cluster, some SNPs may be in LD with other SNPs found on *FADS1* or *FADS3* that may be potentially functional (Table 4).

A study by Brenner has shown that insulin influences the activity of D5D and D6D [35]. According to these results, changes in ADA might be induced by aberrant insulin concentrations in prediabetic states [35]. Kim *et al*. have shown in a study on Korean men in 2011 that SNPs rs174575 (in high LD with rs174579, $r^2 = 0.864$) and rs2727270 (in high LD with rs2072114, $r^2 = 1.0$) were associated with insulin resistance [8]. In the present study, only rs174456 was associated with FI levels. Differences may exist in allele frequencies, environment or the absence of common SNPs between both populations from two different ethnic groups potentially explaining these different SNP association patterns [39].

Many studies have detected a significant relation of the genotype rs174547 (in high LD with rs174546, $r^2 = 1.0$) with FG levels [40,41]. Overall, in the present study, rs174546 was not associated with FG, but was associated with TG levels pre-*n*-3 PUFA supplementation ($p = 0.002$) and a trend was also observed for TG levels post-*n*-3 PUFA supplementation ($p = 0.07$) [13]. Out of the 18 SNPs studied, only rs482548 was associated with FG levels after *n*-3 supplementation.

Regarding the possible effect of variants of rs482548, analysis of the scores obtained with ESE revealed that no variants markedly alter the binding capacity of putative ESE elements, either by reducing their values or by creating new binding site for SR proteins.

A key strength of the study is that it was an interventional study design and that high doses of *n*-3 were administered to participants. Additionally, it would have been interesting to use an oral glucose tolerance test (OGTT) and to measure insulin secretion by dosing C-peptide. The actual study does not allow to go further in the analyses of glycemic control parameters, suggesting that more studies are needed to better understand the impact of the *FADS* gene cluster SNPs on T2DM risk. Also, the short run-in period (2 weeks) may not be sufficient to take into account high basal levels of *n*-3 PUFAs in phospholipids membranes. Compliance was assessed from the return of containers and capsules (87%) to determine the quantity consumed and by measuring membrane phospholipid composition.

Conclusion

In conclusion, the importance of desaturases, which are the key enzymes in the PUFAs metabolism in the development of T2DM, deserves special attention. Our results show that several SNPs involved in the *FADS* gene cluster are associated with glycemic control parameters, mainly with HOMA-IS, in response to an intervention with high doses of *n*-3 PUFA. These SNPs are of particular interest because of their potential effects on glucose metabolism. Dietary *n*-3 supplements could be used as add-ons to a well-balanced diet and can be part of a healthy lifestyle in treating T2DM associated dyslipidemia.

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Hubert Cormier performed statistical analysis, interpreted data and wrote the paper; Elisabeth Thifault, met the participants; Iwona Rudkowska, Simone Lemieux and Marie-Claude Vohl designed research; Patrick Couture was responsible for the medical follow-up; Hubert Cormier and Marie-Claude Vohl have primary responsibility for final content. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Tableau 4.1 Baseline characteristics of the study sample before *n-3* polyunsaturated fatty acids (PUFA) supplementation.

	All[1]	Men[1]	Women[1]
Population. Men/Women	208	96 (46.2%)	112 (53.8%)
Age (years)	30.8 ± 8.7	31.2 ± 8.1	30.5 ± 9.1
Weight (kg)[3]	81.4 ± 13.9	87.2 ± 13.4	76.4 ± 12.3
BMI (kg/m²)[2,3]	27.8 ± 3.7	27.5 ± 3.6	28.2 ± 3.8
Waist circumference (cm)[3]	93.3 ± 10.8	94.8 ± 11.0	92.0 ± 10.4
Cholesterol (mM)[4]			
Total	4.82 ± 1.00	4.80 ± 1.00	4.83 ± 1.02
HDL	1.46 ± 0.39	1.29 ± 0.31	1.61 ± 0.39
LDL	2.79 ± 0.87	2.91 ± 0.87	2.69 ± 0.86
Total chol./HDL ratio[4]	3.49 ± 1.04	3.91 ± 1.13	3.12 ± 0.80
Triacylglycerols (mM)[2,4]	1.23 ± 0.64	1.32 ± 0.74	1.15 ± 0.53
ApoB100 (g/L)[4]	0.86 ± 0.25	0.89 ± 0.25	0.84 ± 0.25
CRP (mg/L)[2,4]	3.13 ± 7.10	1.66 ± 2.45	4.39 ± 9.24
Glycemic controls			
Glucose (mM)[4]	4.95 ± 0.52	5.09 ± 0.44	4.83 ± 0.56
Insulin (ϱ/L)[4]	82.51 ± 35.61	79.50 ± 32.19	85.04 ± 38.20

[1] **Values are means ± SD.**
[2] ***p*-value derived from $\log_{10}$-transformed.**
[3] **Results were adjusted for age.**
[4] **Results were adjusted for age and BMI.**

Tableau 4.2 Effect of the genotype, the *n-3* PUFA supplementation and the interaction (genotype by supplementation) on fasting glucose (FG), fasting insulin (FI) and homeostasis model assessment of insulin sensitivity (HOMA-IS) levels (n = 208).

	FG			FI[1]			HOMA-IS		
	Genotype	**Suppl.**	**Interaction**	**Genotype**	**Suppl.**	**Interaction**	**Genotype**	**Suppl.**	**Interaction**
rs174456	0.28	0.06	0.42	0.04	0.14	0.16	0.84	0.77	0.84
rs174627	0.81	0.002	0.93	0.98	0.97	0.74	0.89	0.52	0.90
rs482548	0.05	<0.0001	0.008	0.18	0.59	0.16	0.11	0.13	0.07
rs2072114	0.07	0.05	0.11	0.25	0.36	0.23	0.65	0.49	0.71
rs12807005	0.66	0.04	0.38	0.30	0.41	0.33	0.90	0.83	0.99
rs174448	0.79	0.002	0.65	0.85	0.66	0.84	0.61	0.27	0.45
rs2845573	0.35	0.03	0.55	0.67	0.81	0.92	0.41	0.19	0.24
rs7394871	0.58	0.05	0.44	0.41	0.55	0.29	0.05	0.04	0.03
rs7942717	0.93	0.004	0.83	0.27	0.67	0.67	0.99	0.40	0.61
rs7482316	0.48	0.003	0.83	0.05	0.39	0.08	0.03	0.09	0.05
rs174602	0.88	0.0006	0.79	0.46	0.72	0.11	0.09	0.12	0.01
rs498793	0.60	<0.0001	0.16	0.50	0.84	0.63	0.26	0.33	0.25
rs174546	0.34	0.03	0.52	0.44	0.58	0.74	0.42	0.28	0.42
rs174570	0.67	0.004	0.80	0.33	0.61	0.18	0.07	0.08	0.03
rs174579	0.58	0.0004	0.77	0.97	0.88	0.71	0.38	0.39	0.35
rs174611	0.88	0.004	0.73	0.75	0.86	0.82	0.54	0.44	0.65
rs174616	0.49	0.0003	0.27	0.44	0.45	0.35	0.21	0.61	0.11
rs968567	0.74	0.0007	0.84	0.51	0.71	0.68	0.68	0.45	0.71

The MIXED procedure implemented in SAS version 9.3 was used to test interaction effects.
All results were adjusted for BMI, age and sex.
[1] ***p*-values derived from $\log_{10}$-transformed for insulin levels.**

Tableau 4.3 Genotype by supplementation interaction effect on HOMA-IS and FG levels after a 6-week *n*-3 PUFA supplementation.

	Pre-*n*-3 PUFA		Post-*n*-3 PUFA		P[1]		β-values ± SE
	11	12 + 22	11	12 + 22			
HOMA-IS	0.067 ± 0.025	0.063 ± 0.027	0.068 ± 0.030	0.056 ± 0.022	0.01	**11**	0.4169 ± 0.0375
rs174602						**12 + 22**	0.4391 ± 0.0379
HOMA-IS	0.066 ± 0.025	0.065 ± 0.029	0.066 ± 0.029	0.057 ± 0.025	0.03	**11**	0.4106 ± 0.0378
rs174570						**12 + 22**	0.4248 ± 0.0384
HOMA-IS	0.065 ± 0.025	0.068 ± 0.033	0.065 ± 0.028	0.059 ± 0.025	0.03	**11**	0.4150 ± 0.0378
rs7394871						**12 + 22**	0.4319 ± 0.0391
FG	4.96 ± 0.43	4.92 ± 0.54	5.02 ± 0.48	5.15 ± 0.52	0.008	**11**	1.5753 ± 0.7327
rs482548						**12 + 22**	1.2278 ± 0.7443

11 stands for major allele homozygotes and 12 + 22 stands for heterozygotes and minor allele homozygotes grouped together.
The MIXED procedure implemented in SAS version 9.3 was used to test interaction effects.
All results were adjusted for BMI, age and sex.
[1] ***p*-values for the interaction (genotype by supplementation).**

Tableau 4.4 Description of the selected polymorphisms in the fatty acid desaturase (*FADS*) gene cluster.

Gene	dbSNP No.[1]	Sequence[2]	Position	MAF	Genotype/Frequency		
***FADS*3**	rs174456	CTAC[A/C]TGGC	intron	0.299	A/A (*n* = 102)	A/C (*n* = 89)	C/C (*n* = 18)
					0.488	0.426	0.086
Intergenic *FADS*2-*FADS*3	rs174627	TCTG[C/T]GTAG	Intergenic	0.124	A/A (*n* = 2)	A/G (*n* = 48)	G/G (*n* = 159)
					0.010	0.230	0.761
***FADS*2**	rs482548	ACAC[C/T]GTGG	3′ UTR	0.126	C/C (*n* = 161)	C/T (*n* = 40)	T/T (*n* = 6)
					0.778	0.193	0.029
***FADS*2**	rs2072114	GTTC[A/G]GGTC	Intron	0.110	A/A (*n* = 167)	A/G (n = 38)	G/G (*n* = 4)
					0.799	0.182	0.019
Intergenic *FADS*1-*FADS*2	rs12807005	CATG[A/G]ATCA	Intergenic	0.012	A/A (*n* = 0)	A/G (*n* = 5)	G/G (n = 204)
					0.000	0.024	0.976
Intergenic *FADS*2-*FADS*3	rs174448	CTGA[C/T]TTCT	Intergenic	0.363	A/A (*n* = 78)	A/G (*n* = 109)	G/G (*n* = 21)
					0.375	0.524	0.101
***FADS*2**	rs2845573	CTCA[C/T]GTTA	Intron	0.081	A/A (*n* = 177)	A/G (*n* = 30)	G/G (*n* = 2)
					0.847	0.144	0.010
***FADS*3**	rs7394871	GGAC[A/C]CCTG	Intron	0.072	A/A (*n* = 2)	A/C (*n* = 26)	C/C (*n* = 181)
					0.010	0.124	0.866
***FADS*3**	rs7942717	AACG[A/G]GTGC	Intron	0.117	A/A (*n* = 161)	A/G (*n* = 47)	G/G (*n* = 1)
					0.770	0.225	0.005
Intergenic *FADS*2-*FADS*3	rs7482316	TCAA[A/G]CTGC	Intergenic	0.103	A/A (*n* = 168)	A/G (*n* = 39)	G/G (*n* = 2)
					0.804	0.187	0.010
***FADS*2**	rs174602	ACCC[A/G]TCCT	Intron	0.184	C/C (*n* = 9)	C/T (*n* = 59)	T/T (*n* = 141)
					0.043	0.282	0.675
***FADS*2**	rs498793	TAAC[A/G]CAGG	Intron	0.456	C/C (*n* = 62)	C/T (*n* = 99)	T/T (*n* = 43)
					0.098	0.717	0.186
***FADS*2**	rs7935946	GTTC[C/T]GGGA	Intron	0.041	C/C (*n* = 195)	C/T (*n* = 11)	T/T (*n* = 3)
					0.933	0.053	0.014
***FADS*1**	rs174546	CTGC[C/T]TTGG	3′ UTR	0.297	C/C (*n* = 103)	C/T (*n* = 86)	T/T (*n* = 19)
					0.498	0.412	0.091
***FADS*2**	rs174570	TTGA[C/T]GTAG	Intron	0.125	C/C (*n* = 159)	C/T (*n* = 46)	T/T (*n* = 3)
					0.764	0.221	0.014
***FADS*2**	rs174579	CTTT[C/T]CAGG	Intron	0.202	C/C (*n* = 127)	C/T (*n* = 78)	T/T (*n* = 3)
					0.611	0.375	0.014
***FADS*2**	rs174611	TGGA[C/T]CCTG	Intron	0.258	C/C (*n* = 12)	C/T (*n* = 84)	T/T (*n* = 113)
					0.057	0.402	0.541
***FADS*2**	rs174616	CTCA[C/T]GTTC	Intron	0.498	A/A (*n* = 51)	A/G (*n* = 108)	G/G (*n* = 50)
					0.244	0.517	0.239
***FADS*2**	rs968567	CCGG[A/G]AGCT	5′ UTR	0.160	A/A (*n* = 2)	A/G (*n* = 63)	G/G (*n* = 144)
					0.010	0.301	0.689

[1] dbSNP No. from HapMap Data Rel 28 Phase II + III, August 10 on NCBI b36 Assembly dbSNP b126 database;
[2] Genes sequences from dbSNP short genetics variations NCBI reference assembly.

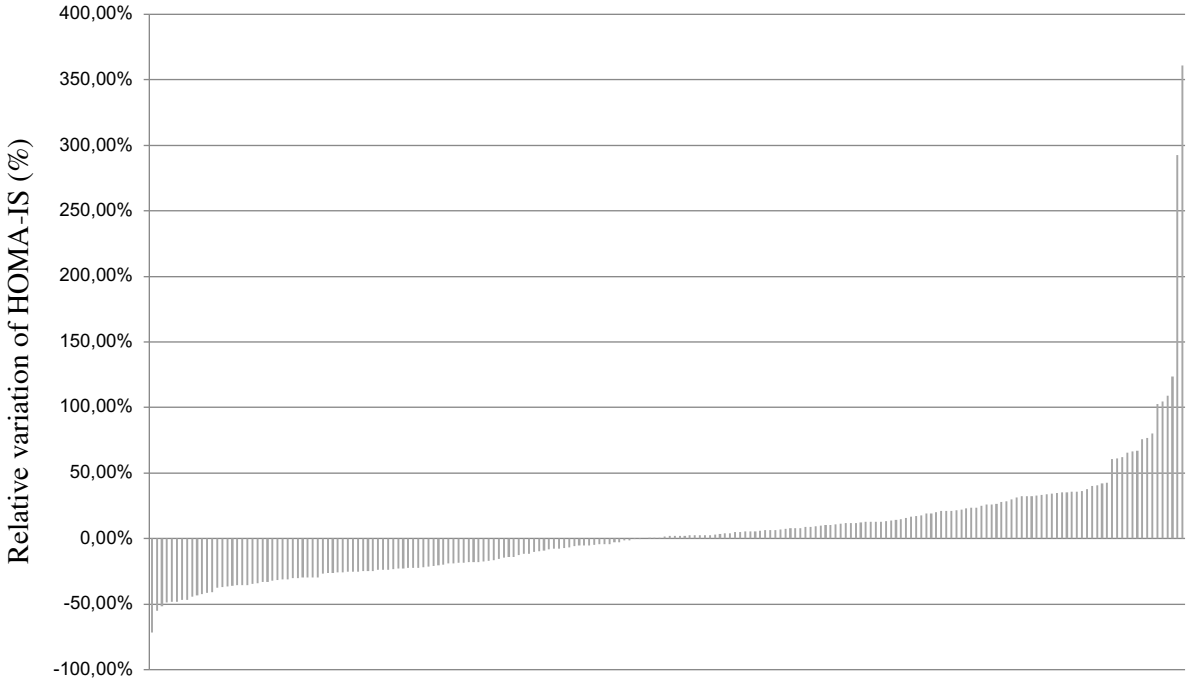

Figure 4.1 Inter-individual variability of HOMA-IS after a 6-week *n*-3 PUFA supplementation (n = 208).

Chapitre 5

Effets des polymorphismes des gènes *FADS1-2-3* et *ELOVL2-5* sur les indices d'activité des désaturases et des élongases : résultats d'une supplémentation en huile de poisson

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Effects of *FADS* and *ELOVL* polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation.

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Running title: *FADS* and *ELOVL* SNPs on desaturase and elongase activities

Résumé

Des SNPs dans le groupe de gènes *FADS1-2-3* et la famille de gènes *ELOVL2-5* influenceraient les activités enzymatiques suite à une supplémentation en AGPI n-3. Les objectifs de l'étude sont de vérifier si une supplémentation en AGPI n-3 est associée aux indices d'activité des désaturases et des élongases et de vérifier si les SNPs dans le groupe de gènes *FADS1-2-3* et la famille de gènes *ELOVL2-5* modulent les activités enzymatiques des désaturases et des élongases. Un total de 208 sujets a complété une période de supplémentation de 6 semaines avec 5 g/jour d'huile de poisson (1,9-2,2 g/jour d'AEP + 1,1 g/jour d'ADH). Le dosage des AG des PPLs plasmatiques a été obtenu par chromatographie en phase gazeuse. Les indices d'activité des désaturases et des élongases ont été estimés en utilisant les rapports produits sur substrats. Vingt-huit SNPs provenant des gènes *FADS1, FADS2, FADS3, ELOVL2* et *ELOVL5* ont été génotypés à l'aide de la technologie TaqMan. Les indices d'activité des désaturases étaient significativement différents après la supplémentation en AGPI n-3 de 6 semaines. L'indice d'activité de la désaturase δ-5 a augmenté de 25,7 ± 28,8% ($p < 0,0001$), tandis que l'indice d'activité de la désaturase δ-6 a diminué de 17,7 ± 18,2% ($p < 0,0001$) après la supplémentation. L'indice d'activité de l'élongase a diminué de 39,5 ± 27,9% ($p < 0,0001$). Des effets d'interactions gène-diète modulant potentiellement les indices d'activité enzymatique des désaturases et des élongases impliquées dans le métabolisme des AGPI n-3 et n-6 ont été observées. Les SNPs du groupe de gènes *FADS1-2-3* et de la famille de gènes *ELOVL2-5* jouent un rôle important dans l'activité enzymatique des désaturases et des élongases, suggérant qu'une supplémentation en AGPI n-3 pourrait affecter le métabolisme des AGPI.

Abstract

Polymorphisms (SNPs) within the *FADS* gene cluster and the *ELOVL* gene family are believed to influence enzyme activities after an omega-3 (n-3) fatty acid (FA) supplementation. The objectives of the study are to test whether an n-3 supplementation is associated with indexes of desaturase and elongase activities in addition to verify whether SNPs in the *FADS* gene cluster and the *ELOVL* gene family modulate enzyme activities of desaturases and elongases. A total of 208 subjects completed a 6-week supplementation period with 5 g/day of fish oil (1.9–2.2 g/day of EPA + 1.1 g/day of DHA). FA profiles of plasma phospholipids were obtained by gas chromatography (n = 210). Desaturase and elongase indexes were estimated using product-to-precursor ratios. Twenty-eight SNPs from *FADS1*, *FADS2*, *FADS3, ELOVL2* and *ELOVL5* were genotyped using TaqMan technology. Desaturase indexes were significantly different after the 6-week n-3 supplementation. The index of δ-5 desaturase activity increased by 25.7 ± 28.8 % ($p < 0.0001$), whereas the index of δ-6 desaturase activity decreased by 17.7 ± 18.2 % ($p < 0.0001$) post-supplementation. Index of elongase activity decreased by 39.5 ± 27.9 % ($p < 0.0001$). Some gene-diet interactions potentially modulating the enzyme activities of desaturases and elongases involved in the FA metabolism post-supplementation were found. SNPs within the *FADS* gene cluster and the *ELOVL* gene family may play an important role in the enzyme activity of desaturases and elongases, suggesting that an n-3 FAs supplementation may affect PUFA metabolism.

Introduction

Elongation and desaturation of long-chain (LC) PUFA of the (n-3) family are made possible by enzymes called desaturases and elongases. Figure 1 shows the metabolic pathway of n-3 and n-6 fatty acids (FA) with an emphasis on enzymes required for synthesis of LC-PUFAs. The use of the δ-6 desaturases (D6D) twice in the conversion of alpha-linolenic acid (ALA) to docosahexaenoic acid (DHA) suggests that this enzyme may play a key regulatory role in the PUFA metabolism (Portolesi et al. 2007).

Fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*, respectively) genes encode for key enzymes in the PUFA metabolism, the δ-5 desaturase (D5D) and D6D, respectively (Malerba et al. 2008). These desaturases are responsible for the double bond formation between two carbons leading to more unsaturated FAs. Elongases are encoded by genes within the *ELOVL* family and catalyzes the elongation of the aliphatic chain of carbons leading to the formation of LC-PUFAs (Jakobsson et al. 2006). Two elongases are involved in LC-PUFA synthesis, the fatty acid elongase-2 and the fatty acid elongase-5, encoded, respectively, by *ELOVL2* and *ELOVL5* genes. A high desaturase activity may lead to an increased bioavailability of ARA with dominant synthesis of ARA-derived proinflammatory eicosanoids, possibly leading to vascular damage, especially in populations eating a Western diet (Martinelli et al. 2009). Excessive amounts of n-6 FAs from the diet and from the endogenous synthesis, as seen in today's Western diets, promote the pathogenesis of many diseases including cardiovascular disease, cancers as well as inflammatory and autoimmune diseases, whereas increased levels of n-3 FA (a lower n-6/n-3 ratio) exert suppressive effects (Simopoulos 2008). In opposition to a diet rich in n-6 PUFA, a diet rich in n-3 FAs could result in a preferential synthesis of anti-inflammatory eicosanoids in addition to a high desaturase activity (Martinelli et al. 2009). A shift from n-6 to n-3 induces changes in the eicosanoid profile, which may lead to a decrease inflammatory state (Calder 2009).

There is a high affinity of D6D for ALA (D'Andrea et al. 2002), thus favoring the n-3 FA pathway at the expense of the n-6 pathway (Sprecher 2002). These two classes of FAs compete for a number of enzyme systems which can influence inflammatory responses,

vascular reactivity, and platelet aggregation (Harris 2006). Moreover, oleic acid (18:1n-9) from the omega-9 (n-9) pathway is also metabolized by D5D and D6D (Das 2007). As a result, all these series of FAs, i.e., n-3, n-6, and n-9, compete for the same set of enzymes, although a preferential affinity has been demonstrated according to the following sequence: n-3 > n-6 > n-9 (Das 2007).

Recent studies suggested that some common SNPs of the *FADS* gene cluster and of the *ELOVL* gene family (Tanaka et al. 2009) are associated with plasma n-6 and n-3 FA levels. Accordingly, Lemaitre et al. (2011) have shown, in a genome-wide association studies in five population-based cohorts comprising 8,866 subjects of European ancestry, that SNPs from the *FADS* gene cluster and from *ELOVL2* gene were associated with plasma eicosapentaenoic acid, DHA and ALA levels measured in plasma phospholipids. SNPs within the *FADS* gene cluster, in particular rs175547, are also believed to alter desaturase activity in two different ethnic populations, Caucasian and Asian, as shown by Merino et al. (2011).

The objectives of the present study are to test whether n-3 FA supplementation is associated with indexes of desaturase and elongase activities as determined by product-to-precursor ratios in addition to verify whether SNPs in the *FADS* gene cluster and the *ELOVL* gene family modulate enzyme activities of desaturases and elongases following an n-3 FA supplementation.

Methods

Sociodemographic

Two hundred and fifty-four unrelated subjects from the greater Quebec City metropolitan area were recruited between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees. To be eligible for the study, subjects had to be aged between 18 and 50 years with a body mass index (BMI) between 25 and 40 kg/m^2. They had to be non-smokers and free of any thyroid or metabolic disorders such as hypo/hyperthyroidism, dyslipidemia, and type 2 diabetes among others. Subjects were excluded if they had taken fish oil supplements for at least 6 months prior to the beginning of the study and if their baseline TG levels were above 4.0 mmol/l. Statistical analyses were performed on 210 individuals who completed the supplementation period.

Study design

The complete study design has been previously reported (Cormier et al. 2012). Briefly, subjects followed a run-in period of 2 weeks where a trained registered dietitian gave individual dietary instructions. Recommendations were drawn from the *Canada's Food Guide to Healthy Eating* (Eating Well with Canada's Food Guide 2007). After a 2-week run-in period, each participant received a bottle containing n-3 FA capsules (Ocean Nutrition, Nova Scotia, Canada) covering the following 6-week period. They had to take five capsules per day, which gave them a total of 3–3.3 g of n-3 FA (1.9–2.2 g EPA and 1.1 g DHA) per day. Subjects had to report any deviations that may have occurred during the protocol. They also had to write down their alcohol and fish consumption on a log sheet. Before each phase of the study, subjects received written and oral dietary instructions by a registered dietitian.

SNPs selection and genotyping

SNPs in *FADS1*, *FADS2*, *FADS3, ELOVL2* and *ELOVL5* were identified using the International HapMap Project SNP database, based on the NCBI B36 assembly Data Rel 28. phase II + III, build 126. The *FADS* gene cluster is made of three genes that are located very close to each other among chromosome 11. Because of the head-to-head orientation of

FADS1 and *FADS2* and the tail-to-tail orientation of *FADS2* and *FADS3*, we added 500-kilo base pairs (kbp) downstream of *FADS1* and 2,500-kbp upstream of *FADS3* to cover the 5′UTR and 3′UTR regions. Intergenic areas comprised between *FADS1* and *FADS2* and between *FADS2* and *FADS3* were also covered. *ELOVL2* and *ELOVL5* genes are both located on chromosome 6. Gene Tagger procedure in Haploview V4.2 was used to determine tag SNPs using a minor allele frequency ≥1 % and pairwise tagging ($r^2 \geq 0.8$). Subsequently, we examined linkage disequilibrium out of the nineteen SNPs of the *FADS* gene cluster area, the 4 tSNPs of the *ELOVL5* gene, and the 6 tSNPs of the *ELOVL2* gene using the LD Plot procedure in Haploview V4.2. The SIGMA GenElute Gel Extraction Kit (Sigma-Aldrich Co. St.Louis. Missouri. USA) has been used to extract genomic DNA. Selected SNPs of the *FADS* gene cluster (rs174456, rs174627, rs482548, rs2072114, rs12807005, rs174448, rs2845573, rs7394871, rs7942717, rs74823126, rs174602, rs498793, rs7935946, rs174546, rs174570, rs174579, rs174611, rs174616 and rs968567) and of the *ELOVL* genes (rs209492, rs2073040, rs2294852, rs9370194, rs13204015, rs12195587, rs4532436, rs3734397, rs2281591 and rs3798710) have been genotyped using validated primers and TaqMan probes (Life Technologies Corporation, Burlington, On.) (Livak 1999). DNA was mixed with TaqMan Universal PCR Master Mix (Life Technologies Corporation, Burlington, On.), with a gene-specific primer and with probe mixture (predeveloped TaqMan SNP Genotyping Assays; Life Technologies Corporation, Burlington, On.) in a final volume of 10 µl. Genotypes were determined using a 7500 RT-PCR System and analyzed using ABI Prism SDS version 2.0.5 (Life Technologies Corporation, Burlington, On.).

Measurement of FA composition in plasma phospholipids

Subjects arrived at the research center following an overnight fast. FA composition of plasma phospholipids was determined by gas chromatography. Venous blood was drawn into EDTA tubes, and plasma was immediately separated by centrifugation at 500 g for 6 min and stored at −80 °C for subsequent analyses. Plasma lipids were extracted with chloroform:methanol (2:1, by volume) according to a modified Folch method (Shaikh and Downar 1981). Total phospholipids were then isolated with isopropyl ether:acetic acid (96:4) by thin layer chromatography (Lepage and Roy 1986). Isolated plasma phospholipids were then methylated (Lepage and Roy 1986). FA profiles were obtained after methylation in

methanol/benzene 4:1 (v/v) (Lepage and Roy 1986) and capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, Canada) equipped with a HP-88 capillary column (100 m × 0.25 mm i.d. × 0.20 μm film thickness; Agilent Technologies, Palo Atto, CA) coupled with a flame ionization detector (FID). Helium was used as carrier gas (split ratio 1:80). FA were identified according to their retention time, using the following standard mixtures as a basis for comparison: the FAME 37 mix (Supelco Inc., Bellefonte, PA) and the GLC-411 FA mix (NuChek Prep Inc, Elysian, MN), as well as the following methylated FAs C22:5n-6 (Larodan AB, Malmö, Sweden) and C22:5n-3 (Supelco Inc., Bellefonte, PA). Phospholipids FA profiles were expressed as the relative percentage areas of total FAs. The database and ontology of Chemical Entities of Biological Interest (ChEBI) has been used to identify FAs herein.

Estimates of D5D and D6D activities were computed using product-to-precursor ratios: (C20:4n-6, ARA):(C20:3n-6, dihomo-γ-linolenic acid (DGLA)), and (C20:3n-6, DGLA):(C18:2n-6, linoleic acid (LA)), respectively, as previously described (Bokor et al. 2010). The index of elongase activity was calculated using the (C22:4n-6):(C20:4n-6, ARA) ratio.

Statistical analysis

All genotype distributions were tested for any deviation from Hardy–Weinberg equilibrium (HWE) using the ALLELE procedure in S.A.S Genetics v9.3 (S.A.S Institute Inc., Cary, North Carolina, USA). Significance testing for linkage disequilibrium coefficient D was obtained using a Chi-square test, likelihood ratio and Fisher's exact test ($p \leq 0.01$). All other statistical analyses were carried out using S.A.S statistical software v9.3 (S.A.S Institute Inc., Cary, North Carolina, USA). Normal distribution was evaluated using the box-plot, as well as skewness and kurtosis ranges. When needed, variables non-normally distributed were $\log_{10}$-transformed. A linear regression using the stepwise bidirectional elimination approach was used to assess which SNPs could explain part of the enzyme activity's variance where the effects of the SNPs, age, sex, and BMI were included in the statistical model and where the effect of baseline enzyme activity was added (in post-supplementation only). The MIXED procedure was used to test for the effects of the genotype, the supplementation (effect of

time) and the genotype × supplementation interaction for each SNP on estimates of enzyme activities when age, sex and BMI were included in the model. The repeated statement was used to indicate the within subjects (repeated) variables. Genotype groups were assessed as three groups expressed as major allele homozygotes, heterozygotes and minor allele homozygotes. For some SNPs, heterozygotes and minor allele homozygotes were grouped if the genotype frequency was under 5 %. The statistical significance was defined as $p \leq 0.05$. Since polymorphisms tested in complex diseases rarely account for a large proportion of the variance, results are thus presented before correction for multiple testing and using a $p \leq 0.05$.

Results

Subjects' characteristics pre- and post-supplementation are presented in Table 1. Surrogate estimates of D5D activity increased by 25.7 ± 28.8 % (Mean ± SD, $p < 0.0001$), whereas those for the D6D activity decreased by 17.7 ± 18.2 % ($p < 0.0001$) after the supplementation. Index of elongase activity decreased by 39.5 ± 27.9 % ($p < 0.0001$) (Table 1). TG, insulin, total-cholesterol and LDL-cholesterol levels were inversely associated with D5D activity and positively associated with D6D activity after the supplementation (data not shown, $p < 0.008$ for all). Moreover, a decrease in plasma levels of n-6 FAs was observed: LA (–9.85 ± 10.76 %, $p < 0.0001$); ARA (–11.20 ± 9.99 %, $p < 0.0001$); as well as a decrease in plasma levels of ALA (–31.44 ± 50.44 %, $p = 0.0002$) following the intervention. On the n-3 FAs side, an increase was observed for plasma levels of EPA (+331.11 ± 214.74 %, $p < 0.0001$) and DHA (+46.31 ± 27.46 %, $p < 0.0001$). The same pattern was also observed for absolute quantities of FAs (μg/ml) where similar rates of change were revealed as shown in Table 1.

All SNPs were in HWE except rs7935946 (*FADS*) that was not considered for further analyses. All selected SNPs are presented in Table 2 and LD tables are presented in Tables 3, 4, and 5. To validate the presence of associations between surrogate estimates of enzyme activities involved in the n-3 FA synthesis pathway with SNPs of the *FADS* gene cluster or *ELOVL* gene family, all SNPs were included in a stepwise regression model adjusted for the effects of age, sex and BMI. Prior to the n-3 FA supplementation, three SNPs were associated with the estimates of D5D activity: rs968567 ($R^2 = 13.83$ %, $p < 0.0001$), rs2845573 ($R^2 = 11.65$ %, $p < 0.0001$) and rs7394871 ($R^2 = 4.25$ %, $p < 0.0001$), one SNP was associated with estimates of D6D activity: rs968567 ($R^2 = 2.86$ %, $p = 0.01$) and two SNPs were associated with the index of elongase activity: rs2281591 ($R^2 = 3.21$ %, $p = 0.008$) and rs3798710 ($R^2 = 2.79$ %, $p = 0.01$). To account for the 6-week supplementation, the same statistical model was used with data post-n-3 FA supplementation as the dependent variable, adjusted for baseline data, age, sex and BMI. With the use of the stepwise bidirectional selection method, five SNPs were associated with the post-supplementation estimates of D5D activity: rs174546 ($R^2 = 8.26$ %, $p < 0.0001$), rs968567 ($R^2 = 2.47$ %, $p = 0.004$), rs7935946 ($R^2 = 2.42$ %, $p = 0.004$), rs2072114 ($R^2 = 2.92$ %, $p = 0.001$) and rs12807005 ($R^2 = 1.20$ %, $p =$

0.04); one SNP was associated with post-supplementation estimates of D6D activity: rs12807005 ($R^2 = 0.92$ %, $p = 0.04$); and none of the SNPs were associated with the index of elongase activity after an n-3 FA supplementation (data not shown) (Tables 6, 7, 8).

In a MIXED model for repeated measures including the effects of time (supplementation), genotype and the interaction (genotype × supplementation) adjusted for age, sex and BMI, ten SNPs of the *FADS* gene cluster (rs174546, rs2072114, rs2845573, rs174570, rs174579, rs174611, rs174616, rs968567, rs174627 and rs174448) were associated with estimates of D5D activity ($p < 0.04$, for all). Three SNPs were associated with surrogate estimates of D6D activity (rs174546, rs175579 and rs968567, $p < 0.04$, for all), and one SNP was associated with the index of elongase activity: rs2281591 ($p = 0.01$) (Tables 6, 7, 8). Results in Tables 6, 7, and 8 were only provided for frequent SNPs with MAFs >20 % and/or for significant gene–diet interaction effects. Some gene–diet interaction effects with SNPs within the *FADS* gene cluster or the *ELOVL* gene family were found and could potentially modulate the enzyme activities of desaturases and elongases following the n-3 FA supplementation. Two gene-diet interactions on D6D activities were found with SNPs of the *FADS* gene cluster for rs174611 ($p = 0.01$) and rs174627 ($p = 0.02$) after the n-3 FA supplementation, and two gene–diet interactions on the elongase activity were also found with SNPs of the *ELOVL* gene family for rs12195587 ($p = 0.02$) and rs4532436 ($p = 0.05$) (Fig. 2).

Discussion

In this study, we tested whether a 6-week supplementation with high doses of n-3 FA above 3 g/day led to differences in enzyme activities expressed as surrogate estimates of D5D and D6D activities and index of elongase activity, as well as if SNPs of genes encoding for these enzymes modulate the enzyme activity following a 6-week n-3 FA supplementation. To do so, 28 SNPs were selected from five genes (*FADS1, FADS2, FADS3, ELOVL2* and *ELOVL5*) covering at least 85 % of the common genetic variations.

In the present study, a decrease in plasma levels of n-6 FAs (LA, DGLA, ARA and adrenic acid) was observed as well as a decrease in plasma levels of ALA, an essential n-3 FA that is also the precursor for the synthesis of EPA and DHA. Subsequently, an increase in the estimate of D5D activity and a decrease in the estimate of D6D activity and the index of elongase activity were observed. D5D activity was negatively associated with cardio-metabolic risk factors, while D6D activity was positively associated. Minor alleles of some SNPs in the *FADS* gene cluster are associated with higher D6D activity and with lower D5D activity in French Canadians while minor allele of SNPs from the *ELOVL* gene family are associated with higher elongase activity following a 6-week supplementation with 5 g/day of fish oil.

The endogenous synthesis of LC-PUFAs is made possible by concerted actions between elongases and desaturases. Index of elongase activity has been much less extensively studied in the context of CVD than desaturase activity was, but Sethom et al. (2011) have shown that elongase and D5D activities tended to decrease according to the number of metabolic syndrome features in Tunisian patients (Sethom et al. 2011). In the current study, we observed a significant increase of D5D activity following an n-3 FA supplementation meaning that the n-3 FA supplementation may exert beneficial effects on CVD risk factors thus potentially impacting on obesity and metabolic syndrome features. A recent study suggested that fatty acid elongase-5 (encoded by *ELOVL5* gene) plays a key role in regulating hepatic lipid and carbohydrate metabolism (Wang et al. 2008). Fatty acid elongase-5 elongates γ-linolenic acid (GLA, C18:3n-6) to form DGLA (C20:3n-6) which is converted

to ARA (C20:4n-6) by the D5D (*FADS1*) in the n-6 metabolism (Moon et al. 2009; Qin et al. 2009).

There is a competition between ALA and C24:5n-3 (tetracosapentaenoic acid) for the D6D, which could lead to decreased levels of DHA after certain intakes of dietary ALA (Gregory et al. 2011). However, in the present study, EPA + DHA was given directly via fish oil supplements, increasing considerably DHA intake and favoring the incorporation of n-3 FA in plasma phospholipids at the expense of the incorporation of n-6 s. Thus, we hypothesized that the D6D (*FADS2*) is more likely to be used to convert ALA in C18:4n-3 as shown by decreased ALA levels in plasma phospholipids (Table 1). Gregory et al. (2011) also observed a saturation of the second FA elongase-2 reaction (DPA, C22:5n-3 → C24:5n3) that could potentially explain the accumulation of DPA when EPA is provided in the diet. In the present study, plasma phospholipid EPA levels increased by 330.0 ± 214.1 % after the 6-week supplementation and DPA increased by 49.0 ± 36.8 % (Table 1).

Since D6D is the rate-limiting enzyme in the PUFA metabolism (Sprecher 2002) and is not specific to a single pathway, one could pretend that giving high doses of n-3 possibly activates the n-3 FA pathway and favors the incorporation of n-3 FAs at the expense of the incorporation of n-6 FAs. Moreover, the use of D6D twice in the conversion of LC-PUFAs suggests that this enzyme may play a key regulatory role in the PUFA metabolism (Portolesi et al. 2008).

In the present study, we found two gene-diet interactions with SNPs from the *FADS* gene cluster and the n-3 FA supplementation modulating surrogate estimates of D6D activity where carriers of the minor allele had the lowest activity. Warensjo et al. confirmed that estimates of D5D activity are inversely related to obesity and insulin resistance, whereas D6D activity shows positive associations (Warensjo et al. 2009). Increased estimates of D6D activities and decreased estimates of D5D and elongase activities were also associated with adverse profiles of several metabolic risk factors in a group of free-living young Japanese women (Murakami et al. 2008). After the 6-week n-3 FA supplementation, we observed an increase in the estimates of D5D activity and a decrease in the estimates of D6D activity

meaning that n-3 FA supplements may be beneficial in the prevention of obesity and insulin resistance. SNPs within the *FADS* gene cluster are believed to alter desaturase activity as shown in a recent paper by Merino et al. (2011), especially rs175547 (in high LD with rs174546, $r^2 = 1.0$) thought to be a causal SNP in the *FADS* gene cluster (Merino et al. 2011). Herein, minor allele homozygotes of all SNPs (or minor allele homozygotes + heterozygotes for SNPs included in the dominant model) that were associated with estimates of D5D activity always exhibited the lowest desaturase activity after the 6-week n-3 FA supplementation, suggesting that they may be at higher risk to develop obesity-related metabolic complications.

Previous studies have also reported associations between SNPs within the *FADS* gene cluster and estimates of desaturase activities (Bokor et al. 2010; Martinelli et al. 2008). Recently, Gillingham et al. (2013) have studied the effects of diets enriched in flaxseed oil or high-oleic acid canola oil and SNPs from *FADS1-2* genes on plasma FAs and found several SNPs associated with [U-^{13}C] ALA metabolism, a precise measure of desaturase activity. Al-Hilal et al. (2013) have shown that genotypes of three SNPs located in the *FADS1/FADS2* gene cluster were strongly associated with proportions of LC-PUFAs and desaturase activities estimated in plasma and in erythrocytes. The same research group has also shown that a higher EPA + DHA dosage reduced n-6 and increased n-3 LC-PUFA proportions and that D5D desaturase activity increased which is concomitant with results observed here (Al-Hilal et al. 2013). In the present study, we have found three significant gene–diet interactions after a 6-week n-3 FA supplementation. In addition, some SNPs were associated with indexes of desaturase or elongase activities independently of the supplementation.

Strengths and limitations

Indexes of desaturase and elongase activities were estimated using product-to-precursor ratios as surrogate markers of desaturase or elongase activities, because direct measures were not possible. The quantification of the GLA FA was not feasible resulting from low proportions of that specific FA in plasma phospholipids. Although there is an elongation step comprised in the (20:3n-6)/(18:2n-6) ratio, it should be noted that this is not considered as the rate-limiting step in the n-6 FAs synthesis. Consequently, the (20:3n-6)/(18:2n-6) ratio

was considered as good estimates and was used to assess the estimate of D6D activity (Bokor et al. 2010; Krachler et al. 2008; Warensjo et al. 2008). Indexes of desaturase and elongase activities depend not only on enzyme activities, but also on PUFA intake.

Conclusion

In summary, a 6-week fish oil supplementation decreased plasma levels of n-6 and increased plasma n-3 FA levels impacting desaturase and elongase activities. Genetic predispositions on genes coding for these enzymes may lead to more or less LC-PUFA conversion depending on the genotype. Some SNPs of the *FADS* gene cluster and the *ELOVL* gene family may play an important role in the enzyme activity of desaturases and elongases, suggesting that a supplementation with n-3 FA may affect PUFA metabolism.

Declarations

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Conflict of interest

Hubert Cormier, Iwona Rudkowska, Simone Lemieux, Patrick Couture, Pierre Julien, and Marie-Claude Vohl declare no conflict of interest.

Ethical standard

The ethics committees of Laval University Hospital Research Center and Laval University approved the experimental protocol. This clinical trial was registered at clinicaltrials.gov (NCT01343342).

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Tableau 5.1 Characteristics of the subjects.

	Pre-n-3 PUFA supplementation	Post-n-3 PUFA supplementation	Δ	*p*-value
Sex (men/women)	97/113			
Age, yrs	30.8 ± 8.7			
Weight, kg	81.3 ± 13.9	81.6 ± 14.2		0.82
BMI, kg/m²	27.8 ± 3.7	27.9 ± 3.8		0.81
Plasma desaturase activity				
D5D (20:4n-6/20:3n-6)	3.57 ± 1.15	4.34 ± 1.25	25.71 ± 28.83 (n=210)	<0.0001
D6D (20:3n-6/18:2n-6)	0.17 ± 0.05	0.14 ± 0.04	-17.68 ± 18.15 (n=210)	<0.0001
Plasma elongase activity				
ELOVL2 (22:4n-6/20:4n-6)	0.0318 ± 0.0064	0.0193 ± 0.0093	-39.52 ± 27.89 (n=209)	<0.0001
Omega-6 (% of total fatty acids)				
Linoleic acid, C18:2n-6, LA	19.71 ± 2.08	17.69 ± 2.22	-9.85 ± 10.76	<0.0001
Arachidonic acid, C20:4n-6, ARA	11.05 ± 1.74	9.74 ± 1.44	-11.20 ± 9.99	<0.0001
Omega-3 (% of total fatty acids)				
Alpha-linolenic acid, C18:3n-3, ALA	0.18 ± 0.15	0.14 ± 0.12	-31.44 ± 50.44	0.0002
Eicosapentaenoic acid, C20:5n-3, EPA	1.12 ± 0.52	4.10 ± 1.28	331.11 ± 214.74	<0.0001
Docosahexaenoic acid, C22:6n-3, DHA	3.53 ± 0.77	5.03 ± 0.86	46.31 ± 27.46	<0.0001
Omega-6 (µg/ml)				
Linoleic acid C18:2n-6, LA	227.26 ± 44.46	199.16 ± 45.38	-11.63 ± 15.82	<0.0001
Arachidonic acid, C20:4n-6, ARA	128.14 ± 32.77	109.63 ± 27.03	-13.04 ± 14.26	<0.0001
Omega-3 (µg/ml)				
Alpha-linolenic acid, C18:3n-3, ALA	2.22 ± 1.89	1.72 ± 1.52	-32.08 ± 51.42	<0.0001
Eicosapentaenoic acid, C20:5n-3, EPA	12.92 ± 6.41	46.15 ± 16.99	328.79 ± 227.51	<0.0001
Docosahexaenoic acid, C22:6n-3, DHA	40.97 ± 12.54	57.04 ± 16.05	43.55 ± 33.50	<0.0001

Measurements made prior to- and after a 6-week n-3 PUFA supplementation; Values are means ± SD
The rate of change (Δ) expressed as the relative variation of plasma FAs as a percentage (%) or absolute quantities (µg/ml) between the pre- and post-n-3 PUFA period. Differences between the pre- and post-n-3 PUFA supplementation were tested using a pairwise Student's T-test.

Tableau 5.2 Characteristics of SNPs within the *FADS* gene cluster and *ELOVL* gene family.

dbSNP No.[a]	Sequence[b]	Position	Alleles (major/ minor)	AA	CC	CA	CG	CT	GA	GG	GT	TT	MAF
							n[c] (%)						
FADS1													
rs174546	TGC[**C/T**]TTG	3′ UTR	C/T		103 (49.8)			86 (41.2)				10 (9.1)	T=29.8
FADS2													
rs482548	CAC[**C/T**]GTG	3′ UTR	C/T		161 (77.8)			40 (19.3)				6 (2.9)	T=12.4
rs2072114	TTC[**A/G**]GGT	Intron	A/G	167 (79.9)					38 (18.2)	4 (1.9)			G=10.9
rs2845573	TCA[**C/T**]GTT	Intron	A/G	177 (84.7)					30 (14.4)	2 (1.0)			G=8.1
rs174602	CCC[**A/G**]TCC	Intron	T/C		9 (4.3)			59 (28.2)				141 (67.5)	C=18.8
rs498793	AAC[**A/G**]CAG	Intron	C/T		62 (9.8)			99 (71.7)				43 (18.6)	T=42.0
rs174570	TGA[**C/T**]GTA	Intron	C/T		159 (76.4)			46 (22.1)				3 (1.4)	T=12.4
rs174579	TTT[**C/T**]CAG	Intron	C/T		127 (61.1)			78 (37.5)				3 (1.4)	T=20.3
rs174611	GGA[**C/T**]CCT	Intron	T/C		12 (5.7)			84 (40.2)				113 (54.1)	C=25.9
rs174616	TCA[**C/T**]GTT	Intron	G/A	51 (24.4)					158 (51.7)	50 (23.9)			G=49.5
rs968567	CGG[**A/G**]AGC	5′ UTR	G/A	2 (1.0)					63 (30.1)	144 (68.9)			A=7.1
rs7935946	TTC[C/T]GGG	Intron			195 (93.3)			11 (5.3)				3 (1.4)	
FADS3													
rs174456	TAC[**A/C**]TGG	Intron	A/C	102 (48.8)	18 (8.6)	89 (42.6)							C=30.0
rs7394871	GAC[**A/C**]CCT	Intron	C/A	2 (1.0)	181 (86.6)	26 (12.4)							A=7.1
rs7942717	ACG[**A/G**]GTG	Intron	A/G	161 (77.0)					47 (22.5)	1 (0.5)			G=11.7
Intergenic regions within the *FADS* gene cluster													
rs174627	CTG[**C/T**]GTA	Intergenic	G/A	2 (1.0)					48 (23.0)	159 (76.1)			A=12.6
rs12807005	ATG[**A/G**]ATC	Intergenic	G/A	0 (0)					5 (2.4)	204 (97.6)			A=1.2
rs174448	TGA[**C/T**]TTC	Intergenic	A/G	78 (37.5)					109 (52.4)	21 (10.1)			G=36.3
rs7482316	CAA[**A/G**]CTG	Intergenic	A/G	168 (80.4)					39 (18.7)	2 (1.0)			G=10.2
ELOVL2													
rs13204015	TTC[**C/T**]TTT	Intron	T/C		0 (0)			17 (8.1)				191 (91.9)	C=4.1
rs12195587	AAC[**A/G**]TAA	Exon	G/A	1 (0.5)					59 (28.2)	149 (71.3)			A=14.6
rs4532436	AGC[**C/G**]AAT	3′ UTR	C/G		43 (21.0)		121 (59.0)			41 (20.0)			G=49.5
rs3734397	CTC[**A/G**]GTA	3′ UTR	C/G		129 (61.4)				69 (32.9)	15 (5.7)			G=22.1
rs2281591	TCT[**A/G**]TTT	Intron	A/G	102 (48.6)					93 (44.4)	15 (7.1)			G=29.3
rs3798710	TTT[**C/G**]AAC	Intron	C/G		139 (66.2)		62 (29.5)			9 (4.3)			G=19.1
ELOVL5													
rs209492	TTG[**C/T**]TTA	Intron	T/C		4 (1.9)			47 (22.4)				159 (75.7)	C=13.1
rs2073040	AGC[**A/G**]GAT	Intron	A/G	82 (39.1)					102 (48.6)	26 (12.4)			G=36.7
rs2294852	CCA[**C/G**]GTT	Intron	C/G		59 (28.1)		103 (49.1)			48 (22.9)			G=47.4
rs9370194	GAC[**C/T**]GTT	Intron	C/T		104 (49.5)			96 (45.7)				10 (4.8)	T=27.6

MAF = Minor allele frequency from the FAS cohort, calculated with the ALLELE procedure in SAS Genetics v9.3;
[a] dbSNP No. from HapMap Data Rel 28 Phase II + III, August 10 on NCBI b36 Assembly dbSNP b126 database.
[b] Genes sequences from dbSNP short genetics variations NCBI reference assembly.
[c] Number of subjects for each genotype.

Tableau 5.3 Linkage disequilibrium (R^2) of the tagging SNPs within the *FADS* gene cluster.

	rs174546	rs12807005	rs968567	rs174570	rs2845573	rs2072114	rs174579	rs7935946	rs174602	rs498793	rs174611	rs174616	rs482548	rs174627	rs174448	rs7482316	rs7942717	rs7394871	rs174456
rs174546		0.016	0.424	0.304	0.159	0.223	0.568	0.054	0.229	0.04	0.452	0.287	0.047	0.232	0.363	0.067	0.021	0.037	0.222
rs12807005	0.016		0.007	0.005	0.003	0.004	0.01	0.001	0.007	0.02	0.003	0.001	0.029	0	0.004	0.003	0.002	0.002	0.001
rs968567	0.424	0.007		0.038	0.02	0.014	0.636	0	0.02	0.038	0.351	0.204	0.02	0.486	0.261	0.009	0.002	0.012	0.292
rs174570	0.304	0.005	0.038		0.522	0.345	0.028	0.005	0.1	0.01	0.033	0.035	0.014	0.013	0.037	0.124	0.008	0.177	0.001
rs2845573	0.159	0.003	0.02	0.522		0.714	0.029	0.003	0.021	0.03	0.026	0.016	0	0.008	0.015	0.154	0.007	0.357	0.002
rs2072114	0.223	0.004	0.014	0.345	0.714		0.04	0.242	0.004	0.001	0.036	0.031	0.008	0.006	0.037	0.191	0.018	0.249	0
rs174579	0.568	0.01	0.636	0.028	0.029	0.04		0.01	0.203	0.025	0.34	0.227	0.029	0.371	0.312	0.001	0.004	0.018	0.292
rs7935946	0.054	0.001	0	0.005	0.003	0.242	0.01		0.066	0.019	0.008	0.014	0.003	0	0.022	0.031	0.011	0.002	0.002
rs174602	0.229	0.007	0.02	0.1	0.021	0.004	0.203	0.066		0.002	0.11	0.123	0.021	0.005	0.199	0.094	0.085	0.013	0.034
rs498793	0.04	0.02	0.038	0.01	0.03	0.001	0.025	0.019	0.002		0.109	0.121	0.033	0.033	0.049	0.025	0.002	0.017	0.022
rs174611	0.452	0.003	0.351	0.033	0.026	0.036	0.34	0.008	0.11	0.109		0.622	0.046	0.475	0.729	0.202	0.014	0.066	0.342
rs174616	0.287	0.001	0.204	0.035	0.016	0.031	0.227	0.014	0.123	0.121	0.622		0.068	0.323	0.68	0.125	0.043	0.035	0.369
rs482548	0.047	0.029	0.02	0.014	0	0.008	0.029	0.003	0.021	0.033	0.046	0.068		0.024	0.053	0.009	0.004	0.005	0.032
rs174627	0.232	0	0.486	0.013	0.008	0.006	0.371	0	0.005	0.033	0.475	0.323	0.024		0.408	0.029	0.017	0.001	0.484
rs174448	0.363	0.004	0.261	0.037	0.015	0.037	0.312	0.022	0.199	0.049	0.729	0.68	0.053	0.408		0.174	0.098	0.053	0.568
rs7482316	0.067	0.003	0.009	0.124	0.154	0.191	0.001	0.031	0.094	0.025	0.202	0.125	0.009	0.029	0.174		0	0.283	0.02
rs7942717	0.021	0.002	0.002	0.008	0.007	0.018	0.004	0.011	0.085	0.002	0.014	0.043	0.004	0.017	0.098	0		0.003	0.161
rs7394871	0.037	0.002	0.012	0.177	0.357	0.249	0.018	0.002	0.013	0.017	0.066	0.035	0.005	0.001	0.053	0.283	0.003		0.02
rs174456	0.222	0.001	0.292	0.001	0.002	0	0.292	0.002	0.034	0.022	0.342	0.369	0.032	0.484	0.568	0.02	0.161	0.02	

Tableau 5.4 Linkage disequilibrium (R^2) of the tagging SNPs within the *ELOVL2* gene.

	rs4532436	r s12195587	rs2281591	rs911196	rs976081	rs13204015
rs4532436		0.169	0.372	0.301	0.237	0.061
rs12195587	0.169		0.063	0.051	0.056	0.297
rs2281591	0.372	0.063		0.08	0.088	0.023
rs911196	0.301	0.051	0.08		0.071	0.019
rs976081	0.237	0.056	0.088	0.071		0.001
rs13204015	0.061	0.297	0.023	0.019	0.001	

Tableau 5.5 Linkage disequilibrium (R^2) of the tagging SNPs within the *ELOVL5* gene cluster.

	rs2073040	**rs2294852**	**rs9370194**	**rs209492**
rs2073040		0.576	0.737	0.153
rs2294852	0.576		0.423	0.067
rs9370194	0.737	0.423		0.02
rs209492	0.153	0.067	0.02	

Tableau 5.6 δ-5 Desaturase indexes (ARA:DGLA) after a 6-week n-3 PUFA supplementation according to genotype for each SNPs of the *FADS* gene cluster. (n = 208)

	Pre-n-3 PUFA D5D activity[a]			Post-n-3 PUFA D5D activity[a]			*p*-values		
	11	**12 or 12+22**	**22**	**11**	**12 or 12+22**	**22**	**SNPs**	**Suppl.**	**Interaction**
FADS1									
rs174546	4.132±1.179	3.166±0.807	2.376±0.572	4.787±1.138[a]	4.091±1.123[b]	3.086±0.739[c]	<0.0001	<0.0001	0.20
FADS2									
rs498793	3.457±1.413	3.567±1.076	3.816±0.815	4.317±1.291	4.383±1.178	4.304±1.391	0.09	<0.0001	0.63
rs174579	3.860±1.222	3.130±0.886	--	4.575±1.282	3.981±1.108	--	<0.0001	<0.0001	0.32
rs174611	2.934±0.760	3.209±0.949	3.919±1.226	4.544±1.152	4.167±1.385	3.735±0.666	<0.0001	<0.0001	0.08
rs174616	3.272±1.203	3.563±1.162	3.904±0.019	4.485±1.098	4.469±1.298	3.948±1.129	0.04	<0.0001	0.09
FADS3									
rs174456	3.706±0.993	3.377±1.106	3.767±1.951	4.468±1.258	4.187±1.154	4.436±1.592	0.07	<0.0001	0.86
Intergenic regions									
rs174627	3.732±1.200	3.080±0.847	--	4.444±1.251	4.017±1.194	--	0.0006	<0.0001	0.14
rs12807005	3.569±1.150	3.700±1.547	--	4.360±1.257	3.609±0.488	--	0.56	0.02	0.07
rs174448	3.859±0.990	3.526±1.268	2.800±0.717	4.539±1.113	4.327±1.372	3.681±0.792	0.001	<0.0001	0.59

[a] Values are Means ± SD

Repeated MIXED procedure implemented in S.A.S statistical software v9.3 adjusted for age, sex, and BMI.

Tableau 5.7 δ-6 Desaturase indexes (DGLA:LA) after a 6-week n-3 PUFA supplementation according to genotype for each SNPs of the *FADS* gene cluster.

	Pre-n-3 PUFA D6D activity[a]			Post-n-3 PUFA D6D activity[a]			*p*-values		
	11	**12 or 12+22**	**22**	**11**	**12 or 12+22**	**22**	**SNPs**	**Suppl.**	**Interaction**
FADS1									
rs174546	0.160±0.043	0.179±0.046	0.180±0.054	0.132±0.038	0.143±0.042	0.142±0.041	0.02	<0.0001	0.23
FADS2									
rs498793	0.172±0.049	0.169±0.042	0.169±0.054	0.138±0.044	0.134±0.037	0.145±0.045	0.64	<0.0001	0.51
rs174579	0.166±0.044	0.176±0.048	--	0.135±0.041	0.141±0.040	--	0.04	<0.0001	0.23
rs174611	0.165±0.050	0.179±0.048	0.163±0.043	0.137±0.039	0.139±0.043	0.132±0.035	0.23	<0.0001	0.01
rs174616	0.178±0.045	0.168±0.048	0.164±0.043	0.139±0.036	0.132±0.041	0.147±0.043	0.34	<0.0001	0.11
FADS3									
rs174456	0.166±0.042	0.172±0.051	0.177±0.044	0.136±0.039	0.137±0.041	0.143±0.048	0.73	<0.0001	0.57
Intergenic regions									
rs174627	0.167±0.045	0.178±0.048	--	0.137±0.041	0.137±0.037	--	0.29	<0.0001	0.02
rs12807005	0.170±0.046	0.177±0.035	--	0.137±0.041	0.163±0.033	--	0.29	<0.0001	0.20
rs174448	0.162±0.041	0.172±0.049	0.184±0.047	0.135±0.037	0.139±0.044	0.140±0.037	0.30	<0.0001	0.09

[a] Values are Means ± SD

Repeated MIXED procedure implemented in S.A.S statistical software v9.3 adjusted for age, sex, and BMI.

Tableau 5.8 Index of elongase activity (22:4n-6/20:4n-6) after a 6-week n-3 PUFA supplementation according to genotype for each SNPs of the *ELOVL* gene family.

	Pre-n-3 PUFA index of elongase activity[a]			Post-n-3 PUFA index of elongase activity[a]			*p*-values		
	11	**12 or 12+22**	**22**	**11**	**12 or 12+22**	**22**	**SNPs**	**Suppl.**	**Interaction**
ELOVL2									
rs13204015	$3.06E^{-2}\pm0.53E^{-2}$	$3.20E^{-2}\pm0.65E^{-2}$	--	$2.09E^{-2}\pm0.64E^{-2}$	$1.92E^{-2}\pm0.96E^{-2}$	--	0.89	<0.0001	0.15
rs12195587	$3.23E^{-2}\pm0.62E^{-2}$	$3.08E^{-2}\pm0.69E^{-2}$	--	$1.88E^{-2}\pm0.96E^{-2}$	$2.04E^{-2}\pm0.86E^{-2}$	--	0.98	0.0005	0.02
rs4532436	$3.01E^{-2}\pm0.49E^{-2}$	$3.18E^{-2}\pm0.66E^{-2}$	$3.40E^{-2}\pm0.68E^{-2}$	$1.89E^{-2}\pm0.75E^{-2}$	$1.97E^{-2}\pm0.96E^{-2}$	$1.86E^{-2}\pm1.04E^{-2}$	0.52	<0.0001	0.05
rs3734397	$3.20E^{-2}\pm0.67E^{-2}$	$3.22E^{-2}\pm0.56E^{-2}$	$2.87E^{-2}\pm0.65E^{-2}$	$1.93E^{-2}\pm0.99E^{-2}$	$2.00E^{-2}\pm0.81E^{-2}$	$1.51E^{-2}\pm0.95E^{-2}$	0.11	<0.0001	0.85
rs2281591	$3.20E^{-2}\pm0.62E^{-2}$	$3.11E^{-2}\pm0.65E^{-2}$	$3.64E^{-2}\pm0.52E^{-2}$	$2.05E^{-2}\pm0.89E^{-2}$	$1.76E^{-2}\pm0.95E^{-2}$	$2.22E^{-2}\pm0.99E^{-2}$	0.01	<0.0001	0.22
rs3798710	$3.14E^{-2}\pm0.61E^{-2}$	$3.29E^{-2}\pm0.69E^{-2}$	--	$1.89E^{-2}\pm0.90E^{-2}$	$2.01E^{-2}\pm1.00E^{-2}$	--	0.06	<0.0001	0.83
ELOVL5									
rs209492	$3.17E^{-2}\pm0.62E^{-2}$	$3.24E^{-2}\pm0.69E^{-2}$	--	$1.91E^{-2}\pm0.92E^{-2}$	$1.99E^{-2}\pm0.98E^{-2}$	--	0.45	<0.0001	0.94
rs2073040	$3.18E^{-2}\pm0.65E^{-2}$	$3.19E^{-2}\pm0.61E^{-2}$	$3.22E^{-2}\pm0.72E^{-2}$	$1.83E^{-2}\pm1.05E^{-2}$	$1.97E^{-2}\pm0.87E^{-2}$	$2.07E^{-2}\pm0.75E^{-2}$	0.63	<0.0001	0.43
rs2294852	$3.15E^{-2}\pm0.73E^{-2}$	$3.22E^{-2}\pm0.60E^{-2}$	$3.16E^{-2}\pm0.61E^{-2}$	$1.84E^{-2}\pm1.01E^{-2}$	$1.95E^{-2}\pm0.96E^{-2}$	$2.00E^{-2}\pm0.78E^{-2}$	0.83	<0.0001	0.62
rs9370194	$3.21E^{-2}\pm0.67E^{-2}$	$3.17E^{-2}\pm0.61E^{-2}$	--	$1.87E^{-2}\pm1.07E^{-2}$	$1.98E^{-2}\pm0.79E^{-2}$	--	0.98	<0.0001	0.20

[a] Values are Means ± SD
Repeated MIXED procedure implemented in S.A.S statistical software v9.3 adjusted for age, sex, and BMI.

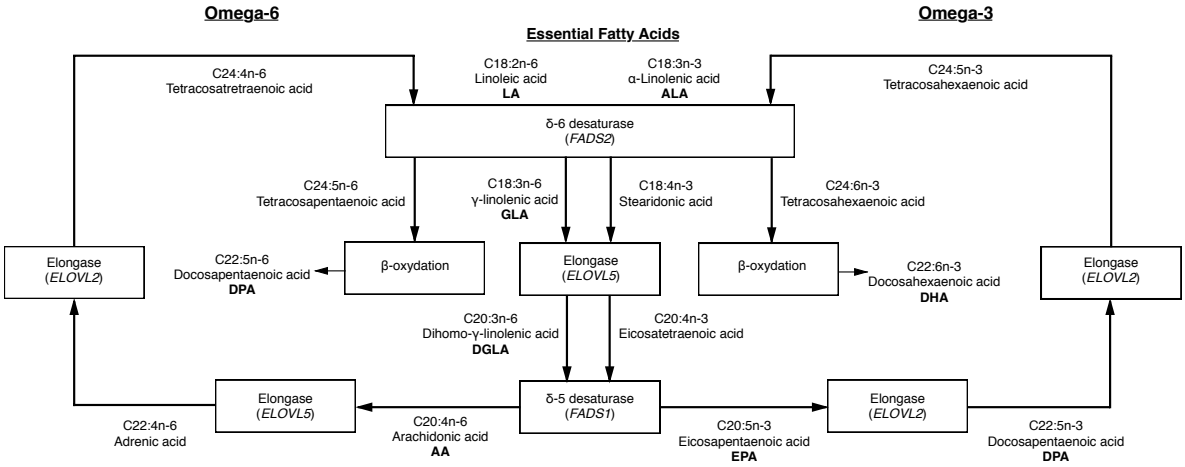

Figure 5.1 Synthesis of n-3 and n-6 PUFAs in humans.

Elongases and desaturases are the enzymes responsible for the conversion of α-linolenic acid (ALA, C18:3n-3) to eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). They are also involved in the synthesis of omega-6′s products, mainly ARA (C20:4n-6) which leads to the production of proinflammatory mediators. Desaturases and elongases are both involved in the fatty acid metabolism. These enzymes are encoded by genes from the *FADS* gene cluster and the *ELOVL* gene family.

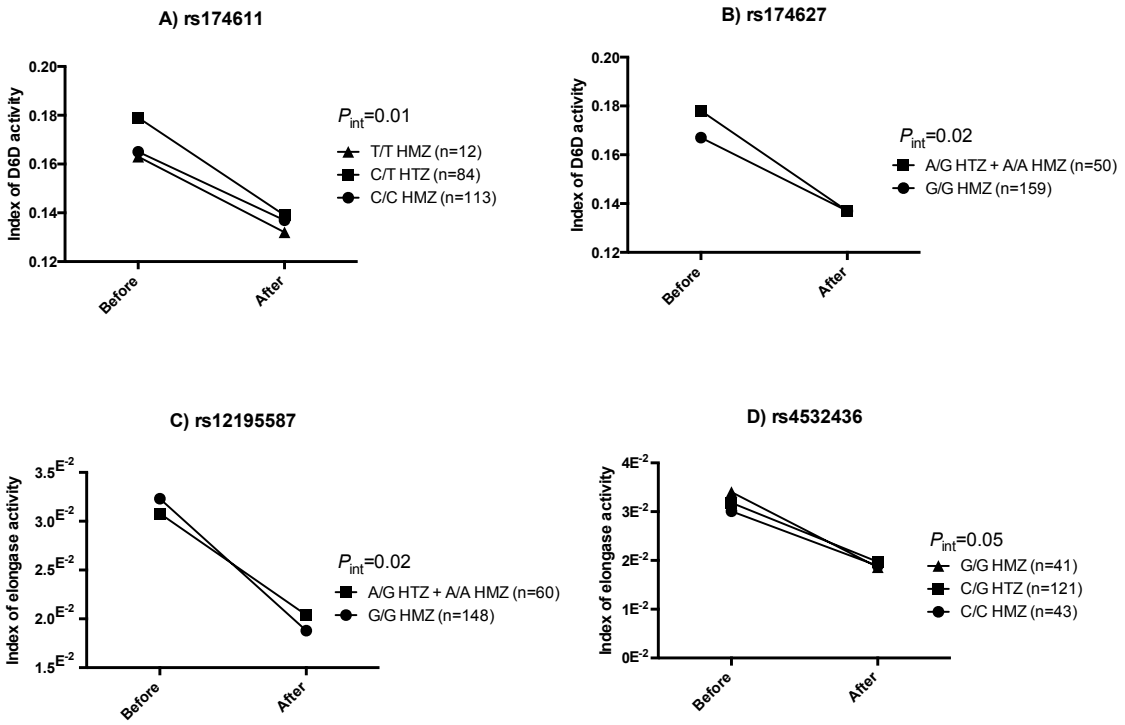

Figure 5.2 Gene-diet interaction effects modulating indexes of enzyme activities after an n-3 fatty acid supplementation.

Chapitre 6 :

Associations entre les polymorphismes du groupe de gènes *FADS1-2-3* et la variabilité des triglycérides plasmatiques en réponse à une supplémentation en AGPI n-3

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Association between polymorphisms in the fatty acid desaturase gene cluster and the plasma triacylglycerol response to an n-3 PUFA supplementation.

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Keywords: triacylglycerol; metabolic pathways; lipids; genotypes; *FADS* gene cluster; polyunsaturated fatty acid omega-3

Résumé

Plusieurs effets bénéfiques sur les facteurs de risque des MCV ont été rapportés pour l'AEP et l'ADH. Cependant, plusieurs études ont observé une variabilité interindividuelle importante dans la réponse lipidique suite à une supplémentation en AGPI n-3. Les variations génétiques peuvent influencer la réponse aux lipides plasmatiques. Le but de cette étude était de vérifier les effets d'une supplémentation en AGPI n-3 sur le profil lipidique plasmatique en relation avec la présence de SNPs dans le groupe de gènes de *FADS1-2-3*. Un total de 208 sujets de la région de Québec a été supplémenté avec 5 g/jour d'huile de poisson sur une période de six semaines. Dans un modèle statistique incluant l'effet du génotype, de la supplémentation et de l'interaction génotype X supplémentation, le SNP rs174546 était significativement associé ($p = 0,02$) aux niveaux de TG plasmatiques avant et après la supplémentation. La supplémentation en AGPI n-3 a eu un effet indépendant sur les niveaux plasmatiques de TG et aucun effet d'interaction génotype X supplémentation n'a été observé. En résumé, nos données supportent la notion que le groupe de gènes *FADS1-2-3* est un déterminant majeur des niveaux de TG plasmatiques. Le SNP rs174546 pourrait être un SNP important associé aux niveaux plasmatiques de TG et à l'expression du gène *FADS1* indépendamment d'une intervention nutritionnelle avec des AGPI n-3.

Abstract

Eicosapentaenoic and docosahexaenoic acids have been reported to have a variety of beneficial effects on cardiovascular disease risk factors. However, a large interindividual variability in the plasma lipid response to an n-3 PUFA supplementation is observed in different studies. Genetic variations may influence plasma lipid responsiveness. The aim of the present study was to examine the effects of a supplementation with n-3 PUFA on the plasma lipid profile in relation to the presence of SNPs in the *FADS* gene cluster. A total of 208 subjects from Quebec City area were supplemented with 5 g/day of fish oil, during six weeks. In a statistical model including the effect of the genotype, the supplementation and the genotype by supplementation interaction, SNP rs174546 was significantly associated ($p = 0.02$) with plasma TG levels, pre- and post-supplementation. The n-3 supplementation had an independent effect on plasma TG levels and no significant genotype by supplementation interaction effects were observed. In summary, our data support the notion that the *FADS* gene cluster is a major determinant of plasma TG levels. SNP rs174546 may be an important SNP associated with plasma TG levels and *FADS1* gene expression independently of a nutritional intervention with n-3 PUFA.

Introduction

High TG levels are associated with cardiovascular disease (CVD) [1]. Population mean TG levels have increased since 1976 in parallel with the constant growing epidemic of obesity, insulin resistance and type-2 diabetes mellitus [2,3]. A meta-analysis of 17 population-based prospective trials including 46,413 men and 10,864 women identified plasma TG levels as an independent risk factor of CVD and estimated that for each increase of 1.0 mmol/L, the relative risk of CVD increased approximately by 30% for men and 75% for women [4,5].

Numerous studies have demonstrated the beneficial effects of *n*-3 PUFA, especially EPA and DHA on reducing CVD risk factors [6,7,8,9]. The intake of EPA and DHA has been associated with a reduced risk of myocardial infarction and the prevalence of recurrence [10,11]. A review of human studies reported that 4 g of marine-derived *n*-3 PUFA per day decreased plasma TG concentrations by 25% to 30% [4]. The AHA recommends an intake of 2 to 4 g of EPA/DHA per day for patients who need to lower their plasma TG levels [12]. The decreased in plasma TG that is observed with high intakes of = *n*-3 PUFA appears to be secondary to the increased hepatic β-oxidation and decreased lipogenesis [13].

The Fish Oil Intervention and Genotype (FINGEN) study showed that TG levels of 31% of the volunteers did not show any reduction after an 8-week supplementation with 1.8 g/day of EPA + DHA [14]. Innate characteristics such as gender, age and genetic factors may contribute to the variability in benefits reported from intervention trials using *n*-3 PUFAs [15]. Therefore, the anticipated effect of such a supplementation on an individual basis does not necessarily match those reported for the general population [16].

The large inter-individual variability observed in the plasma lipid response to a supplementation with *n*-3 PUFA may partly result from genetic variations. Recent data suggest that SNPs found in genes involved in metabolic pathways of *n*-3 PUFA contribute to the variability of PUFA levels [15,17]. The *FADS1* and *FADS2* genes encode

respectively for two desaturases: δ-5 desaturase (D5D) and δ-6 desaturase (D6D) [17]. The D5D and D6D, responsible for double bonds formation in the *n*-3 PUFA pathways, have been associated with differences in FA composition of plasma [18], erythrocyte membranes [19] and adipose tissue [18]. Another potential desaturase, whose function remains to be elucidated, is possibly encoded by the *FADS3* [20].

Concerns regarding the efficiency of *n*-3 PUFA supplementation remain. The TG lowering effects of *n*-3 PUFA using several ratios and doses of EPA and DHA have been reported in different studies. However, these studies do not allow setting the optimal conditions. Conflicting data exist and may arise from inter-individual genetic variations. The purpose of the present study is to test whether the plasma lipid response to a 6-week n-3 PUFA supplementation is influenced by genetic variations in the *FADS* gene cluster.

Methods

Study Population

A total of 254 subjects from the greater Quebec City metropolitan area were recruited between September 2009 and December 2011 through advertisements in local newspapers as well as by electronic messages sent to university students/employees. However, only 208 subjects were eligible for further analyses. Missing values of blood lipid profile pre- and/or post-supplementation did not allow those 46 subjects to be included in statistical analyses. Participants were aged between 18 and 50 years. They were non-smokers, with a body mass index (BMI) between 25 and 40 kg/m^2 and with no current lipid-lowering medications. Subjects were not included if they had taken *n*-3 PUFA supplements for at least 6 months prior, used oral hypolipidemic therapy or had been diagnosed with diabetes, hypertension, hypothyroidism or other known metabolic disorders such as severe dyslipidemia or coronary heart disease. The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University. This trial was registered at clinicaltrials.gov as NCT01343342.

Study Design and Diets

Two hundred and eight subjects followed a run-in period of two weeks where a trained registered dietitian gave individual dietary instructions. Recommendations were drawn from the Canada's Food Guide to Healthy Eating. All subjects were asked to apply these dietary recommendations and to maintain stable body weight throughout the protocol. Among these recommendations, some specifications have been imposed to ensure the success of this study such as not to exceed two portions of fish or seafood per week (maximum 150 g) and to choose, preferably, fish other than oily fish known to be richer in *n*-3 PUFA as fish with white flesh. With the growing popularity of grocery products fortified with *n*-3 PUFA, participants were asked to avoid these products during the study period. Among these enriched products, some eggs, milk, juice, bread and yogurt have been identified. Subjects were also asked to limit their alcohol intakes to no more than two drinks per week. Subjects were not allowed to take *n*-3 PUFA supplements, including those of vegetable sources, and to take vitamins or natural health products during the protocol.

After the run-in period, each participant received a bottle containing *n*-3 PUFA capsules (Ocean Nutrition, Nova Scotia, Canada) covering the following 6-week period. They had to take 5 capsules per day, which gave them a total of 3 g of *n*-3 PUFA (1.9 g EPA and 1.1 g DHA) per day. Compliance was measured by bottles returning and by calculating the number of remaining capsules in the bottles at the end of the supplementation. Subjects had to report any deviations that may have occurred during the protocol. They also had to write their alcohol and fish consumption on a log sheet. Before each phase of the study, subjects received written and oral dietary instructions by a registered dietitian.

A dietitian administrated a validated food-frequency questionnaire (FFQ) before the run-in period to each participant [21]. This FFQ is based on typical food items available in the province of Quebec and contains a total of 91 items; 27 items had between 1 and 3 subquestions. The subjects were asked how often they consumed each item per day, per week, per month, or none at all during the last month. Many examples of portion size were provided for a better estimation of the real portion consumed by the subject. Dietary intake data were analyzed using Nutrition Data System for Research software version 2011 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

Anthropometric Measurements

Body weight, height, and waist girth were measured according to the procedures recommended by the Airlie Conference [22] and were taken before the run-in period, as well as pre- and post-*n*-3 PUFA supplementation. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Biochemical Parameters

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after 12 h overnight fast and 48 h alcohol abstinence. Blood samples were taken to identify and exclude individuals with any metabolic disorders. Afterwards, participants had blood samples taken prior to and after the *n*-3 PUFA supplementation period. Plasma was separated by centrifugation (2500× *g* for 10 min at 4 °C) and samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (TC) and TG concentrations were

measured using enzymatic assays [23]. The high-density lipoprotein cholesterol (HDL-C) fraction was obtained after precipitation of very low-density lipoprotein and low-density lipoprotein particles in the infranatant with heparin manganese chloride [24]. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula [25]. Apolipoprotein B-100 (ApoB100) concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell, as previously described [26]. Plasma C-reactive protein (CRP) was measured by nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously [27].

SNP Selection and Genotyping

SNPs in *FADS1*, *FADS2* and *FADS3* were identified using the International HapMap Project SNP database, based on the National Center for Biotechnology Information (NCBI) B36 assembly Data Rel 28 phase II + III, build 126 (Table 1). Tagger procedure in Haploview V4.2 was used to determine tag SNPs (tSNPs) using a minor allele frequency (MAF) >1% and pairwise tagging ($R^2 \geq 0.8$). Subsequently, we examined linkage disequilibrium (LD) out of the 19 SNPs covering all common variations in the *FADS* gene cluster area, using the LD Plot procedure in Haploview V4.2. Most of the SNPs were in LD ($R^2 \geq 0.8$) and the mean R^2 was 0.953, so 19 SNPs were sufficient to cover the entire area. The SIGMA GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis, MO, USA) has been used to extract genomic DNA. Selected SNPs of the *FADS* gene cluster (rs174456, rs174627, rs482548, rs2072114, rs12807005, rs174448, rs2845573, rs7394871, rs7942717, rs74823126, rs174602, rs498793, rs7935946, rs174546, rs174570, rs174579, rs174611, rs174616 and rs968567) have been genotyped using validated primers and TaqMan probes (Applied Biosystems, Foster City, CA, USA) [28]. DNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems), with a gene-specific primer and with probe mixture (predeveloped TaqMan SNP Genotyping Assays; Applied Biosystems) in a final volume of 10 μL. Genotypes were determined using a 7500 RT-PCR System and analyzed using ABI Prism SDS version 2.0.5 (Applied Biosystems, Foster City, CA, USA).

Gene Expression of the FADS Gene Cluster

cDNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems) and a gene-specific primer and probe mixture (predeveloped TaqMan Gene Expression Assays; Applied Biosystems) in a final volume of 20 μL. The assays used were as follows: Hs00203685ml (*FADS1*), Hs00188654ml (*FADS2*), Hs00222230ml (*FADS3*) and Hs99999905ml (glyceraldehyde-3-phosphate dehydrogenase (GADPH)) as the housekeeping gene. Assays used the same fluorescent reporter probe (FAM™ dye-labeled) and thus each combination treatment and gene were analyzed in individual wells on a 96-well plate. All samples were run in duplicate on an Applied Biosystems 7500 Fast Real Time PCR System (Applied Biosystems) using the following thermal cycling profile: 50 °C (2 min), 95 °C (10 min), followed by 40 steps of 95 °C for 15 s and 60 °C for 60 s. The RT-PCR results were imported into ExpressionSuite Software v1.0 (Life Technologies). Data were adjusted for the endogenous control (GADPH).

Statistical Analyses

Data were analyzed with SAS statistical software V9.2 (SAS Institute, Cary, NC, USA). The ALLELE procedure was used to verify the departure from Hardy-Weinberg equilibrium (HWE) and to calculate minor allele frequency (MAF). Variables not normally distributed were log-transformed before analyses. ANOVA and the type III sum of squares were used to look for significant differences in daily energy and nutrient intakes, at prior and after an *n*-3 PUFA supplementation when age, sex and BMI were included in the model and to test for differences in plasma TG levels among groups divided on the basis of the genotype for rs174546. The repeated MIXED procedure was used to test for the effects of the genotype, the supplementation and the genotype by supplementation interaction on plasma TG and gene expression levels when age, sex and BMI were included in the model. Statistical significance was defined as $p \leq 0.05$. To identify potential effects of variations located in the *FADS* gene cluster region, a transcription factor search was performed using MatInspector 8.0 software from the Genomatix Suite.

Results

All SNPs were in HWE except two: rs7935946 and rs174579 (see Figure 1 for the LD plot). These SNPs were not considered for further analysis. Therefore, associations with 17 SNPs were tested in statistical analyses. The % gene coverage with these 17 SNPs was of 87%.

Baseline characteristics of study participants are presented in Table 2. According to these results, men and women were overweight (BMI > 25 kg/m^2) and had mean plasma TG levels slightly above the cut-point value of 1.129 mmol/L recommended by the AHA for optimal plasma TG levels [29]. Gender differences are evident with respect to weight, TC/HDL-C ratio, CRP, HDL-C and TG levels.

Daily energy intakes were calculated by a food frequency questionnaire validated for healthy French-Canadian men and women [21] and are presented in Table 3. After the supplementation, carbohydrates, saturated fats and proteins were significantly different from the pre-*n*-3 PUFA period ($p = 0.0005$, $p = 0.0008$ and $p = 0.02$, respectively). After the supplementation, the PUFA intake—taking into account food intakes and fish oil capsules used during the supplementation—was significantly increased ($p = 0.003$). In our study, the 6-week average of fish servings/week (a serving = 75 g of fish) was 0.89 servings/week based on the compliance questionnaire given at the end of the study. Furthermore, subjects were asked to limit their fish consumption to no more than 2 servings/week (maximum of 150 g). Based on these results, subjects who had consumed the maximum quantity of fish permitted per week would have added an extra 0.43 g of EPA + DHA/day. With the fish oil supplementation, the total of EPA + DHA was 3 g/day. Common food items eaten by the subjects were seafood, tuna, trout, haddock and salmon.

To test the potential interaction between the *FADS* gene SNPs and the *n*-3 PUFA supplementation on plasma TG levels, the MIXED procedure was used in order to test whether the genotype, the supplementation or the interaction (genotype by supplementation) were associated with plasma TG levels. As shown in Table 4,

independently of the genotype, the supplementation was associated with fasting plasma TG concentrations meaning that the supplementation had an independent effect on plasma TG levels, as expected. One SNP was associated with plasma TG concentrations, rs174546, suggesting that this polymorphism modulates plasma TG levels. No significant genotype by supplementation interaction effects were observed. Further analyses revealed that, in the pre-supplementation period, plasma TG levels were lower in CC homozygotes when compared to carriers of the minor T allele (Table 5, $p = 0.002$). In the post-supplementation period, both genotype groups significantly decreased their plasma TG levels (Figure 2). However, there was no significant difference in post-supplementation plasma TG levels between the genotype groups when age, sex and BMI were included in the model (Table 5). Results remained unchanged after further adjustment for pre-supplementation plasma TG levels (data not shown). Since there was no significant difference between the two groups in post-supplementation plasma TG levels and since the interaction term was not significant in the repeated model, these results suggest that the *FADS* rs174546 is associated with plasma TG levels only and not with the plasma TG response to the *n*-3 PUFA supplementation.

In a model testing the effect of the genotype, the supplementation or the interaction (genotype by supplementation) on *FADS1* gene expression, the SNP rs174546 was associated with *FADS1* gene expression ($p = 0.01$) after adjustments for age, sex and BMI (data not shown). No effect of the *n*-3 PUFA supplementation ($p = 0.54$) and no gene by supplementation effect ($p = 0.56$) explained *FADS1* gene expression.

Discussion

In this study, we tested whether plasma TG levels during an *n*-3 PUFA supplementation varied according to the presence of common polymorphisms in the *FADS* gene cluster. 19 SNPs were initially chosen from the *FADS* gene cluster area covering all common variations of the *FADS* gene cluster. After genotyping, 17 SNPs were in HWE and thus were analyzed in the present study. The *FADS* gene cluster area has been chosen due to the role of D5D and D6D activity in the *n*-3 PUFA metabolic pathway. D5D and D6D are essential parts of PUFA biosynthesis that catalyze a series of desaturation processes [30]. These desaturases are respectively encoded by *FADS1* and *FADS2*. Also, a GWAS has shown that the strongest evidence for an association of genetic contributors of plasma PUFA concentrations was observed in the *FADS* gene cluster area [31]. Some SNPs come from intergenic regions (rs174627, rs12807005. rs174448 and rs7482316) and are part of the *FADS1* and *FADS2* gene promoters because of the head to head orientation. *FADS3* promoter was not considered since no desaturase activity is reported (see all selected SNPs in Table 1). All SNPs were polymorphic for the selected study population and were not in strong LD with each other.

In the present study, we observed an independent genotype effect of the SNP rs174546 on plasma TG levels and on *FADS1* gene expression levels in a model including the SNP, the supplementation effect and the SNP by supplementation interaction. In the literature, SNP rs174546 has been much studied. Numerous studies have attributed beneficial effects to this polymorphism. Indeed. Dumont *et al*. showed that the minor allele of rs174546 was associated with decreased plasma TC and non-HDL-C levels [32]. In another study, Lu *et al*. reported similar results where the common C allele was associated with higher levels of TC non-HDL-C and HDL-C levels, but only in individuals consuming high intakes of omega-6 (>5.26% of total energy intake) [33]. Another study demonstrated that rs174547, in strong-LD with rs174546 (R^2= 1.0), was a dominant SNP in the *FADS* gene cluster that influences desaturase activity and some evidence emerging from human-based research demonstrates that genetic variation in human desaturase genes affects enzyme activity and, consequently, disease risk factors [34]. They also showed that homozygotes for the major allele had a higher estimate of aggregate desaturase activity (ADA) reflected in *n*-3 PUFA while homozygotes

for the minor allele had the lowest ADA [34]. This SNP is also associated with altered desaturase activity reflected in *n*-6 PUFA [34]. Recent studies have also shown that two common and very distinct FADS haplotypes were strongly associated with long-chain PUFAs synthesis levels. Haplotypes A and D, which includes rs174546, may exert differences in transcription levels and the ability to synthesize essential omega-3 and omega-6 long-chain PUFAs [35]. Reduced substrate (*i.e.*, FA) availability leads to a reduction of VLDL TG synthesis [13]. *FADS* gene cluster, especially rs174546, is associated with TG levels and also to ADA, making it a significant SNP when talking about associations between lipids and FA metabolism.

Some SNPs may modulate desaturase activity and lead to changes in *n*-3 PUFA metabolism. We tested SNP rs174546 for potential functional significance using MatInspector 8.0 software, but it did not seem to alter transcription factor binding sites.

After the *n*-3 PUFA supplementation, considerable inter-individual variability in plasma TG levels was observed. It appears that some individuals require higher doses to achieve demonstrable benefits, whereas others are highly sensitive to relatively low doses and individuals with certain genotypes may experience adverse responses with respect to specific risk biomarkers, at least at high doses of *n*-3 PUFA [36]. *FADS* gene expression may modulate specific risk biomarkers in relation to certain genotype. Overall, the *n*-3 PUFA supplementation had no effect on *FADS* gene expression. The SNP rs174546 was a significant predictor of *FADS1* gene expression levels. Caslake *et al*. showed that 31% of all volunteers had no reduction in plasma TG levels after 1.8 g/day of EPA + DHA for 8 weeks [14]. We basically observe similar results in the present study, as 28.8% of the participants had no reduction in plasma TG levels after the 6-week *n*-3 PUFA supplementation. These results demonstrate that intra-individual variability of plasma lipid levels is an important potential source of error enhancing the importance of genetic testing to identify individuals that are more likely to benefit from such therapies [37].

This study presents some limitations. Regarding daily intakes, significant differences for carbohydrates, saturated fats or proteins could be due to recall bias from subjects. Since

subjects were asked to follow recommendations drawn from the *Canada's Food Guide to Healthy Eating*, they could have reported food consumption differences that slightly changed calculated intakes. However, those differences did not affect significantly BMI nor energy intakes. Because carbohydrates intakes were significantly decreased in the post-supplementation period and this could impact on plasma TG levels, the carbohydrate intakes have been added into the statistical model and results remained unchanged (data not shown).

Conclusion

In summary, our data support the notion that the *FADS* gene cluster, especially SNPs from *FADS1*, are major determinants of plasma TG levels. SNP rs174546 may be an important SNP in the *FADS* gene cluster associated with plasma TG levels and *FADS1* gene expression independently of a nutritional intervention with *n*-3 PUFA. These results need to be replicated in other independent studies. A better understanding of the phenomenon could allow the development of personalized dietary advice for prevention of CVD.

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Authors' Contribution to Manuscript

Hubert Cormier performed statistical analysis, interpreted data and wrote the paper; Ann-Marie Paradis, Élisabeth Thifault and Véronique Garneau met the participants; Iwona Rudkowska, Simone Lemieux and Marie-Claude Vohl designed research; Patrick Couture was responsible for the medical follow-up; Hubert Cormier and Marie-Claude Vohl have primary responsibility for final content. All authors read and approved the final manuscript. The authors did not declare any conflicts of interest.

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Tableau 6.1 Selected polymorphisms in the fatty acid desaturase (*FADS*) gene cluster

Gene	dbSNP No.[1]	Sequence[2]	Position	MAF	Genotype/Frequency		
FADS3	rs174456	CTACTAC[A/C]TGGCAGC	intron	0.299	A/A (*n* = 102)	A/C (*n* = 89)	C/C (*n* = 18)
					0.488	0.426	0.086
Intergenic FADS2-FADS3	rs174627	TTATCTG[C/T]GTAGCTA	Intergenic	0.124	A/A (*n* = 2)	A/G (*n* = 48)	G/G (*n* = 159)
					0.010	0.230	0.761
FADS2	rs482548	GGGACAC[C/T]GTGGGGA	3′ UTR	0.126	C/C (*n* = 161)	C/T (*n* = 40)	T/T (*n* = 6)
					0.778	0.193	0.029
FADS2	rs2072114	AGAGTTC[A/G]GGTCTTA	Intron	0.110	A/A (*n* = 167)	A/G (n = 38)	G/G (*n* = 4)
					0.799	0.182	0.019
Intergenic FADS1-FADS2	rs12807005	GATCATG[A/G]ATCACTG	Intergenic	0.012	A/A (*n* = 0)	A/G (*n* = 5)	G/G (n = 204)
					0.000	0.024	0.976
Intergenic FADS2-FADS3	rs174448	ACCCTGA[C/T]TTCTGGG	Intergenic	0.363	A/A (*n* = 78)	A/G (*n* = 109)	G/G (*n* = 21)
					0.375	0.524	0.101
FADS2	rs2845573	TTGCTCA[C/T]GTTACTC	Intron	0.081	A/A (*n* = 177)	A/G (*n* = 30)	G/G (*n* = 2)
					0.847	0.144	0.010
FADS3	rs7394871	AAGGGAC[A/C]CCTGCCC	Intron	0.072	A/A (*n* = 2)	A/C (*n* = 26)	C/C (*n* = 181)
					0.010	0.124	0.866
FADS3	rs7942717	CCAAACG[A/G]GTGCCTG	Intron	0.117	A/A (*n* = 161)	A/G (*n* = 47)	G/G (*n* = 1)
					0.770	0.225	0.005
Intergenic FADS2-FADS3	rs7482316	TTTTCAA[A/G]CTGCCGA	Intergenic	0.103	A/A (*n* = 168)	A/G (*n* = 39)	G/G (*n* = 2)
					0.804	0.187	0.010
FADS2	rs174602	CCAACCC[A/G]TCCTGC	Intron	0.184	C/C (*n* = 9)	C/T (*n* = 59)	T/T (*n* = 141)
					0.043	0.282	0.675
FADS2	rs498793	CTGTAAC[A/G]CAGGCTG	Intron	0.456	C/C (*n* = 62)	C/T (*n* = 99)	T/T (*n* = 43)
					0.098	0.717	0.186
FADS2	rs7935946	AAGGTTC[C/T]GGGAACT	Intron	0.041	C/C (*n* = 195)	C/T (*n* = 11)	T/T (*n* = 3)
					0.933	0.053	0.014
FADS1	rs174546	CCTCTGC[C/T]TTGGCTC	3′ UTR	0.297	C/C (*n* = 103)	C/T (*n* = 86)	T/T (*n* = 19)
					0.498	0.412	0.091
FADS2	rs174570	AACTTGA[C/T]GTAGATC	Intron	0.125	C/C (*n* = 159)	C/T (*n* = 46)	T/T (*n* = 3)
					0.764	0.221	0.014
FADS2	rs174579	TCCCTTT[C/T]CAGGAAG	Intron	0.202	C/C (*n* = 127)	C/T (*n* = 78)	T/T (*n* = 3)
					0.611	0.375	0.014
FADS2	rs174611	TCCTGGA[C/T]CCTGAGA	Intron	0.258	C/C (*n* = 12)	C/T (*n* = 84)	T/T (*n* = 113)
					0.057	0.402	0.541
FADS2	rs174616	GACCTCA[C/T]GTTCCAA	Intron	0.498	A/A (*n* = 51)	A/G (*n* = 108)	G/G (*n* = 50)
					0.244	0.517	0.239

Gene	dbSNP No.[1]	Sequence[2]	Position	MAF	Genotype/Frequency		
					A/A	A/G	G/G
FADS2	rs968567	TCCCCGG[A/G]AGCTCAG	5′ UTR	0.160	(*n* = 2)	(*n* = 63)	(*n* = 144)
					0.010	0.301	0.689

[1] **dbSNP No. from HapMap Data Rel 28 Phase II + III, August 10 on NCBI b36 Assembly dbSNP b126 database;**
[2] **Genes sequences from dbSNP short genetics variations NCBI reference assembly.**

Tableau 6.2 General characteristics of the study sample before *n*-3 PUFA supplementation.

	All[1]	**Men**[1]	**Women**[1]	***p*-values**
Population: Men/Women	208	96	112	
Age (years)	30.8 ± 8.7	31.2 ± 8.1	30.5 ± 9.1	0.55
Weight (kg)[3]	81.4 ± 13.9	87.2 ± 13.4	76.4 ± 12.3	<0.0001
BMI (kg/m^2)[2,3]	27.8 ± 3.7	27.5 ± 3.6	28.2 ± 3.8	0.13
Waist circumference (cm)[3]	93.3 ± 10.8	94.8 ± 11.0	92.0 ± 10.4	0.06
Cholesterol (mM)[4]				
Total	4.82 ± 1.00	4.80 ± 1.00	4.83 ± 1.02	0.75
HDL	1.46 ± 0.39	1.29 ± 0.31	1.61 ± 0.39	<0.0001
LDL	2.79 ± 0.87	2.91 ± 0.87	2.69 ± 0.86	0.08
Total chol./HDL ratio[4]	3.49 ± 1.04	3.91 ± 1.13	3.12 ± 0.80	<0.0001
Triacylglycerols (mM)[2,4]	1.23 ± 0.64	1.32 ± 0.74	1.15 ± 0.53	0.04
ApoB100 (g/L)[4]	0.86 ± 0.25	0.89 ± 0.25	0.84 ± 0.25	0.12
CRP (mg/L)[2,4]	3.13 ± 7.10	1.66 ± 2.45	4.39 ± 9.24	0.02

[1] **Values are means ± SD;**
[2] ***p*-Value derived from log$_e$-transformed;**
[3] **Results were adjusted for age;**
[4] **Results were adjusted for age and BMI.**

Tableau 6.3 Daily intakes from food frequency questionnaire (n = 208).

Nutrients	Pre-*n*-3 PUFA	Post-*n*-3 PUFA	*p*-values
Energy, Kcal	2272 ± 590	2143 ± 566	0.08
Total lipids, g	86.5 ± 29.2	86.6 ± 29.8	0.48
MUFA, g	30.8 ± 11.8	29.6 ± 12.4	0.13
PUFA, g	15.2 ± 6.6	17.1 ± 6.9	0.003
SFA, g	29.0 ± 12.0	25.5 ± 10.4	0.0008
Cholesterol, mg	303.7 ± 147.4	297.3 ± 169.4	0.41
Carbohydrates, g	286.7 ± 78.9	263.4 ± 77.7	0.0005
Protein, g	97.8 ± 30.2	92.6 ± 29.6	0.02
Alcohol, g	3.2 ± 6.0	3.2 ± 6.1	0.81

All values are mean ± SD;
MUFA = monounsaturated fatty acids;
PUFA = polyunsaturated fatty acids;
SFA = saturated fatty acids;
All data were adjusted for sex, age and BMI in ANCOVA;
***p*-values for dietary intakes between pre- and post-supplementation are calculated using ANOVA; Statistical significance was defined as $p \leq 0.05$**

Tableau 6.4 Effect of the genotype, the *n*-3 PUFA supplementation and the interaction (genotype by supplementation) on TG levels ($n = 208$)

SNPs	Genotype	Supplementation	Interaction
	***p*-values**	***p*-values**	***p*-values**
	β	**β**	**β**
rs174456	0.77	0.0001 *	0.67
	0.0013 ± 0.027	0.081 ± 0.027	–0.016 ± 0.038
rs174627	0.23	0.0002 *	0.51
	–0.013 ± 0.031	0.094 ± 0.038	–0.027 ± 0.044
rs482548	0.79	0.0001 *	0.48
	–0.023 ± 0.032	0.048 ± 0.039	0.032 ± 0.045
rs2072114	0.85	0.002 *	0.99
	0.0046 ± 0.034	0.073 ± 0.042	–0.00012 ± 0.047
rs12807005	0.06	0.35	0.68
	–0.13 ± 0.080	0.027 ± 0.11	0.048 ± 0.11
rs174448	0.22	0.0003 *	0.49
	–0.010 ± 0.028	0.083 ± 0.024	–0.027 ± 0.039
rs2845573	0.61	0.01 *	0.57
	–0.028 ± 0.037	0.049 ± 0.048	0.020 ± 0.052
rs7394871	0.46	0.009 *	0.87
	0.017 ± 0.039	0.066 ± 0.051	0.0082 ± 0.055
rs7942717	0.56	0.0007 *	0.9
	0.014 ± 0.032	0.078 ± 0.039	–0.0057 ± 0.045
rs7482316	0.69	0.002 *	0.99
	–0.0070 ± 0.033	0.074 ± 0.042	–0.0013 ± 0.047
rs174602	0.8	0.0001 *	0.5
	0.017 ± 0.029	0.091 ± 0.033	–0.026 ± 0.041
rs498793	0.83	0.01*	0.78
	–0.029 ± 0.029	0.071 ± 0.023	0.0080 ± 0.042
rs174546	0.02 *	<0.0001 *	0.55
	–0.035 ± 0.027	0.084 ± 0.026	–0.023 ± 0.038
rs174570	0.58	0.001 *	0.64
	–0.022 ± 0.032	0.058 ± 0.039	0.020 ± 0.044
rs174611	0.09	<0.0001 *	0.7
	–0.025 ± 0.027	0.081 ± 0.028	–0.014 ± 0.038
rs174616	0.37	0.0005 *	0.84
	–0.022 ± 0.031	0.071 ± 0.022	0.0073 ± 0.044
rs968567	0.13	0.0001 *	0.54
	–0.019 ± 0.029	0.090 ± 0.033	–0.024 ± 0.041

_p_-values are derived from log transformed data;
β represents the effect size ± SE;
All results were adjusted for BMI, age and sex;
The MIXED models implemented in SAS version 9.2 were used to test interaction effects

Tableau 6.5 Triacylglycerol (TG) concentrations according to genotype distributions of the *FADS* gene cluster polymorphisms before and after a 6-week *n*-3 PUFA supplementation.

SNPs		Pre- *n*-3 PUFA supplementation			Post- *n*-3 PUFA supplementation		
		11	**12 + 22**	***p*-values**	**11**	**12 + 22**	***p*-values**
rs174456	Genotype	AA (n = 102)	AC + CC (n = 106)		AA (n = 102)	AC + CC (n = 106)	
	TG levels	1.19 ± 0.61	1.23±0.65	0.45	1.03±0.58	1.01±0.47	0.96
rs174627	Genotype	CC (n = 159)	AC + AA (n = 49)		CC (n = 159)	AC + AA (n = 49)	
	TG levels	1.18±0.58	1.31±0.74	0.06	1.01±0.47	1.05±0.66	0.55
rs482548	Genotype	CC (n = 161)	CT + TT (n = 46)		CC (n = 161)	CT+TT (n = 46)	
	TG levels	1.22±0.64	1.17±0.58	0.62	1.02±0.53	1.03±0.50	0.29
rs2072114	Genotype	AA (n = 166)	AG + GG (n = 42)		AA (n = 166)	AG + GG (n = 42)	
	TG levels	1.20±0.64	1.22±0.59	0.83	1.02±0.55	1.03±0.42	0.87
rs12807005	Genotype	CC (n = 204)	AC + AA (n = 4)		CC (n = 204)	AC + AA (n = 4)	
	TG levels	1.21±0.63	1.26± 0.52	0.15	1.01±0.52	1.21±0.53	0.02 *
rs174448	Genotype	AA (n = 78)	AG + GG (n = 130)		AA (n = 78)	AG + GG (n = 130)	
	TG levels	1.14±0.57	1.25±0.66	0.06	0.99±0.50	1.04±0.54	0.59
rs2845573	Genotype	AA (n = 176)	AG + GG (n = 32)		AA (n = 176)	AG + GG (n = 32)	
	TG levels	1.22±0.67	1.15±0.36	0.98	1.02±0.54	1.05±0.39	0.30
rs7394871	Genotype	CC (n = 180)	AC + AA (n = 28)		CC (n = 180)	AC + AA (n = 28)	
	TG levels	1.22±0.65	1.13±0.42	0.37	1.03±0.54	0.98±0.40	0.53
rs7942717	Genotype	AA (n = 160)	AG + GG (n = 48)		AA (n = 160)	AG + GG (n = 48)	
	TG levels	1.20±0.59	1.25±0.73	0.64	1.02±0.52	1.04±0.53	0.61
rs7482316	Genotype	AA (n = 167)	AG + GG (n = 41)		AA (n = 167)	AG + GG (n = 41)	
	TG levels	1.19±0.58	1.28±0.79	0.73	1.01±0.52	1.07±0.55	0.77
rs174602	Genotype	TT (n = 140)	CT + TT (n = 68)		TT (n = 140)	CT + TT (n = 68)	
	TG levels	1.19±0.61	1.24±0.67	0.65	1.03±0.56	0.99±0.44	0.42
rs498793	Genotype	CC (n = 62)	CT + TT (n = 142)		CC (n = 62)	CT + TT (n = 142)	
	TG levels	1.19±0.67	1.21±0.61	0.30	0.99±0.54	1.03±0.52	0.17
rs174546	Genotype	CC (n = 103)	CT + TT (n = 105)		CC (n = 103)	CT + TT (n = 105)	
	TG levels	1.12±0.51	1.30±0.71	0.002 *	0.97±0.46	1.07±0.58	0.07
rs174570	Genotype	CC (n = 159)	CT + TT (n = 49)		CC (n = 159)	CT + TT (n = 49)	
	TG levels	1.21±0.65	1.19±0.54	0.91	1.01±0.54	1.06±0.46	0.33
rs174611	Genotype	TT (n = 113)	CT + CC (n = 95)		TT (n = 113)	CT + CC (n = 95)	
	TG levels	1.14±0.48	1.29±0.75	0.04 *	0.99±0.45	1.06±0.60	0.19
rs174616	Genotype	AA (n = 51)	AG + GG (n = 157)		AA (n = 51)	AG + GG (n = 157)	
	TG levels	1.24±0.73	1.20±0.59	0.48	1.00±0.53	1.03±0.52	0.32
rs968567	Genotype	GG (n = 143)	AG + GG (n = 65)		GG (n = 143)	AG + GG (n = 65)	
	TG levels	1.17±0.58	1.30±0.71	0.03 *	1.00±0.47	1.06±0.62	0.36

Data are TG levels ± SD; *p*-values are adjusted for age, sex and BMI;
11 stands for major allele homozygote carriers; 12 + 22 stand for minor allele carriers (homozygotes and heterozygotes); Statistical significance was defined as $p \leq 0.05$.

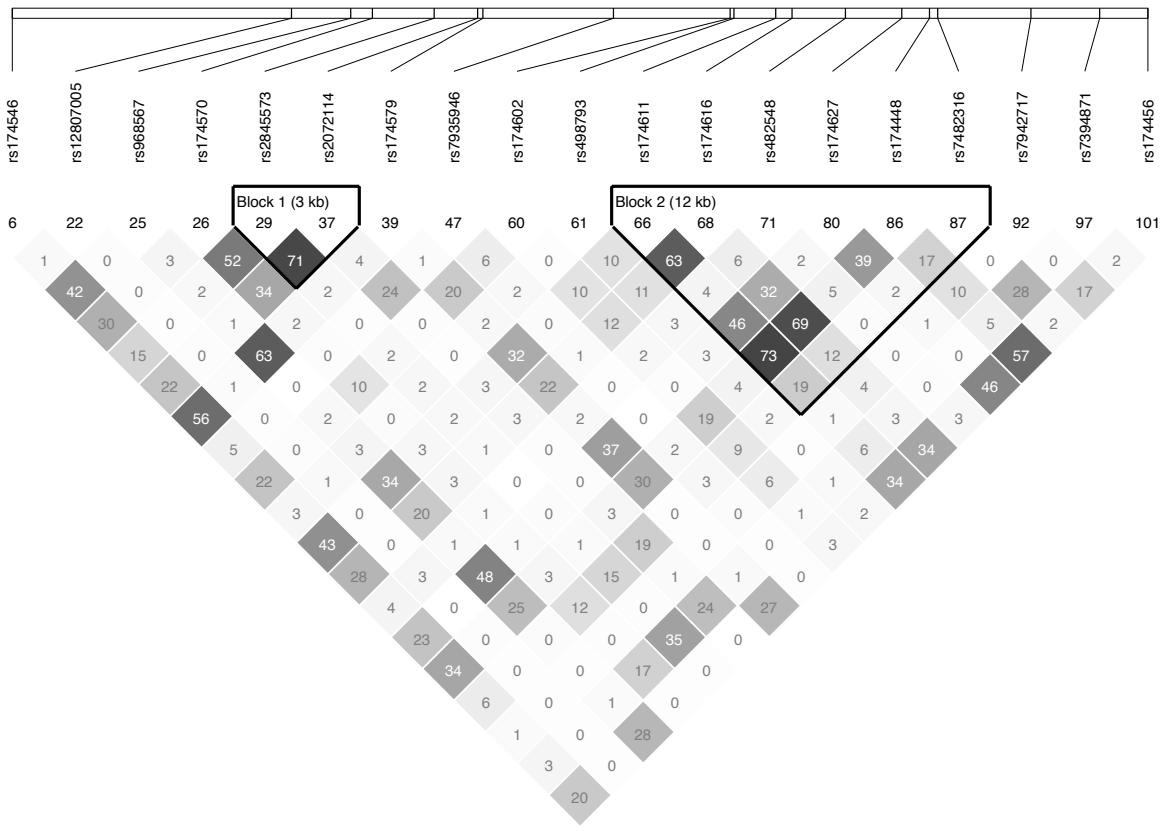

Figure 6.1 Linkage disequilibrium (LD) plot of SNPs of the fatty acid desaturase (*FADS*) gene cluster.

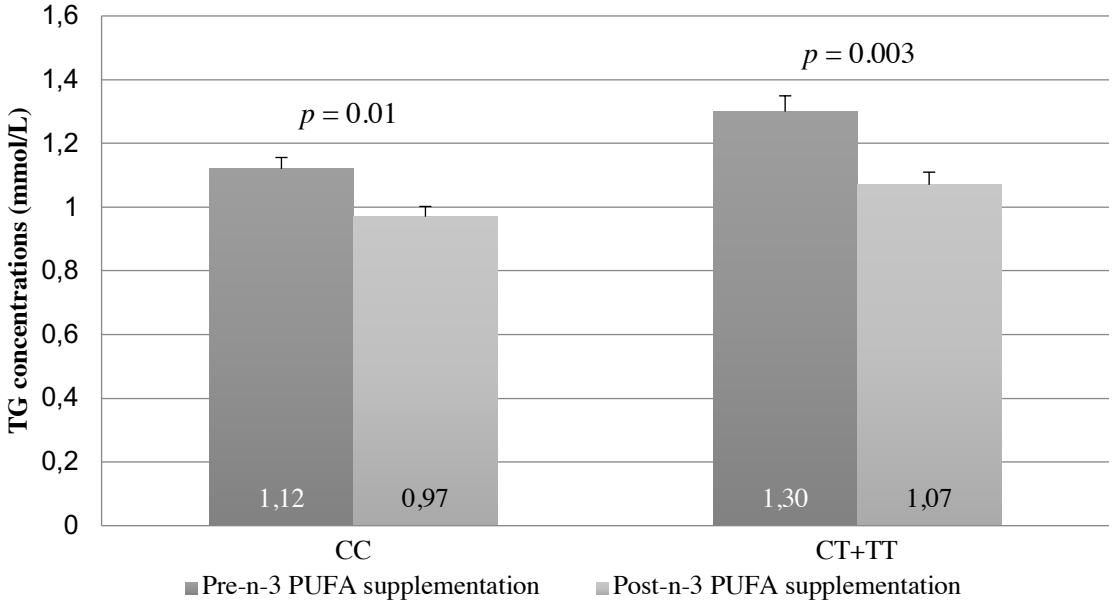

Figure 6.2 TG concentrations before and after a 6-week *n*-3 PUFA supplementation according to the SNP rs174546 in *FADS1*

(Data are means + SE; *p*-Values were determined using the GLM procedure; Statistical significance was defined as $p \leq 0.05$).

Chapitre 7

Expression et polymorphismes des gènes de l'inflammation ; Effets sur les biomarqueurs plasmatiques de l'inflammation suite à une supplémentation de 6 semaines en huile de poisson

Hubert Cormier, Iwona Rudkowska, Simone Lemieux, Patrick Couture et Marie-Claude Vohl.

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Expression and Sequence Variants of Inflammatory Genes; Effects on Plasma Inflammation Biomarkers Following a 6-Week Supplementation with Fish Oil

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Résumé

Plusieurs études suggèrent que certains SNPs provenant de gènes liés à l'inflammation pourraient jouer un rôle dans la production de cytokines, puis interagir avec les AGPI n-3 pour moduler l'inflammation. Le but de la présente étude est de vérifier si l'expression des gènes de l'inflammation est modifiée suite à une supplémentation en AGPI n-3 et de vérifier si des effets d'interactions gène-diète modulent les niveaux plasmatiques des principaux biomarqueurs de l'inflammation (CRP, TNF-alpha et IL-6) sous l'influence de SNPs des gènes de l'inflammation. **Méthodes :** 191 sujets ont complété une supplémentation en AGPI n-3 de 6 semaines avec 5 g/jour d'huile de poisson. L'expression des gènes *TNF-α* et *IL6* a été mesurée dans des cellules mononucléées du sang périphérique (PBMCs) en utilisant la technologie TaqMan. Le génotypage de 20 SNPs du groupe de gènes *TNF-LTA*, des gènes *IL1β, IL6* et *CRP* a été réalisé. **Résultats :** Il n'y a pas eu de réduction significative des niveaux plasmatiques d'IL-6, de TNF-α et de protéine C-réactive (CRP) après la supplémentation en huile de poisson. *TNF-α* et *IL6* ont été légèrement surexprimés dans les PBMCs après la supplémentation (1,05 ± 0,38 et 1,18 ± 0,49, respectivement (n = 191)), mais une quantification relative (QR) entre -0,5 et 2,0 est considérée comme non biologiquement significative. Dans un modèle statistique MIXED ajusté pour les effets de l'âge, du sexe et de l'IMC, des effets d'interaction gène-diète ont été observés pour rs1143627, rs16944, rs1800797 et rs2069840 sur les niveaux d'IL6, pour rs2229094 sur les niveaux de TNF-α et pour rs1800629 sur les niveaux de CRP ($p < 0,05$ pour tous). **Conclusion :** Cette étude démontre qu'une supplémentation de 5 g/jour d'huile de poisson d'une durée de 6 semaines n'a pas modifié les niveaux d'expression des gènes *TNF-α* et *IL6* dans les PBMCs et n'a pas eu d'impact significatif sur les niveaux plasmatiques des principaux biomarqueurs de l'inflammation. Cependant, des effets d'interactions gène-diète ont été observés entre plusieurs SNPs de gènes de l'inflammation modulant les niveaux plasmatiques de biomarqueurs de l'inflammation.

Abstract

A growing body of literature suggest that SNPs from inflammation-related genes could possibly play a role in cytokine production and then interact with dietary *n*-3 fatty acids FAs to modulate inflammation. The aim of the present study was to test whether gene expression of selected inflammatory genes was altered following an *n*-3 PUFA supplementation and to test for gene–diet interactions modulating plasma inflammatory biomarker levels. **Methods:** 191 subjects completed a 6-week *n*-3 FA supplementation with 5 g/day of fish oil. Gene expression of *TNF*-α and *IL6* was assessed in peripheral blood mononuclear cells (PBMCs) using the TaqMan technology. Genotyping of 20 SNPs from the *TNF-LTA* gene cluster, *IL1*β, *IL6* and *CRP* genes was performed. **Results:** There was no significant reduction of plasma IL-6, TNF-α and C-reactive protein (CRP) levels after the 6-week fish oil supplementation. *TNF*-α and *IL6* were slightly overexpressed in PBMCs after the supplementation (fold changes of 1.05 ± 0.38 and 1.18 ± 0.49, respectively (n = 191)), but relative quantification (RQ) within the –0.5 to 2.0-fold are considered as nonbiologically significant. In a MIXED model for repeated measures adjusted for the effects of age, sex and BMI, gene by supplementation interaction effects were observed for rs1143627, rs16944, rs1800797, and rs2069840 on IL6 levels, for rs2229094 on TNF-α levels and for rs1800629 on CRP levels ($p < 0.05$ for all). **Conclusions:** This study shows that a 6-week *n*-3 FA supplementation with 5 g/day of fish oil did not alter gene expression levels of *TNF*-α and *IL6* in PBMCs and did not have an impact on inflammatory biomarker levels. However, gene–diet interactions were observed between SNPs within inflammation-related genes modulating plasma inflammatory biomarker levels.

Introduction

Evidence suggests that fatty acids (FAs) can modulate adipokine production, thus accelerating and influencing an individual's inflammatory response [1]. n-3 FAs have potent anti-inflammatory effects. *n*-3 FAs may affect inflammatory processes through modulation of eicosanoid metabolism and by regulating transcription factor genes involved in inflammation [2]. The resolution of inflammation involves active biochemical compounds, termed resolvins and protectins, which enable inflamed tissues to restore homeostasis [3]. However, these effects have been demonstrated in *in vitro* studies [4,5]. The extent of these effects *in vivo* is not clearly established and remains unclear. Dietary FAs, in particular saturated FAs in addition to *n*-3 and *n*-6 FAs could potentially modulate the expression of genes encoding cytokines, possibly altering plasma cytokine levels as well [6].

A review by Joffe *et al*. suggested that polymorphisms (SNPs) within inflammation-related genes may interact with environmental factors, such as dietary intakes, to modulate an individual's susceptibility to develop obesity and its comorbidities [6].Interaction effects between dietary FAs and variations in inflammation-related genes such as *tumor necrosis factor* α (*TNF*-α) and *interleukin 6* (*IL6*) may influence obesity phenotypes [6]. A growing body of literature suggest that SNPs from inflammation-related genes could possibly play a role in cytokine production and then interact with dietary *n*-3 FAs to modulate inflammation [7].

n-3 FAs are beneficial for inflammation-related diseases such as rheumatoid arthritis, inflammatory bowel diseases and asthma [8]. Accordingly, marine *n*-3 FAs have been shown to decrease expression levels of inflammation-related genes as well as plasma concentrations of cytokine and C-reactive protein (CRP) [6]. However, there are no extensive studies showing a strong relationship between expression of inflammation-related genes and *n*-3 FA supplementation. Previous research from our laboratory has demonstrated changes in inflammatory pathways in human peripheral blood mononuclear cells (PBMCs) after an *n*-3 FA supplementation via a transcriptomic approach [9]. Similarly, Bouwens *et al*. (2009), have examined the effects of high doses of *n*-3 FA supplementation on whole-genome gene

expression profiles in PBMCs, and reported a decrease in expression of genes involved in inflammatory- and atherogenic-related pathways. Although, according to their results, *TNF*-α and *IL6* gene expression levels were not underexpressed after the supplementation [10].

The aim of the present study was to test whether gene expression of inflammation-related genes is altered following an *n*-3 FA supplementation and to test for possible gene–diet interactions with SNPs within these genes modulating plasma inflammatory biomarker levels. We hypothesized that a 6-week marine *n*-3 FA supplementation decreases gene expression of selected inflammation-related genes and also decreases plasma levels of inflammatory biomarkers such as CRP, TNF-α and IL-6 under the influence of SNPs within inflammation-related genes.

Methods

Subjects

A total of 254 unrelated subjects from the greater Quebec City metropolitan area were recruited through emails sent to University students and employees via advertisements in local newspapers. Inclusion criteria were as follow: (1) to be aged between 18 and 50 years; (2) being non-smoker; (3) having a body mass index (BMI) between 25 and 40 kg/m^2; and (4) being free of pharmacologic lipid lowering treatment and/or metabolic disorders. Subjects who had taken *n*-3 FA supplements six months prior to the beginning of the study were excluded. A total of 210 subjects completed the supplementation protocol. Individuals with CRP levels >10 mg/L were excluded, bringing the total down to 191 eligible individuals. This experimental protocol #C09-05-030 was approved by the CHU de Quebec ethics committee on 16 September 2009. This clinical trial was registered at clinicaltrials.gov (NCT01343342).

Study Design and Diets

In order to minimize the intra- and inter-variability in dietary intakes, a 2-week run-in period preceded the supplementation. During this run-in period, a registered dietitian gave individual dietary instructions in order to ensure that participants were in a stable condition before the beginning of the study. Participants received recommendations by a registered dietitian in order to follow the recommendations of the *Eating Well with Canada's Food Guide* [31]. Participants were also asked to maintain their body weight stable throughout the whole research protocol. After the run-in period, each participant received *n*-3 FA capsules in sufficient quantity for the next six weeks. They were instructed to take five capsules/d of fish oil (total of 5 g/day of fish oil), providing a total of 3.0–3.3 g of *n*-3 FAs including 1.9–2.2 g of eicosapentaenoic acid (EPA) and 1.1 g of docosahexaenoic acid (DHA). Before the run-in period, each participant completed a validated food-frequency questionnaire (FFQ) [32] supervised by a registered dietitian. This 91-item FFQ is based on typical food items found in North America. Moreover, they were asked to complete two 3-day food records—prior to and after the *n*-3 FA supplementation period. Dietary intakes data were analyzed

using Nutrition Data system for Research software v.2011 (Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, USA).

Anthropometric Measurements

Body weight, height and waist circumference were measured at every visit in accordance with the Airlie Conference on the Standardization of anthropometric measurements [33]. BMI was calculated as weight per meter squared (kg/m^2).

Biochemical Parameters

Because the study design was not intended to look specifically at inflammation, only a small subset of inflammatory biomarkers were available for this reanalysis. Blood samples were collected after a 12h overnight fast and 48h alcohol abstinence, from an antecubital vein into vacutainer tubes containing EDTA. Plasma CRP was measured by nephelometry (Dade Behring, Deerfield, IL, USA) using a sensitive assay, as described previously [34]. Plasma concentrations of IL6 and TNF-α were measured with high-sensitivity ELISA kits including: Human IL6 Quantikine HS ELISA Kit Minneapolis, MN, USA (R & D Systems, Minneapolis, MN, USA (HS600B)) and Human TNF-α Quantikine HS ELISA Kit (R & D Systems (HSTA00D)) [34].

Measurement of FA Composition in Plasma Phospholipids

FA composition of plasma phospholipids was determined by gas chromatography. Venous blood was drawn into EDTA tubes, then separated by centrifugation at 500 *g* for 6 min and stored at −80 °C for subsequent analyses. Plasma lipids were extracted with chloroform:methanol (2:1, by volume) according to a modified Folch method [35]. Total phospholipids were then isolated with isopropyl ether:acetic acid (96:4) by thin layer chromatography [36]. Isolated plasma phospholipids were then methylated [37]. FA profiles were obtained after methylation in methanol/benzene 4:1 (*v*/*v*) [37] and capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, ON, Canada) equipped with a HP-88 capillary column (100 m × 0.25 mm i.d. × 0.20 μm film thickness; Agilent Technologies, Palo Atto, CA, USA) coupled with a flame ionization detector. Helium was used as carrier gas using a split ratio of 1:80). FA were

identified according to their retention time as well as the following methylated FAs C22:5*n*-6 (Larodan AB, Malmö, Sweden) and C22:5*n*-3 (Supelco Inc., Bellefonte, PA, USA). Finally, phospholipids FA profiles were determined using the relative percentage areas of total FAs.

SNP Selection and Genotyping

SNPs in the *TNF-LTA gene cluster* (6 SNPs), *IL6* (5 SNPs) and *CRP* (4 SNPs) were identified using the International HapMap Project SNP database, based on the National Center for Biotechnology Information (NCBI) B36 assembly Data Rel 28. phase II + III, build 126. The *TNF-LTA* gene cluster is made of two genes that are located very close to each other on chromosome 6 (*LTA* location: Chr6p21.3, 31,572,099 … 31,574,324; *TNF*-α location: Chr6p21.3, 31,575,567 … 31,578,336). *LTA* is also referred to as member 1 of the *TNF*-superfamily. Five hundred kilo-base pairs (kbp) downstream of each gene and 2500 kbp upstream of each gene were added to cover the 5′UTR and 3′UTR regions. Gene Tagger procedure in Haploview v4.2 was used to determine SNPs using a minor allele frequency (MAF) ≥ 5% and pairwise tagging ($r^2 \geq 0.8$). Subsequently, the linkage disequilibrium (LD) was examined for all SNPs using the LD Plot procedure in Haploview v4.2. The SIGMA GenElute Gel Extraction Kit (Sigma-Aldrich Co., St. Louis. MO, USA) has been used to extract genomic DNA. Selected SNPs of the *TNF-LTA* gene family (rs1041981, rs2857706, rs1800629, rs2239704, rs3093662, and rs2229094), *IL6* (rs2069861, rs2069840, rs2069837, rs2069827, and rs1800797), *IL1*β (rs1143633, rs1143634, rs16944, rs3136558, rs1143627) and *CRP* (rs1800947, rs3093059, rs1130864, and rs1205) have been genotyped using validated primers and TaqMan probes (Thermo Fisher Scientific, Waltham, MA, USA) [38]. DNA was mixed with TaqMan Universal PCR Master Mix (Thermo Fisher Scientific), with a gene-specific primer and with probe mixture (pre-developed TaqMan SNP Genotyping Assays; Thermo Fisher Scientific) in a final volume of 10 μL. Genotypes were assessed using a 7500 RT-PCR System and the ABI Prism Sequence Detection System v2.0.5 was used to analyse the data (Thermo Fisher Scientific).

Gene Expression

Gene expression of *IL6* and *TNF*-α genes was measured following the 6-week *n*-3 FA supplementation. Complementary DNA (cDNA) was mixed with TaqMan Universal PCR Master Mix (Thermo Fisher Scientific) and a gene-specific primer and probe mixture in a final volume of 20 μL. All samples were run on a 7500 Fast Real Time PCR System (Thermo Fisher Scientific) using the following thermal cycling profile: 50 °C (2 min), 95 °C (10 min), followed by 40 steps of 95 °C for 15 s and 60 °C for 60 s. The real-time PCR data were imported into Expression Suite Software v1.0. These samples were analysed in triplicate and calibrated to the *GAPDH* gene (endogenous control; *GAPDH*: Hs99999905ml). Relative quantification estimations were achieved using the $2^{-\Delta\Delta CT}$ calculation method [39].

Statistical Analyses

This study is a secondary analysis of the FAS Study that primarily wanted to understand how genes and environment act together on the cardiometabolic risk profile. Data were analyzed with SAS Genetics v9.3. Values that were not normally distributed were $\log_{(10)}$-transformed, negative reciprocal or normalised by a Box-Cox transformation (TNF-α) before analysis. Subjects were categorised as positive ($\Delta < 0\%$) or negative ($\Delta \geq 0\%$) responders based on their relative change of plasma TNF-α, CRP or IL-6 levels after the supplementation. The MIXED procedure for repeated measures was used to test for significant differences in metabolic characteristics between men and women at baseline and for differences between various nutrient intakes prior to and after *n*-3 FA supplementation and to test for the effects of the genotype, the supplementation and the genotype by supplementation (time) interaction on inflammatory marker levels when age, sex and BMI were included in the model. Partial Spearman correlations were calculated between EPA, DHA or total *n*-3 of plasma phospholipids (in % of total FAs) and inflammatory markers. The ALLELE procedure was used to verify the departure from Hardy-Weinberg equilibrium (HWE) and to calculate the minor allele frequency. Individuals with CRP levels > 10 mg/L were excluded bringing the total to 191 eligible individuals. SIFT web-based software was used to predict the effect of amino acid substitution and all tests were run under default threshold values. Finally, the FREQ procedure was used to verify differences in genotype frequency distribution between

positive and negative responders to the *n*-3 FA supplementation. Statistical significance was defined as $p \leq 0.05$.

Results

Population characteristics have been previously described here [11] for the total cohort and here [12] for this specific population included in this reanalysis. Briefly, participants have a higher BMI following the 6-week *n*-3 FA supplementation ($p = 0.006$), but the absolute difference remains extremely low at 0.1 kg/m^2, with no effects on waist circumference. After the supplementation, TG levels decreased, as expected ($p < 0.0001$). Table 1 reports the descriptive characteristics of study participants at baseline.

Pre- and post-supplementation inflammatory marker levels are presented in Table 2. There was no significant difference observed in inflammatory marker levels. However, a large inter-individual variability was observed in the inflammatory response to a fish oil supplementation. For instance, 45.0%, 47.6% and 48.2% of study participants increased their plasma levels of CRP, TNF-α and IL-6 respectively after the 6-week supplementation.

Energy intakes were lower after the *n*-3 FA supplementation ($p = 0.003$) as shown in Table 3. When looking at macronutrient distribution, there was a shift towards an increase in total fat together with a decrease in carbohydrates (absolute difference of 24.8 g/day) and proteins (absolute difference of 5.4 g/day). In fat intake, PUFA intakes were higher ($p = 0.001$) as a result of taking 5 g/day of fish oil supplements while saturated FA intakes were lower with an absolute difference of 3.7 g/day.

Partial Spearman correlations between EPA, DHA or total *n*-3 FA levels (in % of total FA) from plasma phospholipids and inflammatory marker levels adjusted for baseline data (both FAs and inflammatory marker levels), age, sex and BMI are presented in Figure 1. Briefly, total plasma *n-3* FA levels negatively correlates with CRP ($r = –0.15$, $p = 0.04$), TNF-α ($r = –0.17$, $p = 0.02$), and IL-6 levels ($r = –0.15$, $p = 0.04$). Looking only at plasma EPA levels, a negative correlation was found with TNF-α levels ($r = –0.18$, $p = 0.01$) while trends were observed with CRP and IL-6 levels. Plasma DHA levels tended to be negatively correlated with CRP and IL-6 levels ($p < 0.10$, for all).

Figure 2 shows a change in the expression of inflammation-related genes. Indeed, using the $2^{-\Delta\Delta CT}$ calculation method, *TNF*-α and *IL6* were slightly overexpressed in PBMCs after the 6-week *n*-3 FA supplementation (fold changes of 1.05 ± 0.38 and 1.18 ± 0.49, respectively), but relative quantification (RQ) within the –0.5 to 2.0-fold are considered as non-significant.

All selected SNPs were in HWE and LD plots from Haploview v4.2 for each gene are presented in Figure 3. For SNPs within the *TNF-LTA* gene cluster, 6 SNPS covered 93% of the known genetic variability, for *IL6*, 5 SNPS covered 100%, for *IL-1*β, 5 SNPs covered 100%, and for *CRP*, 4 SNPs covered 100%. Table 4 reports all the selected SNPs within the five inflammation-related genes studied.

In a repeated MIXED model adjusted for the effects of age, sex, and BMI, several gene–diet interactions impacting inflammatory marker levels were observed following the *n*-3 FA supplementation, as shown in Table 5. Figure 4 shows the gene–diet interaction on plasma TNF-α levels according to rs2229094 where carriers of the mutated allele increased their plasma TNF-α levels after the 6-week *n*-3 FA supplementation while wild type homozygotes decreased theirs. Also, significant differences were observed in the genotype distribution of rs2229094 between positive and negative responders according to delta TNF-α levels where positive responders decreased their plasma TNF-α levels after the supplementation where an increase was observed in negative responders. There was a higher proportion of C/C HMZ that were negative responders (6.8%) *vs*. positive responders (2.1%) (p = 0.0003). An interaction between rs2229094 (*TNF-LTA*) and the 6-week *n*-3 FA supplementation is of particular interest because that SNP is located in an exon and is responsible for an amino acid change (Cys13Arg). Moreover, analyses with SIFT argue for potential functional effect of this SNP as the amino acid change was considered damaged using homologues in protein alignment (score = 0.04), but tolerated using orthologues in protein alignment

Discussion

We observed in the present study that *n*-3 FAs may interact with SNPs from inflammation-related genes to modulate plasma cytokine levels. There was no reduction of plasma IL-6 and TNF-α as well as CRP levels, but several gene–diet interactions with SNPs within inflammation-related genes and *n*-3 FAs have been found potentially modulating inflammatory marker levels. These findings are consistent with the well-known anti-inflammatory properties of *n*-3 FAs, but the amplitude of the results may differ according to an individual's genotype. In addition to nutrigenetic effects, baseline plasma EPA, DHA or total *n*-3 FA levels are negatively associated with plasma cytokine and CRP levels (Figure 1).

Ferrucci *et al*. have reported that plasma levels of *n*-3 FAs were independently associated with lower levels of pro-inflammatory markers and higher levels of anti-inflammatory markers independent of confounders such as age, sex, BMI, smoking status, education, energy intake, and potentially confounding drug treatment among others [13]. They observed that lower EPA and total *n*-3 FAs were associated with higher IL-6 and TNF-α levels (for total *n*-3 FAs only) [13]. Our results are moving in the same direction as shown by the correlations between baseline FA levels with the principal inflammatory biomarkers (Figure 1).

Trebble *et al*. have shown in 16 healthy subjects, that the dose-response relationships between *n*-3 FA, phospholipid composition and cytokine production by PBMCs is U-shaped meaning that an intermediate level of EPA within plasma and cell membrane phospholipids, resulting from an *n*-3 FA supplementation of <2.0 g/day, may be associated with a greater inhibitory effect on TNF-α release than higher EPA concentrations resulting from *n*-3 FA supplementary intakes of >2.0 g/day [14]. Rees *et al*. have suggested that there is a threshold for an anti-inflammatory effect of EPA somewhere between 1.35 and 2.7 g/day [15]. However, in the present study, there was no significant reduction in plasma CRP, IL-6 or TNF-α levels despite the high doses of *n*-3 FAs used during the protocol (Table 4). This could be explained by low baseline levels of inflammatory markers, by the exclusion of

individuals with plasma CRP levels >10.0 mg/L, or by the intermediate doses of EPA given to study participants. Moreover, there could be a differential effect of each of the *n*-3 FAs impacting inflammatory marker levels.

Moreover, besides the independent influence of dietary FAs on cytokine levels, some SNPs in the *TNF-LTA* gene family may be related to the inter-individual variability observed in plasma TNF-α levels and *TNF*-α gene expression [6]. In this study, rs2229094 (*TNF*-α*)* is associated with plasma TNF-α levels. This SNP is of particular interest due to its exonic location and the presence of a missense mutation (Cys13Arg) that is located at a conserved residue. Recent studies have shown that rs2229094 was associated with type 2 diabetes [16], CRP levels [16], sepsis [17], Crohn's disease [18], and cancer risk [19]. According to Huang *et al*., four functional SNPs of the LTA gene, including rs2229094, may exert possible regulatory effects on gene expression and cytokine production [19].

Another SNPs in the *TNF-LTA* gene family was associated with inflammatory responses. Indeed, we observed an interaction between rs1800629 and *n*-3 FAs modulating CRP levels where carriers of the mutated allele had significantly higher CRP levels than the wild-type genotype after fish oil supplementation (Table 5). Song *et al*. have found that the mutated allele of rs1800629 was associated with increased TNF-α production in PBMCs from healthy subjects after stimulation with LPS [20]. Studies have reported that dietary fat intake could alter the relationship between *TNF* −308G>A (also referred to as rs1800629) with adiposity and serum lipid concentrations. The main results of these studies are that *TNF* −308G>A was associated with an increased risk of obesity and dyslipidaemia, and carriers of the mutated allele appeared to be more responsive to dietary fat intake [21,22]. Meydani *et al*. reported that the production of the cytokines IL-1β, TNF-α, and IL-6 by mononuclear cells was reduced after the consumption of the low-fat, high-fish diet [23].

Evidence have shown that carriers of the mutated allele had a 2-fold increase in the *TNF*-α transcriptional activity, thus playing a role in the altered *TNF*-α gene expression possibly leading to an increase in cytokine production [24]. Moreover, Wilson *et al*. have found that rs1800629 may exert direct effects on *TNF*-α gene regulation, potentially leading to high

TNF-α phenotype (expression levels, transcriptional activity, inflammatory marker levels) and more severe infection diseases in TNF2 homozygotes [25]. For example, Antonicelli *et al*. reported that carriers of *TNF*-α gene –308G>A were more likely to be affected by severe ischemic damage in a case-control study including elderly Italian individuals with and without coronary heart disease [26]. However, in this study, there were no significant differences observed in *TNF*-α gene expression nor in plasma TNF-α levels according to the rs1800629 genotype both in dominant and additive models. Although the relative change in TNF-α levels after the supplementation was not statistically significant between genotype groups owing to the small sample size, the difference was clinically relevant and seems to be in agreement with the actual literature (delta TNF-α→G/G: 28.0% *vs*. A/G: 3.3% *vs*. A/A 1.3%). This heterogeneity observed in the response to fish oil could be partly explained by the replacement of arachidonic acids by *n*-3 FAs into cell membranes leading to the production of diverse eicosanoids. Accordingly, Grimble *et al*. have suggested that the overall effect on TNF-α production (inhibition or stimulation) probably depends on the balance among the different stimulatory and inhibitory eicosanoids produced from arachidonic acid and EPA [7].

Although gene expression of inflammation-related genes is often decreased following an increase of the *n-3* FAs in the diet [27–29], several studies reported no significant decrease in plasmatic levels of inflammatory biomarkers, such as TNF-α, IL-6 or CRP [13,30]. In the present study, *TNF*-α and *IL6* genes were slightly overexpressed in PBMCs after the 6-week *n*-3 FA supplementation, but relative quantification (RQ) within the –0.5 to 2.0-fold are considered not significant. There was no clear effect of the 6-week *n*-3 FA supplementation on the expression of the two selected inflammation-related genes (*IL6* and *TNF*-α) on a metabolically healthy, but slightly overweight population, even with the use of triplicates to ensure a better reduction in biological variance. These results failed to demonstrate changes in expression levels of *TNF*-α and *IL6* when looking at these two genes specifically using a real-time PCR approach. However, Rudkowska *et al*. and Bouwens *et al*. have shown using transcriptomic approaches that inflammation-related pathways in PBMCs were changed to the anti-inflammatory direction after an *n*-3 FA supplementation [9,10].

Strengths and Limitations

Several limitations of the present study need to be addressed. Participants were relatively young (mean age of 30.8 ± 8.7 years) and they had low inflammatory biomarker levels at baseline. The patients were healthy and we excluded participants having plasma CRP levels > 10 mg/L. We did not observe difference in gene expression of inflammation-related genes after the supplementation. This could be attributable to the use of the $2^{-\Delta\Delta CT}$ calculation method assuming that the endogenous control gene and target gene have both similar efficiencies. Also, this study did not allow to isolate the effect of a single FA and its potential gene–diet interactions on inflammatory markers due to the composition of the *n*-3 FA fish oil capsules given to participants that contained EPA and DHA.

Conclusion

Overall, this study shows that a 6-week *n*-3 FA supplementation with 5 g of fish oil daily did not alter gene expression levels of *TNF*-α and *IL6* in PBMCs and did not have an impact on inflammatory biomarker levels. However, significant gene–diet interactions were observed between SNPs within inflammation-related genes modulating plasma inflammatory biomarker levels. These gene–diet interactions may potentially explain the large inter-individual variability observed in plasma inflammatory response following an *n*-3 FA supplementation.

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Author Contributions

Hubert Cormier performed statistical analysis, interpreted data and wrote the paper; Simone Lemieux and Marie-Claude Vohl designed research; Patrick Couture was responsible for the medical follow-up; Iwona Rudkowska participated to the selection of genes; Hubert Cormier undertook laboratory work; Hubert Cormier and Marie-Claude Vohl have primary responsibility for the final content. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Tableau 7.1 Characteristics of the study participants at baseline (*n* = 191)

Characteristics	**All (*n* = 191)**	**Men (*n* = 95, 49.7%)**	**Women (*n* = 96, 50.3%)**
Age	30.8 ± 8.7	30.9 ± 8.1	30.8 ± 9.3
Waist circumference, cm	93.2 ± 10.6	94.8 ± 10.9	91.6 ± 10.1
BMI, kg/m²	27.2 ± 3.6	27.5 ± 3.6	27.9 ± 3.5
Values are means ± SD.			

Tableau 7.2 Pre- and post-supplementation inflammatory marker levels (n = 191).

Biomarkers	Pre *n*-3 FA	Post *n*-3 FA	*p*-Value[1]
CRP levels (mg/L)[2]	1.78 ± 2.09	1.81 ± 2.07	0.95
TNF-α levels (ϱg/mL)[3]	1.70 ± 1.48	1.68 ± 1.30	0.69
IL-6 levels, (ϱg/mL)[2]	1.34 ± 1.13	1.28 ± 0.98	0.54

Values are means ± SD.

*** $p < 0.05$;**

[1] ***p*-value are derived from a MIXED procedure for repeated measures adjusted for age, sex and BMI (except for the BMI and the waist circumference that were adjusted only for age and sex);**

[2] **values were $\log_{(10)}$ transformed;**

[3] **values were normalised using the Box-Cox transformation.**

Tableau 7.3 Dietary intakes pre- and post-supplementation with *n*-3 PUFAs (*n* = 191).

Dietary Intakes	Pre-Suppl.	Post-Suppl. (Including *n*-3 FA Supplements)	*p*-Value[1]
Energy, (kcal)	2290 ± 599	2196 ± 570	0.003
Carbohydrate, (% of TEI)	50.5 ± 7.2	48.5 ± 7.8	0.001
Carbohydrate, (g/day)	288.9 ± 79.8	264.1 ± 78.1	<0.0001
Protein, (% of TEI)	17.4 ± 3.3	16.9 ± 3.1	0.15
Protein, (g/day)	98.6 ± 30.6	93.2 ± 30.1	0.002
Total fat, (% of TEI)	32.5 ± 6.0	35.2 ± 6.3	<0.0001
Total fat, (g/day)	85.0 ± 29.7	86.8 ± 29.9	0.56
SFA, (% of TEI)	11.1 ± 3.1	10.3 ± 3.0	0.001
SFA, (g/day)	29.1 ± 12.1	25.4 ± 10.5	<0.0001
MUFA, (% of TEI)	11.8 ± 2.8	12.0 ± 3.3	0.41
MUFA, (g/day)	30.8 ± 11.8	29.7 ± 12.5	0.17
PUFA, (% of TEI)	5.9 ± 2.1	6.9 ± 2.1	<0.0001
PUFA, (g/day)	15.3 ± 6.7	16.9 ± 6.7	0.001

[1] ***p*-value provided by a paired *t*-test.**
TEI stands for "Total energy intakes"; SFA stands for "Saturated fat".

Tableau 7.4 Selected SNPs within *TNF-LTA*, *IL-6*, *IL-1*β, and *CRP* genes.

Genes	dbSNP No. [a]	Sequence [b]	Position	AA	CC	CA	CG	CT	GA	GG	GT	AT	TT
				n (%)									
TNF-LTA	rs1041981	CA[A/C]CC	Missense [Thr]→[Asn]	22 (10.5)	97 (46.2)	91 (43.3)							
TNF-LTA	rs2857706	TA[A/G]GT	Intron		151 (7.2)			52 (24.8)					7 (3.3)
TNF-LTA	rs1800629	TG[A/G]GG	nearGene-5	2 (1.0)					45 (21.4)	163 (77.6)			
TNF-LTA	rs2239704	GC[G/T]GG	UTR-5	49 (23.3)	72 (34.3)	89 (42.4)							
TNF-LTA	rs3093662	AC[A/G]GA	Intron	178 (84.8)					29 (13.8)	3 (1.4)			
TNF-LTA	rs2229094	TG[C/T]GT	Missense [Cys]→[Arg]		19 (9.1)			74 (35.2)					117 (55.7)
IL6	rs2069861	AA[C/T]AA	nearGene-3		177 (84.3)			33 (15.7)					0 (0.0)
IL6	rs2069840	AA[C/G]TT	Intron		95 (45.2)		92 (43.8)			23 (11.0)			
IL6	rs2069837	TA[A/G]AT	Intron	181 (86.2)					28 (13.3)	1 (0.5)			
IL6	rs2069827	TC[G/T]AT	nearGene-5							172 (81.9)	38 (18.1)		0 (0.0)
IL6	rs1800797	GG[A/G]TG	nearGene-5	73 (34.8)					111 (52.9)	26 (12.4)			
IL1B	rs1143634	TT[C/T]GA	Cds-synon [Phe]→[Phe]		129 (61.4)			70 (33.3)					11 (5.2)
IL1B	rs1143633	CC[A/G]CC	Intron	32 (15.2)					94 (44.8)	84 (40.0)			
IL1B	rs16944	TC[A/G]GG	nearGene-5	28 (13.3)					82 (39.1)	100 (47.6)			
IL1B	rs3136558	GA[C/T]CT	Intron		8 (3.8)			77 (36.7)					125 (59.5)
IL1B	rs1143627	GC[C/T]AT	nearGene-5		29 (13.8)			81 (38.6)					100 (47.6)
CRP	rs1800947	CT[C/G]TC	Cds-synon [Leu]→[Leu]		190 (90.5)		20 (9.5)						0 (0.0)

Genes	dbSNP No. [a]	Sequence [b]	Position	AA	CC	CA	CG	CT	GA	GG	GT	AT	TT
								n (%)					
CRP	rs3093059	AT[C/T]GG	nearGene-5		2 (1.0)			32 (15.2)					176 (83.6)
CRP	rs1130864	AA[C/T]GG	UTR-3		102 (48.6)			84 (40.0)					24 (11.4)
CRP	rs1205	CA[C/T]AG	UTR-3		99 (47.1)			89 (42.4)					22 (10.5)

[a] dbSNP No. from HapMap Data Rel 28 Phase II + III, 10 August on NCBI b36 Assembly dbSNP b126 database;
[b] Genes sequences from dbSNP short genetics variations NCBI reference assembly.

Tableau 7.5 Gene-diet interaction effects on inflammatory markers levels.

Gene	Biomarker	SNPs	Genotype by Suppl.	β ± SE	*p*-Value for the Genotype	*p*-Value for the *n*-3 Suppl.	*p*-Value for the Interaction
IL-1β	IL-6 levels (ρg/ml)	rs1143627	CC × *n*-3 suppl.	-0.014±0.025	0.91	0.78	0.02
			CT × *n*-3 suppl.	0.037±0.014			
			TT × *n*-3 suppl.	-0.014±0.013			
	IL-6 levels (ρg/ml)	rs16944	AA × *n*-3 suppl.	-0.018±0.025	0.87	0.87	0.02
			AG × *n*-3 suppl.	0.038±0.014			
			GG × *n*-3 suppl.	-0.014±0.013			
IL6	IL-6 levels (ρg/ml)	rs1800797	AA × *n*-3 suppl.	0.025±0.015	0.30	0.64	0.05
			AG × *n*-3 suppl.	-0.023±0.013			
			GG × *n*-3 suppl.	-0.016±0.025			
	IL-6 levels (ρg/ml)	rs2069840	CC × *n*-3 suppl.	-0.010±0.013	0.11	0.28	0.04
			CG × *n*-3 suppl.	-0.016±0.014			
			GG × *n*-3 suppl.	0.063±0.029			
TNF-LTA gene family	TNF-α levels, (ρg/ml)	rs2229094	CC × *n*-3 suppl.	0.022±0.012	0.85	0.20	0.03
			CT × *n*-3 suppl.	0.005±0.006			
			TT × *n*-3 suppl.	-0.009±0.005			
	CRP levels (mg/L)	rs1800629	GG × *n*-3 suppl.	-0.012±0.016	0.37	0.17	0.04
			[AA+GA] × *n*-3 suppl.	0.058±0.030			

β: regression coefficient.
The MIXED model for repeated measures included the SNP, the time and the interaction term with adjustments for the effects of age, sex and BMI.

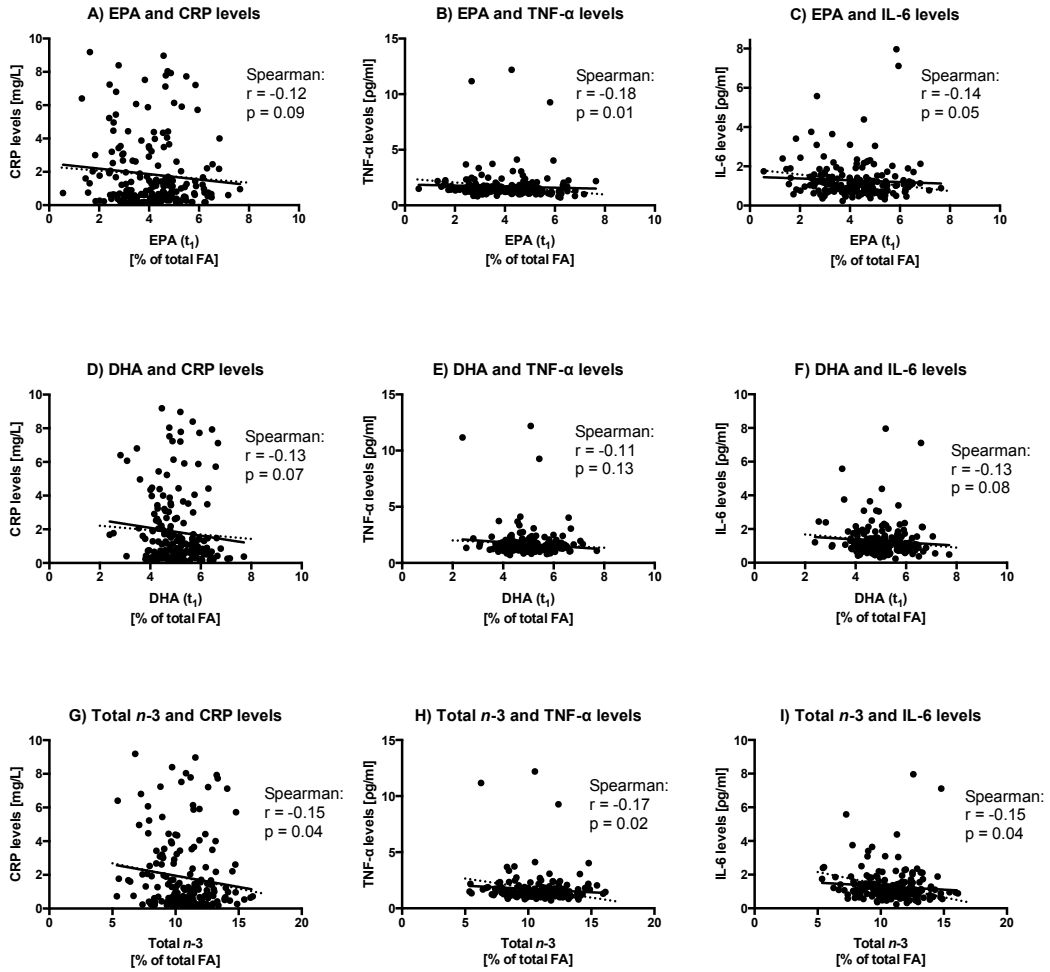

Figure 7.1 Partial Spearman correlations between EPA, DHA or total *n*-3 PUFA (in % of total FA) from phospholipids and inflammatory marker levels controlled for baseline data (both FAs and inflammatory marker levels), age, sex and BMI. Panels (A–C) show correlations between EPA (in % of total FA) and inflammatory markers; Panels (D–F) show correlations between DHA (in % of total FA) and inflammatory markers; and panels (G–I) show correlations between total *n*-3 (in % of total FA) and inflammatory markers; straight lines: unadjusted regression slope, and dotted lines: adjusted regression slope with baseline inflammatory marker levels, baseline *n*-3 from phospholipids levels, age, sex and BMI (n = 191).

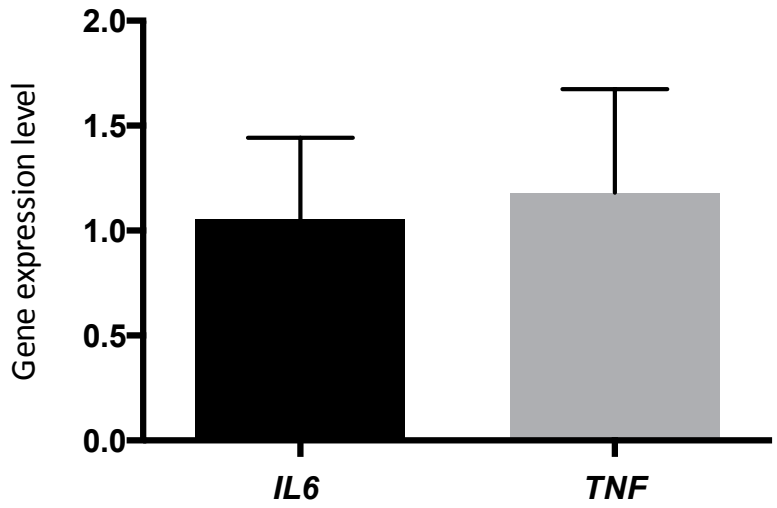

Figure 7.2 *IL6* and *TNF*-α gene expressions after a 6-week *n*-3 FA supplementation (*n* = 191).

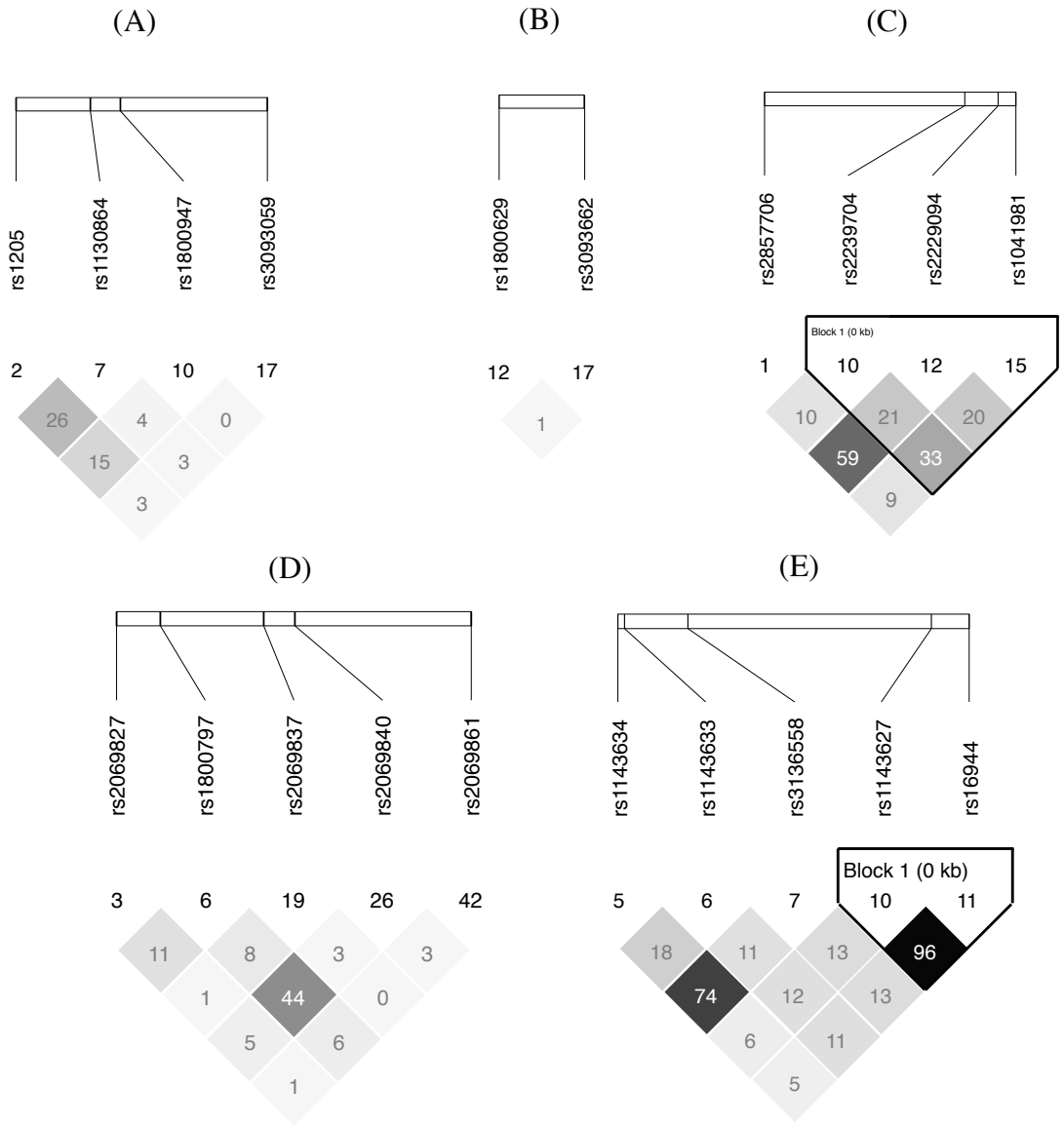

Figure 7.3 LD plots of selected inflammation-related genes.

The lighter the shade of grey, the lesser is the correlation between two SNPs.
(A) *CRP* gene;
(B) *TNF*-α gene;
(C) *LTA* gene;
(D) *IL6* gene;
(E) *IL1B* gene.

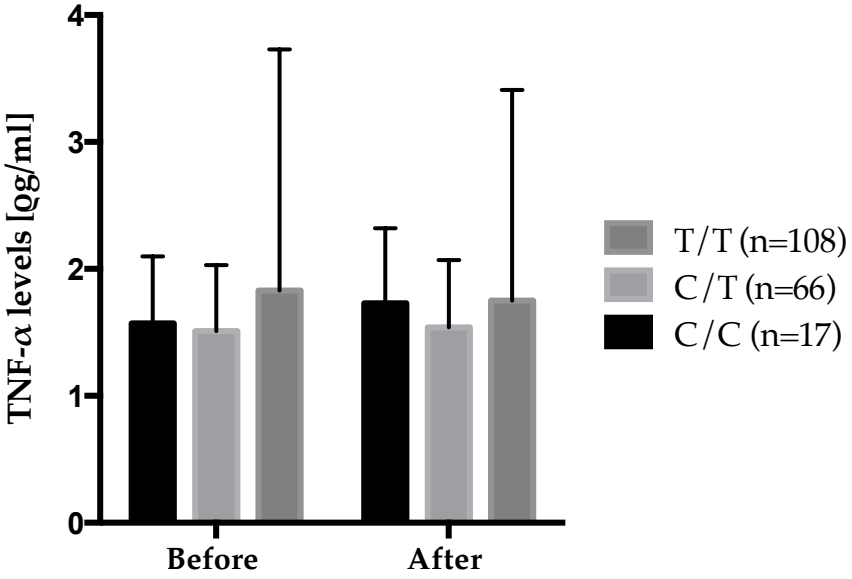

Figure 7.4 Gene–diet interaction on plasma TNF-α levels according to rs2229094 (*TNF-α*) (*n* = 191).

Chapitre 8

Nutrigénomique - Points de vue des diététistes professionnels : Rapport sur la Consultation panquébécoise en ligne sur la nutrigénomique auprès des diététistes membres de l'OPDQ

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Nutrigenomics: Perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians.

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Résumé

Les professionnels de la santé ne sont pas tous familiers avec la nutrigénomique. Cependant, ils reconnaissent que la nutrigénomique a un grand potentiel pour le développement de mesures préventives en santé. La présente étude vise à donner une image globale de la situation actuelle en matière de nutrigénomique dans la pratique des diététistes (Dt.P.) de la province de Québec (Canada). **Méthodes :** Trois cent soixante-treize Dt.P. membres de l'Ordre professionnel des diététistes du Québec ont répondu à un sondage en ligne comportant 34 questions dont la plupart étaient des questions fermées. **Résultats :** Dans l'ensemble, 76,9% des Dt.P. connaissaient la nutrigénomique. Parmi les Dt.P. ayant moins de cinq années d'expérience, 49,2% étaient au courant des tests génétiques liés à la nutrition, appelés tests de nutrigénétique, comparativement à 11,7% des Dt.P. comptant plus de 25 années d'expérience. Actuellement, 75,9% des Dt.P. travaillant dans le domaine de la nutrition clinique dans le secteur public considèrent qu'ils n'ont pas les connaissances de base pour intégrer la nutrigénomique dans leur pratique, comparativement à 62,9% pour les Dt.P. en pratique privée. Lorsqu'interrogés sur les principales limites des tests de nutrigénétique, les Dt.P. considèrent que les tests génétiques ne tiennent pas compte des autres déterminants de la santé, que les tests génétiques et leurs résultats sont peu précis et qu'il existe un manque de preuves scientifiques. Des inquiétudes subsistaient quant aux aspects éthiques et légaux et à son application difficile en raison d'une mauvaise compréhension et/ou interprétation par les professionnels et/ou les clients. Les coûts élevés de ces tests ont également été perçus comme une limitation. **Conclusion :** La majorité des diététistes connaissent et sont intéressés par la nutrigénomique, en particulier ceux qui ont moins d'années d'expérience, bien qu'ils ne se sentent pas suffisamment qualifiés pour intégrer les résultats de la nutrigénomique dans leur pratique actuelle.

Abstract

Background: Not all healthcare professionals are familiar with nutrigenomics. However, they recognize that nutrigenomics has great potential for the development of preventive health approaches. The present study aimed to provide an overall picture of the current situation about nutrigenomics in the practice of registered dietitians (RDs) from the province of Quebec (Canada). **Methods:** Three hundred and seventy-three RDs members of the *Ordre professionnel des diététistes du Québec* completed an online survey that included 34 questions, most of which were closed-ended questions. **Results:** Overall, 76.9% of RDs knew about nutrigenomics. Among RDs with <5 years of experience, 49.2% knew about genetic testing related to nutrition compared to 11.7% for RDs with over 25 years of experience. Currently, 75.9% of RDs working in clinical nutrition in the public sector consider that they do not have the basic knowledge to integrate nutrigenomics in their practice compared to 62.9% for RDs in private practice. When asked about main limitations of genetic testing related to nutrition, RDs considered that genetic testing does not consider the other determinants of health, that genetic testing and their results have poor accuracy, and that there is a lack of scientific evidence. Concerns remained about ethical and legal aspects and its difficult application as a result of poor understanding and/or interpretation by professionals and/or customers. The high costs of these tests were also noted as a limitation. **Conclusions:** Registered dietitians know and are interested in nutrigenomics, especially those with less experience, although they do not feel adequately qualified to integrate findings from nutrigenomics into their practice.

Introduction

For over a decade, nutrigenomics has aimed to identify genes that influence the risk of diet-related diseases across the whole genome, and to understand the mechanisms that link genetic predispositions to diseases (Muller & Kersten, 2003). Personalised nutrition is one of the possible applications of this science, which led to the development and the marketing of genetic tests directly available to consumers on the Internet that are called Direct-to-Consumer tests (DTC-tests). The number of DTC-tests is increasing each year (Genetics & Public Policy Center, 2008; Sterling, 2008; Goddard *et al*., 2009). Consumers' interest in DTC-tests is growing and the commercialisation of these tests, particularly in the field of nutrigenomics, is expected to expand over time as research identifies associations between genotype and phenotype (Ries & Castle, 2008).

Towards the end of the first decade of the 2000s and at the beginning of the 2010s, several trends were moving the use of genetic technologies in clinical settings and encouraging the public to access technologies via the marketplace (2004b), especially with the emergence of the DTC-test. However, Wendel *et al*. (2013) showed that consumers must be accompanied when receiving genetics recommendations, and intermediaries such as general practitioners could play a crucial role. Up to now, the only genetic tests systematically administered in the Quebec's health network (Quebec City, QC, Canada) are made in the context of neonatal screening for 12 metabolic or endocrine diseases (Ouellette, 2011). Blood screening includes tyrosinaemia, phenylketonuria and congenital hypothyroidism, whereas urine screening includes nine other metabolic diseases (Ouellette, 2011).

Studies have shown that healthcare professionals such as physicians, registered dietitians (RDs), nutritionists, pharmacists and naturopaths are not familiar with nutrigenomics and cannot currently integrate findings issued from this science into their clinical practice (2004a, Weir *et al*., 2010). In addition, the majority of healthcare professionals are pessimistic about the idea of integrating this new science in their scope of practice because they do not consider it as useful and find that nutrigenomics does not provide sufficient information to adequately advise the population (Weir *et al*., 2010). However, they recognise the great potential of

nutrigenomics in the development of preventive health approaches (Weir *et al*., 2010). Healthcare professionals are questioning whether it is not too early to incorporate nutrigenomics to the range of health services currently offered (2004a).

The Institute of Medicine identifies RDs as the best qualified professionals for nutrition therapy and they are currently the single identifiable group of healthcare professionals with the appropriate standardised education, clinical training, continuing education and national credentialing requirements necessary to be a directly reimbursable provider of nutrition therapy (2000; American Dietetic Association). The role of RDs has been previously described by the Academy of Nutrition and Dietetics: RDs work to optimise the health of the population and to advance the profession of nutrition by providing quality services, focusing on the safety and quality of life of individuals (The Academy Quality Management Committee and Scope of Practice Subcommittee of the Quality Management Committee 2013). The services provided by RDs are designed to help individuals and the general population to improve their health by adopting eating habits and healthier way of life (2013). The nutrition services cover a wide range of areas, such as clinical nutrition, food service management, education, technology, industry sector, communication, health promotion, disease prevention and the development of public health policy (2013). RDs are considered to be the best-qualified healthcare professionals when it comes to nutrigenomics. However, studies showed that RDs do not consider they have the necessary skills in genetics and genomics to integrate nutrigenomics in their scope of practice (McCarthy *et al*., 2008; Whelan *et al*., 2008).

The present study aimed to provide an overall picture of the current situation of the use of data from nutrigenomics in the professional practice of RDs of the province of Quebec. Specific objectives were to describe the current knowledge of RDs regarding nutrigenomics, to identify training needs in nutrigenomics of RDs and to highlight the perceived limitations of the use of genetic tests in their scope of practice.

Methods

Proceedings

All members of the *Ordre professionnel des diététistes du Québec* (OPDQ) (n = 2754) were invited to participate in this survey as part of the *Quebec-wide e-consultation on nutrigenomics among RDs*. To be eligible, participants had to be a member of OPDQ, be able to answer the questionnaire written in French, and have access to a computer and an Internet connection. They exclusively received the invitation to participate in the survey via e-mail in the OPDQ newsletter. The first invitation was sent on 10 October 2012. Two reminders were then sent every 3 weeks (on 30 October and 12 November 2012). The hyperlink was subsequently closed on 3 December 2012. The IP address of the computer with which the survey was completed was checked, thus reducing the risk of someone completing the survey twice (or more). A total of 421 RDs completed the questionnaire, representing a response rate of 15.3%. A total of 10 questionnaires were excluded from further analysis because undergraduate students or retirees completed them and 38 others were excluded as a result of missing data. The analyses are thus based on questionnaires received from 373 RDs. The composition of the study population was compared with data provided by the OPDQ from December 2012. This project was approved by the Ethics Committee on Research Involving Human Subjects of Laval University.

Questionnaire development

The questionnaire was developed specifically for the present study using Survey Monkey Gold with enhanced security (http://www.surveymonkey.com/). A pretest of the questionnaire was conducted with 11 RDs to assess the time to complete the questionnaire, the clarity of the questions and the relevance of the answer choices. The survey included 34 questions, most of which were dichotomous closed-ended questions. Other issues were multichotomic with one possible answer or multichotomic with multiple possible answers, leaving the respondent the freedom to choose one or more of the answers contained in the list. There was one open question about the limitations of genetic tests and multiple answers were allowed. The answers made by RDs were then computed in eight different categories: (i) don't know; (ii) poor understanding and/or interpretation of the results by professionals

and/or consumers; (iii) the high costs of genetic tests; (iv) ethical and legal aspects; (v) genetic testing does not consider the other determinants of health; (vi) the difficult application of genetic testing in clinical settings; (vii) the lack of evidence; and (viii) genetic testing and their results have poor accuracy. At the very end of the questionnaire, questions for quota sampling (sex, age group, administrative region, scope of practice, year of graduation, number of years of practice) were added to allow comparative statistical analysis between the different subgroups. A Likert scale was used in some issues where, for example, a five-point scale divided the answer choices: 1 = Unlikely; 2 = Less likely; 3 = Neutral; 4 = Likely; and 5 = Very likely.

Statistical analysis

SurveyMonkey allowed the results to be converted and downloaded into excel (Microsoft, Redmond, CA, USA) calculation sheets to import into SAS, version 9.2 (SAS Institute, Cary, NC, USA). Data from the open question was compiled in a document and groups were created to draw the most frequent responses. Areas of practice were divided into six sectors: (i) clinical nutrition in public health settings; (ii) clinical nutrition in private practice; (iii) food services management; (iv) public health nutrition/health promotion; (v) academic/research; and (vi) communications/industry sectors. Frequency distributions were generated. Chi-squared tests were used to test differences in the frequency distribution. Analyses of variance and least squares means were used to determine differences between each group. Scores attributed to metabolic disorders were calculated by summing individuals' answers to the question: 'In your opinion, what is the probability that results from nutrigenetic tests can improve the nutritional recommendations of the following diseases?'. The possible answers ranged from 0 to 5 (0 = I don't know; 1 = Unlikely; 2 = Less likely; 3 = Neutral; 4 = Likely; 5 = Very likely). The overall score is the mean of score that could range from 0 to 5, where a score of 0 means that nutrigenetic tests would not improve the nutritional recommendations of a particular disease and where a score of 5 means that nutrigenetic tests would improve the nutritional recommendations. $P < 0.05$ was considered statistically significant.

Results

Subject characteristics are shown in Table 1. With the profession being predominantly feminine, 95.2% of study participants were women. RDs who participated in the present study were representative of RDs in Quebec with regard to sex, age, area of practice and the administrative region where members are primarily employed (data not shown).

Knowledge in nutrigenomics

Seventy-six percent of RDs working in clinical nutrition in public care settings ($n = 157$) considered they do not have the knowledge necessary to incorporate nutrigenomics into their clinical practice compared to 62.9% for RDs working in clinical nutrition in private practice ($n = 22$). When probed about their interest for training in nutrigenomics, 58.5% of RDs working in clinical nutrition in public care settings stated that they would be extremely interested or very interested in attending training on nutrigenomics compared to 77.1% for RDs working in clinical nutrition in private practice (Table 2). When asked about the way that RDs should be trained/informed on nutrigenomics, the most common answers were education accredited by universities offering the nutrition undergraduate program, videoconferencing, e-Learning, podcasts, conferences, symposia, through articles in the professional journal of the OPDQ, interactive courses on the web, via DVD or through practical workshops in small groups. Currently, 55.4% of RDs consider that training should be mandatory and included in the undergraduate program in nutrition, whereas 19.9% of them consider that training should be optional. Over one-third (34.0%) of RDs consider that it would be appropriate to develop a short graduate program in nutrigenomics. When asked about which instance should offer training in nutrigenomics, 76.3% of RDs said that universities should be the preferred option versus a professional organization (19.7%) and RDs workplace (3.7%).

Among responders, the majority of RDs knew about nutrigenomics (76.9%; $n = 316$). Among RDs working in clinical nutrition in the public sector, 76.8% of them knew about nutrigenomics compared to 91.4% for RDs working in clinical nutrition in private practice ($P < 0.0001$) (Fig. 1). University courses (34.8%; $n = 143$), colleagues (27.0%; $n = 111$),

scientific papers (25.1%; $n = 102$) and scientific meetings (23.1%; $n = 95$) were identified as the best way to acquire knowledge about nutrigenomics from the available choices: newspapers (12.9%; $n = 53$), training (12.2%; $n = 50$), Internet (11.0%; $n = 49$), other healthcare professionals (5.1%; $n = 21$), social networks (5.1%; $n = 21$), television (2.9%; $n = 13$), radio (1.0%; $n = 4$) and patients (0.7%; $n = 3$) (Fig. 2). It is estimated that 14.5% of RDs in clinical nutrition in public sector have heard of nutrigenomics in scientific meetings compared to 55.1% for RDs working in academic and research fields ($P < 0.0001$). University courses were identified as a way for RDs to learn more about nutrigenomics, mostly for RDs who had <5 years of experience of practice and/or had recently graduated. Indeed, 63.0% of RDs who have heard of nutrigenomics in university courses have <5 years of experience compared to 3.0% for RDs who have over 25 years of experience.

As shown in Fig. 1, in the past year, only 6.3% of RDs working in clinical nutrition in the public health settings attended a conference, training or scientific meeting offering nutrigenomic sessions compared to 33.3% of RDs working in the communications/industry sectors, 14.3% for RDs in private practice, 0% for RDs in food services management, 12.5% of RDs in public health nutrition/health promotion, and 55.1% of RDs in academic/research ($P < 0.0001$).

Genetic testing

When asked whether they were aware that DTC-tests were offered to consumers via Internet, 49.2% of RDs with <5 years of experience know that DTC-tests were available in nutrigenomics compared to 11.7% for RDs with more than 25 years of experience.

Registered dietitians' concerns about genetic testing

With regard to preoccupations in nutrigenomics, RDs were very or extremely preoccupied (63.5%, $n = 237$) by genetic discrimination and considered that it is the biggest concern for the public interest. RDs also had other preoccupations regarding genetic testing such as the validity of genetic tests (very or extremely preoccupied; 62.0%; $n = 230$), the high cost associated with DTC-tests (very or extremely preoccupied; 56.6%; $n = 211$), patient anxiety (very or extremely preoccupied; 44.9%; $n = 168$) and confidentiality (very or extremely

preoccupied; 30.3%; $n = 113$). RDs with more than 25 years of experience (48.9%, $n = 45$) indicated that they are very or extremely concerned about privacy issues of DTC-tests in nutrigenomics compared to 21.5% of RDs with <5 years of experience ($n = 130$). Several limitations about genetic testing were also noted by RDs, as shown in Fig. 3. However, <20% of RDs (19.9%, $n = 84$) responded to open-ended questions.

Improvements in nutritional recommendations related to various diseases

Table 3 shows that RDs do not necessarily see nutrigenomics as a window of opportunity for the prevention and/or the nutritional assessment of many diseases. Scores attributed to metabolic disorders were all under 3.00, with a mean score of 2.54 ± 0.10, meaning that RDs were less likely to consider that nutrigenomics could improve nutritional recommendations for these diseases. Associations of nutrigenomics with chronic diseases are much more noticeable. The overall score was >3.00 for all chronic diseases, including coeliac disease, obesity, T2DM and CVD. Associations of nutrigenomics with degenerative diseases are not very strong and this reflects negatively on the overall score (2.52 ± 0.10). Forty-five percent of RDs considered that nutritional recommendations from nutrigenetic tests could actually be beneficial for the treatment of familial hypercholesterolemia, with an overall score of 3.63 ± 0.10. When stratified by their area of practice, scores were generally significantly higher among the Academic/research field (significantly higher for homocystinuria, urea cycle disorder, CVD, cancers, familial hypercholesterolemia and osteoporosis). RDs in public health nutrition/health promotion always displayed the lowest scores.

Discussion

According to results of the present study, more than 75% of RDs knew about nutrigenomics. The results vary considerably from one area of practice to another, which could be explained by the specific needs of patients/clients and the scientific activity required in some areas compared to others. In addition, other areas of practice, such as food services management, do not require knowledge on nutrigenomics, which can explain why RDs evolving in that field did not attend any conferences or scientific meetings on nutrigenomics in recent years.

Regarding DTC-tests, 67.8% of RDs were not aware that such tests exist and RDs with fewer years of experience were better informed, probably because they had completed university courses on nutrigenomics. Among RDs who were aware of the existence of DTC nutrigenomic tests, 49.2% have <5 years of experience, whereas 19.2% of RDs have more than 16 years of experience. Powell *et al*. (2012) showed that only 39% of primary care physicians were aware of the existence of DTC-tests and 85% of them did not feel prepared to answer patients' questions about it. In the present study, when asked whether they have any concerns about DTC-tests, RDs with more than 25 years of experience usually said they were very or extremely concerned about privacy issues. RDs with <5 years of experience were not as concerned as experienced RDs, with only 21.5% feeling worried about privacy. The main limitations of genetic testing identified by RDs are mostly the same as those found in the literature, namely the ethical and legal aspects (Chadwick, 2004; Gorman, 2006; Rosen *et al*., 2006; Morin, 2009; Ahlgren *et al*., 2013); lack of evidence (Ries & Castle, 2008); difficult application of genetic testing in the clinical care settings (Rosen *et al*., 2006; 2004b); validity and predictive value of genetic testing (Ries & Castle, 2008; Morin, 2009); misinterpreted results by healthcare professionals and/or consumers (Morin, 2009); and the high costs of such tests (Morgan *et al*., 2003). In addition, RDs raised the fact that genetic testing would not consider other determinants of health. Rosen *et al*. (2006) reported similar limitations among RDs. Moreover, they also raised other limitations to the application of nutrigenomics among RDs, such as the lack of certainty about insurance reimbursement, background knowledge of RDs, continuing education for RDs, and the need for experts to convey professional expertise.

RDs considered that misunderstanding and/or the misinterpretation of the results by professionals and/or consumers are an actual limit of nutrigenomics, hence the importance of establishing clinical practice guidelines. Weir *et al*. (2010) showed that healthcare professionals including physicians, pharmacists and RDs, raised the fact that clinical practice guidelines would help the development of a standardised approach to counselling. One major barrier to bringing nutrigenomics to the masses is that theory behind this science overwhelms consumers (Isaak & Siow, 2013).

A survey conducted in 2004 in the USA with 995 RDs reported that the majority of RDs (60%) have little confidence in their ability to provide nutrition services based on nutrigenomics (Rosen *et al*., 2006). In the present study, 75.9% of RDs working in clinical nutrition in the public care settings felt they did not have the knowledge to incorporate nutrigenomics in their clinical practice compared to 62.9% for RDs working in clinical nutrition in private practice. Almost 91.4% of RDs working in clinical nutrition in private practice knew about nutrigenomics, which is one of the highest scores with respect to the RDs' area of practice. This could be explained by competition among healthcare providers. Indeed, RDs must now adapt their service offerings based on demand if they wish to remain competitive and this may involve the use of DTC-tests. RDs in clinical nutrition in private practice also consider that they will be using data from nutrigenomics in the future.

RDs do not always perceive the links between genetics and several diseases requiring a nutritional therapy, as shown in Table 3. This is the case of phenylketonuria, where 36.4% of RDs consider it very likely that nutrigenomics will have a positive impact on nutritional recommendations, although there are still 31.1% of RDs who are not convinced that nutrigenomics can be beneficial. Rosen *et al*. (2006) reported that the majority of RDs were positive about the benefits of the application of nutrigenomics. Most agreed or strongly agreed that the application would result in greater individualisation of diet prescriptions (84%), a strong foundation for nutrition recommendations (76%) and dietary prescriptions that would manage/prevent certain diseases (75%). They also reported that RDs with more positive attitudes about the benefits of nutrigenomics were more confident in their ability to apply nutrigenomics, were more likely to indicate previous exposure to nutrigenomics, were

more likely to agree that the application of nutrigenomics will change the practice of dietetics and indicated a greater interest in learning more about nutrigenomics (Rosen *et al*., 2006).

Learning is a key factor with respect to allowing RDs to integrate nutrigenomics findings into their practice. Among responders, university courses appear to be one of the best ways to learn about nutrigenomics. According to Patterson *et al*. (1999), education must play a leading role with regard to learning about nutritional genomics and courses on the interaction between genes and diet must be included in the undergraduate programme. Moreover, according to Vickery & Cotugna (2005) who interviewed programme managers in nutrition, 70.7% of them agree that genetics is an important component of nutrition education and 47.6% of them responded that their institution strongly supported the integration of genetics into the university curricula. Because this is a relatively new science, our study showed that RDs with <5 years of experience are the most familiar with nutrigenomics, possibly because they were exposed to it at some time during their academic background. However, scientific conferences remain an important source of information for RDs who have completed their academic training and are currently in the labour market. As expected, RDs in academic/research sectors attended a conference, training or a scientific meeting in greater numbers compared to any other RDs' scope of practice, and 75.5% of RDs in that group are interested in a training programme in nutrigenomics. Interestingly, RDs working in the communication/industry sectors and RDs working in clinical nutrition in private practice are also mostly interested in a training programme (83.3% and 77.1%, respectively). DeBusk *et al*. (2005) recommended that RDs have a solid foundation in basic sciences and genetics to be prepared to work in the era of nutritional genomics.

Limitations of the present study

Registered dietitians from the present study sample are representative of the distribution of RDs according to their years of experience and their scope of practice, except for RDs under 25 years who represent 15.3% of the participants in the present study, whereas their proportion in OPDQ is only 5.2%. These results suggest that younger RDs have completed the survey in greater numbers compared to older professionals. The fact that the survey was available online could partly explain this difference. According to a report from *Institut de la*

statistique du Québec (*Gouvernement du Québec*, Canada), 95.0% of people aged between 25 and 34 years in the province of Quebec are using the Internet compared to 63.6% for people aged between 55 and 64 years (2011). Also, the response rate (15.3%) is lower than those normally observed in the literature for healthcare professionals. Potts & Wyatt (2002) have shown that response rates may vary depending upon how Internet savvy the sample is. In addition, some professionals may be reluctant to respond to a survey if the questions and/or the subject do not make sense for them and if the investigation takes too much time in a schedule that is already overloaded (Mangus *et al*., 1999).

Conclusion

The findings of the present study show that RDs do not feel sufficiently qualified to integrate nutrigenomics into their clinical practice, although they are interested and see it as an opportunity to expand their scope of practice. RDs are considered to be the most qualified health professional to talk about nutrigenomics with their clients. In that context, it is particularly important that RDs acquire the knowledge and skills to integrate this science into their practice. In light of these results, prelicensure training as part of undergraduate programmes in nutrition appears to be the best way to train future RDs in the field of nutrigenomics. Genomics will help open the door to new approaches in personalised medicine in the near future.

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Conflict of interests, source of funding and authorship

The authors declare that there are no conflicts of interest.

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Hubert Cormier and Bénédicte L.-Tremblay performed statistical analyses and interpreted the data. Hubert Cormier wrote the paper. Ann-Marie Paradis and Marie-Claude Vohl conceived the questionnaire. Marie-Claude Vohl, Sophie Desroches and Julie Robitaille designed the research. Hubert Cormier and Marie-Claude Vohl have primary responsibility

for the final content. All authors read and approved the final manuscript submitted for publication.

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Tableau 8.1 Characteristics of dietitians who participated in the Quebec wide e-consultation on nutrigenomics.

	All n= 373	**Women n=355**	**Men n=18**
Number (%)		95.2	4.8
Age, n (%)			
≤ 25 yr	57 (15.3)	52 (14.7)	5 (27.8)
26-30 yr	91 (24.4)	85 (23.9)	6 (33.3)
31-40 yr	103 (27.6)	99 (27.9)	4 (22.2)
41-50 yr	66 (17.7)	63 (17.8)	3 (16.7)
≥ 50 yr	56 (15.0)	56 (15.8)	-
Level of education, n (%)			
Undergraduate degree in nutrition	243 (65.2)	234 (65.9)	9 (50)
2nd cycle graduate short program in nutrition	12 (3.2)	11 (3.1)	1 (5.6)
Master in nutrition	65 (17.4)	57 (16.1)	8 (44.4)
PhD in nutrition	13 (3.5)	13 (3.7)	-
Other graduate studies	40 (10.7)	40 (11.3)	-
Experience as dietitians, n (%)			
≤5 yr	130 (34.9)	119 (33.5)	11 (61.1)
6 to 15 yr	128 (34.3)	124 (34.9)	4 (22.2)
16 to 25 yr	69 (18.5)	67 (18.9)	2 (11.1)
>26 yr	46 (12.3)	45 (12.7)	1 (5.6)
Area of practice, n (%)			
Clinical nutrition in public health settings	207 (55.5)	199 (56.1)	8 (44.4)
Clinical nutrition in private practice	35 (9.4)	31 (8.7)	4 (22.2)
Food services management	16 (4.3)	16 (4.5)	-
Public health nutrition/Health promotion	48 (12.9)	47 (13.2)	1 (5.6)
Research/Education	49 (13.1)	46 (13)	3 (16.7)
Communications/Industry sector	18 (4.8)	16 (4.5)	2 (11.1)
Urban centers, n (%)			
Quebec City	97 (26.0)	92 (25.9)	5 (27.8)
Montreal	125 (33.5)	119 (33.5)	6 (33.3)
Elsewhere in the province of Quebec	151 (40.5)	144 (40.6)	7 (38.9)

Tableau 8.2 Registered dietitian's interest level to follow training in nutrigenomics.

	Not at all (%)	Little (%)	Neutral (%)	Very (%)	Extremely (%)
Clinical nutrition in public health settings	0 (0)	17 (8.2)	69 (33.3)	82 (39.6)	39 (18.8)
Clinical nutrition in private practice	0 (0)	1 (2.9)	7 (20.0)	16 (45.7)	11 (31.4)
Food services management	0 (0)	5 (31.3)	5 (31.3)	6 (37.5)	0 (0)
Public health nutrition/Health promotion	4 (8.3)	7 (14.6)	13 (27.1)	18 (37.5)	6 (12.5)
Academic/Research	0 (0)	8 (16.3)	4 (8.2)	26 (53.1)	11 (22.5)
Communications/Industry sectors	0 (0)	2 (11.1)	1 (5.6)	9 (50.0)	6 (33.3)
Frequencies calculated using the FREQ procedure in SAS 9.2. Total do not sum to the sample size due to missing data					

Tableau 8.3 Quebec RDs' knowledge in regard to the probability of nutrigenetic tests to improve nutritional recommendations for the following diseases

	I don't know	Less likely	Neutral	Likely	Very likely	P value[1]	Overall scores[2]	Scores, stratified by scope of practice						P value[3]
								Clinic (public sector)	Clinic (private sector)	Food services management	Public health	Academic/Research	Comm.	
Phenylketonuria	31.1% (118)	5.8% (22)	4.2% (16)	22.4% (85)	36.4% (138)	<0.0001	2.93±0.11	ns	ns	ns	ns	ns	ns	0.11
Galactosemia	33.5% (127)	5.8% (22)	4.5% (17)	21.9% (83)	34.3% (130)	<0.0001	2.81±0.11	ns	ns	ns	ns	ns	ns	0.11
Tyrosinemia	37.5% (142)	5.5% (21)	4.5% (17)	20.3% (77)	32.2% (122)	<0.0001	2.64±0.11	ns	ns	ns	ns	ns	ns	0.25
Maple Syrup Urine Disease (MSUD)	49.2% (186)	4.5% (17)	5.6% (21)	15.6% (59)	25.1% (95)	<0.0001	2.12±0.11	ns	ns	ns	ns	ns	ns	0.32
Glycogen storage disease type 1	49.1% (186)	3.7% (14)	3.7% (14)	18.2% (69)	25.3% (96)	<0.0001	2.16±0.11	ns	ns	ns	ns	ns	ns	0.35
Homocystinuria	43.9 (165)	4.3% (16)	5.3% (20)	20% (75)	26.6% (100)	<0.0001	2.35±0.11	2.46^{ab}	2.49^{ab}	2.06^{ab}	1.56^{a}	2.88^{b}	1.94^{ab}	0.07
Urea cycle disorder	36.9% (139)	4% (15)	6.6% (25)	22% (83)	30.5% (115)	<0.0001	2.67±0.11	2.75^{ab}	2.69^{ab}	2.31^{ab}	1.92^{a}	3.33^{b}	2.17^{ab}	0.04
Cystic Fibrosis	34% (128)	3.5% (13)	9.8% (37)	27.1% (102)	25.7% (97)	<0.0001	2.71±0.11	ns	ns	ns	ns	ns	ns	0.17
Metabolic disorders[4]	-	-	-	-	-	-	2.54±0.10	ns	ns	ns	ns	ns	ns	0.10
Coeliac/Crohn's diseases	21.2% (80)	2.9% (11)	6.9% (26)	37.1% (140)	31.83% (120)	<0.0001	3.34±0.10	3.48^{a}	3.40^{ab}	2.56^{ab}	2.63^{b}	3.70^{ab}	3.11^{ab}	0.02
Obesity	19.3% (73)	5.5% (21)	12.1% (46)	35.4% (134)	27.7% (105)	<0.0001	3.27±0.09	3.38^{a}	3.46^{ab}	2.38^{ab}	2.54^{b}	3.67^{a}	3.11^{ab}	0.007

Type 2 diabetes	18.5% (70)	4.2% (16)	10.1% (38)	36.8% (139)	30.4% (115)	<0.0001	3.38±0.09	3.53[a]	3.63[a]	2.50[ab]	2.48[b]	3.80[a]	3.11[ab]	0.0006
Cardiovascular diseases	19.1% (72)	2.1% (8)	6.1% (23)	39.7% (150)	33.1% (125)	<0.0001	3.46±0.09	3.56[ab]	3.54[ab]	2.69[ab]	2.92[a]	3.90[b]	3.22[ab]	0.05
Chronic Diseases[5]	-	-	-	-	-	-	3.36±0.09	3.48[a]	3.54[ab]	2.52[ab]	2.65[b]	3.79[a]	3.15[ab]	0.005
Parkinson	34.5% (130)	6.9% (26)	13% (49)	29.2% (110)	16.5% (62)	<0.0001	2.50±0.10	ns	ns	ns	ns	ns	ns	0.10
Alzheimer	33.2% (125)	7.2% (27)	12.7% (48)	31% (117)	15.9% (60)	<0.0001	2.55±0.10	ns	ns	ns	ns	ns	ns	0.07
Degenerative diseases[6]	-	-	-	-	-	-	2.52±0.10	ns	ns	ns	ns	ns	ns	0.09
Cancer	21.8% (82)	4.5% (17)	10.6% (40)	38.8% (146)	24.2% (91)	<0.0001	3.17±0.09	3.30[ab]	3.03[ab]	2.50[ab]	2.55[a]	3.57[b]	2.94[ab]	0.04
Hypertriglyceridemia	19.3% (73)	2.6% (10)	6.1% (23)	36.2% (137)	35.9% (136)	<0.0001	3.47±0.09	3.62[a]	3.31[ab]	2.63[ab]	2.83[b]	4.04[a]	3.22[ab]	0.006
Familial hypercholesterolemia	18.5% (70)	2.4% (9)	2.9% (11)	31.1% (118)	45.1% (171)	<0.0001	3.63±0.10	3.74[ab]	3.69[ab]	2.81[ab]	2.98[a]	4.24[b]	3.33[ab]	0.007
Osteoporosis	23.8% (90)	3.2% (12)	12.4% (47)	39.8% (151)	20.8% (79)	<0.0001	3.07±0.10	3.15[ab]	3.23[ab]	2.50[ab]	2.42[a]	3.53[b]	2.67[ab]	0.03

Total do not sum to the sample size due to missing data.
"ns" stands for "non-significant".
"Unlikely" and "Less likely" have been grouped together in this table, but statistical analyses have been made for each group separately.
p value derived from a chi-square test for equal proportions using the FREQ procedure in SAS 9.2.
Means ± SE, ranges from 0 to 5; 0=I don't know 1=unlikely, 2=less likely, 3=neutral, 4=likely and 5=very likely.
ANOVA with LSmeans
Metabolic disorders include: phenylketonuria, g**alactosemia, tyrosinemia, MSUD, glycogen storage disease type 1, homocystinuria, urea cycle disorder and cystic fibrosis.**
Chronic diseases include: Celiac/Crohn's disease, obesity, T2DM and CVD
Degenerative diseases include: Parkinson and Alzheimer

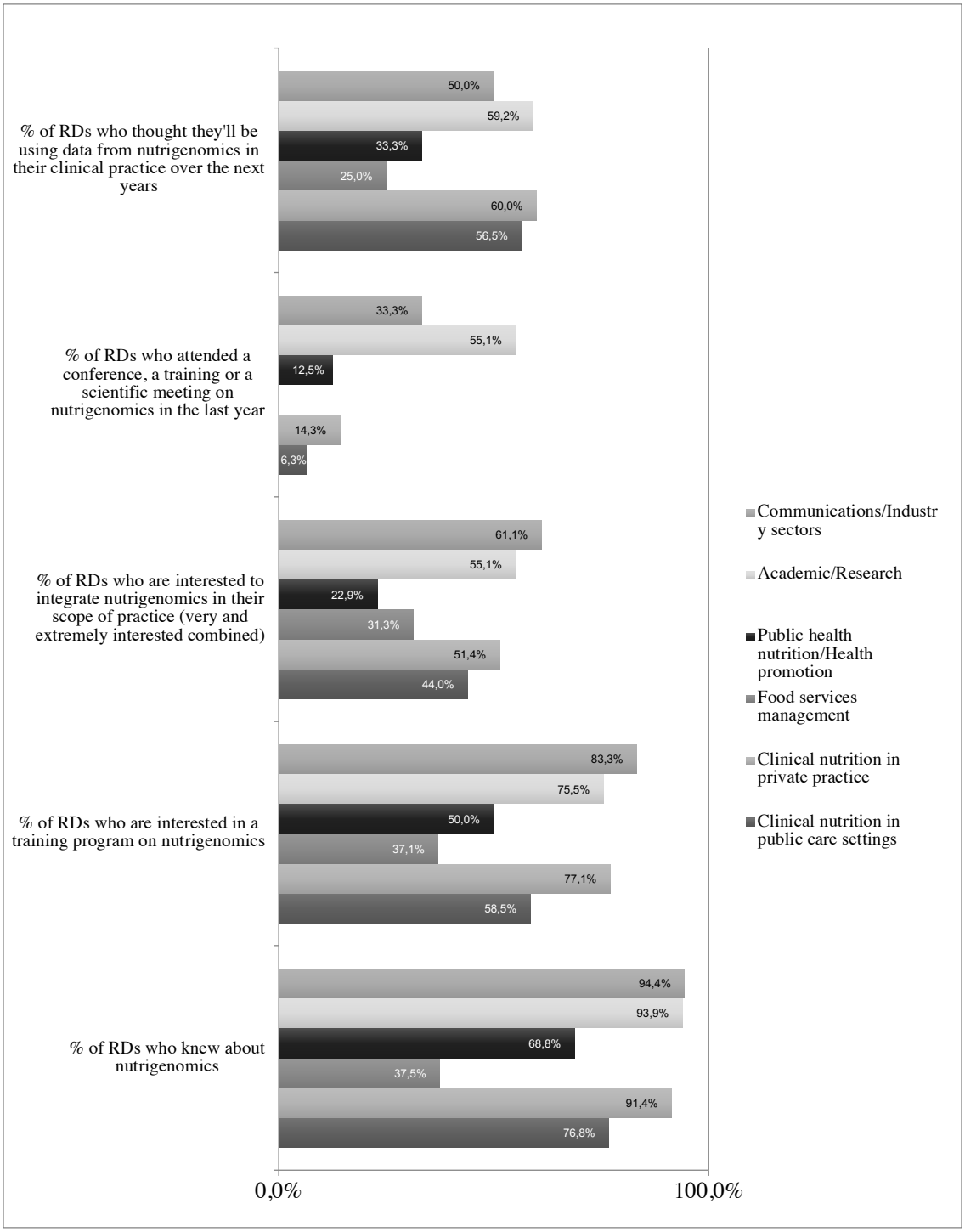

Figure 8.1 Interpretation of the results of the Quebec-wide e-consultation on nutrigenomics by dietitians' scopes of practice.

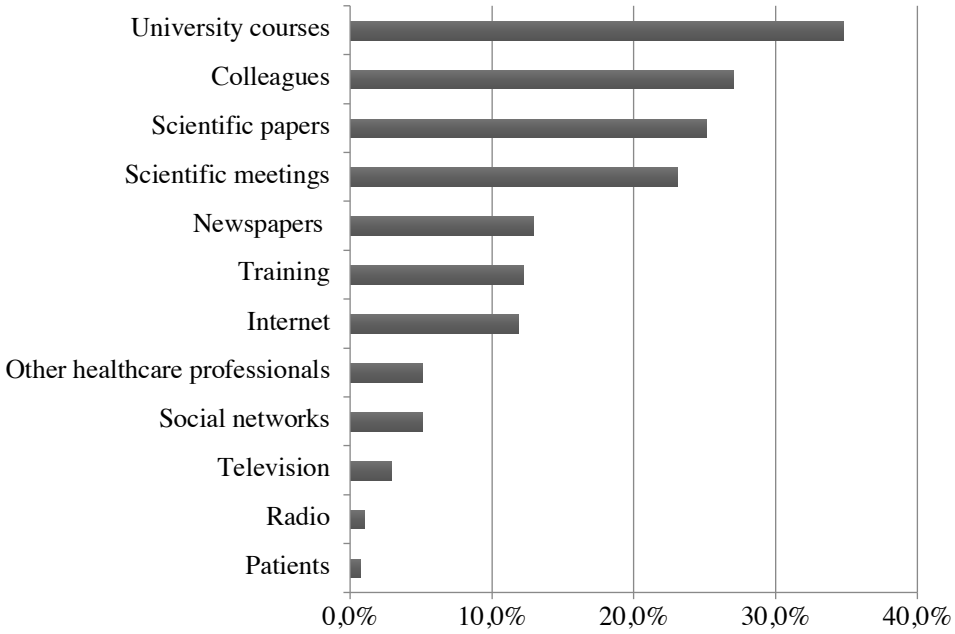

Figure 8.2 Best ways to acquire knowledge in nutrigenomics according to registered dietitians who participated in the Quebec-wide e-consultation on nutrigenomics.

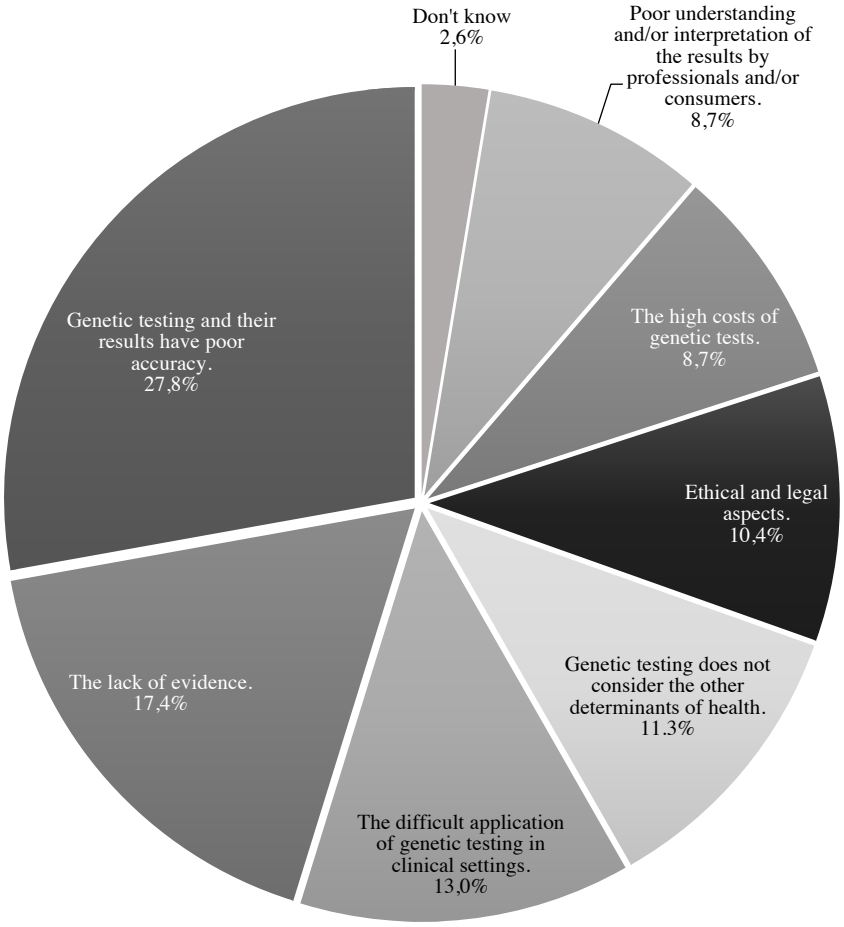

Figure 8.3 Main limitations of genetic testing according to registered dietitians who participated in the Quebec-wide e-consultation on nutrigenomics.

Chapitre 9 :
Conclusion

Ce projet de doctorat avait pour objectif global de vérifier l'impact de SNPs présents sur plusieurs gènes candidats sur la modulation des profils lipidique, glycémique et inflammatoire suite à une supplémentation en AGPI n-3. Pour y arriver, plusieurs approches ont été utilisées dont l'étude de l'expression génique, le dosage des AG dans les PPLs du plasma et des érythrocytes ainsi que l'étude de possibles interactions gène-diète.

La présence d'une grande variabilité interindividuelle dans la réponse à la supplémentation en AGPI n-3 peut être induite par des facteurs environnementaux, par le type d'alimentation ainsi que par une composante génétique. Par exemple, le génotype de l'APOE pourrait affecter la réponse lipidique suite à la consommation d'AGPI n-3 [20, 199-201]. Nos travaux de recherche ont montré une grande variabilité interindividuelle observée dans les niveaux de TG plasmatiques où 28,8% des individus ont connu une hausse de leurs niveaux de TG suite à la supplémentation en APGI n-3 (fournissant 1,9 à 2,2 grammes/jour d'AEP et 1,1 grammes/jour d'ADH) d'une durée de 6 semaines [21]. Ces résultats sont semblables à ceux observées dans d'autres cohortes, dont celle de la *FINGEN study* qui avait un protocole de supplémentation similaire au nôtre où 1,8 grammes d'AEP + ADH/jour pendant 8 semaines était administré aux participants [142]. L'effet hypotriglycéridémiant des AGPI n-3 est bien documenté dans la littérature [7, 202], mais très peu d'études se sont penchées sur la variabilité interindividuelle ou sur les causes pouvant expliquer ces écarts observés entre certains individus. Pour y voir plus clair, notre groupe de recherche a effectué un GWAS afin de comparer la fréquence allélique de SNPs entre les hypo- et hyper-répondeurs à la supplémentation catégorisés sur la base de la variation relative de leurs niveaux de TG plasmatiques suite à la supplémentation en huile de poisson [203]. Basé sur les résultats du GWAS, un score de risque génétique incluant 10 SNPs a été développé et a permis d'expliquer 21,3% de la variation dans la réponse des TG suite à la supplémentation. Ces avancées permettent notamment d'observer que les hypo- et hyper-répondeurs à la supplémentation ont des profils génétiques différents et que l'inclusion d'un score de risque génétique encore plus détaillé et inclusif pourrait s'avérer un outil précieux et complémentaire aux facteurs de risque traditionnels des MCV permettant ainsi de prédire la réponse à un traitement thérapeutique aux AGPI n-3.

Dans le cadre de ce projet de doctorat, le groupe de gènes *FADS* a été largement étudié dans le contexte du métabolisme des lipides. En effet, Tanaka *et al*. (2009), a précédemment identifié, dans un GWAS effectué sur 1 075 participants, la région *FADS1-2-3* comme étant la région la plus fortement associées aux concentrations plasmatiques d'AGPI n-3 et n-6 [131]. Le SNP rs174537 était fortement associé aux niveaux d'ARA et d'AEP [131]. Les gènes *FADS1* et *FADS2* codent pour deux désaturases impliquées dans la biosynthèse des AGPI n-3 et n-6. C'est pourquoi la voie métabolique de la synthèse des AG a été étudiée dans le présent projet de recherche. Pour ajouter à l'ensemble, nous avons décidé d'inclure les gènes *ELOVL2* et *ELOVL5* qui codent pour des élongases afin d'avoir un aperçu réaliste de l'activité enzymatique de cette voie.

Les résultats de ce projet de doctorat démontrent que la variabilité génétique présente dans certains gènes impliqués dans la voie de la biosynthèse des AG, spécifiquement dans les groupes de gènes *FADS1-2-3* et *ELOVL2-5*, affecterait la réponse des niveaux de TG suite à la supplémentation en AGPI n-3. Toutefois, aucun effet d'interaction gène-diète entre les SNPs de *FADS* et la supplémentation en huile de poisson n'a été trouvé, mais des effets du génotype seul affectant les niveaux de départ et les niveaux post-supplémentation de TG plasmatiques ont été trouvés, notamment pour rs174546 qui, selon la littérature, semble être un des SNPs fonctionnels de ce gène [21]. Ce SNPs se situe dans le gène *FADS1* qui, selon Wang *et al*. (2015), serait le gène causal dans la superfamille *FADS1-2-3 [204]*. Les chercheurs ont trouvé des associations pour 6 SNPs de *FADS* avec les niveaux d'expression du gène *FADS1*, les niveaux de protéines dans le foie et le profil lipidomique. Les associations avec *FADS2* et *FADS3* n'ont pas été concluantes, prouvant ainsi que *FADS1* est le gène causal impliqué dans le métabolisme hépatique des lipides et que ses SNPs altèrent la transcription du gène [204]. Selon nos travaux publiés dans le journal *Genes & Nutrition* (2014), plusieurs SNPs de *FADS1* ont également été associés à des effets d'interaction gène-diète modulant l'activité de la D6D où les porteurs de l'allèle mineur avaient la plus faible activité enzymatique [205]. L'activité de la D6D est associée positivement au risque d'obésité et à la résistance à l'insuline [206]. Conformément à la littérature, une augmentation de l'indice d'activité de la D5D et une diminution de l'indice d'activité de D6D ont été observées au sein de la cohorte FAS, laissant penser qu'une supplémentation en AGPI n-3

serait bénéfique en prévention de l'obésité et de l'insulinorésistance. Nos résultats montrent également que les porteurs de l'allèle mineur pour tous les SNPs étant associés à l'indice d'activité de la D5D présentaient toujours des niveaux plus faibles de l'indice d'activité de la désaturase après la supplémentation en huile de poisson, suggérant ainsi que ces individus pourraient être plus à risque de développer des complications métaboliques. La mesure de l'indice d'activité de la D6D a été faite en utilisant le ratio produit/précurseur suivant : (20:3n-6)/(18:2n-6). Or, la quantification du GLA étant plutôt faible dû à sa transformation rapide dans la biosynthèse des AGPI, il nous a été impossible d'obtenir la mesure directe de l'indice d'activité de la D6D sans devoir y inclure une étape d'élongation. Cependant, cette mesure est valide et est reconnue dans la littérature scientifique lorsque les proportions de GLA sont trop faibles [135, 207, 208]. Gillingham *et al*. (2013), ont quant à eux trouvé plusieurs associations entre les SNPs de *FADS1-2* et le métabolisme de l'[U-13 C]ALA, qui est une mesure directe de l'activité des désaturases, suite à l'étude d'une alimentation enrichie en huile de lin, une source d'AGPI n-3 d'origine végétale [209]. En présence de grandes quantités d'AGPI n-6, l'activité enzymatique des désaturases serait axée principalement sur les AGPI n-6 au détriment des AGPI n-3 et favoriserait un profil davantage considéré comme étant pro-inflammatoire. Toutefois, le rôle des AGPI n-6 dans l'augmentation du risque des MCV demeure controversé. Certaines études ont montré une amélioration du profil lipidique par une diminution des taux de cholestérol-total et de cholestérol-LDL, alors que d'autres contredisent clairement ces observations [210-212].

Chaque AG exerce des effets particuliers sur le métabolisme des lipides. Nos résultats de recherche montrent que non seulement les AGPI seuls peuvent moduler les niveaux de TG plasmatiques, mais également lorsqu'ils sont regroupés ensemble pour générer un profil d'AG obtenu à l'aide d'une analyse en composantes principales (PCA). La PCA est utile puisque chaque modification dans les apports en lipides affecte la composition cellulaire des membranes. Les résultats de l'analyse regroupent ainsi les facteurs qui sont fortement corrélés entre eux et qui permettent de mesurer le même construit. De plus, la PCA permet de réduire le nombre d'observations en un plus petit nombre de composantes qui devraient ainsi permettre d'expliquer une plus grande partie de la variance que les AG seuls. Nous avons voulu voir si les AGPI ainsi regroupés en facteurs seraient de meilleurs biomarqueurs

que les AG seuls suite à une supplémentation en huile de poisson. Suite à la PCA, nous avons identifié quatre profils d'AG différents, dont un où le score ainsi obtenu allait en direction opposée entre les hypo- et les hyper-répondeurs aux effets de l'huile de poisson sur les niveaux de TG, laissant penser que la composition en AG des membranes est différente entre ces deux groupes. De plus, plusieurs SNPs du groupe de gènes *FADS* étaient associés aux niveaux d'AG seuls ou à l'un ou l'autre des quatre facteurs précédemment identifiés. Comme les SNPs peuvent représenter entre 40 et 70% de la variabilité interindividuelle observée dans les niveaux d'AG mesurés dans les membranes des érythrocytes, il apparaît évident que des effets génétiques partagés peuvent être soit positifs ou négatifs entre plusieurs AG [213]. Pour les AGPI n-3, les effets rapportés sont généralement positifs. En effet, une augmentation de l'incorporation de l'AEP dans les membranes cellulaires serait inversement associée au risque d'événements coronariens majeurs et l'enrichissement des membranes en ADH confèrerait un profil de risque cardiovasculaire favorable [214, 215].

L'activité des désaturases pourrait également influencer le métabolisme du glucose [216]. De plus, selon Kroger *et al*. (2012), l'activité de la désaturase D6D est la plus fortement associée à l'incidence de DBT2, alors qu'une relation inverse a été vérifiée avec l'activité de la désaturase D5D [217]. Les travaux présentés dans cette thèse ont permis de constater que plusieurs SNPs du groupe de gènes *FADS* étaient associés aux contrôles glycémiques et plus particulièrement à l'indice HOMA-IS [218]. En effet, nous avons observé plusieurs effets d'interaction gène-diète, entre le gène *FADS* (rs7394871, rs174602, rs174570, rs7482316 and rs482548) et la supplémentation en AGPI n-3, affectant l'indice HOMA-IS suite à la supplémentation en huile de poisson. Brenner (2003) a montré que l'insuline pouvait influencer les niveaux d'activité de la D5D et de la D6D, surtout en condition de pré-diabète où la glycémie est plus élevée que la normale, mais pas suffisamment pour établir le diagnostic du DBT2 [216].

La synthèse endogène des AGPI à chaîne longue est possible grâce à l'action concertée des désaturases et des élongases. Or, les AGPI n-3 et AGPI n-6 sont en compétition pour les mêmes systèmes enzymatiques, ayant une influence sur les réponses inflammatoire et vasculaire [219]. C'est pourquoi nous nous sommes intéressés à l'inflammation suite à une

supplémentation en huile de poisson. Plusieurs études ont montré qu'une supplémentation en AGPI n-3 permettait de diminuer les concentrations de CRP, d'IL-6 et de TNFA, mais les résultats demeurent controversés, car d'autres études n'ont pas rapporté d'effets sur ces mêmes biomarqueurs de l'inflammation [220-224]. Dans notre cohorte, il n'y a pas eu de diminution significative dans les niveaux plasmatiques de CRP, d'Il-6 et de TNFA [225]. Toutefois, une interaction gène-diète entre le gène *TNF-alpha (*rs2229094) et la supplémentation modulant les niveaux de TNFA a été observée dans notre cohorte où les porteurs de l'allèle mineur avaient des niveaux significativement plus bas de TNFA après la supplémentation [225]. Le SNP rs2229094 est localisé dans un exon du gène de la super famille *TNF-LTA* et est responsable d'un changement d'acide aminé. De plus, ce SNP aurait un effet fonctionnel potentiel lorsqu'on regarde l'alignement séquentiel de ses nucléotides. Toutefois, selon Trebble *et al*. (2003), il n'y aurait pas de phénomène dose-réponse, mais plutôt une courbe en U avec un effet inhibiteur des AGPI n-3, notamment de l'AEP, sur les niveaux de TNFA à des doses < 2 grammes/jour par rapport à des doses > 2 grammes/jour. Conséquemment, l'impact d'une supplémentation en AGPI n-3 pourrait avoir des effets différents selon le génotype, mais également selon la dose fournie.

Plusieurs limites doivent être prises en considération. Dans un premier temps, l'absence de groupe contrôle dans l'étude FAS a souvent été questionnée par les réviseurs. Or, l'effet hypotriglycéridémiant des AGPI n-3 est bien documenté dans la littérature et il ne fait aucun doute que les résultats obtenus suite à la supplémentation en AGPI n-3 d'une durée de six semaines étaient ceux attendus, soit une réduction significative des niveaux de TG plasmatiques. Les différents questionnaires mesurant les apports alimentaires (journaux alimentaires et questionnaire de fréquence), l'activité physique et l'observance ainsi que l'encadrement par des professionnels de la santé reconnus, ont permis de réduire les écarts qui auraient pu expliquer une partie de la variance obtenue dans les résultats. De plus, toutes les analyses statistiques ont été contrôlées pour une myriade de cofacteurs dont les niveaux de départ d'AG et de TG ainsi que pour le sexe, l'âge, et l'IMC, permettant un maintien de la comparabilité. Conséquemment, il a été possible de mesurer l'impact de la présence de plusieurs SNPs intervenant dans le métabolisme des AG ou du glucose ainsi que dans l'inflammation suite à une supplémentation en huile de poisson. De plus, l'absence de groupe

contrôle aura permis de recruter plus de sujets qui ont pris part au protocole de supplémentation, augmentant du même coup la puissance statistique.

Dans le cadre de ce projet de doctorat, des doses pharmacologiques d'AGPI n-3 ont été administrées aux participants, soit 5 grammes/jour, ce qui est au-delà des recommandations actuelles émises par l'AHA pour le traitement de l'hypertriglycéridémie par les AGPI n-3. Comme l'effet hypotriglycéridémiant est dépendant de la dose d'AGPI n-3 et est fortement dépendant des valeurs de départ, il aurait été bon d'ajuster les critères d'inclusion de l'étude afin de cibler les individus hypertriglycéridémiques, c'est-à-dire des individus ayant des niveaux de départ de TG plasmatiques > 4,00 mmol/L contre une moyenne de 1,23 ± 0,64 mmol/L dans le cadre de l'étude actuelle. Ainsi, davantage d'associations auraient pu être observées. La présence d'individus métaboliquement sains dans l'étude FAS n'a pas permis non plus d'observer des résultats du côté de l'inflammation tel que relatés dans la littérature scientifique.

Bien que les travaux cités précédemment revêtent une importance pour l'enrichissement des connaissances dans le domaine de la nutrigénomique en lien avec le métabolisme des AG, ce projet de doctorat n'aurait pas été complet sans un volet d'application des connaissances. C'est grâce à des recherches de la sorte que l'information issue du monde scientifique puisse se rendre jusqu'aux professionnels de la santé ainsi qu'à la population. Dans le cadre de ce projet de doctorat, nos travaux se sont concentrés sur l'évaluation de l'état actuel des connaissances en nutrigénomique du point de vue des diététistes membres de l'OPDQ. Ainsi, en octobre 2012, les diététistes membres de l'ODPQ ont eu la chance de s'exprimer quant à leurs attentes en matière de formation sur la nutrigénomique via un sondage en ligne transmis dans le bulletin électronique *Contact Courriel*. L'objectif primaire de ce sondage était de fournir une image globale de la situation actuelle sur la nutrigénomique dans la pratique professionnelle des diététistes de la province de Québec. Les résultats de ce sondage ont été publiés dans le *Journal of Human Nutrition and Dietetics*.

Un total de 421 diététistes a répondu au questionnaire en ligne. Toutefois, nous avons dû exclure 10 questionnaires pour des raisons de non-admissibilité (membres retraités ou étudiants) et 38 questionnaires en raison de données manquantes, ce qui porte le total de

questionnaires compilés et analysés à 373. Le questionnaire comprenait 34 questions, dont la plupart étaient des questions fermées.

Dans l'ensemble, 76,9 % des diététistes connaissent ce qu'est la nutrigénomique. Les diététistes ont identifié les cours universitaires (34,8 %, n = 143), les collègues (27,0 %, n = 111), les articles scientifiques (25,1 %, n = 103) et les réunions scientifiques (23,1 %, n=95) comme étant les meilleurs moyens d'acquérir des connaissances sur la nutrigénomique parmi les choix proposés : journaux (12,9 %, n = 53), la formation continue (12,2 %, n = 50), Internet (11,9 %, n = 49), les réseaux sociaux (5,1 %, n = 21), les autres professionnels de la santé (5,1 %, n = 21), la télévision (2,9 %, n = 12), la radio (1,0 %, n = 4), et les patients (0,7 %, n = 3). Actuellement, près de 76 % des diététistes qui travaillent en nutrition clinique (secteur public) estiment qu'ils n'ont pas les connaissances de base pour intégrer la nutrigénomique dans leur pratique professionnelle par rapport à 62,9 % pour les diététistes dans le secteur de la pratique privée.

En ce qui concerne les tests de nutrigénétique actuellement offerts sur le marché, 49,2 % des diététistes avec moins de 5 ans d'expérience affirmaient être à l'affût de la présence de tels tests comparativement à près de 12% chez les nutritionnistes avec plus de 25 années d'expérience. Lorsqu'interrogés sur les principales limites des tests de nutrigénétique, les diététistes affirment que les tests actuels ne tiennent pas compte des autres déterminants de la santé, que les tests génétiques ainsi que leurs résultats ont une précision incertaine, qu'il importe de considérer les aspects éthiques et légaux, qu'il y a un manque de données probantes, que l'application des tests génétiques dans le milieu clinique est difficile, qu'il peut résulter une mauvaise compréhension et/ou interprétation des résultats par les professionnels et/ou par les clients et soulèvent les coûts élevés des tests génétiques. Les résultats de cette étude montrent que les diététistes ne se sentent pas suffisamment qualifiés pour intégrer la nutrigénomique dans leur pratique professionnelle, même s'ils sont intéressés et y voient une occasion d'élargir leur champ de pratique. Les diététistes croient être les professionnels de la santé les plus qualifiés pour parler de la nutrigénomique avec leurs clients/patients. Dans ce contexte, il est particulièrement important que les diététistes acquièrent les connaissances et les compétences nécessaires pour intégrer cette science dans

leur pratique professionnelle. À la lumière de ces résultats, l'intégration de notions sur la nutrigénomique dans le cadre des études de premier cycle en nutrition semble être la meilleure façon de former les futurs diététistes.

Actuellement au Québec, aucune ligne directrice quant à l'utilisation des tests de nutrigénétique ne vient appuyer la pratique actuelle des professionnels. Les professionnels de la santé doivent donc rester à l'affût des publications et s'assurer de mettre à jour leurs compétences dans ce domaine. L'ensemble des conséquences de la génétique sur l'individu et ses proches, mais aussi sur le système de santé n'a pas fait l'objet d'études approfondies. D'ailleurs, selon les conclusions de la prise de position conjointe sur la nutrigénomique par l'OPDQ et le CMQ, les tests disponibles n'ont pas encore démontré une valeur ajoutée sur le plan clinique et plusieurs questions demeurent en suspens dont la couverture d'assurance ou même l'accès à des soins de santé en particulier [180].

Globalement, ce projet de doctorat a permis de mettre en lumière l'impact des SNPs du groupe de gènes *FADS1-2-3*, *ELOVL2-5* ainsi que plusieurs gènes de l'inflammation dans le métabolisme des AG et leurs effets d'interaction permettant entre autres de moduler les niveaux de TG plasmatiques ou de biomarqueur de l'inflammation suite à une supplémentation en huile de poisson. Ces résultats s'ajoutent à ceux déjà présents dans la littérature sur ces gènes qui semblent être des gènes d'intérêt pour plusieurs pathologies, y compris les MCV. Or, le pourcentage de variance expliqué par chacun des SNPs demeure faible et son utilité clinique n'est pas pertinente pour le moment. Des haplotypes, des modèles additifs, des modèles de prédiction ou des scores de risque génétique semblent toutefois être des voies potentiellement intéressantes à explorer afin d'expliquer un plus grand pourcentage de la variance dans la réponse des niveaux de TG plasmatiques suite à une supplémentation en huile de poisson. Les résultats devront toutefois être répliqués dans d'autres cohortes et avec d'autres populations/ethnies. De plus en plus d'études se penchent sur les adaptations génétiques à l'environnement. Comme les AGPI n-3 et n-6 à longue chaîne peuvent être facilement obtenus à partir d'un régime omnivore ou via une supplémentation en huile de poisson, il en est autrement dans une diète majoritairement végétarienne ou végétalienne. La synthèse endogène des AGPI n-3 et n-6 est donc essentielle chez cette population et passe notamment par l'implication des désaturases encodées par les gènes *FADS*. Une indel de 22

bp (rs66698963) a récemment été identifiée dans le gène *FADS2* chez les populations d'Asie du Sud-Est et en Afrique, une adaptation conséquente de leur régime alimentaire traditionnel et riche en aliments d'origine végétale [226, 227]. Cette indel est associée à une biosynthèse des AG plus efficace, leur conférant ainsi un avantage adaptatif contrairement aux Inuits du Groenland, une population qui consomme de grandes quantités d'AGPI n-3 d'origine marine, où plusieurs allèles de *FADS* sont associés à une biosynthèse beaucoup moins efficace [228].

En conclusion, la recherche en génomique nutritionnelle évolue rapidement. Plusieurs questions demeurent sans réponse malgré les grandes avancées dans ce domaine. Ainsi, chaque nouvelle publication résulte en une amélioration des connaissances qui mèneront un jour au concept de médecine/nutrition personnalisée. D'ici-là, de gros défis demeurent dont la formation des professionnels, les enjeux éthiques reliés aux banques de données génétiques et à leur utilisation ainsi que la transposition des résultats de recherche en des recommandations nutritionnelles tangibles. Le travail est colossal, puisque des adaptations génétiques modulées par la diète et/ou l'environnement apparaissent chez certaines populations, d'où la présence d'une grande variabilité interindividuelle. Néanmoins, il est justifié de croire que la nutrigénomique permettra un jour d'optimiser la santé des individus et des populations.

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